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ABSTRACTS

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Comparative MHC Genetics Workshop: Populations and Polymorphism

W100 Characterization of the functional and transcriptional variation of cattle MHC class I alleles. J. C. Schwartz*[1], G. Maccari[1,2], D. Heimeier[1], and J. A. Hammond[1], [1]*The Pirbright Institute, Guildford, UK,* [2]*Anthony Nolan Research Institute, London, UK.*

The major histocompatibility complex (MHC) class I region of cattle is both highly polymorphic and, unlike many species, highly variable in gene content between haplotypes. Historically, cattle MHC class I alleles were phylogenetically grouped based on sequence similarity in the more conserved 3′ end of the coding sequence, which formed the basis of cattle MHC class I nomenclature. We have annotated 5 complete cattle MHC class I haplotypes from the recent cattle and yak genome assemblies and compared them to a previous assembly of an A14 haplotype. Of the 5 likely pseudogenes previously described in the class I region, we found that one is putatively functional in all haplotypes and transcription was confirmed using allele-specific expression analysis of transcriptomic data. Two further previously identified pseudogenes are also putatively functional in some haplotypes, but transcription has yet to be confirmed. Based on full gene sequences as well as 3′ coding sequence, we identified subgroups of BoLA-3 and BoLA-6 that represent distinct genetic loci. Transcriptomic analysis reveals that certain allele groups within all loci appear to be consistently weakly expressed compared with others, which may reflect functional characteristics and a promiscuous affinity for peptides as evidenced in other species. These observations will help to inform further studies into how MHC class I region variability influences T cell and NK cell functions in cattle.

Key Words: cattle, yak, recombination, pseudogenes, BoLA

W101 Association of bovine leukemia virus-induced lymphoma with BoLA-DRB3 polymorphisms at the DNA, amino acid, and binding pocket property levels. C.-W. Lo*[1], S.-N. Takeshima[2,3], K. Okada[4], E. Saitou[5], T. Fujita[6], Y. Matsumoto[1], S. Wada[7], H. Inoko[8], and Y. Aida[1,2], [1]*Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan,* [2]*Viral Infectious Diseases Unit, RIKEN, Saitama, Japan,* [3]*Department of Food and Nutrition, Jumonji University, Saitama, Japan,* [4]*Iwate University, Iwate, Japan,* [5]*Hyogo Prefectural Awaji Meat Inspection Center, Hyogo, Japan,* [6]*Livestock Research Institute of Oita Prefectural Agriculture, Forestry and Fisheries, Research Center, Oita, Japan,* [7]*Photonics Control Technology Team, RIKEN Center for Advanced Photonics, Saitama, Japan,* [8]*Genome Analysis Division, GenoDive Pharma Inc., Kanagawa, Japan.*

Bovine leukemia virus (BLV) causes enzootic bovine leucosis, a malignant B-cell lymphoma in cattle. The DNA sequence polymorphisms of bovine leukocyte antigen (BoLA)-*DRB3* have exhibited a correlation with BLV-induced lymphoma in Holstein cows. However, little is known about the relationship between BLV-induced lymphoma and DRB3 at the amino acid and structural diversity levels. Here, we aim to comprehensively analyze the correlation between BLV-induced lymphoma and DRB3 at DNA, amino acid, and binding pocket property levels. Genomic DNAs from 106 BLV-infected but clinically normal Japanese black cattle and 227 BLV-infected Japanese black cattle with lymphoma which were collected from a nationwide survey in Japan were used in this study. BLV infection was determined using enzyme-linked immunosorbent assays targeting BLV-gp51. *BoLA-DRB3* genotyping was based on PCR-sequence-based typing method. BoLA-DRB3 molecules 3D structures and electrostatic surface potential modeling were analyzed based on the crystal structure of HLA-DRB1. Association study based on Fisher's exact test was performed by comparing the allele, genotype, or amino acid frequencies between asymptomatic and lymphoma cows. The results were penalized with the Bonferroni correction procedure to correct for false positive rate. In allele level, among 382 alleles, *DRB3*011:01* was identified as a resistance allele, whereas *DRB3*005:02* and *DRB3*016:01* were susceptibility alleles. Amino acid association studies showed that positions 9, 11, 13, 26, 30, 47, 57, 70, 71, 74, 78, and 86 were associated with lymphoma susceptibility. Structure and electrostatic charge modeling further indicated that binding pocket 9 of resistance DRB3 was positively charged. In contrast, alleles susceptible to lymphoma were neutrally charged. Altogether, this is the first association study of *BoLA-DRB3* polymorphisms with BLV-induced lymphoma in Japanese black cattle. In addition, our results further contribute to understanding the mechanisms regarding how *BoLA-DRB3* polymorphisms mediate susceptibility to BLV-induced lymphoma.

Key Words: bovine leukemia virus, lymphoma, *BoLA-DRB3*, peptide-binding pockets, association study

W102 Application of MHC sequencing to vaccine development: Proteome-wide analysis of zoonotic bacterium *Coxiella burnetii* for conserved T-cell epitopes presented by multiple host species. L. M. Wright Piel[1], C. J. Durfee[1], and S. N. White*[1,2], [1]*USDA-ARS Animal Disease Research, Pullman, WA, USA,* [2]*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA,* [3]*Center for Reproductive Biology, Washington State University, Pullman, WA, USA.*

Immunoinformatic methods can leverage both host and pathogen genetic variation, but most proteome-wide T-cell epitope studies have focused on viral applications [11]. The technology is maturing for leveraging MHC sequences from a wide variety of host species and analysis of the bacterial proteome requires greatly increased calculations (by multiple orders of magnitude). *Coxiella burnetii* is a globally distributed (except New Zealand) gram-negative bacterium responsible for human Q fever and coxiellosis in ruminant livestock. Previous vaccines with whole cell inactivated bacteria can confer protection but can also produce reactogenic immune responses. A protective vaccine is required that does not cause excessive immune reactions. T-cell immunity plays a critical role in *C. burnetii* control, since either $CD8^+$ or $CD4^+$ T cells can empower clearance. We sought to identify *C. burnetii* epitopes that can interact with a range of major histocompatibility complex (MHC) alleles from multiple relevant host species (including human, mouse, and cattle). We screened 1,815 proteins from the reference Nine Mile phase I (RSA 493) *C. burnetii* assembly and we removed 402 proteins due to a lack of inter-isolate conservation. We eliminated an additional 391 proteins due to the presence of host homology to avoid potential autoimmune responses. We analyzed the 1,022 remaining proteins for ability to produce peptides that bind MHCI or MHCII. MHCI and MHCII predicted epitopes were filtered and compared between species yielding 777 MHCI epitopes and 453 MHCII epitopes. There were 31 epitopes with overlap between MHCI and MHCII across host species. Of these, there were 9 epitopes within epitope-dense proteins containing ≥ 5 total epitopes. Overall, 55 proteins contained high scoring T-cell epitopes. In addition to Com1, most proteins were novel compared with previously interrogated vaccine candidates. These data contain the first proteome-wide assessment of *C. burnetii* peptide epitopes. Furthermore, we captured a range of hosts for this zoonotic pathogen through the inclusion of human, mouse, and bovine data, demonstrating opportunities for widely useful *C. burnetii* vaccine construction. This work identified new vaccine targets and enhanced opportunities for selecting T-cell epitopes in vaccine design.

Key Words: *Coxiella burnetii*, T-cell epitope, proteome-wide, cross-species

W103 Withdrawn

W104 Molecular characterization of swine leukocyte antigen (SLA) gene diversity in European farmed pigs. S. E. Hammer*[1], T.

Duckova[1], S. Groiss[1], M. Stadler[1], M. Jensen-Wearn[2], W. T. Golde[3], U. Gimsa[4], and A. Saalmueller[1], [1]*University of Veterinary Medicine Vienna, Vienna, Austria,* [2]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [3]*Moredun Research Institute, Edinburgh, Scotland, UK,* [4]*Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

In Europe, swine represent economically important farm animals and furthermore have become a preferred preclinical large animal model for biomedical studies, transplantation, and regenerative medicine research. The need for typing of the swine leukocyte antigen (SLA) is increasing with the expanded use of pigs as models for human diseases and organ-transplantation experiments, their use in infection studies, and for design of veterinary vaccines. In this study, we characterized the SLA class I (SLA-1, SLA-2, SLA-3) and class II (DRB1, DQB1, DQA) genes of 549 farmed pigs representing 9 commercial pig lines by low-resolution SLA haplotyping. In total, 50 class I and 37 class II haplotypes were identified in the studied cohort. The most common SLA class I haplotypes Lr-04.0 (SLA-1*04XX-SLA-3*04XX(04:04)-SLA-2*04XX) and Lr-32.0 (SLA-1*07XX-SLA-3*04XX(04:04)-SLA-2*02XX) occurred at frequencies of 11.02 and 8.20%, respectively. For SLA class II, the most prevalent haplotypes Lr-0.15b (DRB1*04XX(04:05/04:06)-DQB1*02XX(02:02)-DQA-*02XX) and Lr-0.12 (DRB1*06XX-DQB1*07XX-DQA*01XX) occurred at frequencies of 14.37 and 12.46%, respectively. Meanwhile, our laboratory contributed to several vaccine correlation studies (e.g., PRRSV, CSFV, FMDV, swine influenza A virus) elucidating the immunodominance in the T-cell response with antigen-specificity dependent on certain SLA-I and SLA-II haplotypes. Moreover, these SLA-immune response correlations could facilitate tailored vaccine development, as SLA-I Lr-04.0 and Lr-32.0 as well as SLA-II Lr-0.15b and Lr-0.12 are highly abundant haplotypes in European farmed pigs.

Key Words: *Sus scrofa*, sequence-specific primer PCR, polymorphism, swine leukocyte antigen (SLA)

W105 Expression of genes related with immunomodulation and immunogenicity of equine mesenchymal stem cells: Influence of major histocompatibility complex. A. Cequier*[1], S. Fuente[1,2], A. Vitoria[1,2], A. Romero[1,2], F. Vázquez[1,2], C. Rodellar[1], and L. Barrachina[1,2], [1]*Laboratorio de Genética Bioquímica LAGENBIO (Universidad de Zaragoza), Instituto Agroalimentario de Aragón (IA2), Universidad de Zaragoza-CITA; Instituto de Investigación Sanitaria de Aragón (IIS), Zaragoza, Spain,* [2]*Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, Zaragoza, Spain.*

Mesenchymal stem cells (MSCs) can be used for therapy due to their immunomodulatory properties and the horse is a suitable animal model to develop them. Allogeneic application presents several advantages over autologous therapy, but MSC immunogenicity should be considered. The immune response to equine allogeneic MSCs can be influenced by donor-receptor MHC-matching/mismatching and MHC-expression level, which can be modified by inflammation. The aim of this study was to analyze the gene expression of molecules related to the immunoregulatory and immunogenic profiles of equine MSCs, basal (MSC-B) and proinflammatory primed (MSC-P), after their coculture in vitro with lymphocytes from either autologous or allogeneic MHC-matched/mismatched animals. MHC-haplotypes were determined by analyzing 10 intra-MHC microsatellites regions associated with Equine Leukocyte Antigens. MSCs from 3 MHC-homozygous horses were exposed to activated lymphocytes from autologous and allogeneic horses for 3 d. Afterward, MSC gene expression was assessed by RT-qPCR for immunomodulatory (vascular cell adhesion molecule 1, VCAM-1; cyclooxygenase 2, COX-2; indoleamine 2–3-dioxygenase, IDO; inducible nitric oxide synthase, iNOS; interleukin 6, IL-6) and immunogenicity (MHC-I, MHC-II, CD40, CD80) genes. Gene expression of both immunomodulatory and immunogenicity-related genes was higher in all MSC-P combinations over MSC-B. Specifically, IL-6 was significantly upregulated in MSC-P exposed to both MHC-matched and mismatched lymphocytes, whereas only MSC-P exposed to MHC-mismatched lymphocytes also overexpressed VCAM-1, MHC-I and CD40. These results suggest that inflammation increases both the immunogenic and immunomodulatory MSC profiles. Importantly, the cellular response in an MHC-mismatched setting would promote a further upregulation of these genes. Further studies are needed to clarify how these changes may be translated in vivo into an immune recognition with clinical implications. The MHC-matching between donor and recipient is closely related to the immune recognition of allogeneic MSCs and thus, to the effectiveness and safety of the therapy.

Key Words: horses and related species, cell biology, immunology, microsatellite, qPCR

W106 Sequencing of LEI0258 marker reveals populations' specific alleles and new repeat motif patterns. P. Manjula*[1], T. Kalhari[2], S. Cho[1], M. Kim[2], E. Cho[3], and J. Lee[1,2], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea,* [2]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea,* [3]*Department of Bio big Data, Chungnam National University, Daejeon, Republic of Korea.*

Chicken MHC-*B* diversity can be assessed using MHC-linked microsatellite markers including LEI0258. The LEI0258 is a variable number tandem repeat (VNTR) marker characterized by 2-repeat sequences of 13 bp and 12 bp. Sequence information of LEI0258 alleles exemplifies the repeat motif variations, upstream and downstream insertion/deletion, and SNPs (single-nucleotide polymorphisms). This method provides a clearer interpretation for the particular VNTR marker while implicitly indicating the MHC allele diversity in chicken breeds around the world. In this study, we analyzed a total of 604 LEI0258 sequences from Asian, African, and Americas and commercial chicken populations. As the allele size (bp) is mainly determined by its R13 and R12 copy number variations, the repeat motif combination patterns that correspond to each allele size and their additional polymorphisms were evaluated. A total of 86 allele sizes (182 – 552 bp) were reported. Asian and African chicken possess a higher number of alleles, numerically 61 and 57 than that of Americas and commercial breeds. Eighteen shared alleles, and varied private alleles were identified. Accordingly, 46 repeat motif combinations were reported in 86 allele sizes, including 14 novel combinations in Asian, 2 in the Americas, and one in African chickens. This number was evidently higher than the previously reported, and greatly supports the extreme polymorphism of the LEI0258 marker. In 26 alleles, these combinations consisted of a single copy of R13 with different R12 repeats (2 to 28), and remained alleles consist of varied copies of R13 and R12 repeats. Moreover, the results indicate that the same allele size can occur with different combinations due to the homoplasy. Additional allele variations were observed as different fragment sizes that possess the same repeat combination due to the occurrence of various indels and SNPs in the upstream and downstream of VNTR. As per the results, the loss or gain of VNTR and additional polymorphisms that collectively determine the allele variations for LEI0258, claim higher MHC diversity in Asian and African chickens.

Key Words: LEI0258, variable number tandem repeat (VNTR), MHC, allele diversity

W107 Evaluation of polymorphisms in BLB-2 gene in Korean Ogye chicken using NGS data. T. Kalhari*[1], P. Manjula[2], S. Cho[2], M. Kim[1], E. Cho[3], and J. Lee[1,2], [1]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Korea,* [2]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea,* [3]*Department of Bio-big data, Chungnam National University, Daejeon, Korea.*

The chicken MHC region contains highly complex and polymorphic genes that are involved in adaptive immune responses. Its competency primarily depends on the ability to identify specific pathogens. Consequently, the exon 2 of *BLB2* gene located at the MHC-B class II region, heavily responsible for the formation of Peptide Binding Regions (PBRs) of class II glycoproteins which detects such pathogens becomes crucial.

In this study, the Korean Ogye chickens, a national treasure in South Korea, were evaluated for the polymorphisms in exon 2 of the *BLB2* gene. The Ogye genome was sequenced using Illumina Next-Generation Sequencing followed by variant calling, utilizing NGS GATK best practices pipeline. Next, the targeted exon of *BLB2* was sequenced using Sanger sequencing, to confirm the in silico obtained variants in the same population. As per the NGS data, a total of 20 variants were called for the entire exon 2 (270 bp) of *BLB2*, including 15 missense, 2 frameshift, 1 synonymous, 1 conservative inframe insertion, and 1 disruptive inframe deletion. Focusing on the confirmation of derived variants, they were compared with the Sanger sequencing data and all most all the variants were well confirmed. This characterizes a higher polymorphism in Ogye and this fairly manifests that especially these SNPs might involve in attributing unique immunity in Korean Ogye chicken. However, further studies are warranted to analyze the selection on variants and potential structure alterations in PBRs.

Key Words: NGS, Sanger sequencing, Ogye, MHC, variant

W108 The IPD-MHC Database: Novel tools for the study of the major histocompatibility complex. G. Maccari*[1,2], J. Robinson[2,3], J. A. Hammond[1], and S. G. E. Marsh[2,3], [1]*The Pirbright Institute, Pirbright, Woking, Surrey, UK,* [2]*Anthony Nolan Research Institute, Royal Free Campus, London, UK,* [3]*UCL Cancer Institute, Royal Free Campus, London, UK.*

The IPD-MHC Database provides a centralized resource for the collection and analysis of sequences found within the major histocompatibility complex (MHC) of nonhuman species. Overseen by the Comparative MHC Nomenclature Committee, it is therefore the primary source of data for the study of the nonhuman MHC. Since 2015 the IPD-MHC project has been through a structured program of improvement, aimed at expanding the content and improving the utility of the database in line with improved sequencing methods and community demand. As a result, the IPD-MHC database has been redesigned to provide a unified resource for the inter- and intra- species comparison of genomic and nongenomic data from different taxonomic groups. This work has been performed in synergy with the Comparative MHC Nomenclature Committee to draft a unified and improved set of guidelines for the allele nomenclature to cover MHC variation at genomic level. The project has grown in both content and impact, now hosting 95 different species and almost 12,000 alleles. The increasing availability of high-quality, manually curated data spurred the need for advanced tools for the analysis and interpretation of allele variation. This included the redesign of existing tools to provide new pathways to access and consume the expanded volume of data. The IPD project has recently introduced a centralized API allowing the programmatic interrogation of the databases, and a more sophisticated extrapolation of the available data. A primer design and virtual PCR tool has recently been integrated, providing a resource for the ad hoc design of inter- or intra- species, locus-specific or allele-specific probes. As a consequence of improving the bioinformatic framework, additional species-specific metadata is hosted, including a taxonomic-specific haplotype section. Structural information from homology models will be included for class I MHC alleles, providing insight on the overall structure and the specific impact of variation, including the ability to allow users to analyze and compare peptide binding pockets. With the latest improvements and expansions, we hope that the future of the IPD-MHC database has been secured by increasing the utility of the high-quality data being hosted.

Key Words: bioinformatics, MHC, comparative genomics, databases/repositories, polymorphism

Plenary I

W109 Combining quantitative genetics and population genomics to improve beef sustainability. J. E. Decker*[1,2,3], T. N. Rowan[1,2,4,5], S. M. Nilson[1], H. J. Durbin[1,2], C. U. Braz[1], R. D. Schnabel[1,2,3], and C. M. Seabury[6], [1]*Division of Animal Sciences, University of Missouri, Columbia, MO, USA,* [2]*Genetics Area Program, University of Missouri, Columbia, MO, USA,* [3]*Institute for Data Science and Informatics, University of Missouri, Columbia, MO, USA,* [4]*Department of Animal Science, University of Tennessee, Knoxville, Knoxville, TN, USA,* [5]*College of Veterinary Medicine, Large Animal Clinical Science, University of Tennessee, Knoxville, TN, USA,* [6]*Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA.*

Quantitative genetics and population genetics are related, but often disconnected, scientific disciplines. Whereas applied quantitative genetic focuses on creating genetic change in the future, population genetics looks at what genetic changes have occurred in the past. However, combining theories, concepts and open questions from these 2 disciplines allows us to better understand phenotypic variation and to create tools to select more sustainable cattle populations. Cattle poorly adapted to their environment loose revenue and jeopardize sustainability of the food supply. Genomic data allows us to rigorously analyze local adaptation and genotype-by-environment interactions. We use local adaptation selection scans, genotype-by-environment genome-wide association analyses, and ecoregion-specific genomic predictions of growth traits to predict local adaptation in beef cattle. Analyzing ~160,000 cattle from 2 breed associations with ~850,000 high-accuracy imputed SNPs, we used linear mixed model selection mapping methods (envGWAS) to identify genomic loci associated with adaptation. Among these data sets, we identify 114 genes responding to local adaptation selection. When genotype-by-environment interactions exist, ranking animals using an ecoregion-specific genetic evaluation will be different from national cattle evaluations. We developed ecoregion-specific genomic predictions using both a genomic additive relationship matrix and a genotype-by-environment (GxE) relationship matrix. When using this model, prediction accuracy for additive effects remained the same or slightly improved, and we were also able to predict random GxE effects. Across all analyses, genes related to immunity and the circulatory system were enriched. Prototype region-specific genomic predictions will identify cattle best suited to an environment.

Small Ruminant Genetics and Genomics Workshop

W110 Invited Workshop Presentation: High-quality assembly of Tibetan sheep genome helps reveal high-altitude hypoxia adaptation's genetic mechanism. X. Li*[1,2] and M.-H. Li[3,1], [1]*CAS Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences (CAS), Beijing, China,* [2]*University of Chinese Academy of Sciences (UCAS), Beijing, China,* [3]*College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Tibetan sheep, a hypoxia-tolerant species that live in extremely high-altitude environment, is a remarkable model to help investigate genetic mechanism of high-altitude hypoxia adaptation. In this study,

we assembled a high-quality genome of Tibetan sheep with a contig N50 length of 77.47 Mb using a combination Illumina and Pacbio high-throughput sequencing system (https://www.ncbi.nlm.nih.gov/assembly/GCA_017524585.1/). After the contigs were scaffolded and then clustered through Hi-C data, a final 2.65 Gb genome was de novo assembled consisting of 27 chromosomes. After annotation, we obtained 20,919 gene models, and BUSCO analysis showed a high degree of completeness of genome, with 911 out of 954 (95.49%) complete eukaryotic universal genes. Gene family clustering analysis of 9 mammal taxa (Tibetan sheep, Marco Polo sheep, goat, cattle, yak, pig, horse, dog and human) identified 19,092 gene families. The unique genes in Tibetan sheep are over represented in cellular component (e.g., mitochondrial respiratory chain) and pathways (e.g., oxidative phosphorylation, thermogenesis and cardiac muscle contraction), suggesting a role of these genes in high-altitude hypoxia adaptation. Furthermore, selective analysis through comparing 15 Tibetan sheep (>3,000 m above sea level) with 10 Hu sheep (<100 m) using whole-genome SNP data, and comparative genomic analysis between 2 assembly genomes (Tibetan versus Rambouillet sheep) also help us identify a series of candidate genes, nonsynonymous SNPs and SVs associated with high-altitude hypoxia adaptation.

Key Words: Tibetan sheep, de novo, gene family clustering, comparative genomics, high-altitude hypoxia adaptation

W111 Invited Workshop Presentation: The genome landscape of worldwide sheep reveals genetic mechanism of multiple morphological and agronomic traits. M.-H. Li*, *College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Sheep (*Ovis aries*) have become well adapted to a diverse range of agroecological zones with radical phenotypic, morphological and behavioral divergency after natural and human selection. Here, through comparing the resequencing genomes of 16 Asiatic mouflon and 5 old landrace populations (i.e., Dutch Drenthe Heathen, Hu sheep, Altay sheep, Djallaonké and Karakul sheep) originated from different geographic and genetic origins, we identified 261 candidate domesticated genes (e.g., *SLC11A1*, *HOXA11*, *CAMK4*, *LEF1*, *TET2*, *KDR*, *CTBP1*, *GAK*, *CPLX1*, *PCGF3*, *FLT1*, *BCO2*, *CHGA*, *HTRA1*, *IGF2BP2*, *RFX3*, *MRPL41*, *KITLG*, *HERC5*, *MAN2B2*, *FGFRL1*, *PDE6B*, *EDN3*, *RALY*, *GTF2I*, *GTF2IRD1*) associated with functions including female reproductive traits, resistance to infection, bone formation, fat deposition, pigmentation and behavior. Furthermore, we detect strong signatures of selection in genes involved in the hypoxia and UV signaling pathways (e.g., HIF-1 pathway and HBB and MITF genes) using resequencing data of Tibetan sheep on the Qinghai–Tibetan Plateau and northern Chinese sheep from low-altitude region. Particularly, we identified PDGFD as a likely causal gene for fat deposition in the tails of sheep through performing separate pairwise-population selection tests using comparisons of fat-rumped (Altay sheep) and long fat-tailed (Large-tailed Han sheep) breeds with short fat-tailed (Tan sheep) short thin-tailed (Shetland sheep) breeds. Transcriptome, RT-PCR, qPCR and western blot analyses demonstrated the gene and protein expression level of PDGFD were consistently correlated negatively with fat deposition. This gene functions by activating PDGF receptor PDGFRβ which has an essential role in inhibiting differentiation of white adipocytes. In addition, we also found various selected regions, novel functional genes and nonsynonymous SNPs which may be responsible for traits such as reproduction (e.g., *BMPR1B*), milk yield (e.g., *ARHGEF4* and *IQGAP3*), wool fineness (e.g., *KRT1*, *KRT2*, *KRT71*, *KRT72*, and *KRT74*), body size (e.g., *ATM* and *LINGO2*), numbers of horns (e.g., *RXFP2*, *HOXD1*, *HOXD3*, *HOXD8*, *HOXD9*, and *HOXD10*) and nipples (e.g., *SYNDIG1L*), pigmentation (e.g., *MITF*) and ear size (e.g., *LEMD3* and *MSRB3*).

Key Words: worldwide sheep, whole-genome resequencing, morphological and agronomic traits, genome-wide selection tests

W112 Comparative transcriptome analysis between suckling lambs with different levels of perirenal adipose tissue in the carcass. M. Alonso-García[1], A. Suárez-Vega[1], J. Mateo[2], H. Marina[1], R. Pelayo[1], C. Esteban-Blanco[1], J. J. Arranz[1], and B. Gutiérrez-Gil*[1], [1]*Departamento de Producción Animal, Facultad de Veterinária, Universidad de León, Campus de Vegazana, León, León, Spain,* [2]*Departamento de Higiene y Tecnología de los Alimentos, Facultad de Veterinária, Universidad de León, Campus de Vegazana, León, León, Spain.*

Suckling lamb meat is very appreciated in the European-Mediterranean region. This meat is tender, juicy and has a smooth texture. Suckling lamb carcass quality is positively related to the amount of perirenal adipose tissue, which is the predominant carcass internal fat depot. Quality traits of interest in this production show a strong influence of maternal effects as the lambs are fed exclusively of milk and slaughtered between 21 and 30 d of age. RNA-sequencing (RNA-seq) has proven to help expand our understanding of the relationships between the transcriptome and the phenotype across different physiological, treatment, or disease conditions. The objective of the present study was to compare the perirenal fat transcriptome between lambs with high and low percentages of perirenal fat in the carcass. For that, 18 male Spanish Assaf lambs born in the same flock and lambing season from primiparous ewes were initially considered. After birth the lambs had colostrum access for 4 to 8 h, and they were then fed ad libitum with reconstituted milk replacer powder. The animals were slaughtered when they reached the market live-weight (9–12 kg). At slaughter, perirenal adipose tissue samples were collected from each lamb for RNA extraction. After a phenotypic characterization of the carcass composition, RNA samples from the 4 lambs with the highest percentage of perirenal fat (high-PFP group) and the 4 lambs with the lowest PFP (low-PFP group) were analyzed through RNA-seq. The differential expression analysis performed with *DESeq2* identified a total of 101 differentially expressed genes (DEGs) ($P_{adj} < 0.05$). Enrichment analyses for the 58 genes with higher expression levels in high-PFP lambs than in low-PFP lambs highlighted terms related the fatty acid metabolism such as *fatty acid derivative biosynthetic process* and *response to fatty acid (GO Biological Process)*, *Sterol Regulatory Element-Binding Proteins* (SREBP) *signaling* (*Pathway*) and *Obesity (Disease)*. This study provides a preliminary evaluation of genes and physiological pathways that may underlie phenotypic variation of the levels of perirenal adipose tissue in Assaf suckling lambs.

Key Words: sheep and related species, transcriptome, fat/lipid, meat production

W113 Exploring differentially expressed genes in hypothalamic transcriptome in different sexual behavior phenotypes in rams using RNA-seq. K. Lakhssassi*[1,2], I. Ureña[3], B. Marín[4], M. P. Sarto[1], B. Lahoz[1], J. L. Alabart[1], J. Folch[1], M. Serrano[3], and J. H. Calvo[1,5], [1]*Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)-IA2, Zaragoza, Spain,* [2]*Institut National de la Recherche Agronomique, Rabat, Morocco,* [3]*Instituto Nacional de Investigación y Tecnología Agrária y Alimentaria (INIA), Madrid, Spain,* [4]*Universidad de Zaragoza, Zaragoza, Spain,* [5]*Fundación Agencia Aragonesa para la Investigación y el Desarrollo (ARAID), Zaragoza, Spain.*

The ram effect is commonly used to improve the out of season reproduction, showing that males with greater sexual behavior (mounts and services) produce a greater stimulus during seasonal anestrus, which lead into a higher percentage of mated ewes and higher fertility. However, there is a considerable variation in sexual behavior among rams. The main objective of this study was to investigate transcriptional changes in the hypothalamus (HT), key tissue in sexual activity regulated by photoperiod, in 2 groups of rams with different sexual behavior using RNA-seq. First, 59 rams were submitted to individual sexual behavioral pen tests, twice for each ram. Each ram was exposed to 3 estrous females for 20 min to observe their behavior, and count the frequency of mounts and ser-

vices. Therefore, 2 homogeneous groups of rams were identified: active (A) (7.93 ± 3.56, average mounts ± SD) and not active (NA; without any mount). Six rams of each group were killed and total RNA was extracted from HT. Sequencing was carried out generating Illumina paired-end reads of 151 bp. Gene level quantification was estimated using HTSeq, while differential expression and pathway analysis to find regulated functional groups were performed with EdgeR and Gene Set Enrichment Analysis (GSEA), respectively, using the OmicsBox package from BioBam. In the comparison A *vs* NA, 52 differentially expressed genes (DEGs) were found being 41 and 11 genes up- and downregulated, respectively. One of the most outstanding upregulated genes was the *PDYN* that has been related to sexual motivation in male European starlings. Therefore, it has been proposed as one of the neuropeptides that are involved in the control of sexual behavior at the central level. Finally, enrichment analysis including 17,003 DEGs expressed in the HT yielded 130 overrepresented pathways at FDR q-value 5%. The most interesting GO was related to behavior (GO:0007610) with 133 enriched genes including *Neuropeptide Y* (*NPY*) and *Pro-Melanin Concentrating Hormone* (*PMCH*) genes, also involved in the control of sexual behavior whereas others genes such as *CIART* and *NR1D1*, belong to circadian rhythm pathways.

Key Words: sheep, RNA-seq, ram, sexual behavior

W114 Unveiling genomic regions that underlie footrot resistance in Portuguese sheep Merino. D. Gaspar*[1,2], A. Usié[1,3], C. Leão[3,4], C. Matos[5], L. Padre[3], C. Dias[3], C. Ginja[2], and A. M. Ramos[1,3], [1]*CEBAL – Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo, Beja, Portugal,* [2]*CIBIO/InBIO – Research Centre in Biodiversity and Genetic Resources, University of Porto, Vairão, Porto, Portugal,* [3]*MED-Mediterranean Institute for Agriculture, Environment and Development, University of Évora, Évora, Portugal,* [4]*INIAV (Instituto Nacional de Investigação Agrária e Veterinária), Santarém, Portugal,* [5]*ACOS – Agricultores do Sul, Beja, Portugal.*

Footrot is an acute necrotic and highly contagious disease, caused by a co-infection of 2 g-negative anaerobic bacteria, *Dichelobacter nodosus* and *Fusobacterium necrophorum*. It affects the interdigital skin and hooves of sheep, being the main cause of lameness and a major animal welfare and economical concern for the wool, milk and meat sheep industries worldwide. Current effective strategies to control footrot are costly and rely on the use of antibiotics, which could result in the development of parasite resistance mechanisms in the long term. The development of genomic markers associated with footrot resistance can provide a more reliable strategy for classifying and selecting sheep with increased resistance, besides enhancing our understanding of the biology of this disease. We aimed to identify genomic regions and molecular mechanisms associated with resistance to footrot in Portuguese native Merino breeds. For this, a set of 50k single-nucleotide polymorphisms (SNPs) was specifically designed based on whole-genome data obtained for 39 sheep (depth of coverage >22X). A total of 1,466 Portuguese Merino sheep were genotyped using this SNP array. Genome-wide association analysis was performed using a quantitative trait approach based on the modified Egerton system (scores from 0 to 5) for foot integrity and footrot lesions. Genome-wide significance was determined using corrected p-values for multiple testing and SNPs significantly associated with footrot resistance were filtered at a genome-wise false discovery rate of 5%. Our results revealed a set of promising SNPs associated with resistance to footrot that overlaps candidate genes related to immune response and wound healing. These findings contribute to better understanding the architecture of footrot resistance in Merino sheep and to enhance the development of genomic tools to control infections. Also, the whole-genome data were used to investigate the underlying population structure of these native Iberian Merino breeds in the context of worldwide sheep, which is useful to define conservation and management programs.

Key Words: sheep, Merino, footrot, GWAS

W115 Identification of a novel loss-of-function variant in the ovine *TMCO6* gene associated with motor neuron disease of North Country Cheviot sheep. A. Letko*[1], I. M. Häfliger[1], E. Corr[2], F. Brulisauer[2], S. Scholes[2], and C. Drögemüller[1], [1]*Institute of Genetics, Bern, Switzerland,* [2]*SRUC Consulting Veterinary Services, Penicuik, Midlothian, UK.*

Motor neuron diseases (MND) occur sporadically in farm animals including sheep. The aim of our study was to characterize the phenotype and the genetic etiology of an early-onset neurodegenerative disorder observed in several lambs of purebred North Country Cheviot sheep, a native Scottish breed. Affected lambs showed progressive ataxia and subsequent histopathological analysis revealed motor neuronal degeneration including cytoplasmic vacuolation. By whole-genome sequencing of 4 affected lambs, we identified a shared homozygous loss-of-function frameshift variant in exon 6 of the ovine *TMCO6* gene on chromosome 5. Herein we present evidence for the occurrence of a familial novel form of a recessively inherited MND in sheep due to a likely pathogenic 4bp deletion that is assumed to lead to a dysfunction of a transmembrane and coiled-coil domain-containing protein 6 (TMCO6: p.Leu215PhefsTer34). The uncharacterized TMCO6 protein is proposed to interact with the ubiquilin-1 (UBQLN1) protein, which plays an important role in the regulation of different protein degradation mechanisms and pathways and is reported to be associated with sporadic forms of amyotrophic lateral sclerosis (ALS). Therefore, these findings implicate an important role of TMCO6 for proper function and survival of motor neurons and provide a novel candidate gene for human ALS or similar motor neuron disease. Furthermore, these results enable selection against the fatal disorder in sheep population.

Key Words: neurogenetic disorder, rare disease, precision medicine, whole-genome sequencing, membrane trafficking

W116 A homozygous frameshift variant in *MFSD2A* associated with congenital brain hypoplasia in a Kerry Hill sheep family. G. Lühken*[1], A. Letko[2], M. Häfliger[2], M. J. Schmidt[3], C. Herden[4], L. Herkommer[4], J. Müller[4], and C. Drögemüller[2], [1]*Institute of Animal Breeding and Genetics, Justus Liebig University, Giessen, Germany,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [3]*Clinic for Small Animals, Neurosurgery, Neuroradiology and Clinical Neurology, Justus Liebig University, Giessen, Germany,* [4]*Institue of Veterinary Pathology, Justus Liebig University, Giessen, Germany.*

In several consecutive years, a German breeder observed male and female purebred Kerry Hill sheep lambs with severe ataxia and in some cases with convulsions. The lambs died after some days to weeks. All affected lambs descended from the same sire or one of his sons. We hypothesized an autosomal recessive inherited brain disorder underlying the observed deaths and aimed to identify the potential causal allele. Imaging with CT and MRT of 2 affected lambs revealed a reduced skull circumference compared with age matched controls. The forebrain appeared unusually small in relation to the cerebellum and midbrain. The cerebral cortical surface pattern was simplified (pachygyria). There was moderate ventriculomegaly. The cortical and subcortical white matters were thin and the contrast between white and gray matter was diminished. Pathological examination confirmed these findings. Additionally, a multifocal mild cortical dysplasia was found. CNS infection was ruled out. Genotyping 5 affected lambs on the 50k ovine SNP array allowed us to localize the critical genome regions harboring the causative variant to 3 shared IBD segments of totally 8 Mb on different chromosomes by homozygosity mapping. By whole-genome sequencing of an affected lamb, homozygous private variants called in this single case were identified by comparison with 86 publicly available control sheep genomes. This yielded 101 private homozygous protein-changing variants affecting 73 different genes. Within the critical intervals there was only a single variant predicted to be nonsynonymous. Genotyping using Sanger sequencing showed perfect co-segregating of this variant with the observed disorder in the studied

Kerry Hill family, consistent with autosomal recessive inheritance. This private homozygous loss-of-function frameshift variant in exon 3 of *MFSD2A* (c.285dupA; p.Asp96fs) is predicted to truncate the encoded protein. MFSD2A is part of the blood-brain barrier. In humans, missense mutations in *MFSD2A* are associated with progressive microcephaly, spasticity, and brain imaging abnormalities.

Key Words: sheep and related species, genome sequencing, genetic disorder, animal health

W117 Identification of novel SNPs associated with litter size in Rasa Aragonesa sheep breed. K. Lakhssassi[1,2], J. Grimplet[1], M. P. Sarto[1], B. Lahoz[1], J. L. Alabart[1], J. Folch[1], M. Serrano[3], and J. H. Calvo*[1,4], [1]*Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) – IA2, Zaragoza, Spain,* [2]*Institut National de la Recherche Agronomique (INRA), Rabat, Morocco,* [3]*Instituto Nacional de Investigación y Tecnología Agrária y Alimentaria (INIA), Madrid, Spain,* [4]*Fundación Agencia Aragonesa para la Investigación y el Desarrollo (ARAID), Zaragoza, Spain.*

Rasa Aragonesa is an autochthonous Mediterranean sheep breed, mainly reared in extensive or semi-extensive farming systems and oriented to meat production. Three *FecX*-mutated alleles called $FecX^{R}$, $FecX^{Gr}$ and $FecX^{Ra}$ in *BMP15* gene have been described in this breed up to now. In a previous research, a genome-wide association study (GWAS) using 158 ewes (73 high prolific *vs* 85 low prolific ewes) genotyped with the Illumina AgResearch Sheep HD (680K) SNP array was performed, identifying the $FecX^{Gr}$ allele at genome-wide level, and also 11 significant SNPs at chromosome-wide level. Therefore, the main objective of this study was to investigate the GWAS significant regions at chromosome level and identify new SNPs/genes associated with litter size in Rasa Aragonesa sheep breed. First, we annotated the genes identified in the 500 kb region on both sides of the significant SNPs at chromosome-wide level. Genes and SNPs were mapped on the Oar v3.1 (Texel) sheep genome. We identified 8 (*MTFMT*, *PTGER2*, *SLC51B*, *RASL12*, *KBTBD13*, *TXNDC16*, *GNPNAT1* and *ERO1A*), 1 (*ARID1B*), 4 (*CCT6B*, *NLE1*, *RAD51D* and *LIG3*) and 6 (*HK1*, *TACR2*, *AIFM2*, *TYSND1*, *ZMIZ1* and *POLR3A*) genes in chromosomes 7, 8, 11 and 25 respectively, related to reproduction. To identify putative SNPs related to litter size, we chose 8 prolific ewes without the *FecX* alleles to be sequenced using low coverage whole-genome sequencing (lcWGS; 10x). Analysis was run through Galaxy project: Trimmomatic was used to trim reads and remove adapter sequences, Bowtie2 to align reads to the reference genome Oar v3.1 and finally FreeBayes was used for variant calling. In total, we selected 14 SNPs within the genes described above based on the impact of amino acid substitution on the structure and function of the protein using the PolyPhen-2 and Variant Effect Predictor (VEP) tools. The genotype of these SNPs obtained by lcWGS were validated using Sanger sequencing. The lcWGS results were confirmed for 7 SNPs, located within *TXNDC16*, *ZMIZ1* and *POLR3A* genes. Finally, 4 SNPs located in the *ZMIZ1* and *POLR3A* genes were genotyped to study their effect in the GWAS population.

Key Words: litter size, whole-genome sequencing (WGS), SNP, sheep

W118 Machine learning algorithm to predict coagulating milk factor through milk traits in 2 sheep breeds. H. Marina*, B. Gutiérrez-Gil, R. Pelayo, A. Suárez-Vega, C. Esteban-Blanco, and J. Arranz, *Departamento de Producción Animal, Facultad de Veterinária, Universidad de León, Campus de Vegazana s/n, León, Spain.*

Sheep milk is mainly used to manufacture high-quality cheeses. In the last years, breeding programs aimed at increasing total milk solids due to the economic importance of cheese yield for the sheep dairy industry. Consequently, several studies focused on the milk coagulation properties (MPC) and cheese production parameters have been performed in dairy sheep. At the practical level, noncoagulation of sheep milk samples has been an important problem for the dairy industry. Previous studies in Spanish Assaf and Churra sheep breeds identified about 13% and 4% of noncoagulating samples within 60 min from the addition of the clotting enzyme, respectively. The routine measurement of MCP in commercial populations for inclusion as selection criteria in sheep breeding programs is expensive and not practical. Therefore, this study aimed to employ machine learning (ML) approaches to integrate the phenotypes registered by the official milk recording system and the pH of the milk to predict the coagulation behavior of individual milk samples as a binary trait (coagulating vs. noncoagulating samples) in the 2 dairy sheep breeds. A total of 1,039 and 973 milk samples were analyzed for Assaf and Churra breeds, respectively. The prediction ability of the selected traits was tested with ML applied to random forests through a 10-fold cross-validation procedure per breed using the R software. Our results revealed a global statistical sensitivity of 90% and 98% for Assaf and Churra breeds, respectively, on the validation set. In both breeds, the pH of the milk was the trait with the highest prediction ability for the coagulation behavior. Moreover, the second most relevant trait for coagulation prediction was the somatic cell count for Assaf and milk yield for Churra breeds. These results support the implementation of the ML methodologies to predict the coagulation behavior of milk samples without the need to measure MCP traits specifically. Future studies should increase the number of samples to achieve higher prediction efficiencies and assess whether this approach can be implemented in different dairy sheep breeds.

Key Words: sheep and related species, animal breeding, complex trait, machine learning, product quality

W119 Identification of selection signatures on the X chromosome in East Adriatic sheep. M. Shihabi[1], B. Lukic[2], I. Drzaic[1], M. Ferencakovic[1], V. Brajkovic[1], L. Vostry[3], V. Cubric-Curik[1], and I. Curik*[1], [1]*University of Zagreb, Faculty of Agriculture, Zagreb, Croatia,* [2]*J.J. Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia,* [3]*Czech University of Life Sciences, Prague, Czech Republic.*

Sheep farming is one of the most important livestock sectors in the region of East Adriatic. Therefore, the identification of genomic regions showing signals of a positive selection signature is of economic importance. The aim of this study was to analyse regions showing selection signals in 59 sheep individuals representing the East Adriatic sheep populations (Dalmatian Pramenka; Dubrovnik Ruda Sheep; and Pag Island sheep) using the Ovine Infinium HD SNP BeadChip. Our analysis was limited to the X chromosome only (21,748 SNPs), as such analyses are rarely performed, with the exception of a few studies. Among sheep populations, the Balkan sheep group was never represented in such an analysis. After quality control, we performed 2 different methods, identification of extremely frequent SNPs in ROHs (eROHi) and Integrated Haplotype Score (iHS), based on the analysis of genomic variation within a large meta-population. Using iHS, we detected a weak selection signal in a narrow region (6 SNPs at position 25.6 Mb) indicative of the interleukin-1 receptor accessory protein-like 1 (*IL1RAPL1*) gene. In humans, *ILRAPL1* has been linked to the hippocampal memory system and learning ability. Using eROHi (1Mb), we identify strong selection signals in a large region extending from position 69.9 to 74.6 Mb with a number of potential gene candidates (*POU3F4*, *CYLC1*, *RPS6KA6*, *HDX*, *APOOL*, *ZNF711*, *POF1B*, *TRNAC-GCA*, *CHM*, and *DACH2*). *CHM*, *DACH2*, *RPS6KA6*, *ZNF711*, and *IL1RAPL1* were also identified as selection signals in some Chinese and European breeds. Nevertheless, the pattern of selection signals in the X chromosome when comparing iHS and eROHi was somewhat different from analyses performed on autosomes and needs to be further analyzed using other methods and on large samples.

Key Words: adaptation, sheep and related species, genetic improvement, genotyping, evolutionary genomics

W120 Study on fiber characteristics of different Inner Mongolia Cashmere goats. Z. Chongyan, X. Yuchun, G. Juntao, S. Xin, Z. Cun, Q.

Qing, D. Dongliang, W. Zhixin, L. Jinquan, and L. Zhihong*, *Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China.*

Cashmere goat hair structure belongs to heterogeneous fleece, composed of long and thick medullary wool produced by primary follicle and short and thin medulla-less cashmere grown by secondary follicle. Inner Mongolia Cashmere goat is a big export province of cashmere. Its cashmere cellulose is called "soft gold" and "fiber gem." Therefore, this study will compare the fiber of Inner Mongolia cashmere goats of Alpas and Alashan type. The strength, fineness, Elongation at break and coefficient of variation of fiber diameter of 2 varieties cashmere goats were compared. The results show that the strength, elongation at break and coefficient of variation of fiber diameter of Alashan cashmere are higher than those of Alpas cashmere; the fineness of Alpas cashmere is higher than Alashan cashmere. In strength, fineness and coefficient of variation of fiber diameter, Alpas wool is higher than Alashan; The breaking elongation of Alashan wool is higher than that of Alpas. This shows that different types and kinds of fibers have their own characteristics and advantages and disadvantages, and it is most sensible to choose according to different production needs.

Key Words: fiber, strength, cashmere, wool

Ruminant Genetics and Genomics Workshop

W121 A comprehensive catalog of regulatory variants in the cattle transcriptome: A case study for the FarmGTEx Project. G. E. Liu*, *Animal Genomics and Improvement Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, MD, USA.*

The systematic characterization of genetic regulatory variants on the transcriptome of livestock is essential for interpreting the molecular mechanisms underlying traits of economic value, and to increase the rate of genetic gain through artificial selection. The Farm Animal Genotype-Tissue Expression (FarmGTEx) Consortium is a collaborative endeavor to provide a comprehensive atlas of tissue-specific gene expression and genetic regulation in livestock species. In its pilot phase, by uniformly analyzing publicly available sequence data using our newly-developed transcriptome pipeline as described in the cattle GTEx preprint (https://www.biorxiv.org/content/10.1101/2020.12.01.406280v1), we build a cattle Genotype-Tissue Expression atlas (cGTEx, http://cgtex.roslin.ed.ac.uk/) for the research community, based on 11,642 RNA-seq data sets from over 100 cattle tissues. We describe the landscape of bovine transcriptome across tissues and report thousands of *cis* and *trans* genetic variants (like expression QTL, or eQTL), associated with gene expression and alternative splicing for 24 tissues. We evaluate the specificity/similarity of these genetic regulatory effects across tissues, and functionally annotate them using a combination of multi-omics data. Finally, we link gene expression in different tissues to 43 economically important traits using a large transcriptome-wide association (TWAS) study to provide novel biological insights into the molecular regulatory mechanisms underpinning agronomic traits in cattle. This study provides a showcase for the FarmGTEx Project in other major farm animal species including pigs, chickens, sheep, and goats. The research plan for the next phases of FarmGTEx in ruminants will be discussed and highlighted. *This is a presentation about Cattle Genotype-Tissue Expression Atlas on behalf of the FarmGTEx Consortium. The contributors include Shuli Liu, Yahui Gao, Oriol Canela-Xandri, Sheng Wang, Ying Yu, Wentao Cai, Bingjie Li, Erola Pairo-Castineira, Kenton D'Mellow, Konrad Rawlik, Charley Xia, Yuelin Yao, Xiujin Li, Ze Yan, Congjun Li, Benjamin D. Rosen, Curtis P. Van Tassell, Paul M. VanRaden, Shengli Zhang, Li Ma, John B. Cole, George E. Liu, Albert Tenesa, Lingzhao Fang, etc from multiple international institutions]

Key Words: cattle, expression QTL, GWAS, RNA-seq, transcriptome-wide association (TWAS)

W122 Comparison of sequencing and assembly strategies for the cattle pangenome effort. A. Leonard*[1], Z.-H. Fang[1], B. Rosen[2], D. Bickhart[2], T. Smith[2], and H. Pausch[1], [1]*ETH Zürich, Zürich, Switzerland,* [2]*ARS, USDA, Beltsville, MD, USA.*

Selective breeding and natural selection have resulted in over a thousand diverse cattle breeds, grouped into 2 subspecies: *Bos taurus taurus* and *Bos taurus indicus*. Currently, the cattle reference genome is based on a single Hereford-breed animal, limiting comparisons of diversity from more distantly derived lineages. As efforts continue to increase the number of breeds with reference-quality assemblies, we examined various sequencing technologies and assembly methods for cattle trios to identify efficient approaches. We assembled 5 distinct breed-specific cattle genomes, including a low-heterozygosity purebred Original Braunvieh, a medium-heterozygosity F1 cross of Nellore and Brown Swiss, and a high-heterozygosity interspecies cross of gaur and Piedmontese. Both PacBio's High-Fidelity (HiFi) and Oxford Nanopore Technology's (ONT) long reads produce high-quality assemblies across multiple tested assemblers. However, we highlight several differences between the sequencing technologies, like the quantity of telomeric or centromeric sequence and expected gene content found in the assemblies. We also demonstrate the effectiveness of graph-based over direct trio-binning assembly approaches across different scales of heterozygosity, with average N50 values of 70 Mb and 30 Mb respectively. Effect of sequence coverage depth on assembly completeness, contiguity, and accuracy was assessed to optimize the balance of quality and cost. These assemblies also represent the first reference-quality long-read assemblies for their respective breeds. Using the minigraph tool and the current cattle reference genome as the backbone, we selected the best HiFi and ONT assembly for each of the 5 breeds for iterative incorporation into the assembly graph. Through the graph, we identified approximately 70k breed-specific structural variants that were used to construct a relationship dendrogram among the breeds that showed high agreement with previous SNP-based phylogenies. The results provide insight into predicted effects of sequencing platform and experimental design selection for pangenome projects in cattle and other diploid species.

Key Words: cattle, genome assembly, sequence variation, bioinformatics, phylogeny

W123 Dissection of the scurs phenotype to refine the mapping of scurs. G. Wang* and C. Gill, *Texas A&M University, College Station, TX, USA.*

Scurs and horns are 2 types of headgear in cattle. Scurs are corneous growths in the same location of the skull as horns, which range in size from buttons to horn-like structures, but generally do not fuse with the frontal skull. Expression of scurs is epistatic to horns and only heterozygous polled cattle can grow scurs. The objective of this study was to dissect the scurs phenotype and refine the mapping of scurs. *Bos taurus-Bos indicus* F_2 and reciprocal backcross mapping populations were used for this study. The status of headgear (horns, scurs, polled) was recorded on live animals or at 18 mo of age when cattle were harvested. Photographs were taken of the headgear and skulls. Blood samples were extracted for DNA and 50K genotypes were obtained for the F_2 and backcross cattle and HD genotypes were obtained for parents and grandparents that contributed to more than 10 offspring. Genotypes were imputed to HD scale using eagle and minimac. The Celtic *polled* locus was either directly gen-

otyped or inferred from haplotypes. Only heterozygous polled cattle (n = 560) were used in this study. Scurs were categorized based on anatomy. We identified at least 3 distinct types of scurs, which were classified as "buttons," "sheaths," and "bony scurs," which reflect 3 milestones of headgear development. The frequency of the different types of scurs differs between sexes and different families tended to segregate for a single type of scurs. Genome-wide association studies were conducted within each sex for presence or absence of scurs and for the different types of scurs. We identified a major locus for presence of scurs (FDR-corrected *P*-value $<1 \times 10^{-15}$) on BTA 12 that is close to RXFP2, a well-known regulator of headgear in other ruminants. Other significant loci were found on BTA 17 and 22 for the different classifications of scurs. Based on differences in the GWAS results between sexes, the role of RXFP2 may differ in males and females, which could explain the sexual dimorphism of the scurs phenotype.

Key Words: cattle, scurs, GWAS, sexual dimorphism

W124 Genomic breeding values from low-coverage Nanopore sequencing. H. J. Lamb, B. J. Hayes, L. T. Nguyen, and E. M. Ross*, *The Queensland Alliance for Agriculture and Food Innovation, St Lucia, Queensland, Australia.*

Turnaround time has been a major limitation to the use of genomic prediction in Australia's northern beef industry where cattle are often only handled once a year. Therefore, Australia's northern producers require an alternative to traditional SNP array genotyping, which takes 6–8 weeks. We have previously proposed using portable DNA sequencing technology to sequence animals on-farm to produce rapid genomic estimated breeding values (GEBVs). To investigate the feasibility of this approach we sequenced 19 Droughtmaster cattle to an average coverage of 5.5x on the minION (Oxford Nanopore Technologies; Guppy v4.2.2). The sequence was aligned to ARS v1.2 and SNP were called using a custom algorithm that accounts for variable read depth across loci. Genotypes at loci that overlapped with 641k high-quality markers from the Illumina Bovine 777k HD SNP array were extracted. Missing genotypes were imputed based on the weighted likelihood of each genotype given the allele frequency of the population. Marker effects at the same loci were taken from a population of 30k beef cattle (Brahman and crossbreeds) in Northern Australia for body weight. The marker effects were then used in a SNP-BLUP to calculate 2 EBVs for each animal, one using the genotypes obtained from the sequencing data, and one using genotypes from the 50K SNP array (imputed up to 641K). The SNP array and the sequencing based EBVs were then compared with determine the level of concordance between the 2 methods. We observed a correlation between the Nanopore sequencing derived GEBVs and SNP array GEBVs of 0.96 for body weight and a regression slope of 1.02 when missing genotype calls in the sequencing data were not imputed. When missing genotypes were imputed based on the population allele frequencies the correlation increased to 0.97 with a regression slope of 1.06. This indicates that accurate GEBVs can be calculated at low sequencing depths using portable sequencing technologies. Methods to combat sequencing errors and regions of ultra-low coverage (1x-2x) could be implemented to further increase the accuracy of GEBVs derived from on-farm sequencing. Furthermore, investigations to reduce the cost of sequencing are underway.

Key Words: sequencing, Nanopore, cattle, GEBV

W125 Can SNPs associated with variation in the level of stress biomarkers be used for the selection of stress-resilient dairy cows? M. M. Passamonti*[1], M. Milanesi[4], J. Ramirez Diaz[1], A. Stella[2], M. Barbato[1], M. Premi[1], R. Negrini[1], A. Cecchinato[3], E. Trevisi[1], J. L. Williams[1], and P. Ajmone Marsan[1], [1]*Universitá Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*Consiglio Nazionale della Ricerca, Milan, Italy,* [3]*Universitá di Padova, Padua, Italy,* [4]*Università della Tuscia, Viterbo, Italy.*

Despite progressive improvements in management practices, animals are still exposed to physiological and environmental stressors, which are exacerbated by ongoing climate change. Selecting stress-resilient animals could increase animal welfare and production efficiency. At the physiological level, stress causes a change in homeostasis. Biomarker' levels for metabolism, liver functionality and immune system which are modulated during stress response can be measured in the plasma [J1]. In a previous study we identified significant association of SNP markers with the plasma level of 3 proteins (ceruloplasmin, CP; paraoxonase, PON; and gamma-glutamyl transferase, GGT) in Italian Holstein and Italian Red Pied breeds sampled around mid-lactation. In all cases variation in the levels of these biomarkers was mainly driven by genetic variants mapping within or nearby genes coding for the proteins themselves. The aims of the present study were to confirm these associations in a different set of animals, and to understand if the SNPs associated with the level of biomarkers are also predictive of the animal response to stress. A total of 1,000 Italian Holstein dairy cattle were sampled at one farm in Italy and genotyped with the GGP bovine 100K SNP panel (Neogen). Single-SNP, gene and haplotype based GWAS were conducted using plasma-biomarker levels as intermediate phenotypes for stress response. Results confirmed genetic association between SNPs and plasma levels of paraoxonase (BTA4) and gamma-glutamyl transferase (BTA17). A novel association was discovered between SNPs and alkaline phosphatase (BTA2), while the association with CP was not confirmed. 100 cows having opposite homozygote genotypes at SNPs previously associated with CP, GGT and PON have been identified and are being sampled during the stressful peripartum period. Postpartum animals will be assessed for their metabolic response to parturition and early lactation stresses. The results of this investigation will shed light on the utility of intermediate phenotypes as proxies of complex traits and on the value of including them in genomic assisted breeding programs as novel traits.

Key Words: cattle, stress, genetics, GWAS

W126 The distinct morphological phenotypes of domestic sheep are shaped by introgressions from their sibling wild species. H. Cheng*, J. Wen, Z. Zhang, and Y. Jiang, *Northwest A&F University, Yangling, Shaanxi, China.*

Domestic sheep, including thousands of breeds worldwide, exhibit distinct phenotypes. However, how are these phenotypic variations affected by introgression has not been systematically investigated. Here, we analyzed 1,403 whole-genome sequences of sheep containing 69 samples from all 7 wild species (snow sheep, bighorn, thinhorn, argali, urial, Asiatic and European mouflon) within *Ovis* genus, and 1,334 domestic sheep representing more than 170 worldwide breeds to assess the extent of introgression in domestic sheep. We identified on average 7.1%, 1% and 0.19% introgressive sequence for each domestic sheep from Asiatic mouflon, urial and argali, respectively. Introgression signals were detected in *RXFP2* from Asiatic mouflon and *MSRB3* from argali. *RXFP2* was well-known associated with horn status in sheep. *MSRB3* was proved to be strongly associated with the width of ear by using hybrid populations in our study. Furthermore, we found that introgressions in *RXFP2* and *MSRB3* resulted in 3 distinct haplotypes, which showed strong signature of selection among diverse domestic populations. Our results suggest that distinct haplotypes originating in wild species and introgressed into domestic sheep have played an important role in phenotype diversity and local population adaptation, and contribute to a depth understanding of occurrence timing, area and process of introgression events among *Ovis* species.

Key Words: domestic sheep, introgression, horn status, ear morphology

W127 Genome-wide local ancestry and direct evidence for cytonuclear disequilibria in hybrid African cattle populations (*Bos taurus*/*indicus*). J. A. Ward*[1], G. P. McHugo[1], M. J. Dover[1], T. J. Hall[1], S. I. Ng'ang'a[2,3], T. S. Sonstegard[4], D. G. Bradley[5], L. A. F. Frantz[2,3], M. Salter-Townshend[6], and D. E. MacHugh[1,7], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [2]*Palaeogenomics Group, Department of Veteri-*

nary Sciences, Ludwig Maximilian University, Munich, Germany, [3]*School of Biological and Chemical Sciences, Queen Mary University of London, London, UK,* [4]*Acceligen, Eagan, MN, USA,* [5]*Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland,* [6]*UCD School of Mathematics and Statistics, University College Dublin, Dublin, Ireland,* [7]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland.*

Cattle play an important role in African economies, culture, and society. Today, most African cattle are hybrids of the humpless *Bos primigenius taurus* (taurine) and the humped *Bos primigenius indicus* (indicine) types. These subspecies originated from independent domestications of *Bos primigenius primigenius* and *Bos primigenius namadicus*, respectively. The most recent common ancestor (MRCA) for the taurine and indicine lineages is estimated to have lived between 200,000 to 500,000 years ago, and as such there are significant differences between the *B. p. taurus* and *B. p. indicus* genomes. Despite substantial indicine nuclear genomic admixture in African cattle, they only carry taurine mitochondrial DNA (mtDNA). The efficient function of the vertebrate mitochondrion relies on fine-tuned interactions that exist between the products of over 1,000 nuclear genes and 37 mitochondrial genes. This bi-genomic system presents a challenge when there may be a mismatch between the nuclear and mitochondrial genomes, as may be the case in hybrid populations. Using high-density SNP data from over 500 cattle, representing 10 hybrid African cattle breeds, 4 pure taurine breeds and 4 pure indicine breeds we tested the hypothesis that there has been adaptive introgression of taurine ancestry at regions of the nuclear genome containing genes that encode proteins functionally associated with the mitochondrion. Our results support this hypothesis, demonstrating that mitonuclear disequilibria exists in hybrid African cattle populations. Using local ancestry analysis to infer ancestry at individual SNPs and then employing a bootstrap approach, we generated distributions of mean ancestry deviation at groups of nuclear-encoded mitochondrial genes. This analysis indicated that there is a significant retention of taurine alleles at nuclear-encoded mitochondrial genes in hybrid African cattle populations.

Key Words: cattle and related species, population genomics, admixture, mitochondrial DNA

Avian Genetics and Genomics Workshop

W128 Genetic diversity and population structure of Myanmar native chickens using double digest restriction-site associated DNA sequencing (ddRAD-seq). S. L. Y. Mon*[1], M. Lwin[2], A. A. Maw[3], L. L. Htun[3], S. Bawm[3], K. Kawabe[4], Y. Nagano[5,1], A. J. Nagano[6], Y. Wada[5,1], S. Okamoto[1], and T. Shimogiri[1], [1]*The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan,* [2]*Livestock Breeding and Veterinary Department, Yangon, Myanmar,* [3]*University of Veterinary Science, Nay Pyi Taw, Myanmar,* [4]*Education Center, Kagoshima University, Kagoshima, Japan,* [5]*Faculty of Agriculture, Saga University, Saga, Japan,* [6]*Faculty of Agriculture, Ryukoku University, Otsu, Shiga, Japan.*

Myanmar native chickens are main protein source in Myanmar. The breeding progress must be promoted to meet the demand of the increasing human population. However, the genetic information on Myanmar native chickens is limited. Therefore, in this study, we investigated the genetic diversity and population structure of Myanmar native chickens using ddRAD-Seq. A total of 343 chicken and junglefowl genomic DNA samples consisting of 9 Myanmar native populations (n = 171), red junglefowls (n = 17), 7 Asian native populations (Bangladesh, Cambodia, China, Indonesia, Japan, Laos, Thailand) (n = 95), and 4 commercial chickens (Barred Plymouth Rock, broiler, layer, Rhode Island Red) (n = 60) were used. The ddRAD-Seq was conducted as described by Sakaguchi et al. (2015). Genomic DNAs were digested with *Bgl*II and *Eco*RI. Sequencing was done using HiseqX. The SNPs were called with Stacks v2.53, filtering SNPs over 80% matching samples, cut off 0.05% minor allele frequency. Genetic diversity indices such as expected (HE) and observed heterozygosity (HO) were calculated using Plink v1.07. Principal component analysis (PCA) was drawn by SNPRelate R package. Population structure was analyzed by Admixture v1.3. We used 20,993 filtered autosomal SNPs for analysis. The HE and HO of 21 chicken populations ranged from 0.144 and 0.145 in Japan (JP) to 0.277 and 0.261 in YGN. Those of Myanmar native populations ranged from 0.255 and 0.241 in FCN to 0.277 and 0.261 in YGN, showing higher genetic diversities. Two-dimensional plot of the first 2 PCs revealed that commercial and JP chickens were clearly differentiated from native chickens and red junglefowls. Population structure analysis suggested appropriate 8 clusters for 21 populations. Four commercial and JP populations formed the respective homogeneous clusters. The remaining 3 clusters were observed in native chickens and red junglefowls: The first cluster was homogeneously grouped by red junglefowls and China, second by the fighting chickens. The third was distributed only in Myanmar native populations in varying proportions.

Key Words: animal breeding

W129 Taxonomy classification of Nigerian local turkey using 12S mitochondrial rRNA gene. D. I. Ibiwoye*[1,2], F. E. Sola-Ojo[1], D. O. Aremu[1], I. A. Abubakar[1], N. B. Afolabi-Balogun[3], C. A. Adeniyi[4], and A. O. Oni[3], [1]*University of Ilorin, Ilorin, Kwara, Nigeria,* [2]*Huazhong Agricultural University, Wuhan, China,* [3]*Fountain University, Osogbo, Osun, Nigeria,* [4]*Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China.*

Taxonomic classification plays a key role in the evolutionary history of animal species. They are commonly observed within several orders in wild birds. The local turkey (*Meleagaris gallopavo* f. *domestica*) is a common species that is raised for human consumption and use. This study was conducted using 40 poults (20 males and 20 females of white and black lavenders) of 8 weeks age, blood samples were collected for DNA extraction, the components exon of the poults were amplified, it was sequenced and aligned against each other and against NCBI outputs from orthologs spanning Aves mammals, Chordata, and other vertebrates. Results from the study showed some mutations in the form of insertions and substitutions. The phylogenetic tree was constructed from the aligned sequences to show how closely related the local turkey is with insect and vertebrate sequences; the result shows that male and female local turkeys are closely related to *Gallus gallus*.

Key Words: taxonomic classification, 12S gene, turkey, phylogenetic tree, mutation

W130 Transcriptomic analysis of the *Musculus complexus* in naked neck broiler chickens. A. C. Mott*, C. Blaschka, A. Mott, A. R. Sharifi, and J. Tetens, *Georg-August University, Göttingen, Lower Saxony, Germany.*

The locus for naked neck (*Na*) in chickens reduces feather coverage and leads to increased heat dissipation from the body surface resulting in better adaptability to hot conditions. However, the *Na* gene is linked to significantly lower hatchability due to an increased late embryonic mortality. This preliminary study analyses the transcriptome of the hatching muscle (*M. complexus)* and attempts to elucidate the reasons behind reduced hatching rates of *Na* chickens. This was carried out by the sampling of embryos at 20 d of embryonic development (ED20) from

12 chicken embryos (6 × wildtype and 6 × *Na/Na*). RNA was extracted from the *M. complexus* of each embryo using an RNeasy Plus Universal Mini Kit (Qiagen) and was sequenced using NovaSeq 6000 (Illumina) 2x50bp v1.5. Raw sequencing reads were processed for adapter removal, trimming and removal of low quality reads. Differential expression (DE) between experimental groups was then analyzed using DESeq2 (V. 1.28.1), with volcano plots being created with R package EnhancedVolcano (V. 1.6.0). Gene set analyses were also conducted using R package clusterProfiler (V. 3.16.0). Protein interaction maps were created using STRING (V. 11.0). The results of DE analyses led to the discovery of 221 DE genes in the *M. complexus* of the experimental animals, with 51.1% downregulated in the *Na*/*Na* animals (LFC > 1, $P < 0.05$). Among those, 119 genes were of uncertain function (LOC symbols), with 80 were classified as uncharacterized. To identify potential functional protein clusters a protein interaction network analysis was performed on the top 20 up and downregulated genes. This analysis revealed 2 protein clusters: (1) FABP1, GAL8, and ORM1, and (2) CYP7A1 and AKR1D1. Through gene cluster analysis, several Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were observed, steroid hormone biosynthesis, linoleic acid metabolism, retinol metabolism, arachidonic acid metabolism, and primary bile acid synthesis. As these pathways are essential in the development of the chicken embryo, this study indicates that the changes in regulation of these pathways could play a significant role in the increased late mortality of *Na* broilers.

Key Words: chicken, naked neck, hatchability, RNA-seq, transcriptome

W131 Study on differentially expressed genes in granular layer and theca layer of laying Silky Fowl and White Leghorn. Y. Tai*[1], X. Yang[1], D. Han[2], and X. Deng[1], [1]*China Agricultural University, Lab of Animal Genetic Resource and Molecular Breeding, Beijing, China,* [2]*College of Veterinary Medicine, China Agricultural University, Beijing, China.*

Under similar genetic conditions and the same feeding conditions, the egg production of local breed Silky Fowl is significantly lower than that of the White Leghorn. Egg laying performance is related to follicle growth and development, and follicle development is closely related to the granulosa cells and the theca cells. Therefore, transcriptome sequencing was used to analyze differential expression genes of the follicular granular layer and the theca layer of the Silky Fowl and White Leghorn. Silky Fowl and White Leghorn were raised in the ranch of China Agricultural University. We choose 4 of each breed and collected the granular layer and theca layer for high-throughput sequencing. Compared with the White Leghorn, in hierarchical granular layer tissues, the Silky Fowl has 389 upregulated genes and 270 downregulated genes. Downregulated genes were mainly associated with cell division, cell cycle and hormone receptor while the upregulated genes were related to ribosomal function, lipid metabolism and protein synthesis. Compared with the White Leghorn, in hierarchical theca layer tissues, the Silky Fowl has 232 upregulated and 139 downregulated genes. Downregulated genes were mainly associated with cell proliferation, steroid hormone synthesis, and angiogenesis while the upregulated genes were related to melanin synthesis. Different expression genes in granular layer and theca layer were provided in our study for the first time, related to follicular development of different chicken breeds. It is helpful to improve the understanding of the molecular mechanism of low egg production of local chickens.

Key Words: chicken, granular layer, theca layer

W132 Hypothalamic and ovarian transcriptome profiling reveals potential candidate genes in low and high egg production of White Muscovy ducks (*Cairina Moschata*). S. Bello*, H. Xu, and Q. Nie, *South China Agricultural University, Guangzhou, Guangdong, China.*

In China, the low egg production rate is a major challenge to Muscovy duck farmers. Hypothalamus and ovary play essential role in egg production of birds. However, there are little or no reports from these tissues to identify potential candidate genes responsible for egg production in White Muscovy ducks. A total of 1,537 laying ducks was raised; the egg production traits which include age at first egg (days), number of eggs at 300days and number of eggs at 59weeks were recorded. Moreover, 4 lowest (LP) and 4 highest producing (HP) were selected at 59 weeks of age, respectively. To understand the mechanism of egg laying regulation, we sequenced the hypothalamus and ovary transcriptome profiles in LP and HP using RNA-seq. The results showed that the number of eggs at 300days and number of eggs at 59weeks in the HP is significantly more ($P < 0.001$) than the LP ducks. In total, 106.98G clean bases were generated from 16 libraries with an average of 6.68G clean bases for each library. Further analysis showed 569 and 2,259 differentially expressed genes (DEGs) were identified in the hypothalamus and ovary between LP and HP, respectively. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis revealed 114 and 139 pathways in the hypothalamus and ovary, respectively which includes Calcium signaling pathway, ECM-receptor interaction, Focal adhesion, MAPK signaling pathway, Apoptosis and Apelin signaling pathways that are involved in egg production. Based on the GO terms and KEGG pathways results, 10 potential candidate genes (*P2RX1*, *LPAR2*, *ADORA1*, *FN1*, *AKT3*, *ADCY5*, *ADCY8*, *MAP3K8*, *PXN*, and *PTTG1*) were identified to be responsible for egg production. Further, protein-protein interaction was analyzed to show the relationship between these candidate genes. Therefore, this study provides useful information on transcriptome of hypothalamus and ovary of LP and HP Muscovy ducks

Key Words: egg production, transcriptome, gene expression, candidate genes, Muscovy duck

W133 Nextflow IsoSeq (nf-isoseq) pipeline provides a first insight into the chicken transcript landscape. S. Guizard*, J. Smith, R. Kuo, K. Miedzinska, J. Smith, M. Davey, and M. Watson, *The Roslin Institute, Edinburgh, Scotland, UK.*

The EU H2020 Gene-Switch project is a collaborative endeavor that will produce new genome information to enable the characterization of genetic and epigenetic determinants of complex traits in the 2 monogastric species (chicken and pig) that are the primary sources of meat worldwide. The characterization of genome functional elements is being made through a wide diversity of analysis methods (RNA-seq, miRNA-seq, ATAC-seq, WGBS, ChIP-seq, Iso-seq, Promoter Hi-C), on 7 tissues at 3 developmental stages (pig: 30, 70 d post-fertilization, new born and chicken: E8, E15, hatched). Functional annotation of the genome partly relies on RNA sequencing. Illumina short-read sequencing has been extensively used, as it is low cost, labor saving and rapid. However, short sequence lengths make isoform reconstruction challenging. This drawback can be overcome with long-read data. Iso-Seq, with circular cDNA sequencing, generates accurate full-length transcripts (<1% error). It allows direct identification of gene intron-exon structure and better isoform detection. This method has growing utility and has been used to re-annotate genomes but no standard pipeline has yet been released. We have thus developed the nf-isoseq pipeline, which enables insight into the chicken transcriptomic landscape. The nf-core is a community initiative working on the development of standard, portable and reproducible analysis pipelines. The pipeline generates CCS (circular consensus sequence), maps transcripts to the genome and reduces gene model redundancy with TAMA collapse. Thanks to the Nextflow language and containerization technologies, it can be easily run on a wide range of cluster configurations. We processed the Iso-Seq data for each chicken sample to reveal the transcriptome for a given tissue at a given time point. Comparison of annotations gives a first look at transcriptional landscapes across tissues and the dynamic over time development. This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the grant agreement no. 817998. .

Key Words: poultry and related species, bioinformatics, computational pipeline, transcriptome, meat production

W134 A new chromosome-level turkey genome. C. P. Barros*[1], M. F. L. Derks[1], J. Mohr[2], B. J. Wood[2,3], M. C. A. M. Bink[4], and M. A. M. Groenen[1], [1]*Wageningen University and Research, Wageningen, the Netherlands,* [2]*Hybrid Turkeys, Kitchener, ON, Canada,* [3]*School of Veterinary Science, University of Queensland, Gatton, QLD, Australia,* [4]*Hendrix Genetics Research, Technology and Services, Boxmeer, the Netherlands.*

The domesticated turkey (*Meleagris gallopavo*) is a species of large agricultural importance, as the second largest contributor to world poultry meat production. A high-quality reference genome is essential for research as well as for the turkey breeding industry. Our aim was to create a new chromosome-level turkey genome with improved genome completeness and continuity, gene annotation, mapping of functional sequences and structural variation discovery. Adopting the trio-binning approach, we sequenced 3 individuals (2 parents and one F_1), using PacBio long-read sequencing technology, to create a high-quality chromosome-level turkey assembly, as well as 2 high-quality chromosome-level parental haplotype assemblies. We produced a chromosome-level F_1 assembly with a total length of 1,001,818,376 bp captured in 151 scaffolds. The assembly covers 35 complete chromosomes in a single scaffold (N50: 70,339,173 bp), which even outperforms the current Gal6 chicken genome in terms of continuity. In addition, we assessed the completeness using BUSCO, showing that we cover more than 96% of the genes in the avian and vertebrate sets. The new turkey assembly provides a significant improvement in terms of gene space and contig orientation and continuity compared with the previous turkey build. Comparative analysis revealed a large inversion on the Z chromosome. We used synteny to compare the Z chromosome in turkeys to other Galliformes species and confirmed that this inversion is unique to turkeys. We assessed structural variance in the F_1 assembly and in the parental assemblies (which are of equally high quality). Although being highly similar, the parental haplotypes show a small number of structural differences, such as a large duplication on chromosome 8. Most of the assembled turkey chromosomes (2n = 80) are micro-chromosomes. We found that micro-chromosomes have a higher gene density and level of expression than macro-chromosomes. We also observed statistically significant differences in the length of several repeat and gene features. Collectively, we present a new high-quality chromosome-level turkey genome, which will significantly contribute to turkey and avian genomics research and benefit the turkey breeding industry.

Key Words: poultry and related species, genome assembly, genome sequencing, comparative genomics, genome annotation

W135 Bridge 60k SNP panel for the chicken genome-wide study. D. Seo*[1,2], S. Cho[1], D. Lee[1], M. Kim[1], P. Manjula[1], J. Shin[1], D. Lim[3], H. Choo[4], J. Cha[4], K. Kim[4], I.-S. Jeon[4], K.-T. Lee[3], B. Park[4], S. H. Lee[1,2], and J. H. Lee[1,2], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, South Korea,* [2]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, South Korea,* [3]*Animal Genomics and Bioinformatics Division, National Institute of Animal Science, RDA, Wanju, South Korea,* [4]*Poultry Research Institute, National Institute of Animal Science, RDA, Pyeongchang, South Korea.*

The native chicken is a unique asset held by countries worldwide, and various types of research are needed to preserve genetic diversity and utilize their potential characteristics. In particular, Korean native chicken (KNC) is well known for its excellent meat quality and endemic adaptability. But there are limited scientific investigations for KNC, and less profitable than commercial broilers warrant unique genetic information for their rapid genetic improvement. This study developed the Bridge 60k SNP panel to find economic trait-related genetic markers and apply them to genomic selection. This SNP panel was designed to link Illumina 60k array and Affymetrix 600k array information by genomic imputation. Illumina 60k data can impute to Bridge 60k panel using 12,289 SNPs, and Bridge 60k panel was imputed to Affymetrix 600k array data using 37,076 SNPs. The average genotype accuracy of imputed SNPs was confirmed as 0.88~0.94 (r^2 = 0.76–0.80). In addition, 11,880 SNPs were included to detect trait-related candidate SNPs from the GWAS for meat quality and growth-related traits in KNC, a comparative study for the signature of selection between KNC with commercial chickens, and an economic trait-related marker obtained from the chicken QTLdb. Moreover, the utilization was expanded by adding 492 markers for the MHC variation study and the identification of KNCs. It is expected that the SNP panel developed in this study will be the best implement for finding candidate genes for economic traits and improve KNC with various characteristics by applying genomic selection to conventional chicken utilization.

Key Words: single nucleotide polymorphism, imputation, Korean native chicken, economic traits, genomic selection

W136 Genomic signatures of selection for egg production rate using whole-genome sequence in Hinaidori chickens. T. Goto*[1], S. Fukuda[2], K. Rikimaru[2], R. A. Lawal[3], J. Pool[4], and O. Hanotte[5,6], [1]*Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan,* [2]*Akita Prefectural Livestock Experiment Station, Akita, Japan,* [3]*The Jackson Laboratory, Bar Harbor, ME, USA,* [4]*University of Wisconsin-Madison, Madison, WI, USA,* [5]*University of Nottingham, Nottingham, UK,* [6]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

Phenotypic variation of egg production rate is regulated by multiple quantitative trait loci (QTLs) in chickens. Though several QTLs in Animal QTLdb affecting egg production are mostly found in commercial layers and European breeds, little is known about the genetic control of egg production rate in indigenous chicken breeds. After checking egg production rates from 671 hens in Hinaidori, a Japanese indigenous breed, we selected high egg production (65.7% in average; n = 8) and low egg production (25.8% in average; n = 8) populations. We newly collected Illumina whole-genome sequence data from Hinaidori (HNI, n = 16) and added publicly available red jungle fowl genome sequences (RJF, n = 29). Using the GATK Best Practices, 31,381,234 bi-allelic single nucleotide polymorphisms (SNPs) on chromosomes 1–33 (GRCg6a) were called. To detect signatures of selection, F_{ST} was calculated for 20-kb sliding windows (total 73,918 windows). In addition, the Population Branch Excess (PBE) statistic was calculated from 3 F_{ST} values among High-HNI, Low-HNI, and RJF to focus more directly on loci under positive selection in the focal population (High-NHI) only. We identified 87 windows (PBE >0.4) which show High-HNI-specific genetic differentiation. These windows define 24 candidate positively selected genome regions on chromosomes 1–4. These regions contain 15 protein-coding genes and 13 long noncoding RNA genes. These results support the use of population genomics approaches for the identification of candidate regions links to egg production in parallel to pedigree-based analysis.

Key Words: chicken, population genomics, egg production, genes, F_{ST}

W137 Dissecting the polygenic genetic architecture of growth using genotyping by low-coverage sequencing in a deep intercross of the Virginia body weight lines: Novel loci revealed by increased power and improved genome coverage. T. Rönneburg*[1], Y. Zan[2,1], C. Honaker[3], P. Siegel[3], and Ö. Carlborg[1], [1]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden,* [2]*Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Science, Umeå, Sweden,* [3]*Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.*

Dissecting the basis of quantitative traits is a central topic in genetics and highly polygenic traits are particularly challenging due to the power necessary to confidently identify loci with minor effects. Experimental crosses are valuable resources for mapping such traits, though genome-wide analyses generally target major loci using a F_2 population, with additional individuals only generated for replication and fine mapping. This was the case in attempts to dissect the genetic basis of the long-term, divergent bi-directional selection responses for 56-d body weight in the Virginia chicken lines. An 18-generation intercross line was developed

from the low and high selected lines. Dissecting the genetic architecture of the 9-fold difference in 56-d body weight after 40 generations of selection revealed that although selection acted on a highly polygenic genetic architecture, only a handful of the 20 reported candidate loci reached individual significance levels. Making use of current methods, this study was designed to dissect this highly polygenic genetic architecture further, re-genotyping all available samples genome-wide and performing an integrated QTL mapping analysis across chicken from generations F_2-F_{18}. A cost-efficient low-coverage sequencing based approach was implemented to obtain high-confidence genotypes for >3,300 individuals. In total, 12 genome-wide significant QTL and 10 additional suggestive QTL were mapped. Of these, only 3 reached genome-wide significance or suggestive thresholds in the earlier F_2-based genome-wide scan. Five of the significant and 4 of the suggestive QTL overlapped with the 20 candidate loci reported in the fine mapping and replication analyses of the later generations in the cross. Novel QTLs were primarily mapped due to an overall increase in power, with minor contributions from increased genome coverage and improved marker information content. Significant and suggestive QTL are estimated to explain 60.7% of the difference between the parental lines, 3 times as much as previously reported, resulting in a more confident, comprehensive view of the individual loci that form the genetic basis of the highly polygenic, long-term selection responses for 56-d body weight in the Virginia chicken lines.

Key Words: poultry and related species, complex trait, genotyping, genome-wide association

Cattle Molecular Markers and Parentage Testing Workshop

W138 Impact of genomic breed composition on production traits in crossbred dairy cattle. M. Jaafar*[1], B. Heins[2], C. Dechow[3], and H. Huson[1], [1]*Cornell University, Ithaca, NY, USA,* [2]*University of Minnesota, Morris, MN, USA,* [3]*Penn State University, University Park, PA, USA.*

Understanding breed composition (BC) gives us insight into population structure and breeding history, informs crossbreeding programs, and provides mechanisms to correct population stratification in across-breed genetic evaluations and genome-wide association. This study examined variation in BC using Illumina BovineSNP50 genotypes and correlated BC to key performance traits. Ancestry was localized to chromosomal regions to determine the relationship between ancestry and trait selection. The intent was to determine how breed selection in crossbred dairy cattle influences traits and to determine if specific ancestral haplotypes are selected for at key performance QTL. To this end, 2 rotational crossbred populations, Procross and Grazecross were assessed. Procross is a product of rotational cross-breeding of Viking Red (VKR), Holstein (HOL), and Montbéliarde (MON) whereas Grazecross consists of Viking Red (VKR), Normande (NOR), and Jersey (JER). Both breeding programs capitalized on the positive effect of heterosis. Genomic breed composition was generated on 610 crossbred cattle incorporating genotypes of the respective purebred breeds for genomic ancestry estimation using Admixture. The average genome-based prediction estimates for Procross were 22% VKR, 45% MON, and 33% HOL while Grazecross were 21% VKR, 49% NOR, and 30% JER. BC were correlated with the performance traits of milk, fat, protein, and somatic cell score, by comparing ancestry of extreme high and low-performance groups. Analysis showed that both MON and HOL breed composition plays a significant role in higher milk and fat production in Procross while VKR and NOR are related to improved health performance in Grazecross. This inference was supported by the local ancestry analysis using RFMIX software. The results showed higher Procross milk production animals more commonly have HOL ancestry on BTA 14 where the DGAT gene resides, while Grazecross cattle more commonly have NOR ancestry on BTA 4, 6, and 10 at known health QTL. In conclusion, we believe that it is crucial to select and maintain a particular variation in breed composition to ensure optimal trait performance in crossbred cattle.

Key Words: genomic breed composition, crossbred dairy cattle, local ancestry, admixture, production traits

W139 A high-throughput Applied Biosystems Axiom Bovine Genotyping array with 100,000 markers optimized for dairy evaluation. A. Pirani*, D. Oliver, C. Bertani, and M. Patil, *Thermo Fisher Scientific Inc., Santa Clara, CA, USA.*

While the world population increases at an unprecedented rate, meeting the growing food needs continues to be a challenge. For more than a decade, the bovine dairy industry has employed the genetics of their cattle to improve production traits, such as milk yield and protein percentage. These methods have shown to be critical for the improvement of dairy cattle productivity. This breeding strategy is achieved by genotyping thousands of biallelic SNPs, interrogating loci well-distributed across the entire genome, potentially capturing all relevant quantitative trait loci (QTL). These genotypes are converted to genomic estimated breeding values (GEBVs) using a method called genomic selection (GS). The application requires the interrogation of a fixed set of markers rapidly over thousands of samples, so medium-density, 25,000 to 100,000 marker microarrays are an ideal fit. For genotyping dairy cattle, Thermo Fisher Scientific provides numerous Applied Biosystems Axiom microarrays measuring around 65,000 markers. These arrays, such as the Axiom Bovine Genotyping v3 Array includes 44,000 markers recognized by the Council on Dairy Cattle Breeding (CDCB). Recently, the CDCB released a list of 80,000 markers used for genetic evaluation. Thermo Fisher Scientific has developed a 100,000-marker microarray to interrogate all 80,000 CDCB relevant makers. In addition, this array includes markers for even genomic coverage, economically valuable traits-associated markers, sex-linked markers, microsatellite imputation markers, and parentage verification, such as the International Society for Animal Genetics (ISAG) 200 and ICAR 354 markers. This higher-density panel can also be useful in tracking undesirable genetic trends, such as inbreeding depression, to drive overall genetic improvement of dairy cattle in commercial breeding programs.

Key Words: cattle and related species, animal breeding, bioinformatics, microarray, genomic selection

W140 Investigating the accuracy of imputing variants on chromosome X in admixed dairy cattle using the ARS-UCD1.2 assembly of the bovine genome. Y. Wang*[1,2], K. Tiplady[1,2], T. J. J. Johnson[2], C. Harland[2], M. Keehan[1,2], T. J. Lopdell[2], R. G. Sherlock[2], A. Wallace[2], B. Harris[2], M. D. Littlejohn[2], R. Spelman[2], D. Garrick[1], and C. Couldrey[2], [1]*AL Rae Centre for Genetics and Breeding, School of Agriculture, Massey University, Hamilton, Waikato, New Zealand,* [2]*Research and Development, Livestock Improvement Corporation, Hamilton, Waikato, New Zealand.*

Sequence level imputation in dairy cattle to date has mainly focused on autosomes. The aim of this study was to evaluate the imputation performance of chromosome X (chrX) from low-density to high-density (HD) genotypes and subsequently to sequence level. Three low-density genotyped study populations: 41,551 animals genotyped on GeneSeek GGP panels, 55,465 animals genotyped on an Illumina BovineSNP50 panel (50k), and 28,315 animals genotyped on a GeneSeek GGP50k panel were separately imputed to Illumina BovineHD level (777k) using a reference of 3,769 animals. Subsequently, these animals were imputed to sequence level using 1,298 sequenced animals as a reference. In addition, a 10-fold

cross-validation experiment was conducted to evaluate the imputation performance of Beagle 5.1. ChrX was divided into the pseudoautosomal (PAR) and non-PAR regions. Imputation of these regions was performed separately using Beagle 5.1. Imputation for the non-PAR was also undertaken separately for males and females using Minimac 3. For HD imputation, Beagle 5.1 achieved an average dosage R-squared (DR2) of 0.96 for both non-PAR and PAR for all study populations. For the PAR, Minimac 3 achieved an allelic R-squared (AR2) of 0.95 for all 3 populations. In the non-PAR, Minimac 3 produced an average AR2 for females of 0.95 for all study populations, however, for males AR2 of 0.81 for 50k study, 0.74 for GGP study and GGP50k panels were returned. For sequence imputation, Beagle 5.1 achieved a DR2 of 0.76 for non-PAR region and 0.87 for PAR region, whereas Minimac 3 achieved an AR2 of 0.69 for male non-PAR region, 0.46 for female non-PAR region and 0.53 for PAR region. For the 10-fold cross-validation groups, the average genotype concordance is 0.99 for non-PAR and 0.98 for PAR region. The average genotype correlation is 0.98 for non-PAR and 0.97 for PAR. Beagle 5.1 is able to perform non-PAR imputation for males or females, whereas Minimac 3 cannot. Using information of both sons and the dams imputed simultaneously allows for improved imputation accuracy given that chrX of the son is directly inherited from the dam and provides the perfect phase. The chrX variants at the imputed sequence level will facilitate future genome-wide association studies and genomic prediction.

Key Words: imputation, cattle, X chromosome, sequence variation

Horse Genetics and Genomics Workshop

W141 Will selection for elasticity maintain the allele causing fragile foals? M. Ablondi*[1,2], M. Johnsson[2], S. Eriksson[2], A. Sabbioni[1], Å. Viklund[2], and S. Mikko[2], [1]*Department of Veterinary, Università degli Studi di Parma Science, Parma, Italy,* [2]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Warmblood Fragile Foal Syndrome (WFFS) is an autosomal monogenic disease leading to a defective connective tissue, which in turn causes skin and mucosa lacerations, fragile skin, hyperextension of the articulations and hematoma. The WFFS is caused by a recessive lethal missense point mutation in the procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 gene (*PLOD1*, c.2032G>A). Foals homozygous for the WFFS recessive allele are not viable, and either aborted during late gestation, or have to be euthanized shortly after birth. Despite its harmful effect, a relatively high WFFS carrier frequency has been found among Warmblood breeds, suggesting a heterozygote advantage. Thus, the aims of this study were 1) estimate WFFS carrier frequency in the Swedish Warmblood breed (SWB), 2) evaluate the effect of WFFS genotype on estimated breeding values, and 3) simulate the potential effects of balancing selection and different selection strategies on future carrier frequency. The WFFS carrier frequency calculated from a cohort of 511 randomly selected SWB horses born in 2017 was equal to 7.4%, whereas it ranged from 0.0% to 14.0% among the whole set of tested SWB (1,811 horses) divided into 8 birth year classes starting from 1980 till 2019. Overall, we found a favorable effect of the WFFS allele for movements and dressage traits, highlighting potential balancing selection on the WFFS allele in SWB horses bred for dressage purposes. Via simulations, we proved that balancing selection could maintain a recessive lethal over generations in populations similar to the SWB breed. The allele frequency of a recessive lethal allele is expected to slowly decline over generations but will decrease slower in the presence of balancing selection. Finally, we demonstrated that selection against carrier sires can over time give a more rapid decrease of the mutant allele frequency. Further research is needed to confirm the noticeable association between equine performance and the WFFS genotype. Identification of such associated genetic markers or novel causative mutations to horse performance traits might serve as new tools in horse breeding to select for healthy, sustainable, and better performing horses.

Key Words: *PLOD1*, mobility, movements, genotyping, candidate gene

W142 Quantitative trait loci associated with alternative gaits in Colombian Paso horses. M. Novoa-Bravo*[1,3], F. Serra-Bragança[2], R. Naboulsi[3], M. Sole[3], M. Rhodin[4], and G. Lindgren[3], [1]*Genética Animal de Colombia SAS, Bogotá, Colombia,* [2]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands,* [3]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [4]*Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

The lateral footfall pattern in gaited horses is mainly caused by the DMRT3 mutation. However, some horse gaits are not explained by this mutation suggesting that other loci could affect the footfall pattern and other gait parameters. The Colombian Paso Horses show different gaits including the Paso Fino, Trocha, and Colombian Trot. Usually, these horses perform just one of the gaits per horse. The DMRT3 mutation is fixed in horses performing Paso Fino, but not in the other gaits. The Trocha is a 4-beat diagonal coupled gait with a lateral footfall pattern not explained by the DMRT3 mutation. Whereas the Colombian Trot is a 2-beat diagonal coupled gait. We hypothesize that other loci may explain the footfall pattern and other differences in gait parameters between Colombian Trocha horses and Colombian Trot horses. To evaluate that, we selected 217 Colombian Paso horses, in several Colombian regions, performing Trocha (100 horses) or Colombian Trot (117 horses) according to the owner/trainer criterion. We measured these horses using objective gait analysis with inertial measurement units (Equimoves) to estimate gait parameters and to classify the horse gaits by using a machine learning approach. In addition, we genotyped 95 horses (DMRT3 CC genotypes) from the 217 phenotyped horses using 670K ThermoFisher Axiom Equine array and we did a preliminary GWAS by each parameter estimated and with the objective classification output. We calculated 40 gait parameters. Also, the classification algorithm objectively differentiated 53Colombian Trot horses (45% from the owner/trainer classification) and 158 Colombian Trocha horses from the 217 horses (6 horses were not classified). We used 85 horses and 360,755 markers per horse after quality controls. The GWAS employing different genomic models shows QTLs associated with the stride frequency ($P < 9.2 \times 10^{-9}$ after Bonferroni correction) and the Trot/Trocha classification ($P < 2.2 \times 10^{-9}$ after Bonferroni correction) in the chromosomes 10 (ECA10) and 16 (ECA16) respectively. In this preliminary study, we found other loci than DMRT3 associated with the lateral footfall pattern in horse gaits and with the stride frequency in Colombian Paso horses. This finding could be extrapolated to similar gaits to the Trocha in other horse breeds as Foxtrot in Missouri Foxtrotter, and Marcha batida in Mangalarga-Marchador breed, revealing genetic variants that potentially affect the locomotion of the horses. This needs further investigation.

Key Words: locomotion, horses, gait, QTL

W143 Genetic structure of maternal lines in Przewalski horses based on mtDNA variation. A. D. Musial*[1], K. Ropka-Molik[1], M. Stefaniuk-Szmukier[2], G. Mycka[2], A. Fornal[1], and N. Yasynetska[3], [1]*National Re-*

search Institute of Animal Production, Balice, Poland, [2]University of Agriculture, Krakow, Poland, [3]Biosphere Reserve, Askania-Nova, Ukraine.

Przewalski's horses (*Equus ferus przewalskii*) are considered the last living population of wild horses, however, according to recent reports, modern Przewalski's horses may have descended from a feral line of horses domesticated about 5,000 years ago. Currently, the Przewalski horse population consists of no more than 2.000 individuals worldwide. Based on pedigree data, 14 founders of the species are distinguished, but the population cannot be completely genetically pure, because the mothers of 2 of the founders belonged to the *Equus caballus* species. To date, studies on mitochondrial DNA (mtDNA) identified 3 unique haplotypes in Przewalski's horse, none of which are found in modern horses. The polymorphism of 2 hypervariable fragments of the mitochondrial DNA D-loop region, which contains about 10% of all nucleotide changes present in the mtDNA molecule, plays a unique role in verifying the origin of horses in terms of female genealogy. The aim of the study was the analysis of the mtDNA variability within the selected population of Przewalski's horses, using the sequence of the mitochondrial DNA hypervariable region. The hair samples were collected from 23 Przewalski horses living in the reserve Askania-Nova (Ukraine). All horses belonged to the 3 dam lines (Munich, Prague, Askanian) were tested on 46 SNP loci (single nucleotide polymorphism). The mitochondrial DNA hypervariable region with a total length of 1,165 bp were analyzed using Sanger method. Bioinformatic analysis allowed for assigning the examined Przewalski's horses to 3 distinctly different haplotypes. The first haplotype showed the greatest similarity to the *Equus caballus* reference, the second mtDNA profile was clearly separates from the genus *Equus*, while the third haplotype was similar to *Haringtonhippus*, an extinct species living in the Pleistocene in North America. This result may indicate an extensive history of the origins of the Przewalski horse species and requires further research. This research was funded by "Diamentowy Grant" no. 0211/DIA/2019/48, Ministry of Science and Higher Education, Poland.

Key Words: *Equus ferus przewalskii*, mitochondrial DNA, SNP, origin

W144 Epigenetic characterization of horse centromeric domains in different tissues and individuals. E. Cappelletti*[1], F. M. Piras[1], R. Hijaz[1], L. Sola[1], J. L. Petersen[2], R. R. Bellone[3,4], C. J. Finno[3], T. S. Kalbfleisch[5], E. Bailey[5], S. G. Nergadze[1], and E. Giulotto[1], [1]*Department of Biology and Biotechnology, University of Pavia, Pavia, Italy,* [2]*Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE, USA,* [3]*University of California-Davis, School of Veterinary Medicine, Department of Population Health and Reproduction, Davis, CA, USA,* [4]*University of California-Davis, School of Veterinary Medicine, Veterinary Genetics Laboratory, Davis, CA, USA,* [5]*University of Kentucky, Gluck Equine Research Center, Lexington, KY, USA.*

The centromere is the locus required for chromosome segregation during cell division whose function is epigenetically specified by CENP-A, the centromere specific histone H3 variant. The typical presence of satellite DNA at mammalian centromeres hampers a comprehensive molecular analysis of this locus. Our discovery that, in equid species, several centromeres are devoid of satellite DNA represented an important step forward and made equids a powerful model system for unraveling the epigenetic marks related to centromere function at the molecular level. In the horse, the centromere of chromosome 11 (ECA11) is the only one devoid of satellite DNA. We previously demonstrated that the position of its CENP-A binding domain is not fixed but slides within an about 500 kb region in different individuals, giving rise to positional alleles. These epialleles are inherited as Mendelian traits but their position can slide in one generation. This was the first demonstration that, in a native mammalian centromere, the position of the functional CENP-A domain can slide within a relatively wide region suggesting that centromeric domains are characterized by positional instability which may be physically limited by epigenetic boundaries. As members of the equine community of the FAANG (Functional Annotation of ANimal Genomes) consortium, our first goal was to unravel whether centromere sliding can occur during development. To this purpose we characterized the position of the centromeric domains of ECA11 in tissues of different embryonic origin from 2 adult Thoroughbred mares. Our results demonstrated that the centromere is located in the same region in all tissues, suggesting that the position of the centromeric domains is maintained during development. We then evaluated the epigenetic and transcriptional profile of the centromeric locus, taking advantage of ChIP-seq, RNA-seq and microRNA-seq data sets produced by the consortium. This analysis demonstrated that the ECA11 centromere is transcriptionally silent across tissues and individuals, indicating that transcription is not a key feature of centromeric chromatin.

Key Words: horses and related species, Functional Annotation of Animal Genomes (FAANG), ChIP-seq, chromatin

W145 Genomic improvement of the horse X chromosome and characterization of the pseudoautosomal boundary. M. Jevit*[1], B. Davis[1], C. Casanteda[1], D. Miller[2], and T. Raudsepp[1], [1]*Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA,* [2]*Cornell University, Ithaca, NY, USA.*

The horse reference genome was improved with the release of *EquCab3* and the first Y chromosome reference. The most complex sequences, such as those in the sex chromosomes, are still unresolved. These include large amplicons, repeats, and the pseudoautosomal boundary (PAB). We initiated a comprehensive study specifically to improve the assembly of the horse sex chromosomes. To refine the assembly of complex regions, we are utilizing 3 new technologies: trio-binning, Hi-C and Bionano optical mapping. Trio-binning uses long-read sequences from F1 interspecific hybrids and short reads from parent species. High molecular weight blood DNA was extracted from a female hinny and sequenced on 2 PacBio Sequel cells. Paired-end short reads (150bp) for horse (*Twilight*) and donkey (*Willy*) were obtained from SRA. These sequences as well as the hinny long reads were assembled with trio-binning function of the Canu assembler program. The initial assembly was scaffolded with Hi-C data as well as a Bionano optical maps one of a Thoroughbred stallion (*Bravo*). The resulting assembly is 2.4 Gb in total separated into 162 scaffolds. Our initial goal was to use these assembly to better define the PAB - the end of the pseudoautosomal region (PAR) where X-Y recombination stops. Despite the evolutionary and biological importance of the PAB, the region has been characterized at molecular level in only a few species, and is not well-defined in horse. Previously, we identified and Sanger sequenced 4 BAC clones - 2 spanning PAB-X and PAB-Y. To identify the PAB of the horse, BAC sequences were aligned to the Y assembly, EquCab3 and a 42 Mb-size contig from trio-binning assembly which corresponds to the short arm of the X. We identified a region on both X and Y where X-Y homology drops from over 97% (PAR) to almost zero, indicative of the PAB. This region corresponds to the location of the *XKR3Y* gene in the Y but is not well-annotated in the X. We identified a duplication and an inversion in EquCab3 which was not consistent with the corresponding region in the X-BACs, or the new 42 Mb Xp contig, suggesting a mis-assembly in EquCab3. We believe that these approaches combined will also resolve other complex portions in the horse sex chromosomes.

Key Words: trio-binning, HiC, sex chromosomes, bionano, pseudoautosomal boundary

W146 Integration of long-read sequencing technology improves transcriptome annotation of the equine genome. S. Peng*[1], T. S. Kalbfleisch[2], R. Bellone[1,3], J. L. Petersen[4], and C. J. Finno[1], [1]*1Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,* [2]*Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA,* [3]*Veterinary Genetics Laboratory, School of Veterinary*

Medicine, University of California-Davis, Davis, CA, USA, [4]Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE, USA.

The publication of the EquCab3.0 genome assembly has resulted in several improvements to the equine genome assembly. The current focus of the equine community is to construct a complete atlas including the tissue-specific transcriptome and regulome to inform studies of complex genetic traits. By incorporating improved genome sequences, in silico gene predictions, and available short-read mRNA sequencing data, the new Ensembl gene annotation for EquCab3.0 (release 102) now contains 44,891 transcripts from 20,955 genes, with a 98.4% BUSCO complete score. However, the inherent limitation in short-read sequencing technology prevents accurate identification of tissue-specific isoforms, compromising the quality of the annotation of the tissue-specific transcriptome. To address this issue, we performed a pilot study incorporating long-read sequencing technology to identify novel isoforms and improve annotation of untranslated regions (UTRs) of the equine genome. Cerebral cortex samples from 4 animals with RIN scores >8 were sequenced using PacBio Iso-seq protocol in one SMRT cell. A total of 21,376 nonredundant transcripts were expressed in at least 3 of the 4 animals. Annotation of these transcripts revealed both novel transcripts and isoforms in the equine genome. The incorporation of long-read sequencing data will be pivotal to the completion of a tissue-specific equine transcriptome.

Key Words: horse, FAANG, transcriptome, functional annotation, long-read sequencing

W147 Transcriptome analysis of 8 priority tissues in 2 Thoroughbred stallions for the Functional Annotation of Animal Genomes project. A. Barber*[1], S. Peng[2], A. Fuller[1], E. Giulotto[3], T. Kalbfleisch[4], C. Finno[2], R. Belone[2], and J. Petersen[1], [1]*University of Nebraska–Lincoln, Lincoln, NE, USA,* [2]*University of California-Davis, Davis, CA, USA,* [3]*University of Pavia, Pavia, Italy,* [4]*University of Kentucky, Lexington, KY, USA.*

The functional annotation of genomes will provide the tools necessary to perform applied research into complex traits such as disease, reproduction, and performance. The Functional Annotation of Animal Genomes (FAANG) project aims to identify all functional elements of the genome in domestic species, including the horse. The objective of this study was to utilize RNA-sequencing data (RNA-seq) to determine tissue-specific gene expression from healthy Thoroughbred stallions across tissues. Transcriptome data are necessary to correlate gene activity with regulatory elements identified using other methodologies (e.g., ChIP-seq). To build a transcriptome database in the adult male horse, 102 tissues were collected from 2 Thoroughbred stallions and flash frozen. Eight tissues were prioritized for cross-species and between-sex comparisons: adipose, lamina, left ventricle, lung, liver, parietal cortex, skeletal muscle, and testis. RNA was isolated from tissues with RIN scores ranging from 7.6 to 9.7 (average = 8.8). Stranded, Poly-A$^+$ libraries were sequenced, and the resulting reads were trimmed and mapped to EquCab3.0. An average of 34.1M reads per sample were obtained (range 30.7M (testis) to 38.8M (adipose)). On average, 92% of reads uniquely mapped to the genome (range 87.9% (left ventricle) to 93.6% (testis)). Combined with data previously collected from 2 adult Thoroughbred mares, these transcriptome data will allow for analysis of gene expression across tissues, between sexes, and among species. Integrating these data with other functional annotation data will provide a basis for future studies. Furthermore, these data provide an understanding of the transcriptome in these prioritized tissues, which will aid in identification of aberrant gene function related to various traits of interest in the horse.

Key Words: horses and related species, RNA-seq, gene expression, genome annotation

W148 Rare and common variant discovery by whole-genome sequencing of 101 Thoroughbred racehorses. T. Tozaki*, A. Ohnuma, M. Kikuchi, T. Ishige, H. Kakoi, K.-I. Hirora, and S.-I. Nagata, *Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

The Thoroughbred is formed by crossing Arab breeds and British native horses and is currently used in horseracing worldwide. In this study, we constructed a genome-wide variant database from 101 unrelated Thoroughbred horses born in Japan, or born in the USA, the UK, Ireland, or France and then imported to Japan. whole-genome sequencing (WGS) data were obtained using Illumina paired-end (150 bp) sequencing technology with 36.8-fold coverage on average (range 29.5–54.2). WGS of 101 Thoroughbred racehorses revealed 11,570,312 and 692,756 SNVs from autosomal (1–31) and X chromosomes, respectively, in a total of 12,263,068 SNVs, and that around 11% of these are rare variants. Individual horses had a maximum of 25,554 rare variants; several of these were functional variants, such as nonsynonymous substitutions, start-gained, start-lost, stop-gained, and stop-lost variants, suggesting that rare functional variants affecting protein functions reflect individual phenotypes. In humans, rare variants are known to play a key role in many complex diseases, and rare variants in horses may also play a key role in Thoroughbred disease and/or racing performance, which could explain the missing heritability. The Equine Genetics and Thoroughbred Parentage Testing Standardization Committee of ISAG is investigating a move from STRs to SNVs as markers for determining parentage in Thoroughbreds. In the present study, we identified many SNVs as common variants and found duplicated regions in the horse genome and multiple mapped regions of sequence reads. Therefore, candidate SNVs for parentage verification should be selected from the common variants and exclude SNVs detected at these duplicated regions. The SNV database obtained in the present study can be used to confirm this. Currently, the generation and use of genetically modified racehorses is banned by the ISBC and the IFHA in horseracing. In the present study, we targeted only Thoroughbreds and determined the extent of Thoroughbred genomic diversity among the population of racehorses. Our findings will be useful as baseline information for gene-doping tests that use whole-genome and targeted resequencing.

Key Words: horse, whole-genome sequencing, Thoroughbred, rare variant, gene doping

Animal Epigenetics Workshop

W149 Micrococcal nuclease sequencing of pig sperm suggests a relationship between nucleosome retention and both semen quality and early embryo development. M. Gòdia[1], S. S. Hammoud[2], M. Naval-Sánchez[3], I. Ponte[4], J. E. Rodriguez-Gil[4], A. Sánchez[4,1], and A. Clop*[1,5], [1]*Centre for Research in Agricultural Genomics CRAG, Cerdanyola del Valles, Catalonia, Spain,* [2]*University of Michigan, Ann Arbor, MI, USA,* [3]*CSIRO, St Lucia, Brisbane, Australia,* [4]*Universitat Autonoma de Barcelona, Cerdanyola del Valles, Catalonia, Spain,* [5]*CSIC, Barcelona, Catalonia, Spain.*

In animals, the chromatin structure of the mature spermatozoon is ultra-compacted due to the replacement of histones by protamines during spermatogenesis. However, a small fraction of nucleosomes remains bound to DNA at specific sites of the genome and it has been linked to sperm biology and embryogenesis. The genomic characterization of nucleosome occupancy in the sperm chromatin could help identifying molecular markers for sperm quality and fertility traits. Nonetheless, these

maps are not yet available for most livestock species, including swine. In this study, we performed micrococcal nuclease digestion followed by high-throughput sequencing on pig ejaculated spermatozoa and mapped the mono-nucleosomal and sub-nucleosomal chromatin fractions. We found 25,293 mono-nucleosomal and 4,239 sub-nucleosomal peaks covering 0.3% and 0.02% of the porcine genome, respectively. We detected positional conservation of the nucleosome-associated DNAs in sperm between human and pig. We also carried gene ontology analysis of the genes mapping nearby the mono-nucleosomal peaks and also searched for putative transcription factor binding motifs within the mono-nucleosomal peaks and found an enrichment for sperm function and embryo development related processes. Remarkably, we detected enrichment for the canonical binding site of Znf263. In humans, this transcription factor has been suggested as a key regulator of the genes with paternal preferential expression during early embryo development. In addition, we also observed co-occupancy of the RNAs present in pig sperm and these RNAs related to sperm quality, with the mono-nucleosomal peaks. We also found a co-location trend between GWAS hits for semen quality in swine and the mono-nucleosomal sites. The results obtained in this study clearly indicate that there is a relationship between nucleosome positioning in sperm with sperm phenotypes and embryo development.

Key Words: pig, sperm, chromatin nucleosome, micrococcal nuclease

W150 ISO-seq data reveals allele-specific isoform expression. S. Bardoloi*, L. Nguyen, B. Engle, B. Hayes, and E. Ross, *University of Queensland, Brisbane, Queensland, Australia.*

Allele-specific expression (ASE) is the imbalance in transcription of paternal and maternal alleles at a locus. The few ASE studies using RNA-sequencing data have been reported in cattle. Since RNA-seq produces short reads much of the data is unable to be associated with a haplotype of origin, as it does not overlap a SNP. Follow in from this, the isoform that is being expressed by each haplotype has not been considered. We hypothesized that some of the ASE observed in transcriptomes was not due to different levels of expression between haplotypes, but rather that each haplotype was expressing a different isoform. Allele-specific isoform expression (ASIE) can only be identified when transcriptome data is able to be characterized both in terms of haplotype of origin, and isoform. To address this hypothesis, we conducted an ASIE analysis in Brahman cattle using data generated from isoform sequencing technology (ISO-seq, PacBio) of liver. A phased haplotype level assemble of the animal was first used to assign each ISO-seq read to the haplotype of origin. Isoforms were then identified in the same data [LN1] using TAMA. For each gene, a contingency table of haplotype of origin (columns) and isoform (rows) was calculated. The values in the table were the number of ISO-seq reads for each isoform from each haplotype. A Fisher's exact test was used to test for independence between the isoform and the haplotype of origin. After filtering and correcting for multiple testing using a Bonferroni correction, 12 of the 36 genes showed a significant relationship between isoform and haplotype of origin. The significant genes are Proteosome subunit alpha 1, B. indicus complement factor H, Fibrinogen alpha chain, Glycoxalase 1, Aldo keto reductase, UDP glucuronosyltransferase, Uncharacterized, Aldolase, Acyl-CoA dehydrogenase, Proteosome subunit alpha 2, CXXC repeat containing interactor of PDZ3 domain (CRIPT)[LN2]. This is the first observation of ASIE reported in Brahman cattle. Further studies on more tissues and examining the relationship between ASIE and ASE are currently under way.

W151 A comprehensive RNA editome reveals RNA editing sites affecting the function of *HSPA12B* in myogenesis via altering binding ability for *miRNA-181b*. A. A. Adetula*[1,2], X. Fan[1], Y. Zhang[1], Y. Yao[1], J. Yan[1], M. Chen[1], Y. Tang[1], Y. Liu[1], G. Yi[1], K. Li[1,2], and Z. Tang[1,2], [1]*Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China,* [2]*Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

One of the most prevalent forms of post-transcriptional RNA modification is the conversion of adenosine (A) nucleotides to inosine (I), mediated by the ADAR family of enzymes. RNA editing generates diversity in mammals and results in amino acid transitions and changes in gene expression. However, the extent to which RNA editing affect gene expression via modifying microRNA (miRNA) binding site remains unexplored. In this study, we systematically profiled the RNA editome across 10 tissues from Duroc and Luchuan pigs and identified RNA editing sites. A total of 171,909 editing sites were discovered, of which 4,552 were differentially edited sites across tissues between the 2 breeds. The RNA-edited gene sets were enriched in known developmental pathways essential for physiological function and organ development, such as TGF-β, PI3K-Akt, AMPK, and Wnt signaling pathways. Moreover, we found that RNA editing events at the miRNA binding sites in the 3′-UTR of HSPA12B mRNA could prevent the miRNA-mediated mRNA downregulation of HSPA12B in the muscle-derived satellite (MDS) cell, consistent with the results obtained from the Luchuan skeletal muscle. This study demonstrates the importance of RNA editing in regulating gene expression and offers a novel approach to understand the genetic mechanisms underlying phenotypic variation in animals.

Key Words: RNA editing, gene expression, HSPA12B, microRNA, skeletal-muscle-derived satellite cell

W152 Livestock methylomics: Systematic evaluation of DNA methylation profiling assays for industry. A. Caulton*[1,2], R. Brauning[1], K. G. Dodds[1], A. Hagani[3], J. Zoller[4], C. Couldrey[5], S. Horvath[3], and S. M. Clarke[1], [1]*AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand,* [2]*University of Otago, Dunedin, New Zealand,* [3]*Department of Human Genetics, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA,* [4]*Department of Biostatistics, Fielding School of Public Health, University of California Los Angeles, Los Angeles, CA, USA,* [5]*Livestock Improvement Corporation, Hamilton, New Zealand,* [6]*University of Idaho, Moscow, ID, USA.*

DNA methylation plays a fundamental role in the regulation of growth and development in mammals and serves as a biomarker of chronological age. While the importance of DNA methylation is well established, its potential for breeding applications within the livestock sector is yet to be realized. To successfully incorporate DNA methylation data into current genetic merit predictions, high-throughput, robust and cost-effective assays should be employed. Here we compare 4 sequence-based approaches to determine genome-wide methylation signatures across 8 different tissue types in sheep (1) whole-genome bisulfite sequencing (WGBS); (2) reduced representation bisulfite sequencing (RRBS); (3) methylation sensitive restriction enzyme sequencing (MRE-seq) and; (4) direct detection of methylation with Nanopore single-molecule sequencing technology. The concordance between the methodologies is evaluated and benchmarked against WGBS, the gold standard methylation profiling assay. The benefits, and disadvantages of each method are examined with emphasis on industry implementation. In addition to sequence-based approaches, we have used the custom mammalian methylation array "HorvathMammalMethyl40" to assay DNA methylation levels at approximately 37 thousand CpG sites across key livestock species. From this work we have constructed the first 'epigenetic clock' for domesticated goat, cattle, red and wapiti-breed deer, and composite-breed sheep. This livestock epigenetic clock uses only 214 CpG sites to estimate age (relative to the maximum lifespan of the species) with high accuracy across all 4 species ($r > 0.93$), using a single mathematical model. The applications of this livestock epigenetic clock could extend well beyond the scope of chronological age estimates. Many independent studies have demonstrated that a deviation between true age and clock derived molecular age is indicative of past and/or present health (including stress) status. There is, therefore,

untapped potential to utilize epigenetic clocks in breeding programs as a predictor for age-related, production traits.

Key Words: methylation, epigenetic clock, livestock, epigenetics, ovine FAANG

W153 Withdrawn

W154 Identifications of epigenetic regulation mechanism according to the growth of pig in abdominal fat tissue through multi-omics integration analysis. D.-Y. Kim* and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Republic of Korea.*

Fat is a major organ involved in the synthesis of new fatty acids (FA), FA circulation, and lipid metabolism. Various genetic studies have been conducted on pig fat, but understanding the growth and specific adipose tissue is insufficient. The purpose of this study is to investigate the epigenetic difference of abdominal fat according to the growth of pigs. Abdominal fat was collected at each time point of 10 and 26 weeks from cross-breeding F1 pigs between KNP and Yorkshire breeds, and then methylome and transcriptome data were produced using MBD-seq and RNA-seq. Differentially methylated genes (DMG) and differentially expressed genes (DEG) were identified as 2,251 and 5,768, respectively. DMG and DEG have been shown to be primarily involved in immune responses, such as the chemokine signaling pathway and the B-cell receptor signaling pathway, and functions related to lipid metabolisms, such as the PPAR signaling pathway and fatty acid breakdown. When we investigated the effect of DNA methylation on gene expression through cis-regulation and trans-regulation analysis, cis-regulation related to immune responses and trans-regulation associated with fat metabolism were identified. Genes in these pathways are associated with immunity, lipolysis, and synthesis during growth, suggesting that they may be key biomarkers. Thus, we provide a systematic investigation of changes in the epigenetic regulation of porcine abdominal fat with aging to broaden our understanding of the regulatory mechanisms involved in fat metabolism and immune response growth.

Key Words: pigs and related species, epigenomics, functional genomics, genome sequencing, fat/lipid

W155 Epigenetic marks in the promoter of *GNAS* and *EBF3* are associated with meat tenderness in *Bos indicus*. M. M. de Souza[1,2], S. C. M. Niciura[1], M. I. P. Rocha[1,3], W. J. S. Diniz[1,4], J. J. Bruscadin[1,3], J. Afondo[1], P. S. N. de Oliveira[1], G. B. Mourão[5], A. Zerlotini[6], L. L. Coutinho[5], J. E. Koltes[2], and L. C. A. Regitano*[1], [1]*Embrapa Pecuária Sudeste, Empresa Brasileira de Pesquisa Agropecuária, São Carlos, São Paulo, Brazil,* [2]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [3]*Department of Genetics and Evolution, Federal University of São Carlos, São Carlos, São Paulo, Brazil,* [4]*Department of Animal Sciences, North Dakota State University, Fargo, ND, USA,* [5]*Department of Animal Science, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil,* [6]*Embrapa Informática Agropecuária, Empresa Brasileira de Pesquisa Agropecuária, Campinas, São Paulo, Brazil.*

Tenderness is a complex trait with economic value for the beef market. Understanding the genetics and epigenetics mechanisms underlying this trait may help improve the accuracy of breeding programs and deliver a better quality product to the consumers. However, little is known about epigenetic effects in the muscle of *Bos taurus* and their implications in tenderness, and no study is available in *Bos indicus* so far. Therefore, we searched for differences in the methylation profile of *Bos indicus* muscle with extreme values for meat tenderness (tender = 6, tough = 6). For this, we analyzed reduced representation bisulfite sequencing (RRBS) and identified 123 differentially methylated CpGs (DMCs) and 42 regions (DMRs) ($P < 0.05$ and methylation difference >25%), although the global methylation profile had low variation in the population. Most of the DMR (70.73%) and DMC (83.72%) were hypermethylated in the tender group. Enrichment analysis of previously predicted target genes suggested that signal transduction pathways may be affected by differences in methylation between tender and tough meat, with G protein-coupled receptor signaling being a key pathway. Our analysis suggested that different methylation levels related to tenderness may regulate the expression of *GNAS* and *EBF3* (RNA-seq; Pearson correlation, $P < 0.05$). *GNAS* showed expression positively correlated with CpG methylation level in the DMC23 (r = 0.75) while *EBF3* expression was negatively correlated with DMC89 (r = −0.72), DMC90 (r = −0.75) and DMR40 (r = −0.81). These elements were in CpG islands, located in the promoter of the gene *EBF3* and intron 1 of *GNAS*, an alternative promoter of this gene. *GNAS* is known to have a complex imprinted status and is a member of the G protein-coupled receptor signaling pathways. This pathway and the gene *EBF3* function in muscle homeostasis, relaxation, and muscle cell-specificity. We present DMCs and DMRs that may be of interest to decipher the epigenetic mechanisms affecting tenderness. Further, *EBF3* and *GNAS* were identified as potential candidate genes associated with tenderness via methylation.

Key Words: RRBS, cattle, Nelore, methylation, imprinting

W156 Characterization of the adipose tissue DNA methylation framework between male and female suckling lambs. A. Suárez-Vega, C. Esteban-Blanco, H. Marina, R. Pelayo, M. Alonso-Garcia, C. Hervas-Rivero, B. Gutierrez-Gil, and J.-J. Arranz*, *Universidad de León, León, Spain.*

Epigenetics is emerging as a cutting-edge area of research in livestock nutrition, genetics, and breeding. The epigenetic variation is due to epigenetic marks, including DNA methylation, chromatin remodeling, histone modification, long noncoding RNA, and microRNAs. For animal breeding, epigenetics may help in understanding the genetic architecture of complex traits. For example, in the sheep industry, the quantity and composition of adipose deposits influence the quality of lamb carcasses. Moreover, one determining factor influencing adipose deposit distribution, physiology, and cell signaling is sex. However, little is known about how DNA methylation marks in males and females can contribute to sex differences in adipose metabolism in livestock species. To explore the functional importance of genome-wide DNA methylation sex differences in adipose tissue of lambs, we proposed a systematic identification of the genomic DNA methylation patterns in perirenal fat tissue between 6 male and 6 female sucking lambs (~30 d of age) using high-throughput whole-genome bisulfite sequencing. Read mapping, and DNA methylation calling was performed using the Oar_rambouillet_v1.0 reference assembly. The average percentage of reads mapped to the reference genome was 79%. The pattern of methylated sites in males and females was very similar, with the majority of sites being CG sites (>95%), whereas some few CHH (~3%) and CHG (<1%) sites (C = cytosine; G = guanine; H = adenine, cytosine or thymine) were also found. The genomic regions with the highest methylation levels were 3′ UTRs, introns, repeated regions, and exons. Preliminary results on differential methylated regions between male and female lambs opened new insights on a potentially relevant role of sex in adipose epigenetic regulation. These results suggest that changes in methylation patterns could trigger fat deposition in both sexes.

Key Words: sheep and related species, epigenomics, fat/lipid, product quality

W157 Maternal methionine supplementation alters alternative splicing and DNA methylation in bovine skeletal muscle. L. Liu* and F. Peñagaricano, *University of Wisconsin-Madison, Madison, WI, USA.*

The evaluation of alternative splicing, including differential isoform expression and differential exon usage, can provide some insights on the transcriptional changes that occur in response to environmental perturbations. Maternal nutrition is considered a major intrauterine regulator of fetal developmental programming. The objective of this study was to assess potential changes in splicing events in the longissimus dorsi muscle of beef calves gestated under control or methionine-rich diets.

RNA sequencing and whole-genome bisulfite sequencing were used to evaluate muscle transcriptome and methylome, respectively. Alternative splicing patterns were significantly altered by maternal methionine supplementation. Most of the altered genes were directly implicated in muscle development, muscle physiology, ATP activities, RNA splicing and DNA methylation, among other functions. Interestingly, there was a significant association between DNA methylation and differential exon usage. Indeed, among the set of genes that showed differential exon usage, significant differences in methylation level were detected between significant and nonsignificant exons, and between contiguous and noncontiguous introns to significant exons. Overall, our findings provide evidence that a prenatal diet rich in methyl donors can significantly alter the offspring transcriptome, including changes in isoform expression and exon usage, and some of these transcriptomic changes are mediated by changes in DNA methylation.

Key Words: differential isoform expression, differential exon usage, fetal programming

Plenary II

W158 Harnessing the power of genomics and AI to breed new species for aquaculture. M. Wellenreuther*[1,2], [1]*The New Zealand Institute for Plant and Food Research Ltd., Nelson, New Zealand,* [2]*School of Biological Sciences, University of Auckland, Auckland, New Zealand.*

Aquaculture production holds immense promise to create food for future generations, but a lack of species diversity presents a challenge for sustained growth of the sector. Here I report on our recent work to develop 2 native finfish species for aquaculture in New Zealand, the Australasian snapper (*Chrysophrys auratus*) and the silver trevally (*Pseudocaranx georgianus*). Specifically, I will focus on recent developments to genomically enable aquaculture breeding of new species by (1) harnessing genomic insights about their biology, with a focus on growth; and (2) developing smart AI models to enable rapid phenotyping and individual fingerprinting. I will end my talk by discussing the promises and challenges of these technologies to diversify the species that we can farm.

Pig Genetics and Genomics Workshop

W159 Pig genome functional annotation enhances biological interpretations of complex traits and comparative epigenomics. Z. Pan*[1], Y. Yao[2], H. Yin[3], Z. Cai[4], Y. Wang[1], L. Bai[3], C. Kern[1], M. Halstead[1], K. Chanthavixay[1], N. Trakooljul[5], K. Wimmers[5], G. Sahana[4], G. Su[4], M. Sandø Lund[4], M. Fredholm[6], P. Karlskov-Mortensen[6], C. W. Ernst[7], P. Ross[1], C. K. Tuggle[8], L. Fang[2], and H. Zhou[1] [1]*Department of Animal Science, University of California, Davis, Davis, CA, USA,* [2]*MRC Human Genetics Unit at the Institute of Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh, UK,* [3]*Agricultural Genome Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China,* [4]*Center for Quantitative Genetics and Genomics, Faculty of Technical Sciences, Aarhus University, Tjele, Denmark,* [5]*Leibniz-Institute for Farm Animal Biology, Dummerstorf, Germany,* [6]*Animal Genetics, Bioinformatics and Breeding, Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark,* [7]*Department of Animal Science, Michigan State University, East Lansing, MI, USA,* [8]*Department of Animal Science, Iowa State University, Ames, IA, USA.*

The functional annotation of livestock genomes is crucial for understanding the molecular mechanisms that underpin complex traits of economic importance, adaptive evolution and comparative genomics. Here, we provide the most comprehensive catalog to date of regulatory elements in the pig (*Sus scrofa*) by integrating 223 epigenomic and transcriptomic data sets, representing 14 biologically important tissues. We systematically describe the dynamic epigenetic landscape across tissues by functionally annotating 15 different chromatin states and defining their tissue-specific regulatory activities. We demonstrate that genomic variants associated with complex traits and adaptive evolution in pig are significantly enriched in active promoters and enhancers. Furthermore, we reveal distinct tissue-specific regulatory selection between Asian and European pig domestication processes. Compared with human and mouse epigenomes, we show that porcine regulatory elements are more conserved in DNA sequence, under both rapid and slow evolution, than those under neutral evolution across pig, mouse, and human. Finally, we provide novel biological insights on tissue-specific regulatory conservation and demonstrate that, depending on the traits, mouse or pig might be more appropriate biomedical models for different complex traits and diseases in humans through integrating comparative epigenomes with 47 human genome-wide association studies.

Key Words: pig, epigenome, comparative genomics, chromatin state, complex trait

W160 Alteration of expression of miRNA and mRNA transcripts in fetal muscle tissue in the context of sex, mother and variable fetal weight. S. Ponsuksili*[1], A. Ali[1], F. Hadlich[1], E. Murani[1], and K. Wimmers[1,2], [1]*Leibniz Institute for Farm Animal Biology (FBN), Institute for Genome Biology, Dummerstorf, Germany,* [2]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany.*

Prenatal embryonic and fetal development are important processes that are closely linked to birth weight and piglet survival, as well as subsequent growth performance and final carcass quality. In particular, skeletal muscle growth and mass are largely determined during the prenatal period, when the number of muscle fibers is almost fixed. Prenatal muscle growth is regulated by complex molecular pathways that are not well understood. MicroRNAs (miRNAs) have emerged as key regulators of vital pathways and biological processes, including developmental processes such as myogenesis. The study aimed at elucidating molecular routes of developmental processes mediated by miRNAs and the expression of mRNA targets in fetal muscle tissue at the background of variable fetal weights. Analyses were performed in an experimental population based on reciprocal crossing of German Landrace (DL) and Pietrain (Pi) to obtain a 3-generation pig F_2 population. The sows were slaughtered and the F_2 fetuses (n = 118) were extracted from the uteri and their weight was recorded. Expression profiling for mRNAs and miRNAs of M. longissimus dorsi was done using microarrays. The effect of sex, dam and fetal weight on the expression levels of transcripts was analyzed using a linear model (GLM procedure, SAS 9.4 Software, SAS Inc., Cary, USA) containing the fixed effects (sex and mother) and fetal weight as a covariate. Transcripts with FDR <0.05 were considered as a significant threshold. The abundance of 13 mRNA transcripts was found to be significantly affected by sex, most of them were located on the sex chromosome, except ANKS1B and LOC100155138. Moreover 853 and 275 probe-sets were influenced by the dam and fetal weight at 63 dpc, respectively. Only miR-153 was differentially expressed due to sex, the expression of 13 miRNAs was

influenced by dam and 6 miRNAs by fetal weight. In addition, we identified 343 pairs of 12 miRNAs and 152 in silico predicted and negatively correlated mRNA target transcripts that showed correlation with fetal weight. The correlated miRNAs and their target genes were enriched in key Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and biological processes. These results demonstrate triangular relationships between miRNA expression in fetal skeletal muscle tissues, their mRNA targets and fetal weight at 63 dpc of prenatal development in pigs.

Key Words: fetal weights, pig, miRNA, mRNA

W161 Time serial ovarian transcriptome analysis for entire porcine estrous cycle reveals changes of steroid metabolism and corpus Luteum development. Y. Park*, Y.-B. Park, S.-W. Lim, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

The estrous cycle (estrus, metestrus, diestrus, and proestrus) is a physiological process that occurs in most mammalian females under the effect of reproductive hormones. This cycle affects reproduction and causes many changes, especially in the reproductive organs. Among them, an ovary is an important place where ovulation, luteinization, CL development, and luteolysis take place. For a more in-depth study of the dynamic changes in gene expression, the transcriptome of the porcine ovary was observed in the estrous cycle at intervals of 3 d from d 0 to d 18. A total of 4,414 DEGs were identified at 7 time points of the estrous cycle, and these were classified into 3 clusters according to the transcriptome expression pattern. During diestrus, the expression of the transcriptome increased rapidly, and cluster 1 was upregulated at that period, whereas clusters 2 and 3 tended to be downregulated. We performed functional analysis of the genes included in each cluster, selected Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways related to the gene ontology (GO) terms, and identified significant genes among them. In cluster 1, GO results were found that included terms such as intestinal absorption and sterol biosynthesis, and based on this, steroid biosynthesis was selected for the significant KEGG pathway. In cluster 2, cytokine-cytokine receptor interaction was chosen as important KEGG pathways based on GO outcomes such as neutrophil chemotaxis and regulation of timing of cell differentiation. Finally, morphogenesis and embryo development-related terms were shown in cluster 3, and the hedgehog signaling pathway was selected. Our study exhibited the dynamic changes and a comprehensive understanding of the porcine ovary during the estrous cycle through DEG profiling and transcriptome analysis. Especially, we found several genes that were affected to hedgehog signaling pathway and consequently, this suggests that genes that influence embryonic development during the diestrus are expressed in the ovary. Further studies should be conducted with every estrous cycle to understand the mechanism of the porcine ovary.

Key Words: pigs and related species, functional genomics, gene expression, RNA-seq, reproduction

W162 Identifying muscle transcriptional regulatory elements in the pig genome. D. Crespo-Piazuelo*[1], O. González-Rodríguez[1], M. Mongellaz[2], H. Acloque[2], M.-J. Mercat[3], M. C. A. M. Bink[4], A. E. Huisman[5], Y. Ramayo-Caldas[1], J. P. Sánchez[1], and M. Ballester[1], [1]*Animal Breeding and Genetics Program, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Spain,* [2]*Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Génétique animale et biologie intégrative (GABI), Jouy-en-Josas, France,* [3]*IFIP-Institut du porc and Alliance R&D, Le Rheu, France,* [4]*Hendrix Genetics Research Technology and Services B.V., Boxmeer, the Netherlands,* [5]*Hypor B.V., Boxmeer, the Netherlands.*

The present work is part of H2020 GENE-SWitCH project which aims to deliver new functional genome information of two main monogastric farm species (pig and chicken) to better understand the genetic determinants of complex traits. Specifically, in this work, we aimed to identify regulatory elements of muscle transcriptome in the genome of 300 pigs (100 Duroc, 100 Landrace, and 100 Large White) using expression genome-wide association studies (eGWAS). For that purpose, muscle samples were obtained at slaughter and RNA was extracted using spin column-based kit and sequenced on the Illumina NovaSeq6000 platform. Counts were quantified by RSEM/1.3.0 and normalized by TMM (trimmed mean of M-values). Low expressed genes and those not present in at least 5% of the animals were removed, remaining 13,887 genes for further analysis. Through whole genome sequencing (NovaSeq6000 platform), 44,127,400 polymorphisms (SNPs and indels) were found among all the individuals. A total of 25,315,878 polymorphisms were kept after filtering out those with missing genotype data >0.1 and minor allele frequency <0.05. eGWAS were conducted between the filtered polymorphisms and the normalized expression data using the fastGWA tool from GCTA/1.93.2. After Bonferroni correction, a total of 8,099,604 significant associations were found between 4,814,732 polymorphisms and the expression of 7,496 genes. A total of 25,642 polymorphisms were associated with more than 10 genes and were considered hotspot regulatory elements. Regarding their genomic position, 3,283,835 polymorphisms (68%) were annotated as cis-regulatory elements, as they were located at 1Mb or less than their associated gene. The most significantly associated variant (adj. P-value = 2.66×10^{-178}) was located on an intronic region of the SRSF protein kinase 3 (*SRPK3*) gene, which encodes a protein associated to muscle development. Our results identified key regulatory elements associated with gene expression in muscle that may be included in new predictive models to increase the accuracy of genomic predictions, speeding up the rate of genetic improvement of economically important traits in pigs. This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the grant agreement no. 817998.

Key Words: pigs and related species, system genetics (eQTLs), RNA-seq, muscle, regulatory element

W163 Characterization of circulating microRNA profile in Iberian pigs with and without heat stress. M. Muñoz*[1], A. Fernández-Rodríguez[2], F. García[1], A. García-Cabrero[1], C. Caraballo[1,3], G. Gómez[4], G. Matos[4], C. Óvilo[1], and J. García-Casco[1,3], [1]*Animal Breeding Department, INIA (CSIC), Madrid, Spain,* [2]*Unit of Viral Infection and Immunity, National Center for Microbiology, Institute of Health Carlos III, Majadahonda (Madrid), Spain,* [3]*Centro de Investigación en cerdo Ibérico INIA-Zafra (INIA, CSIC), Zafra (Badajoz), Spain,* [4]*Sánchez Romero Carvajal—Jabugo, SRC, Huelva, Spain.*

In the last century, the average surface temperature of the planet rose almost one degree, being 2016 the warmest year since records are available. One of the consequences of climate warming is heat stress, that alters the physiology of animals reducing female and male reproduction. Iberian pigs have been adapted to high temperatures during centuries, however, a seasonal effect on reproductive traits such as the number of piglets born alive or the number of weaned piglets has been observed. Circulating microRNAs (ECmiRNAs) are small regulatory RNAs (size <22 nt) proposed as biomarkers reporting physiological estates. We have analyzed the circulating microRNA profile in plasma samples of 9 Iberian sows and 6 sires at 2 different months: June (heat stress; HS) and November (without heat stress; NHS) with an average temperature of 23.84°C and 9.84°C, respectively. Small RNA libraries were built with NEBNext Small RNA Library kit using 1x50nts single ends and sequenced in an Illumina HiSeq2500. After quality control and trimming, the sequences were processed using miRDeep2 (v. 0.0.7) software which characterizes known and novel microRNAs. Afterward, differentially expressed ECmiRNAs between HS and NHS months were detected using edgeR software. DIANA-miRPath v3.0 was used to detect the in silico targets of the differentially expressed ECmiRNAs and a pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG). We obtained a total of 11.63 million of reads per sample on average and 8.64 million of reads after trimming and quality control. On average, a total of 5.32

million reads (60.67%) mapped against the reference porcine genome assembly (Sscrofa11.1), being a 14.14% identified as microRNAs. A total of 249 known and 4 novel ECmiRNAs were identified in the sample set. Differential expression analyses stratified by sex were carried out, being 4 ECmiRNAs differentially expressed in females (ssc-miR-30c-5p, ssc-let-7a, ssc-miR-361-5p, and ssc-let-7e) and none in males. A total of 39 KEGG pathways such as oocyte meiosis or fatty acid biosynthesis and metabolism affected by these miRNAs were observed. These results suggest the 4 differentially expressed ECmiRNAs could be used as biomarker for heat stress in Iberian pigs.

Key Words: pigs and related species, microRNAs, environment, reproduction

W164 Historical biogeography of Philippine native pigs and the perplexing mitochondrial DNA variation in Philippine wild pigs. J. Layos*[1,2], C. Godinez[1,3], L. Liao[4], Y. Yamamoto[1], and M. Nishibori[1], [1]*Laboratory of Animal Genetics, Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan,* [2]*College of Agriculture and Forestry, Capiz State University, Burias Campus, Mambusao, Capiz, Philippines,* [3]*Department of Animal Science, Visayas State University, Visca, Baybay City, Leyte, Philippines,* [4]*Laboratory of Aquatic Ecology, Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan.*

The Philippines is an archipelago of 7,641 islands situated in Island Southeast Asia at the crossroads of past human migrations in the Asia-Pacific region. It was believed to have never been connected to the Asian continent, even during the severe Quaternary sea-level drops. As a result, the history of pig dispersal in the Philippines remains controversial due to the limited molecular studies and the absence of archeological assemblages that exhibit signs of pig domestication. This study provides the first comprehensive screening of the mitochondrial DNA of Philippine native pigs (n = 175) and Philippine wild pigs (n = 9) to resolve their earlier dispersal history by conducting phylogenomic analysis altogether with domestic pigs and wild boars corresponding roughly to their geographic origin. Results revealed a demographic signal of pig ancestry exhibiting a close genetic affiliation from mainland Southeast Asia and Northeast Asia Regions which corroborates a gene flow that might have arisen through human migration and trade. Here, we proposed 2 possible dispersal routes. One is through Northeast Asia paralleled with the Neolithic expansion in Island Southeast Asia and Oceania, and the other is through mainland Southeast Asia, which may have traversed through the Sundaic Region to Palawan and the Sulu Archipelago. Despite geographical barriers to migration, numerous genetic lineages have persisted on various Philippine islands and even warrants the recognition of a Philippine Lanyu-type. The prehistoric population size dynamics predate a demographic expansion during the Late Pleistocene ages as the Southern regions to be the probable origin, eventually expanded toward the Central regions. The intriguing signal of disparity detected among the molecular result, morphology, and distribution range of the numerous Philippine endemic wild pigs opens a new challenging approach in shedding the complexities between these animals.

Key Words: genetic diversity, historical biogeography, mitochondrial DNA, Philippine native pigs, phylogenomics

W165 The genomic inbreeding trends in Italian heavy pig breeds over the last 25 years. G. Schiavo*[1], S. Bovo[1], A. Ribani[1], S. Tinarelli[1,2], V. Utzeri[1], M. Cappelloni[2], M. Gallo[2], and L. Fontanesi[1], [1]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy,* [2]*Associazione Nazionale Allevatori Suini (ANAS), Rome, Italy.*

The control of inbreeding is fundamental in managing livestock species. A high level of inbreeding leads to a decline in performance and fitness, a phenomenon known as inbreeding depression, and to the emergence of deleterious or lethal alleles that, in absence of inbreeding, would remain at a low frequency in the population. The Italian pig industry, mainly oriented to the production of the Protected Designation of Origin (PDO) dry-cured ham, is based on 3 heavy pig breeds, namely Italian Large White (ILW), Italian Landrace (IL) and Italian Duroc (ID). The breeding program of these breeds started about 25 years ago and information about pedigree has been recorded in the last decades. Inbreeding coefficient (F_{PED}) is traditionally calculated using pedigree records. With the advent of high-throughput genotyping platforms, new methods to calculate the genomic inbreeding have been developed, directly using genome information. One of the most effective methods is the detection of long stretches of the genome that is homozygous at each adjacent locus, called runs of homozygosity (ROH). The proportion of the autosomal genome covered by ROH can be used to estimate the level of genomic inbreeding (F_{ROH}) and the history of the population. In this work, we retrospectively analyzed F_{PED} and F_{ROH} over this period in these 3 breeds. A total of 3,400 ILW, 1940 ILA and 1,100 ID pigs born over the last 25 years have been genotyped with the 70K Illumina GGP Porcine HD and Illumina Porcine60K SNPchips. F_{PED} was computed with Inbupgf90 software from pedigree data. ROH were identified with PLINK version 1.9. Then F_{ROH} and F_{PED} were averaged over all animals born by year. Averaged F_{ROH} over all considered years was higher in ID; the trend of F_{PED} and F_{ROH} was increasing during years for all breeds. It is worth to notice that F_{PED} trend had a strongest slope with respect to F_{ROH}, indicating that, at a biological level, decades of selection did not worsen the inbreeding level of the breeds. The results indicated that both F_{ROH} and F_{PED} can be used to manage inbreeding levels in Italian heavy pig breeds and provided information that could be useful to manage these pig genetic resources.

Key Words: pigs and related species, population genomics, genotyping, inbreeding, single nucleotide polymorphism (SNP)

W166 The common warthog (*Phacochoerus africans*) reference genome and sequence variation. L. Eory[1], P. Wiener[1], H. A. Finlayson[1], K. Gharbi[2], S. Girling[3], C. Palgrave[1], E. Okoth[4], T. Burdon[1], M. Watson[1], and A. L. Archibald*[1], [1]*The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, UK,* [2]*Edinburgh Genomics, University of Edinburgh, Edinburgh, UK,* [3]*The Royal Zoological Society of Scotland, Edinburgh, UK,* [4]*International Livestock Research Institute, Nairobi, Kenya.*

The common warthog (*Phacochoerus africanus*) is an endemic, omnivorous species of savanna and woodlands of sub-Saharan Africa with conservation status of 'least-concerned'. The species harbor genotypes adapted to the environmental challenges posed by the hot and arid environment as well as by endemic pathogens. Common warthogs are known to be reservoirs of African Swine Fever Virus that causes a hemorrhagic disease with high mortality in domestic pigs while warthogs tolerate ASFV infections. High molecular weight DNA from a female individual was sequenced using Pacific Biosciences Sequel instruments yielding 150Gbp of raw data with a read N50 of 13.9kbp (ca. 60x coverage of the genome). A contig level assembly was generated using Falcon and Falcon-unzip. The contigs were scaffolded using Bionano optical mapping data and Bionano Solve software and proximity ligation data from a Hi-C library created using Dovetail Genomics kit. The scaffolds were refined with Dovetail Genomics' HiRise pipeline. Gaps in the assembly were filled with PBJelly using the PacBio long-read data. Error correction was done with Pilon using Illumina short-read data from the reference individual. The final assembly (GCA_016906955.1) comprises 2.4 Gbp in 718 contigs (contig N50 10.6 Mbp) with 1 scaffold for each of the 16 autosomes (PAF1–16) and PAFX plus 177 unplaced scaffolds (scaffold N50 141.88 Mbp). Comparisons with the pig (*Sus scrofa*) Sscrofa11.1 genome assembly confirmed the expected homologies including PAF1 representing a fusion of SSC13 and SSC16 and PAF3 representing a fusion of SSC15 and SSC17. RNA-seq data from 15 tissue samples from the reference individual have been used to identify the gene content of the genome. BUSCO analysis indicates that the assembly is highly complete with 94.9% BUSCOs (Benchmarking Universal Single-Copy Orthologs)

complete. Whole-genome shotgun short-read sequence data were generated from 6 further warthogs at ca. 50x genome coverage. Analysis of these data plus ca. 100x coverage of the reference animal and 7x coverage of a further individual in the public databases with the GATK pipeline revealed 23,278,680 high-quality single nucleotide polymorphisms (SNPs).

Key Words: common warthog, *Phacochoerus africans,* genome sequence, single nucleotide polymorphisms (SNP)

W167 A pan-genome of commercial pig breeds. M. Derks*[1,3], B. Harlizius[2], M. van Son[2], M. Lopes[1], E. Grindflek[2], E. Knol[1], E. Sell-Kubiak[4], and A. Gjuvsland[2], [1]*Topigs Norsvin Research Center, Beuningen, the Netherlands,* [2]*Norsvin SA, Hamar, Norway,* [3]*Wageningen University and Research, Wageningen, the Netherlands,* [4]*Poznan University if Life Sciences, Poznan, Poland.*

In recent decades, high-quality reference genomes have become available for most important livestock species. The availability of the reference genome (of Duroc origin) together with gene annotation have revolutionized pig genomics and genetics research over the past decade. However, sequences deviating considerably from the reference (i.e., from other breeds) will be interpreted as low-quality, so-called reference bias. One consequence of this is that a lot of (structural) variation not present in the reference genome is often missed. Structural variation includes various types of variation in which part of the DNA is altered (e.g., genomic deletions, duplication). Structural variants can have a large effect on phenotypes but they are often ignored or remain unidentified. Hence, the extensive degree of variation in pigs shows that a single reference genome does not represent all genomic variation within pigs, and a pig pan-genome will be the future standard. The pig pan-genome is particularly useful to identify presence/absence variations, structural variation, and other, miscellaneous variations. In this study we produced a pig pan-genome by sequencing 4 pigs from different genetic backgrounds using the Nanopore long-read sequencing technology. The breeds comprise 2 dam lines, Large White and Landrace and 2 sire lines, Duroc and a synthetic line. We generated ~150X coverage Nanopore sequencing data (read N50: 40 kb) to produce the assemblies. We produced chromosome-level assemblies (using the Flye software) comparable to the current 11.1 in terms of completeness and continuity. We further identified between breed structural variation (using Syri and Sniffles tools), which gives a unique insight in the genomic structural variation that define and differentiate the breeds. In addition, we generated lower coverage long-read sequence data for 29 animals distributed over the 4 breeds to facilitate the discovery of within breed structural variation. Together we have produced a pig pan-genome covering 4 elite breeds from Topigs Norsvin. The pig pan-genome will facilitate in the discovery of novel (structural) variation which provides a unique fundamental insight into breed genomic characteristics, which can subsequently be utilized to improve pig breeding.

Key Words: pan-genome, Nanopore sequencing, structural variation

Plenary III

W168 Diversification and sustainability of aquaculture production: What can (and cannot) we do as geneticists? F. Bertolini*, *Technical University of Denmark, National Institute of Aquatic Resources, Lyngby, Denmark.*

Diversification of aquaculture production is a key issue to reduce the pressure on fisheries derived by the increasing demand of proteins from aquatic species. In this context, the aquaculture sectors can now take full advantage of the progress in genomic technologies and of the availability of genomic information that allows enhancing the investigation of an increasing number of aquatic species. However, the majority of aquaculture practices are still capture-based for several relevant species and the advancement in term of selective breeding and implementation of genetics is still limited to a few species despite the enormous potential due to the large number of many other species that, in theory, can be cultivated. The talk will provide an overview and specific examples that start from the domestication processes of some of these species, with the subsequent implementation of selective breeding, its common and peculiar targeted traits and the major not-genetically-related obstacles that a geneticist has to face and that will need to be solved before any genetic programs can be applied. Key examples will be discussed, particularly the emblematic cases of Anguillidae, peculiar animals with a unique life cycle and a clear commercial interest that should be considered in the context of innovative production systems that take into consideration the complex ecosystem in which this species has evolved.

Livestock Genomics for Developing Countries Workshop

W169 Genetic basis of thermo-tolerance in African indigenous chickens. A. A. Gheyas*[1], M. Rachman[2], A. Vallejo-Trujillo[2], O. Bamidele[3,4], A. Kebede[3,5], T. Dessie[3], J. Smith[1], and O. Hanotte[2,3], [1]*Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Midlothian, Scotland, UK,* [2]*School of Life Sciences, University of Nottingham, Nottingham, UK,* [3]*LiveGene – CTLGH, International Livestock Research Institute, Addis Ababa, Ethiopia,* [4]*Kings University, Ode Omu, Nigeria,* [5]*Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia.*

Indigenous chickens in Africa are adapted to their harsh tropical environments. Heat stress is a major challenge to poultry survival and productivity in the tropical climate. The African continent, however, is not homogeneous in its temperature regimen but can show extreme conditions (High and Low) due to large variations in altitude (e.g., in Ethiopia). Elucidating the genetic basis of thermo-tolerance in African indigenous chickens has important implications for enhancing adaptive performance of dual-purpose improved breeds in tropical smallholder backyard poultry farming systems. Here we investigate the genomic signatures of positive selection in Nigerian and Ethiopian indigenous chickens to identify the genetic basis of thermo-tolerance. Genome sequence data from 12 Nigerian populations (87 samples), representing high-temperature agro-ecologies (annual means: 24–29°C, max: 35°C) were analyzed in combination to identify genomic regions of low heterozygosity (using *Hp* method) as a signature of positive selection for heat-tolerance. Contrarily from Ethiopia, we compared 4 populations (38 samples) from extreme low and high-temperature regions (1–37°C; negatively correlated with altitude) to find selective sweeps showing genetic differentiations (using *XPEHH* and *Fst* approaches). Several highly plausible candidate genes for heat tolerance were detected from the Nigerian analysis but few from extreme population comparisons from Ethiopia. Instead, the Ethiopian analysis detected several strong candidates for high altitude/cold tolerance adaptation. Important heat stress candidate genes from the literature were also checked in the studied populations. Heat shock protein genes (*HSP70* and *HSP90)*, which have shown differential expression between heat tolerant and nontolerant chicken breeds, failed to show any genetic differentiation between extreme populations from Ethiopia. This lack of genetic differentiation between extreme groups indicates another mechanism

of regulation of heat tolerance genes (e.g., epigenetic). Future studies should, therefore, aim toward assessing the role of epigenetic regulation of heat-tolerance traits.

Key Words: poultry and related species, genome biology, genome sequencing, adaptation, genetic improvement

W170 Whole-genome sequence analysis to detect potential candidate genes for reproduction in South African beef cattle. K. Nxumalo*[1,2], M. B. Malima[1], J. Grobler[2], M. Makgahlela[1,3], J. Kantanen[4], C. Ginja[5], D. R. Kugonza[6], N. Mohamed[7], R. P. M. A. Crooijmans[8], and A. A. Zwane[1], [1]*Animal Breeding and Genetics, Agricultural Research Council-Animal Production, Pretoria, South Africa,* [2]*Department of Genetics, University of the Free State, Bloemfontein, Free State, Bloemfontein, South Africa,* [3]*Department of Animal, Wildlife and Grassland Sciences, University of Free State, Bloemfontein, Bloemfontein, South Africa,* [4]*Animal Production Research, Agricultural Research Centre (MTT), Jokioinen, Finland.,* [5]*CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal,* [6]*Department of Agricultural Production, School of Agricultural Sciences, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda,* [7]*Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt,* [8]*Animal Breeding and Genomics Group, Wageningen University and Research, Wageningen, the Netherlands.*

Indigenous cattle of South Africa are well known for their great production under seasonally harsh environmental conditions. The breeds consist of unique morphological features that differentiate them from other breeds, which includes the color patterns and horn shape. However, there is need to account for the changing climate, therefore, new and better adaptation improvements are required for various livestock species. This study investigated genes associated with reproduction traits in South African indigenous cattle breeds namely; Afrikaner, Bonsmara, Nguni and Tuli. The Illumina whole-genome sequences from 43 individuals (Afrikaner (n = 10), Tuli (n = 8), Bonsmara (n = 10), Nguni (n = 15)) were generated at 10X coverage. The iHS statistical method was used to detect the selective regions within the breeds. Selection of signatures analysis revealed 54, 72, 86, and 92 genomic regions under positive selection in the Nguni, Afrikaner, Tuli and Bonsmara cattle, respectively. Within these regions, there were genes involved in the regulation of reproductive performance. From 322 genes identified, the analysis revealed 4 common significant genes (*YWHAZ*, *KHDRBS2*, *KDM4B and SEMA5B*) associated with cow fertility under the threshold of 4%. Gene ontology enrichment analysis revealed that several biological pathways could be involved in the variation of fertility in female cattle. The identification of genes responsible for fertility may facilitate the better understanding of reproduction-associated traits in this South African indigenous species.

Key Words: fertility traits, candidate genes, South African cattle, whole-genome sequencing

W171 Population structure, inbreeding and admixture for indigenous goats within a pilot community-based breeding program in Pella, North West, South Africa. T. Mtshali*[1,3], F. Muchadeyi[2], O. Mapholi[3], E. Dzomba[4], and K. Hadebe[2], [1]*Agricultural Research Council, Vegetable and Ornamental Plants, Pretoria, South Africa,* [2]*Agricultural Research Council, Biotechnology Platform, Onderstepoort, Pretoria, South Africa,* [3]*University of South Africa, Florida, Johannesburg, South Africa,* [4]*University of KwaZulu-Natal, Scottsville, Pietermaritzburg, South Africa.*

The level of inbreeding, genetic relatedness and population structure of goats within a community-based breeding program (CBBP) are crucial to guide decision making and sustainability of any breeding program. The current study investigates population structure, admixture, levels of inbreeding and runs of homozygosity (ROH) for indigenous goats within a pilot CBBP in Pella village, North West province, South Africa. Sixty-three goat samples from 38 participating households were genotyped using Illumina Goat50K SNP array. Principal Component Analysis and admixture ($K = 3$) revealed at least 2 genetic backgrounds which are linked to geographic distances between the different households. Inbreeding levels ranged from 0 to 0.36. A total of 637 ROH with a mean of 29.97 ± 10.99 was observed in the study. Chromosome 1 had the highest number of ROH (n = 49), followed by chromosome 8 (n = 36) and the lowest was observed for chromosome 6. The smallest length classes < 5 Mb were more abundant (48%) than the longest segments (>40) which occurred less frequently (3%). High ROH coverage within the short category may indicate an ancient family relatedness. The low number of breeding bucks in the area should be considered a threat to the population's diversity. The study findings provided insights into the demographic history and the diversity of the goats within the CBBP and will guide future buck selection and exchange.

Key Words: relatedness, goat improvement, community-based breeding program

W172 Genetics of base coat color variations and coat color patterns of the South African Nguni cattle investigated using high-density SNP genotypes. L. Kunene*[1], F. Muchadeyi[2], K. Hadebe[2], G. Mészáros[3], J. Sölkner[3], and E. Dzomba[1], [1]*University of KwaZulu-Natal, Scottsville, South Africa,* [2]*Agricultural Research Council, Onderstepoort, South Africa,* [3]*University of Natural Resources and Life Sciences, Gregor-Mendel-Straße 33 A-1180 Vienna, Austria.*

Nguni cattle are a hardy breed with diverse coat colors. Nguni coats have found a niche market in the leather industry leading to breeding objectives toward the promotion of such diversity. However, there is limited studies on the genomic architecture underlying the coat color and patterns and the absence of such information hampers any potential breeding and improvement of such trait. This study investigated the genetics of base coat color, color-sidedness and the white forehead stripe in Nguni cattle using coat color phenotyped Nguni cattle and Illumina Bovine HD (770K) genotypes. Base coat color phenotypes were categorized into black (n = 39), brown (n = 6) and red (n = 19). The black and brown were put into one class called the eumelanin (n = 45) while the red belonged to the second class of pheomelanin (n = 19). Animal were categorized into color-sided (n = 46) and non-color-sided (n = 94) animals and similarly into presence (n = 15) or absence (n = 67) of white forehead stripe. Genome-wide association tests were conducted using 622,103 SNPs that remained after individual and SNP quality filtering and the Efficient Mixed Model Association eXpedited method implemented in Golden Helix SNP Variation Suite. The GWAS for base coat color (eumelanin vs pheomelanin) resulted into 4 indicative SNPs on BTA18 and a well-known gene, *MC1R*, was observed within 1MB from the indicative SNPs ($P < 0.00001$) and found to play a role in the melanogenesis and the MAPK signaling pathway. GWAS for color-sidedness resulted in 4 indicative SNPs, none of which was associated with the *KIT* candidate gene for color-sidedness. GWAS for the white forehead stripe resulted in 17 indicative SNPs on BTA6. Linkage disequilibrium analysis between the *KIT* SNPs and indicative SNPs yielded low r^2 (<0.007 ± 0.22) indicative of weak correlations. Other than the *KIT* gene, 4 genes *MAPK10*, *EFNA5*, *PPP2R3C* and *PAK1* were found to be associated with the white forehead stripe. These genes are part of the MAPK, adrenergic and Wnt signaling pathways that are synergistically associated with the synthesis of melanin, suggestive of a different genetic mechanism for presence or absence of forehead stripes in the South African Nguni cattle. Overall, our results prove prior knowledge of the role *MC1R* in base coat colors in cattle and suggested a different genetic mechanism for forehead stripe phenotypes.

Key Words: coat color, patterns, GWAS, Nguni cattle, SNP genotypes

W173 Correlation between resilience and tolerance in Angus females exposed to *Rhipicephalus* (*Boophilus*) *microplus*. C. D. S. Arce*[1], F. R. Araújo Neto[2], A. M. Maiorano[1], L. G. Albuquerque[1], and H. N. Oliveira[1], [1]*Universidade Estadual Paulista "Júlio de Mesquita Filho," Ja-*

boticabal, Sao Paulo, Brazil, [2]Instituto Federal Goiano, Rio Verde, Goias, Brazil.

Resilience (R) is a function of resistance and tolerance (T). This study aimed to estimate the correlation between R and T traits of female Angus exposed to *Rhipicephalus* (*Boophilus*) *microplus* using preweaning weight gain (PWG) as phenotype. Records of 546 animals were collected in 2014. Tick counts (TC) were performed as described in Wharton and Utech (1970). Two TC were performed with an interval of 41 d to avoid medical treatment residual between the counts. Animals were genotyped with the GPP Bovine 150k Illumina panel. Samples with call rate below 0.90 and SNPs located in the same positions were excluded. We used only SNPs located in autosomal chromosomes with call rate values greater than 0.98, minor allele frequency greater than 0.03, and Hardy–Weinberg values greater than 10^{-7}, totaling 71,237 SNPs. Phenotypic observations that deviated from the mean by ± 3 standard deviations were removed. Contemporary groups (CG), based on the CG average for PWG and post-weaning weight gain, were formed considering management group, year, and farm. R was computed in a 2-step analysis: first, the environmental gradients (EGs) were computed by applying a mixed regression to the log(TC + 1) values, based on estimated CGs effects; second, EGs were normalized using Z-Score and measured as resilience with a reaction norm model (RNM). T was measured using log(TC + 1) as slope in an RNM. Genomic breeding values (gEBVs) were estimated using GBLUP method. Pearson correlation test was applied to the obtained gEBVs for R and T. The correlation coefficient was significant, high, and positive (0.95; $P < 0.05$), indicating that resilient animals are also tolerant. However, this result should be interpreted with caution since the maternal effect was not included in the analysis of PWG. Therefore, it is not possible to state that the linear association between these traits is due entirely to the direct genetic effect. The inclusion of maternal effect is recommended to elucidate its influence on the studied traits.

Key Words: animal breeding, cattle and related species, environment, genetic improvement

W174 Signature of stress-related characteristics according to changes in pig breeding condition through transcriptome analysis. S.-W. Lim*, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

Pig breeding condition, such as density-stress, is one of the important factors in terms of pig productivity. However, the molecular mechanisms for the level of whole-genome expression depending on the pig breeding condition has not been well studied. The purpose of this study is to identify functional mechanisms according to changes in pig breeding condition through transcriptome analysis. In this study, we accepted samples from 3 conditions (control, welfare and density) and observed their transcriptomic changes using the RNA-seq method. Gene alignment and gene transfer format of the entire pig genome was annotated using Sus scrofa 11.1.102. Whole gene expression profiling was performed using a general linear model using edgeR of R package. The differentially expressed genes (DEGs) were extracted by the *P*-value <0.01 with absolutely expressed for double changes to each comparison group. As a result, for each group genes were identified into the DEGs (welfare vs. control; a total of 109 genes, downregulated genes 62 and upregulated genes 47, density vs. control; a total of 199 genes, downregulated genes 126 and upregulated genes 73, welfare vs. density; a total of 135 genes, downregulated genes 55 and upregulated genes 80). Gene Ontology (GO) functional enrichment analyses distinguished the DEGs primarily associated with metabolism, signaling molecules and circulatory systems. KEGG pathway enrichment analyses revealed the DEGs primarily associated with oxidative stress signaling and immune signaling. Therefore, the biological process in breeding condition suggests that the expression of stress-related genes through metabolic and immune signals were high. We believe that further research would be required to understand more precise mechanisms.

Key Words: pigs and related species, functional genomics, RNA-seq, animal welfare, behavior

Applied Genetics of Companion Animals Workshop

W175 Supplementation of the AgriSeq Canine SNP Parentage and ID panel with additional ISAG and sex determination markers. A. Burrell*, K. Gujjula, H. Suren, and R. Conrad, *Thermo Fisher Scientific, Austin, TX, USA.*

Parentage testing and genomics-assisted breeding are critical aspects of successful veterinary management. Due to its highly accurate and reproducible results, targeted GBS is becoming an increasingly favored technology for SNP genotyping. With the utilization of next-generation sequencing, labs can test hundreds of samples across thousands of SNPs simultaneously in a simple high-throughput workflow starting from either extracted nucleic acid or crude lysis samples. The AgriSeq Canine SNP Parentage and ID Panel, released in 2019, is an amplicon-based next-generation sequencing panel for parentage determination in dogs. In 2020 ISAG finalized the list of 233 recommended markers for canine parentage determination. The final recommendation contained 5 autosomal and 3 sex chromosome markers not present on the original AgriSeq panel. The AgriSeq Canine SNP Parentage and ID Panel was quickly updated to include not only the 8 missing markers, but 5 additional sex determination markers to ensure robust and repeatable sex determination results. The final panel contains 392 SNPs, including 4 markers targeting both the X and Y chromosome and 4 markers targeting the Y chromosome exclusively, and 2 deletions. Utilizing the AgriSeq HTS Library Kit, a high-throughput targeted amplification and resequencing workflow, performance was validated on a panel of > 100 samples. Libraries were sequenced on the Ion S5 using an Ion 540 chip with genotyping calling generated using the Torrent Variant Caller (TVC) plugin. Mean call rates, the percentage of markers on a panel generating a genotype call, was >98%. Concordance with orthogonal testing, including the Axiom Canine HD array and CE sequencing, was > 99.4%. Sex determination accuracy was 100% for all samples tested and parentage determination was accurately assigned when testing the ISAG Comparison test samples. The data demonstrates the utility of the AgriSeq targeted GBS approach for canine parentage and sex determination applications. For Research Use Only. Not for use in diagnostic procedures.

Key Words: AgriSeq, parentage, GBS, NGS, genotyping

W176 Development of a highly informative SNP panel for parentage assessment in dogs. K. R. Gujjula*, H. Suren, A. Burrell, and S. Chadaram, *Thermo Fisher Scientific, Austin, TX, USA.*

AgriSeq targeted Genotyping-By-Sequencing (GBS) is successfully being used as a high-throughput, customizable and cost-effective genotyping solution in animal breeding, parentage testing, and genetic purity testing. Traditionally, parentage identification was performed with microsatellites markers, also known as short tandem repeats (STRs). Genetic testing with STRs is difficult in closely related sires due to limited allelic variation, these minor variations in allele sizes are difficult to capture. For parentage determination and identification of canines, we have developed the Applied Biosystems AgriSeq panel containing 394 markers, including 386 autosomal markers and 8 sex chromosome markers. These markers were contributed by groups in canine parentage testing (Neogen, Oriv-

et, and Vetgenomics) and contain all 233 ISAG recommended parentage markers. To evaluate the information content of the panel, we combined the published genotype data from 7,381 unique dog samples. Further, we retained only those breeds which had more than 20 uniquely genotyped samples which resulted in 83 breed group representations. For each SNP marker, we calculated allele frequencies within a breed only if the "marker x breed" combination had at least 20 unique genotyped samples which resulted in confident allele frequency estimation. Per breed 290 markers, on average, met the allele frequency estimation criteria, the lowest being 237 markers and the maximum being 382 markers. The results show that per breed 134 markers, on average, had a MAF (Minor Allele Frequency) ≥ 0.30. The mean MAF for markers per breed varied from 0.15 to 0.35, with an average of 0.26. Out of 83 breeds, 79 breeds had a mean MAF for markers above 0.20. Another observation for SNPs with a MAF ≥ 0.30 is that the minor allele nucleotide is often different among breeds. The panel also contains 8 sex determination markers, 4 out of 8 are XY-based markers and 4 are Y-based markers. The panel evaluation shows that the markers are highly informative for use in parentage testing and traceability. For Research Use Only. Not for use in diagnostic procedures.

Key Words: canine, AgriSeq, ISAG, parentage, GBS

W177 AgriSum Toolkit Plugin 2.0: Enabling multi-species panel analysis for AgriSeq. H. Suren*[1], S. Daly[2], and K. R. Gujjula[1], [1]*Thermo Fisher Scientific, Austin, TX, USA,* [2]*Thermo Fisher Scientific, Lissieu, France.*

The AgriSum Toolkit (AST) is a data summarization and visualization plugin for the Torrent Suite Software (TSS). AST was primarily developed for analyzing targeted genotyping-by-sequencing (tGBS) solutions for AgriSeq. ATS provides overall panel, markers and samples summary metrics with visualizations. ATS also reports the actual genotype alleles, which can be filtered and exported in multiple formats (Matrix, TOP/BOTTOM, and ISAG) compatible for downstream analyses. In the AST plugin 2.0, we have added a new feature which enables our customers to analyze multi species panel easily. Conventionally, only one single report was created for the entire panel aggregating all the metrics. Aggregated summary metrics for multi species panel were often confusing customers due to confounding effect (e.g., a very poor performing sub panel dragging the overall average metrics significantly down). In multi species report, key performance metrics are calculated and reported explicitly for each individual sub panel within the run. Customer can navigate back and forth accessing key performance metrics and data files for each sub panel as well as the overall panel within the report with ease. AgriSum Toolkit 2.0 enables our customers to genotype multi species samples on the same chip. Thereby increasing the multiplexing capabilities without worrying about interpretation of the sequencing data. AST is publicly available and can be downloaded via Thermo Fisher Cloud and installed on TSS at no additional cost. For Research Use Only. Not for use in diagnostic procedures.

Key Words: AgriSeq, genotyping by sequencing (GBS), Ion S5, NGS, genotyping

W178 Whole-genome sequencing analysis of a cat family with radial hemimelia. N. Bilgen*[1], M. Y. Akkurt[1], B. Çinar Kul[1], R. M. Buckley[2], L. A. Lyons[2], and Ö. S. Çildir[1], [1]*Faculty of Veterinary Medicine, Department of Genetics, Ankara University, Ankara, Turkey,* [2]*Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA.*

Hemimelia is a congenital condition characterized by complete or partial absence of one or more bones. If malformation is affecting radius bones it is called radial hemimelia (RH). Thumbs may also be affected by this malformation. In nature, cats with RH cannot survive due to the defect on their forelimb and the mother tends not to nurture defective kittens, thus the kittens die. Sporadic cases of RH from around the world in unrelated stray cats were reported. Although it has been suggested that RH in Siamese and domestic shorthair cats may be a hereditary trait, it has not been possible to determine neither the inheritance pattern nor the genetic background of the disease. In this study, 4 Siamese cats, from an extended family of 24 cats, consisting of father, mother, bilaterally affected female kitten, and unilaterally affected male kitten were subjected to genetic analysis. First detailed radiological examinations were performed, and swab samples were taken for DNA extraction. To determine the possible genetic drivers of the malformation, extracted DNAs were subjected to WGS using the Illumina HiSeq platform. The variant call files for the 99 Lives WGS and WES cats were imported into VarSeq. Ensembl annotation 101 and NCBI annotation 99 were used for annotation. 336 domestic cats representing various breeds and populations from the 99 Lives consortium were available for comparison to the 4 Siamese cats. Assuming an autosomal recessive condition, candidate variants were defined as heterozygous in the 2 parents and homozygous in the 2 affected offspring. All other cats in the 99 Lives data set were considered homozygous reference. A total of 2,508,262 SNPs were determined. Of them, 336,763 SNP mutations were identified as causing large effects in the severely affected kitten. After filtering based on inheritance and the condition being specific to the 4 cats, only 22 variants were identified as candidates for RH. Most variants (n = 16) clustered between 140 and 146 Mb on cat chromosome A1. Candidate mutations validated by Sanger sequencing is proceeding. This is the first study describing the genetic background and inheritance model of the RH in cats depending on pedigree. Study was supported by Ankara University Scientific Research Projects Coordination Unit (Project no: 18B0239001).

Key Words: cats and related species, candidate gene, genome-wide association, high-throughput sequencing (HTS), radial hemimelia

W179 Breed, trait, locus, and allele nomenclature standardization for the domestic cat. L. A. Lyons*, *College of Veterinary Medicine, University of Missouri, Columbia, MO, USA.*

The X-linked coloration in domestic cats, the *Orange* locus, represents one of the first loci assigned to a chromosome in the field of genetics. Considering new traits under novelty selection for cat breed development, overall cats have approximately 30 phenotypic loci, including 24 genes and 55 allelic variants. Early coloration and coat length variants for the loci *A* (*Agouti*), *B* (*Brown*), *C* (*Color*), and *L* (*Long hair*), followed the nomenclature of mice coat color phenotypes, indeed demonstrating cat phenotypic variants in homologous genes for *ASIP*, *TYRP1*, *TYR*, and *FGF5*, respectively. However, some allelic variants have been incorrectly assigned. For example, *Hairless* (*Hr*) was assigned to the nude Sphynx phenotype as like the nude mouse, but Sphynx hairless is actually a variant in the gene *KRT71*. True *Hr* variants have been more recently identified for a novelty breed called, Lykoi. In addition, cats have patterning loci that are not represented in mice, such as *LVRN* variants for the *Tabby* locus and *DKK4* variants for the *Ticked* locus. Therefore, a need for nomenclature standardization and reconciliation is required as cat breeders often use terms that are inconsistent with the scientific literature and representing non- homologous loci across species. Commercial testing services also should be using a standardized nomenclature so that researchers, testing laboratories and cat breeders can all be making the same interpretations regarding cat phenotypes. To support standardization of genetic nomenclature in the cat, a group of researchers focused on cat studies, as well as breeders and cat judges with scientific backgrounds, have formed a working group to define loci and allele nomenclature, as well as breed nomenclature, for the domestic cat. The committee is reviewing the scientific literature, specifically of other mammals, and will be using standards set by other nomenclature committees to define cat loci and variant alleles. Novel nomenclature rules are being established in cats to consider alleles from different species, which occurs in popular hybrid cat breeds, such as the Bengal, which are crosses of Asian leopard cats (*Prionailurus bengalensis*) and domestic cats (*Felis silvestris catus*). A table of historical

and standardized nomenclature will be presented for the domestic cat for phenotypic traits and blood type.

Key Words: *Felis*, feline, domestic cat, coat color, nomenclature

W180 Online Mendelian Inheritance in Animals (OMIA): Standardized vocabularies for breeds and traits. I. Tammen[1], N. Vasilevsky[2], C. A. Park[3], Z. Hu[3], M. Haendel[4], and F. W. Nicholas*[1], [1]*Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, Australia,* [2]*Oregon Clinical and Translational Research Institute, Department of Medical Informatics and Clinical Epidemiology, Oregon Health and Science University, Portland, OR, USA,* [3]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [4]*Center for Health AI, University of Colorado Anschutz Medical Campus, Aurora, CO, USA.*

Online Mendelian Inheritance in Animals (OMIA, https://omia.org) provides up-to-date summary information on all known harmful and beneficial variants in animals, together with background information on all known inherited disorders and non-disease traits, which are called 'phenes'. OMIA curation focuses on phenes with confirmed and suspected Mendelian modes of inheritance. Several phenes caused by somatic mutations, chromosomal abnormalities, genetic modifications, or phenes with unknown or complex modes of inheritance are also included. As OMIA developed over the past 25 years, breed and phene names were entered as published. Sometimes phene names were altered to reflect similarities to phenes in the hyperlinked database Online Mendelian Inheritance in Man (OMIM; https://omim.org). The ever-increasing inter-connectedness of the internet has highlighted the need for OMIA to adopt standardized vocabularies that will enable OMIA to be reciprocally hyperlinked with relevant internet resources. The most powerful of these vocabularies are ontologies such as the Mondo Disease Ontology developed as part of the Monarch Initiative (https://mondo.monarchinitiative.org/). Starting with breeds, and using the existing Livestock Breed Ontology (https://www.animalgenome.org/bioinfo/projects/lbo/) as a model, we are using the Ontology Development Kit (https://ontology-development-kit.readthedocs.io/en/latest/) to combine existing breed lists of all OMIA species, thereby creating a Universal Breed Ontology that will be made available for general use. As a first step toward an OMIA Phene Ontology, we are enhancing OMIA's existing reciprocal links with OMIM. Because OMIM phenes are already incorporated into the Mondo Disease Ontology, we can then use the enhanced OMIA/OMIM links to introduce the Mondo standardized phene vocabulary into OMIA. Subsequent efforts will involve incorporating OMIA phenes that do not have human homologues, using (where possible) existing vocabularies such as those in the VeNom codes (http://venomcoding.org/). These efforts will help to standardize OMIA vocabularies, and will improve interconnectivity between databases for unambiguous information links.

Key Words: databases/repositories, bioinformatics tools, nomenclature, breed standardization

Domestic Animal Sequencing and Annotation Workshop

W181 Bovine genome annotation using integration of multi-omics data. H. Beiki[1], C. Gill[2], H. Jiang[3], W. Liu[5], Z. Jiang[4], S. McKay[6], B. M. Murdoch[7], J. Koltes[1], M. Rijnkels[2], T. P. L. Smith[8], P. Ross[9], H. Zhou[9], and J. Reecy*[1], [1]*Iowa State University, Ames, IA, USA,* [2]*Texas A&M University, College Station, TX, USA,* [3]*Virginia Tech University, Blacksburg, VA, USA,* [4]*Washington State University, Pullman, WA, USA,* [5]*Penn State University, State College, PA, USA,* [6]*University of Vermont, Burlington, VT, USA,* [7]*University of Idaho, Moscow, ID, USA,* [8]*US Meat Animal Research Center, Clay Center, NE, USA,* [9]*University of California–Davis, Davis, CA USA.*

The diversity of RNA and miRNA transcripts among 47 different bovine tissues/cell types was assessed. Poly(A) selected RNA-seq and miRNA-seq data were generated from tissues of Hereford cattle closely related to Dominette L1, the individual represented in the reference bovine genome. A total of approximately 4.1 trillion RNA-seq reads and 1.9 billion miRNA-seq reads were collected, with a minimum of 27.5 million (M) RNA-seq and 4.2 M miRNA-seq reads from each tissue (average 87.8 M ± 49.7 M and 26.6 M ± 13.3 M, respectively). A total number of 171,985 unique transcripts (50% protein-coding) and 35,150 unique genes (64% protein-coding) were identified across tissues. A total of 159,033 transcripts (92% of predicted transcripts) were structurally validated by independent data sets such as Pacific Biosciences single-molecule long-read isoform sequencing, Oxford Nanopore Technologies sequencing, *denovo* assembled transcripts from RNA-seq, Ensembl and NCBI gene sets. In addition, all transcripts were supported by extensive independent data from different technologies such as WTTS (transcriptome termini site sequencing), RAMPAGE (RNA annotation and mapping of promoters for the analysis of gene expression), several different types of histone modification data (H3K4me3, H3K4me1, H3K27ac and CTFC) and ATAC-seq (assay for transposase-accessible chromatin using sequencing). A large proportion of transcripts (69%) were novel, although mostly produced by known protein-coding genes (87%), while 13% (9% of all transcripts) corresponded to novel genes. The median number of transcripts per known gene (tpg) was 4, which was higher than that was observed in either the Ensembl (1.5 tpg) or NCBI (2.3 tpg) annotated gene sets. Our new bovine genome annotation extended more than 11,000 known gene borders (5′ end extension, 3′ end extension, or both) compared with EBI or NCBI annotations. Furthermore, we detected 12,698 novel genes (80% noncoding), which are not reported in current bovine genome annotations. Most these genes were structurally validated by independent data (85%) or were replicated in multiple tissues (96%). These validated results show significant improvement over current bovine genome annotations.

Key Words: bovine, genome annotation, functional genomics

W182 BovReg: A high-resolution functional annotation of the cattle genome using novel breeds/crosses. G. Costa Monteiro Moreira*[1], S. Dupont[1], D. Becker[2], M. Salavati[3], R. Clark[4], E. L. Clark[3], G. Plastow[5], C. Kühn[2,6], C. Charlier[1], and BovReg Consortium[6], [1]*Unit of Animal Genomics, GIGA Institute, University of Liège, Liège,Belgium,* [2]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany,* [3]*The Roslin Institute, University of Edinburgh, Edinburgh, UK,* [4]*Genetics Core, Edinburgh Clinical Research Facility, The University of Edinburgh, Edinburgh, UK,* [5]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada,* [6]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Transcriptome (mRNA, total RNA and small-RNA) and ChIP-seq assays (antibodies H3K4me3, H3K4me1, H3K27me3, H3K27ac and CTCF) were compiled in a comprehensive catalog of 129 tissue samples collected from 6 individuals of both sexes, different ages, kept in different environments and from 3 divergent breeds/crosses: Belgian dairy (Holstein), Canadian composite (Kinsella crossbred) and German beef/dairy cross (Charolais x Holstein). Libraries were built, and sequenced on the Illumina NovaSeq 6000 (150nt paired-end - mRNA and total RNA; 100nt/50nt single-end - small-RNA; 100nt paired-end - ChIP-seq). Preliminary data analyses for transcriptome assays were performed using Nextflow/nf-core pipelines (RNA-seq-1.4.2, smrnaseq-1.0.0) adopting a guided transcript assembly approach (Stringtie) for mRNA and total RNA. For ChIP-seq analysis, mapping (ARS-UCD1.2, 1000bulls), read processing and peak calling were performed using BowTie2, SAMtools and MACS2 software, respectively. mRNA and total RNA predicted

transcripts were compared with the annotation from Ensembl v.102. All annotated loci (Ensembl v.102) with exon-overlapping transcripts were represented in the new annotation. Moreover, 14,893 genes had at least one potentially novel transcript model and 133,226 novel transcripts were predicted. Overall, miRNAs were the predominant small-RNA class detected across the tissues (81.1% corresponded to miRNAs, 0.4% rRNA, 0.3% tRNA, 3.02% snoRNA, 0.93% snRNA, 14.25% either mitosRNA, unknown or artifacts). piRNAs were detected in testis, representing the majority of small-RNAs in adult testis and about 30% in neonate testis. Combining the ChIP-seq results from a subset of tissue samples, 73,739 putative active promoter and transcription start site (TSS) states (H3K4me3), 27,408 active enhancer states (H3K27ac and H3K4me1), 12,365 repressive states (H3K27me3) and 68,505 insulators bound by CTCF were detected. The data generated, combined with the new map of TSS already available (CAGE) and ATAC-seq, Hi-C, ChIRP, RRBS, lncRNA and circRNA which will be generated in collaboration with other partners in the BovReg Consortium, will yield improved functional annotation of the cattle genome.

Key Words: cattle and related species, Functional Annotation of Animal Genomes (FAANG), functional assay, regulatory element

W183 Annotation of transcription start sites in the bovine genome reveals novel breed-specific complexity. M. Salavati*[1], R. Clark[2], D. Becker[3], C. Kühn[3,4], G. Plastow[5], G. Costa Monteiro Moreira[6], C. Charlier[6,7], E. L. Clark[1], and BovReg Consortium [4], [1]*The Roslin Institute, University of Edinburgh, Edinburgh, UK,* [2]*Genetics Core, Edinburgh Clinical Research Facility, The University of Edinburgh, Edinburgh, UK,* [3]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany,* [4]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [5]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada,* [6]*Unit of Animal Genomics, GIGA Institute, University of Liège, Liège, Belgium,* [7]*Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.*

Mapping of transcription start sites (TSS) in multiple tissues is a key first step in understanding transcript regulation and diversity and how these might influence phenotypic plasticity in cattle. TSS mapping can provide information about complex promotor activity, pervasive transcription and tissue-specific promotor usage. Data from multiple tissues and nonreference breeds or crosses will increase our understanding of TSS usage and complexity, further improving the annotation of the bovine genome (ARS-UCD1.2). Using cap analysis gene expression (CAGE) sequencing of 105 samples (from 24 different tissues) from dairy (Holstein, n = 43), composite beef (KC-composite, n = 31) and beef/dairy cross (Charolais × Holstein, n = 31) breeds, we aimed to capture additional TSS complexity in cattle. CAGE libraries were prepared, sequenced and mapped to ARS-UCD1.2_Btau5.0.1Y (1000bulls run 9). The mapped reads were analyzed using the CAGEfightR package to generate unidirectional (TSS) and bidirectional (TSS-Enhancer) clusters. We identified more than 4.5 million putative TSS and 57,412 TSS-Enhancer clusters in total across all samples. Tissue-specific analysis captured, on average per tissue (±SE), 253,852 ± 24,713 TSS clusters, 41.6% of which were novel. On average per tissue 12,138 ± 889 TSS-Enhancer clusters were captured, of which 27.6% were novel. The greatest number of clusters were observed in lung (TSS) and spleen (TSS-Enhancer). Breed-specific analysis revealed differences in TSS complexity between the breeds and crosses analyzed. The highest number of breed-specific TSS were detected in the KC-composite (3,102) followed by 1,152 in Holstein and 1,092 in Charolais × Holstein. The same pattern was observed in the TSS-Enhancer clusters (419 in KC-composite, 286 in Charolais × Holstein and 202 in Holstein). These differences indicate that in this study the total number of TSS observed was greater in crossbred relative to purebred cattle. The data we have generated will provide a breed- and tissue-enriched map of TSS that combined with RNA-seq and small RNA-seq, generated by BovReg partners, will create a new high-resolution transcriptional map for the bovine genome.

Key Words: CAGE-seq, regulatory element, transcriptome, cattle and related species, Functional Annotation of Animal Genomes

W184 The Ovine Functional Annotation of Animal Genomes project. B. M. Murdoch*[1,6], K. M. Davenport[1], M. Salavati[2], E. Clark[2], A. Archibald[2], A. T. Massa[3], M. R. Mousel[4,5], M. K. Herndon[3], S. N. White[3,4,6], K. C. Worley[7], S. Bhattarai[8], S. D. McKay[8], B. Dalrymple[9], J. Kijas[10], A. Caulton[11], S. Clarke[11], R. Brauning[11], T. Hadfield[12], T. P. L. Smith[13], and N. E. Cockett[12], [1]*Department of Animal, Veterinary, and Food Science, University of Idaho, Moscow, ID, USA,* [2]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, Scotland, UK,* [3]*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA,* [4]*USDA, ARS, Animal Disease Research Unit, Pullman, WA, USA,* [5]*Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, USA,* [6]*Center for Reproductive Biology, Washington State University, Pullman, WA, USA,* [7]*Baylor College of Medicine-Human Genome Sequencing Center, Houston, TX, USA,* [8]*University of Vermont, Burlington, VT, USA,* [9]*University of Western Australia, Crawley, Western Australia, Australia,* [10]*CSIRO Agricultural Flagship, St. Lucia, Brisbane, Australia,* [11]*AgResearch, Hamilton, New Zealand,* [12]*Utah State University, Logan, UT, USA,* [13]*USDA, ARS, U.S. Meat Animal Research Center (USMARC), Clay Center, NE, USA.*

Annotation of regulatory and transcribed elements is important in understanding complex phenotypes related to production and health in livestock species. The Ovine Functional Annotation of Animal Genomes (FAANG) Project aims to characterize transcriptional regulatory elements across the sheep genome. Approximately 100 tissues were collected from the Rambouillet ewe, Benz 2616, used to assemble the ovine reference genome ARS-UI_Ramb v2.0. Functional assays, including sequencing of messenger RNA (mRNA-seq), microRNA (miRNA-seq), and full-length RNA transcripts (Iso-seq), cap analysis gene expression (CAGE), chromatin immunoprecipitation with sequencing (ChIP-seq), assay for transposase-accessible chromatin with sequencing (ATAC-seq), whole-genome bisulfite sequencing (WGBS) and reduced representation bisulfite sequencing (RRBS) were performed on a subset of these tissues. Fourteen chromatin states depicting promoters, enhancers (active, poised, and repressed), and accessible chromatin across the genome were defined with ChromHMM using ChIP-seq and ATAC-seq data and compared across tissues. These chromatin states in combination with DNA methylation, transcription start site identification, and RNA expression provide a very high resolution annotation of the expressed and regulatory regions of the ovine genome. Characterizing regulatory elements in sheep will provide a valuable resource to facilitate a deeper understanding of how gene regulation control influences complex traits in this globally important livestock species.

W185 AQUA-FAANG: Genome functional annotation of the 6 major European farmed fish species. D. J. Macqueen*[1], S. Lien[2], and AQUA-FAANG Consortium[3], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, Scotland, UK,* [2]*Centre for Integrative Genetics (CIGENE), Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway,* [3]*AQUA-FAANG Consortium, Europe.*

High-quality reference genome sequences can be readily generated using modern sequencing technologies, but alone are rarely sufficient to identify the basis for complex biological traits. For many scientific and commercial reasons, we need to understand how phenotypes are being shaped by functional and regulatory features within farmed animal genomes, including aquaculture species. European aquaculture produces 3 million tonnes of fish (worth > €9 billion) annually and employs around 50,000 people. AQUA-FAANG is a Horizon 2020 funded project that

aims to promote sustainable growth within this sector by creating a major up-step in our ability to exploit the genomic basis for complex traits in the 6 major farmed fish species in Europe. Alongside BovReg and GENESWitCH, AQUA-FAANG is one of 3 consortia funded under the same Horizon 2020 call, which are formally linked by the EuroFAANG initiative. AQUA-FAANG is annotating functional and regulatory regions within the genomes of the target species using sequencing assays defined by the FAANG initiative; RNA-seq, ATAC-seq, and ChIP-Seq. The work includes standardized biological samples representing a common panel of tissues at juvenile and sexually mature stages ("BodyMaps"), multiple key landmarks in embryogenesis (from zygotic genome activation to post-Pharyngula stage) ("DevMaps") and immune tissues/cells stimulated with viral and bacterial PAMPs to mimic infection. AQUA-FAANG is also exploring novel approaches to understand the genomic basis for disease resistance, including single-cell transcriptomics and genome editing. A major goal of the project is to predict the genetic basis for disease resistance traits using genome functional annotation, identifying and prioritizing genetic variants responsible for resistance to problematic diseases in fish aquaculture, and developing tools to support uptake of functional genomic data for selective breeding in European aquaculture done in partnership with industry. The project includes a major comparative objective to reveal the evolutionary conservation of functional and regulatory elements in farmed fish genomes. We are currently 24 mo into a 54-mo project, with approximately 2,000 libraries sequenced to date across the major functional annotation maps. This talk will provide an overview of the AQUA-FAANG project and latest status toward achieving our ambitious objectives.

Key Words: farmed fish, FAANG, genome, functional annotation, H2020 project

W186 The Farm Animal Genotype-Tissue expression (FarmGTEx) consortium. L. Fang*, *The University of Edinburgh, Edinburgh, UK.*

In domesticated animals, genome-wide association studies (GWAS) have discovered hundreds of thousands of loci associated with complex traits and diseases of economic value. However, most associated variants are noncoding, hindering our understanding of the underlying molecular mechanisms. The Farm Animal Genotype-Tissue Expression (FarmGTEx) consortium aims to study the genetic regulatory determinants of the transcriptome (e.g., expression and splicing QTLs) in a range of tissues and cells for farmed animals by performing meta-analysis of all available data, and to develop the resource database for the scientific community. The FarmGTEx consortium is an international collaborative endeavor that includes over 20 Universities and Institutes around the world. The pilot phase of FarmGTEx (2018–2021) aims to add value to publicly available sequence data by uniformly analyzing ~40K samples of RNA sequence and ~10K samples of whole-genome sequence. The next phases of FarmGTEx will open a more comprehensive effort globally, adding data not currently in the public domain and increasing the number of samples/individuals/breeds/species. An enhanced FarmGTEx will systematically integrate additional molecular phenotypes (e.g., epigenetic modifications, protein profiles, metabolites and microbiota) by collaborating with other consortia like FAANG. The FarmGTEx consortium will serve as a primary source of genetic regulatory variants for animal genetics and breeding, evolutionary biology, veterinary medicine and comparative genetics. This is a presentation is on behalf of the FarmGTEx Consortium.

Key Words: expression QTLs, FarmGTEx, domesticated animals, regulatory variants

W187 The Bovine Pangenome Consortium. B. D. Rosen*[1], D. M. Bickhart[2], T. P. L. Smith[3], D. Boichard[4], G. A. Brockmann[5], A. J. Chamberlain[6], C. Couldrey[7], H. D. Daetwyler[6], A. Djikeng[8], C. Drögemüller[9], S. Elzaki[5], R. K. Gandham[10], D. Hagen[11], O. Hanotte[12], M. P. Heaton[3], Y. Jiang[13], Z. Jiang[14], D. Larkin[15], G. Liu[1], W. Y. Low[16], P. Ajmone Marsan[17], B. M. Murdoch[18], F. C. Muchadeyi[19], J. Mwacharo[20], H. L. Neibergs[14], H. Pausch[21], S. Demyda-Peyrás[22], J. Prendergast[23], P. J. Ross[24], R. D. Schnabel[25], J. Sölkner[26], A. Soudre[27], A. Tijjani[12], J. L. Williams[17], and Bovine Pangenome Consortium [28], [1]*USDA ARS AGIL, Beltsville, MD, USA,* [2]*USDA ARS DFRC, Madison, WI, USA,* [3]*USDA ARS MARC, Clay Center, NE, USA,* [4]*INRAE Animal Genetics and Integrative Biology, Jouy-en-Josas, France,* [5]*Humboldt-Universität zu Berlin, Berlin, Germany,* [6]*Agriculture Victoria, Melbourne, Victoria, AU,* [7]*LIC, Hamilton, New Zealand,* [8]*Centre for Tropical Livestock Genetics and Health, Midlothian, Scotland, UK,* [9]*University of Bern, Bern, Switzerland,* [10]*National Institute of Animal Biotechnology, Hyderabad, India,* [11]*Oklahoma State University, Stillwater, OK, USA,* [12]*International Livestock Research Institute, Addis Ababa, Ethiopia,* [13]*Northwest A&F University, Yangling, China,* [14]*Washington State University, Pullman, WA, USA,* [15]*Royal Veterinary College, University of London, London, UK,* [16]*The University of Adelaide, Adelaide, South Australia, Australia,* [17]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [18]*University of Idaho, Moscow, ID, USA,* [19]*Agricultural Research Council, South Africa, Pretoria, South Africa,* [20]*Scotland's Rural College, Midlothian, Scotland, UK,* [21]*ETH Zürich, Zürich, Switzerland,* [22]*Universidad de Córdoba, Córdoba, Spain,* [23]*The Roslin Institute, Midlothian, Scotland, UK,* [24]*STgenetics, Navasota, TX, USA,* [25]*University of Missouri, Columbia, MO, USA,* [26]*University of Natural Resources and Life Sciences, Vienna, Austria,* [27]*Université Norbert ZONGO, Koudougou, Burkina Faso,* [28]*Bovine Pangenome Consortium.*

Cattle are thought to have been domesticated over 10,000 years ago in 2 independent events, giving rise to the taurine (*Bos taurus taurus*) and indicine (*Bos taurus indicus*) subspecies. Their spread across the world through human migration, subsequent selection for multiple purposes, and adaptation to varying local conditions has written a complex history into their genomes. This story is further complicated by interspersed instances of genetic bottlenecks and hybridization with wild relatives. As such, a single linear reference genome is insufficient to fully describe and interrogate the extent of genetic variation in cattle. We have launched a global Bovine Pangenome Consortium (BPC) to generate reference-quality genomes of cattle breeds and closely related wild species from the *Bos* genus. Our goal is to capture the breadth of global diversity, including underrepresented cattle breeds, and integrate it into a single pangenome reference. The BPC includes over 60 members representing 40 institutions in 20 countries. We have already released multiple breed-specific and wild-relative reference assemblies using the latest DNA sequencing technologies and genome assembly tools. We are refining methods for incorporating these assemblies into a single reference and developing workflows for data utilization and visualization. This work will significantly increase the global cattle genomics community's ability to accurately identify and select for health and production traits in target populations around the world.

Key Words: cattle, genome assembly, pangenome, global diversity

W188 An improved, high-quality ovine reference genome to facilitate functional annotation of gene regulatory elements. K. M. Davenport*[1], D. M. Bickhart[2], K. C. Worley[3], S. C. Murali[3], N. E. Cockett[4], M. P. Heaton[5], T. P. L. Smith[5], B. M. Murdoch[1], and B. D. Rosen[6], [1]*Department of Animal, Veterinary, and Food Sciences, University of Idaho, Moscow, ID, USA,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI, USA,* [3]*Baylor College of Medicine, Houston, TX, USA,* [4]*Utah State University, Logan, UT, USA,* [5]*US Meat Animal Research Center, USDA-ARS, Clay Center, NE, USA,* [6]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD, USA.*

The domestic sheep is an important agricultural species raised for food and fiber across the world. A high-quality reference genome for this species allows for precise functional annotation of genetic regulatory elements. Further, better quality reference genomes lead to improved mappability of a variety of sequence data and facilitate the discovery of genetic mechanisms influencing biological traits in sheep. The rapid advances

in genome assembly algorithms and emergence of increasingly long sequence read length provide the opportunity for an improved de novo assembly of the sheep reference genome. Tissue was collected postmortem from an adult Rambouillet ewe (Benz 2616) selected by USDA-ARS for the Ovine Functional Annotation of Animal Genomes project. Nanopore sequence (50x coverage) generated from lung tissue was combined with publicly available short-read Illumina (55x coverage) and long-read PacBio (75x coverage) sequence and assembled with canu v1.9. Assembled contigs were scaffolded with Salsa v2.2 using Hi-C data, followed by gap filling with PBsuite v15.8.24 and polishing with Nanopolish v0.12.5. Duplicates were removed with PurgeDups v1.0.1 and chromosomes were oriented by identification of centromeres and telomeres with RepeatMasker. Final polishing was performed with 2 rounds of a pipeline consisting of freebayes v1.3.1 to call variants, Merfin to validate them, and BCFtools to generate the consensus fasta. The ARS-UI_Ramb_v2.0 assembly has improved continuity (contig N50 of 43.18 Mb) compared with Oar_rambouillet_v1.0 (contig N50 of 2.57 Mb) and Oar_v4.0 (contig N50 of 0.15 Mb). In addition, the ARS-UI_Ramb_v2.0 assembly has reduced contig L50, fewer total number of scaffolds, greater mappability, and fewer insertions and deletions identified by RNA-seq data than other ovine assemblies. This sheep reference assembly provides a basis for regulatory element annotation and offers a resource for the greater scientific community to facilitate further research in sheep and comparative genomics.

Key Words: sheep and related species, genome assembly, genome sequencing

W189 Annotation of full-length transcripts including alternative splicing from 19 chicken tissues using Oxford Nanopore long-read sequencing. D. Guan*[1], M. M. Halstead[1], A. D. Islas-Trejo[1], D. E. Goszczynski[1], H. H. Cheng[2], P. Ross[1], and H. Zhou[1], [1]*Department of Animal Science, University of California-Davis, Davis, CA, USA,* [2]*Avian Disease and Oncology Laboratory, USDA-ARS, East Lansing, MI, USA.*

Alternative splicing of transcripts is a major factor affecting phenotypic variability of farm animals. Thus, it is important to obtain a comprehensive annotation of transcript isoforms across tissues to enhance precise genetic improvement. In this study, we utilized Oxford Nanopore Technology (ONT) to identify and annotate full-length transcript and alternative splicing in diverse chicken tissues derived from 68 biological samples, comprising 19 tissues from adult males and females of a line 6 × line 7 F1 cross. ONT sequencing from a single flow cell resulted in more than 19.4 million unique mapped reads with mean read length of 648 bases, and average quality of 18.2. Using the StringTie computational pipeline, we annotated 79,885 transcripts with mean length of 1,664 bases at 54,590 genetic loci, representing ~1.5 transcripts per locus. There were 48,464 multi-exon and 31,421 single-exon transcripts. Compared with the Ensembl database (GRCg6a version 102), we annotated 2.7- and 3.3-fold more transcripts and gene loci, respectively. The sensitivity and precision of our annotation were 38.8% and 14.5% higher at the transcript level, and 57.7% and 17.5% higher at the locus level, respectively. Among them, 14.6% (11,624) fully matched to the reference, 35.4% (28,276) partially matched to known genes, and 50% (39,985) annotated transcripts had no match. Further analyses are underway to identify tissue-specific novel isoforms and their respective biological functions in chickens. In summary, identification of transcript isoforms across diverse tissues has significantly improved the annotation of the chicken genome, and provides important knowledge in connecting genotype to phenotype in livestock species.

Key Words: full-length transcript, alternative splicing, long-read sequencing, annotation, chicken

W190 Uncovering abundant missing genes in the chicken reference genome solves the avian gene depletion puzzle. M. Li*[1], N. Xu[1], P. Bian[1], X. Hu[2], Y. Jiang[1], and N. Yang[3], [1]*Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of Animal Science and Technology, Northwest A&F University, Yangling, China,* [2]*State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, China,* [3]*National Engineering Laboratory for Animal Breeding and Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Agriculture and Rural Affairs, China Agricultural University, Beijing, China.*

In contrast to the rich biodiversity of the avian clade, both the gene number and evolutionary rate of birds appear far lower than in mammals, which has engendered long-standing controversy. By performing 20 de novo genome assemblies for chickens worldwide, we identified ~1,300 novel protein-coding genes in 159 Mb nonredundant novel sequences compared with the reference genome (GRCg6a). In the novel sequences, tandem repeats and secondary structures such as G-quadruplexes are associated with low read depth, which has previously prevented their assembly. However, we found that most of the novel sequences and coding genes are shared across the panel of sequenced genomes. The novel genes are mainly located on sub-telomeric regions with a much-elevated substitution rate, which could date back to the common ancestor of birds. Our study provides a framework for constructing a comprehensive avian reference genome and suggests that the integrated evolutionary rate of birds is underestimated and the true gene number of birds is comparable to that of other tetrapods, hence resolving a long-standing puzzle regarding avian evolution.

Key Words: chicken, de novo assembly, DNA secondary structures, novel genes, substitution rate

W191 Chromatin accessibility and regulatory vocabulary in indicine cattle. P. Alexandre*[1], M. Naval-Sánchez[1,2], M. Menzies[1], L. Nguyen[3], L. Porto-Neto[1], M. Fortes[4], and A. Reverter[1], [1]*CSIRO Agriculture and Food, St. Lucia, QLD, Australia,* [2]*Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia,* [3]*Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD, Australia,* [4]*School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia.*

The non-uniform topological organization of nucleosomes across the genome reflects a dynamic process that controls chromatin accessibility, particularly at regulatory loci, ultimately influencing cell differentiation and response to the environment. In indicine cattle (*Bos indicus*), identifying regulatory elements in open chromatin regions across different tissues can shed light on the regulation of health and production outcomes, with specific relevance to adaptation to tropical environments. In this study, we generated open-chromatin profiles for liver, muscle, and hypothalamus of 3 Brahman heifers through ATAC-seq (Assay of Transposase Accessible Chromatin sequencing). Briefly, ATAC-seq data processing and alignment were completed using the Harvard pipeline (https://informatics.fas.harvard.edu/atac-seq-guidelines.html) and MACS2 v2.1.1. was used to call peaks from merged bam files per tissue. Peaks were compared between tissues using bedtools v. 2.29.2 to define tissue-specificity. To identify enriched transcription factor binding sites and master regulators in each tissue, peaks were converted to human coordinates and motif discovery was performed using i-*cis*Target. To validate the relationships between the master regulator and its predicted targets at transcriptional level, previously described RNA-seq data of liver from the same animals was used to build a co-expression network using the Partial Correlation and Information Theory (PCIT) algorithm. We identified HNF1, MEF2, and NFYA as candidate master regulators of the epigenomic profile in liver, muscle, and hypothalamus, respectively. Integration with transcriptomic data in liver allowed us to validate direct targets of HNF1, a master regulator of hepatocyte differentiation, with 22% of precited target genes also presenting significant co-expression. Our findings provide insights into the identification and analysis of regulatory elements in non-model organisms and the evolution of regulatory elements within indicine cattle subspecies. Ongoing research aims at comparing our results with *Bos tau-*

rus data, aiming at uncovering the functional genomic basis of climatic adaptation in beef cattle.

Key Words: cattle and related species, genome annotation, ATAC-seq, regulatory element, climatic adaptation

Animal Forensic Genetics Workshop

W192 Invited Workshop Presentation: Allelic ladder design and production for short tandem repeat (STR) genotyping. M. E. D'Amato*, *Forensic DNA Laboratory, Dept. Biotechnology, Faculty of Natural Sciences, University of the Western Cape, Bellville, South Africa.*

Short tandem repeat (STR) genotyping systems require of a technical framework to secure precision, accuracy and reproducibility of results and standardized nomenclature to communicate and compare results. The proficiency of the adopted practice would determine the impact of results in the global scientific community. STR genotyping practices in non-model organisms suffer from shortcomings on the technical framework, and preclude the comparative use of data beyond the borders of a single laboratory. This presentation is focused on strategies to produce an allelic ladder to genotype a STR multiplex. This allelic ladder is one of many components designed for a customized human Y-STRs prototype kit UniQ-Typer Y-10. Both the large-scale strategy using cloned products and the simplified in-house protocol are presented. These strategies can be applied to STRs of any other organism. The ladder was produced for a total of 143 alleles from 10 loci with di-, tri-, tetra-, and pentanucleotide repeats with numerous cases of incomplete repeats. The basic principle is to obtain a PCR product for each allele or pool of alleles per locus, normalize their concentration, repeat PCR if necessary, and pool the locus-specific allelic ladders from all loci to obtain an all-loci allelic ladder. The industrial protocol starting from cloned alleles and the in-house method starting from genomic DNA PCR products are compared. One set of 10 fluorescently labeled primers at 20-nm scale is enough to produce 2 mL of allelic ladder after one batch amplification of alleles. Approximately four 96-well plates were necessary to conduct QC and normalize a ladder from cloned DNA and 2 plates when working from genomic DNA.

W193 SNP marker combination for discrimination of Korean native chickens using a machine learning model. S. Cho*[1], D. Seo[1,2], M. Kim[2], E. Cho[3], P. Manjula[1], T. Kalhari[2], and J. Lee[1,2], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea,* [2]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea,* [3]*Department of Bio-big data, Chungnam National University, Daejeon, Republic of Korea.*

Korean native chicken (KNC) is a unique genetic resource in Korea and an important pure breed for the conservation and development of commercial native chicken. KNCs are classified into 5 lines according to their plumage colors. This study aims to develop an optimal SNP marker combination that can distinguish KNCs from other chicken breeds. The 600K high-density SNP chip data from 443 Korean native chicken purebred (NG: 81, NL: 70, NR: 115, NW: 90, NY: 87) were used and compared with the SYNBEED SNP data set. A total of 400 candidate markers for breed identification were selected by case-control genome-wide association test using PLINK 1.9 software from 544,830 quality-controlled SNPs. For the candidate markers selection, one SNP per block with an interval of 50 haploblocks was considered based on the linkage disequilibrium (LD) information. After filtering the candidate markers through the feature selection process by the random forest machine learning model, 40 SNP markers were finally selected as the minimum number of marker sets. To evaluate the discrimination power of selected markers, total data were divided into 70% for training and 30% for test data sets. A total of 8 machine learning models were tested to derive and evaluate their respective accuracy levels. As the results, all the tested models confirmed more than 99% of accuracies. In particular, 100% sensitivity was obtained in all machine learning models. Therefore, the selected SNP marker combination can provide an effective genomic identification tool for the KNC native chicken lines.

Key Words: breed identification, Korean native chicken, machine learning, single nucleotide polymorphisms

W194 Development of 14-short tandem repeat (STR) panel for forensic DNA analysis of red fox. A. E. Hrebianchuk*[1], N. S. Parfionava[1], V. N. Lukashkova[1], S. A. Kotava[1], and I. S. Tsybovsky[2], [1]*Scientific and Practical Centre of the State Forensic Examination Committee of the Republic of Belarus, Minsk, Republic of Belarus,* [2]*Republican unitary service enterprise "BelJurZabespechenne", Minsk, Republic of Belarus.*

The study of samples of animal origin for forensic purposes of the scientific and practical center conducts in the investigation of the facts of illegal hunting, theft of domestic animals or animal abuse. The development of a test system for the DNA identification of individuals of the red fox was carried out on the basis of short tandem repeat (STR) loci developed for the red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*), and domestic dog (*Canis lupus familiaris*). The sample included 242 foxes from all regions of the Republic of Belarus. As a result of statistical analysis, we formed a 14-locus panel, including 3 loci of species affiliation (internal control for differentiation of individuals of a dog, wolf, raccoon dog, which can be found on material evidence), 9 identifying loci and 2 markers for sex determination. In the analyzed STR loci, allelic diversity ranged from 5 to 21 alleles. In total, 111 alleles were identified in this study. To calculate the efficiency of the panel for individual identification and paternity testing, we estimated the cumulative probabilities of parentage exclusion, when one parent is known (CPE1) 2 parents are known (CPE2), the combined power of discrimination (CPD), and the combined probability of identity (CPID; theoretical). The cumulative probabilities of parentage exclusion CPE1and CPE2 for 14 loci averaged 0.888 and 0.932, respectively. The power of discrimination for each marker showed high values, and varied from 0.825 to 0.979; CPD values were near 1.0 for this panel. The theoretical estimates of CPID for 14 markers were7.95×10^{-13}. The test system is validated according to the SWGDAM protocol and tested on collection samples of red fox and on real forensic objects. The developed test system will be used in expert practice when investigating the facts of illegal hunting in the Republic of Belarus.

Key Words: dogs and related species, forensics, STR profiling

W195 Genetic profiling of horses in forensic cases. A. Fornal*, K. Kowalska, T. Zabek, A. Piestrzynska-Kajtoch, and K. Ropka-Molik, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

DNA from a crime scene does not always have to come from a human. Animal DNA can also be evidence in a case. Forensic laboratories in human crime cases are highly specialized units; however, in cases involving animals, they may not have enough information, tools and experience to identify genetic evidence from animals. Horse pedigree testing and individual identification can be a valuable tool in forensics and in cases of mysterious horse death. In the presented cases, we used biological traces like hair follicles and blood stains (materials were collected at the crime scenes), and animal remains like macerated hoof found in the ground. Biological traces had various quality. We obtained DNA with different degree

of degradation. The hoof was well-preserved, however, we received DNA only from the hoof wall but not from the solar surface of the hoof. DNA isolated from biological traces and the hoof was used for DNA profiling of horses (we used 17 microsatellite markers for routine equine genotyping). Determining the DNA profile of a horse from biological material from a crime scene can be difficult and is dependent mainly on the quality of the biological material collected. The degree of degradation of the biological material can impede testing. Statistically, for biological traces of horses in our lab, 77.08% of the core set markers and 83.72% of the additional set markers were obtained for all materials (regardless of material quality). Biological trace analysis is usually feasible and allows for at least a minimal set of microsatellite loci to establish a concordance with a comparison sample. The chances of success in obtaining a DNA profile from animal remains (like a hoof or tooth) largely depends on the degree of decomposition of the material tested. Isolation of DNA from such material as a hoof wall is sufficient. We have obtained a complete genetic profile from DNA isolated from hoof wall. Based on herd structure analyses, we successfully selected the parental pair of the missing offspring and identified the remains as missing foal. A study of the cases presented demonstrates that DNA profiling of horses can be a valuable tool in criminal cases and missing individual cases in wild or semi-wild horse herds.

Key Words: pedigree testing, horse

W196 Design of a low-density panel of SNPs to detect fraud in cured goat cheese. A. M. Martínez*[1], A. Canales[1,2], M. Macri[1,2], and J. V. Delgado[1], [1]*University of Cordoba, Cordoba, Spain,* [2]*Animal Breeding Consulting S.L., Cordoba, Spain,* [3]*Instituto Canario de Investigaciones Agrarias, Tenerife, Spain.*

Palmera goat is a local breed from La Palma (Canary Islands, Spain) whose main and most valuated product is the Palmero cheese, that is protected by a quality mark that guarantees that only milk from the Palmera goat is included in the elaboration process. The genotyping of 24 DNA samples of each Canarian breed (Majorera, Tinerfeña and Palmera) and other 6 Spanish and international breeds was carried out with the Goat SNP50 BeadChip in accordance with the instructions of the manufacturer. TRES software was used to stablish the minimum number of SNPs necessary to discriminate the Palmera, Majorera and Tinerfeña breeds, resulting a 3,385 SNPs set. Using this number of SNPs was possible to differentiate Palmera from the rest of the breeds included in the study as well. The reduced panel was tested in 110 samples of experimental cheeses containing different proportions of Palmera, Majorera and Tinerfeña milk, ranging from 10% to 100%. Furthermore, 22 samples of commercial Palmero cheeses were analyzed. PLINK v. 1.90 software was used to calculate MAF values and the package Adegenet for the R software and fastStructure were used for inferring population structure. The results show that this panel is useful to distinguish the cheeses prepared with 100% milk of Palmera, 100% Tinerfeña, and 100% Majorera with assignment coefficients of 0.9962, 0.9999 and 0.9999 respectively. However other proportions of milks are not so clearly differentiated because it is difficult to discriminate mixtures of Tinerfeña and Majorera breeds. Anyway, this panel is highly efficient for identifying variable proportions of Palmera milk in all the experimental cheeses. In conclusion, the panel of 3,385 SNPs designed is a powerful and objective tool to detect milk from other genetically related goat breeds such us Majorera and Tinerfeña. The systematic analysis of milk or cheese with this set of markers can be used by the Palmero Cheese Denomination of Origin Regulating Council to ensure the quality and the authenticity of this product. This study was funded by the RTA2014-00047-00-00 Project (INIA).

Key Words: Palmera goat, quality, Majorera

W197 Genomic DNA extraction from canine feces for genotyping and identification with targeted GBS application. Q. Hoang, K. Kice, C. Carrasco, S. Chadaram*, and R. Conrad, *Thermo Fisher Scientific, Austin, TX, USA.*

The desire to use canine feces as a source of canine genomic DNA is growing due to local governments wanting to identify those pet owners that do not pick up after themselves in public spaces. Unfortunately, obtaining genomic DNA from canine feces that is high enough in quality and quantity to use in many genomic sequencing applications is often difficult and expensive. Feces is known to contain high amounts of PCR inhibitors that must be effectively removed from extractions. Additionally, canine DNA is found mostly on the surface of the fecal material and must be swabbed from the surface. Finally, the high amount of bacterial DNA that is extracted from the feces makes the total percentage of canine DNA very low in fecal DNA extractions. As a result of these factors, sequencing data from canine feces give poor results that are often unusable. Here, we look at genomic DNA extracted by swabs using the MagMAX CORE Nucleic Acid Purification Kit as a method for obtaining DNA from canine feces that is suitable for sequencing applications. We compare DNA obtained from feces with DNA obtained from a saliva swab. We found that though the call rates are lower using feces, the calls for both sample types are the same. We conclude that the MagMAX CORE Nucleic Acid Purification Kit yields genomic DNA from canine feces that is suitable for identifying individual dogs from sequencing results.

Key Words: fecal DNA, canine genotyping, DNA extraction, identification, targeted GBS

W198 A *Bos indicus* epigenetic clock predicts age from tail hair. L. T. Nguyen*, M. Forutan, B. J. Hayes, and E. M. Ross, *Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Queensland, Australia.*

Methylation pattern changes associated with aging have been documented in a range of species including humans, mice, and dogs. Age-associated epigenetics markers have been used to generate algorithms that predict the individual's age based on methylation patterns, these predictions are referred to as epigenetic clocks. Here we aimed to derive the first epigenetic clock for indicine cattle. Indicine cattle are extensively grazed in low-input systems and often only mustered once a year, which results in very few or inaccurate birthdate records. Deriving an age prediction epigenetic clock for indicine cattle could increase the accuracy of recorded birthdates, which are often estimates based on size when mustered. We used minIONs (Oxford Nanopore Technologies) to sequence the genomes of 100 cattle with ages ranging from 5 d to 17 years. Methylation sites were called on 56 of the samples using fc5. Sites that were called in at <80% of animals or with a standard deviation <0.5 were removed from the analysis. A 5-fold cross-validation was then used to predict the age of each animal using BLUP (Best Linear Unbiased Prediction), where the relationship matrix was calculated from the methylation matrix and represented similarity of methylation patterns. A second relationship matrix was also calculated that contained genes associated with age in both humans and dogs identified from the literature. In all cases, the animals being predicted were removed from the reference population. The correlation between predicted age and actual age was 0.65 when genome-wide markers were used, and the limited panel of genes identified in human and dog epigenetic clocks. The mean absolute deviation (MAD) was 1 year for animals aged less than 3 years and 1.5 years for animals aged 3–10 years. This is the first reported epigenetic clock in cattle. Work is continuing to increase the accuracy of the clock. Accurate prediction of age will have implications for breed registrations (which require birthdates), herd management and genomic selections for economically important traits reliant on accurate ages such as growth rate and age at puberty.

Key Words: epigenetic clock, indicine cattle, best linear unbiased prediction, Oxford Nanopore technology, age prediction

W199 Response to selection on fecal microbiota composition in Large White piglets. C. Larzul*[1], M. Borey[2], Y. Billon[3], M.-N. Rossignol[2], G. Lemonnier[2], J. Estelle[2], and C. Rogel-Gaillard[2], [1]*Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France,* [2]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [3]*INRAE, GenESI, Surgères, France.*

Pig gut microbiota displays high inter-individual variability and it remains an open question to determine to what extent its taxonomic composition relies on host genetic determinism and not only on environmental conditions. We carried out a study to demonstrate coevolution of the host and its gut microbiota established 1 mo postweaning, by directional selection over 2 generations. The gut microbiota was characterized by sequencing the V3-V4 variable region of the 16S rRNA gene from fecal samples collected on 60-d-old Large White piglets. Amplicon sequence variants were inferred from amplicon data and the microbial community was further studied at the genus level. Based on the stratification of the initial population (generation G_0) according to the 2 major pig enterotypes, characterized by relative overabundance of either *Prevotella* and *Mitsuokella* or *Ruminococcus* and *Treponema*, we used the relative abundance of these 4 genera as selection criteria. From the G_0 population of 317 piglets, we selected 6 males and 30 females per line and produced 2 successive generations (G_1 and G_2) of approximately 130 pigs per line. We consistently confirmed a moderate heritability for each of the selected genera (h^2 = 0.3 to 0.4). We also estimated the heritability values of the relative abundances for 64 additional bacterial genera, which ranged from 0.1 to 0.5. We showed significant differences between the 2 lines in the relative abundance of the 4 bacterial genera at G_1 ($P < 0.001$, from 0.6 genetic standard deviation for *Treponema* to 1.3 for *Prevotella*). In the following generation G_2, response to selection was maintained for *Prevotella* and was even increased for the 3 other genera. The observed contrasts were in the expected direction for the genera under direct selection, and we extended the analysis to the 64 other bacterial genera with estimated heritabilities higher than 0.1. All these results confirm a significant influence of host genetics on the composition of gut microbiota at 60 d of age in pigs, and a capacity of directional selection over generations that will be further explored together with early and late host traits.

Key Words: pigs, microbiomics, heritability, genetic improvement

W200 The impact of host genetics, independently of environmental factors, on porcine gut microbiota composition. A. Heras-Molina*[1], J. Estellé[2], A. López-García[1], J. L. Pensantez-Pacheco[1,3], S. Astiz[1], C. Garcia-Contreras[1], M. Vazquez-Gomez[4,5], B. Isabel[4], A. Gonzalez-Bulnes[6], and C. Ovilo[1], [1]*INIA (CSIC), Madrid, Spain,* [2]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [3]*School of Veterinary Medicine and Zootechnics, Faculty of Agricultural Sciences, University of Cuenca, Cuenca, Ecuador,* [4]*Faculty of Veterinary Medicine, UCM, Ciudad Universitaria, Madrid, Spain,* [5]*Nutrition and Obesities: Systemic Approaches Research Unit (NutriOmics), INSERM, Sorbonne Université, Paris, France,* [6]*Departamento de Producción y Sanidad Animal, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, Valencia, Spain.*

Pig microbiota is associated with the host's breed, with lean and fatty breeds showing relevant differences in their productive parameters. However, in previous studies aiming to investigate the microbiota differences between both breed groups, involved animals were gestated in their corresponding lean or fatty mother, leading to maternal influences. The present study aimed to elucidate the importance of host's genotype on the gut microbiome composition without maternal confounding factors. Sixteen Iberian (IB; fatty breed) sows were inseminated with heterospermic semen (from Iberian and Large White [LW; lean breed] boars). Offspring was sampled at 60 d old (n = 36; 22 IB×IB and 14 IB×LW) and at 210 d old (n = 31; 18 IB×IB and 13 IB×LW) to obtain fecal microbiota composition which was analyzed by sequencing the 16SrRNA gene (V3-V4 amplicon) in an Illumina MiSeq. Bioinformatic analyses were done with QIIME2, and biostatistics analysis were performed using phyloseq and metagenomeSeq R packages. Firmicutes and Bacteroidetes were majority at the phylum level, while *Prevotella* and *Treponema* were the most abundant genera. Observed α diversity was only affected by age ($P < 0.0001$; higher at 210 d). For β diversity there was a genotype × age interaction ($P < 0.01$) and at 210 d, IB×IB animals showed higher β diversity than IB×LW ($P < 0.05$). The differential abundance analysis showed 156 significant (q <0.05) over-abundant amplicon sequence variants (ASVs) in IB×IB animals and 71 in IB×LW. At the genus level, *Anaerovibrio* and *Lachnospiraceae* were more abundant in IB×LW. At 60 d, most over-abundant ASVs observed in IB×IB animals belong to *Ruminococcus* genus, and at 210 d, IB×LW had the most over-abundant ASVs belonging to *Prevotella*. At the genus level, *Agathobacter*, *Parasutterella* and *Lachnospiraceae* were more abundant at 60 d old and *Ruminococcus* at 210 d old, all in IB×IB. These different abundant bacteria are involved in adipogenesis, feed efficiency, digestibility and inflammation, and could be related to breeds' phenotypic differences. Thus, the host genotype affected pig's gut microbiota composition, in a model that exclude maternal confounding factors.

Key Words: pigs and related species, metagenomics, breed diversity, pregnancy, meat production

W201 Rumen eukaryotes are the main risk factors for larger methane emissions in dairy cattle. A. Saborío-Montero*[1,2], M. Gutiérrez-Rivas[1], R. Atxaerandio[3], A. García-Rodríguez[3], I. Goiri[3], J. López-Paredes[4], J. A. Jiménez-Montero[4], and O. González-Recio[1,5], [1]*Departamento de Mejora Genética Animal, Instituto Nacional de Tecnología Agraria y Alimentaria, Madrid, Spain,* [2]*Centro de Investigación en Nutrición Animal y Escuela de Zootecnia, Universidad de Costa Rica, San Pedro, San José, Costa Rica,* [3]*Department of Animal Production NEIKER - Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Campus Agroalimentario de Arkaute s/n., Vitoria, País Vasco, Spain,* [4]*Departamento técnico de Confederación de Asociaciones de Frisona Española (CONAFE), Valdemoro, Madrid, Spain,* [5]*Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.*

Mitigation of methane emissions from dairy cattle is relevant to reduce environment impact and increase profitability through improvement of energy usage. The objective of this study was to estimate how microbiome composition determines large methane concentration (MET) and methane intensity (MI, ppm CH_4/kg of milk) in comparison to more traditional proxies (*i.e.* milk yield and conformation traits). A total of 1,359 Holstein cows from 17 herds in 4 northern regions of Spain were included in this study, the microbiome composition data were a subset containing 437 cows from 14 herds. Cows were classified in quartiles for MET and MI, according to individual records of methane measurements during the cow's visit to the automatic milking system unit. A probit approach under a Markov chain Monte Carlo (McMC) Bayesian framework was used to determine risk factors for high MET and high MI. Genetic merit for methane concentration and microbiome composition (86 phylum and 1,240 genus) were the main drivers for a cow to be classified as high MET and MI. Reducing MET and MI genetic merit by one SD decreased the probability of being classified in the upper quartile by 35.2% (33.9% to 36.4%) and 28.8% (27.6% to 29.6%), respectively. A reduction in probabilities was observed as the relative abundance of most bacteria increased (i.e., Firmicutes 9.9% (8.3 to 11.3) for MET and 7.1% (6.2 to 8.2) for MI, per unit of SD). An opposite effect occurred with Eukaryotes. Larger abundance of most eukaryote became a risk factor to be classified as a

high emitter animal (*i.e.*, Oomycetes 14.2% (11.7% to 16.4%) for MET and 11.8% (9.4% to 14.0%) for MI, per unit of SD). An increment of one unit of SD in milk yield increased the probability of being classified in the upper quartile for MET by 3.7% (2.3% to 4.2%) and reduced the probability for MI by 12.6% (12.2% to 13.3%). Structure and capacity traits were not main drivers of being classified in the higher quartile of methane emission and intensity, with risk odds lower than 2% per unit of SD. After genetic merit, microbiome composition was the most relevant risk factor for larger methane emissions. This study suggests that mitigation of MET and MI could be addressed through animal breeding programs including genetic merits and strategies that modulate the microbiome.

Key Words: genetic merit, microbiome, methane, dairy cow, risk factor

W202 Cecal microbiota composition of experimental laying hens infected with infectious bronchitis virus differs according to genetics and vaccination. M. Borey*[1], B. Bed'Hom[1,2], N. Bruneau[1], J. Estellé[1], F. Larsen[3], F. Blanc[1], M.-H. Pinard-van der Laan[1], T. Dalgaard[3], and F. Calenge[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, UMR GABI, Jouy-en-Josas, France,* [2]*Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France,* [3]*Department of Animal Science, Aarhus University, Tjele, Denmark.*

Interactions between the gut microbiota and the immune system may be involved in the responses to vaccination and infection. We studied the correlations between cecal microbiota composition and parameters describing the immune response in 6 different experimental laying hen lines vaccinated against the infectious bronchitis virus (IBV) and further infected with the pathogen. A cohort of 96 animals was vaccinated at 2 and 5 weeks of age (w.o.a) before an IBV infectious challenge at 8 w.o.a. We quantified peripheral leukocyte subsets and expression of cell surface markers through flow cytometric assay. We characterized the cecal bacterial communities with a 16S rRNA gene amplicon sequencing approach performed on animals killed 1 wk after the IBV infectious challenge. Based on the tracheal IBV load measured the first 5 d after the infectious challenge, 2 lines were considered as high responders to IBV vaccination. The host genetic background was only slightly associated with microbiota composition. In contrast, the effect of vaccination on cecal microbiota composition was strong and similar in all the lines, with a reduced abundance of OTU from the *Ruminococcaceae* UCG-014 and *Fecalibacterium* genera, and an increased abundance of OTU from the *Eisenbergiella* genus. The main association between the cecal microbiota and the immune phenotypes involved $TCR_{V\delta}$ expression on $TCR_{V\delta}^{+}$ T cells, which especially shared negative associations with OTU from the *Escherichia-Shigella* genus. These results confirm the existence of a complex interaction between cecal microbiota and immunity after vaccination.

Key Words: microbiota, IBV, infection, vaccine, chicken

W203 Could the gut microbiome modulate environmental variance and animal resilience? C. Casto-Rebollo*[1], M. Argente[2], M. García[2], A. Blasco[1], and N. Ibáñez-Escriche[1], [1]*Institute for Animal Science and Technology, Universitat Politècnica de València, València, Spain,* [2]*Departamento de Tecnología Agroalimentaria, Universidad Miguel Hernández de Elche, Orihuela, Spain.*

The gut microbiome and their derived metabolites could influence the immune response and affect the host health. Environmental variance of traits (VE) has been related to the immune system and the animal resilience. Animals with a low VE seems to cope better with environmental disturbances, being more resilient. The aim of this study was to identify the metabolites from gut microbiota with different concentration levels between 2 divergent rabbit lines selected for a high and a low VE of litter size (LS). Untargeted metabolites from cecum samples of 28 does (14 from each rabbit line) were obtained using the Discovery HD4 platform of Metabolon. A total of 725 metabolites were identified. After quality control, 631 from 26 animals were maintained in the data set. We combine a 2-sided Mann-Whitney test and the logarithm to base 2 of the fold change to identify the metabolites with differences in means concentration levels between lines. Two partial-least square-discriminant analyses (PLS-DA) were performed to identify the relevant metabolites showing the largest contribution to the classification of the rabbit lines. The classification performance of the PLS-DA was computed using a 4-fold cross-validation. A total of 16 metabolites allowed a classification performance for the PLS-DA higher than 95%. From them, we highlighted the metabolites behenoylcarnitine, equol, ethyl-glucopiranoside, glycerophosphoglycerol, and dimethylglycine because also showed relevant differences in mean between the rabbit lines. These metabolites are involved in the lipid metabolism, xenobiotic metabolism, and amino acid metabolism. These could be relevant to modulate the VE and the animal resilience. However, further studies are needed to understand the effect of this metabolites in these rabbits. Currently, a shotgun metagenomic analysis from the same cecum samples are being performance to identify the bacteria and genes with differences in abundances between the rabbit lines. The final aim is to understand how the selection for the VE of LS has affected the gut microbiome and what if the gut microbiome can be an important factor to modulate the animal resilience.

Key Words: gut microbiome, environmental variance, metabolites, resilience, rabbit

W204 The difference of lipid metabolism based on intestinal microbiome and transcriptome between Dorper and Tan sheep. Y. Ma*, X. Yang, G. Hua, and X. Deng, *National Key Laboratory of Animal Genetics, Breeding and Reproduction, China Agricultural University, Beijing, China.*

There are differences in metabolism between different breeds of sheep, which can be reflected in changes in fat content. Our research found that the IMF content of Tan sheep is significantly higher than Dorper. We infer that lipid metabolism plays a key role in the different fat content. Dorper and Tan sheep are kept in the same environment. We collected 4 Dorper and 4 Tan sheep cecum, colon contents samples from 8-mo-old ewes. First, we sequenced the 16sDNA of the bacteria in the intestinal contents to obtain the specific composition of the microorganisms in each sample at each classification level. Second, the metabolome uses LC-MS to analyze the intestinal contents, and differentially expressed metabolites are represented by Variable importance for the projection (VIP). Third, we performed mRNA sequencing on the liver, muscle, and brain tissues, and used FC >2 and $P < 0.05$ as screening criteria to obtain differentially expressed genes (DEG). In our study, we found that there are 700 OTUs shared by the cecum and colon tissues. The ratio of *Firmicutes* to *Bacteroides* in Tan sheep is higher than Dorper. LEfse analysis found that the proportion of *Rikenellaceae* in Tan sheep is lower. Spearman correlation analysis found that bile acid metabolites were significantly associated with different genus. In metabolomics analysis, the pathway of Bile Secretion was significantly enriched. The bile secretion in the intestine of Tan sheep is significantly higher than Dorper. Bile acids are closely related to liver lipid metabolism and circadian rhythm. The differential expression of *ABCB11*, *SREBF1* and *CYP1A1* genes in the liver plays a key role in lipid metabolism and bile acid secretion. *PPARGC1A* gene in muscle tissue and *NR1D1*, *PER1*, and *PER3* gene in brain tissue are also thought to be related to metabolic rhythm. Our research shows that the difference in fat content between Dorper and Tan sheep may be caused by lipid metabolism, and key bacteria, bile acid metabolism and liver tissue DEG jointly cause this phenomenon.

Key Words: fat content, microbial diversity, metabolome, transcriptome, bile secretion

W205 The potential of using rumen microbial profiles for the prediction of enteric methane emissions traits for commercial livestock breeding. T. Bilton*[1], M. Bastiaanse[1], M. Hess[1], J. Budel[2], G. Noronha[2], H. Henry[1], S. Hickey[3], G. Pile[1], P. Janssen[4], J. McEwan[1], and S. Rowe[1],

[1]*AgResearch, Mosgiel, New Zealand,* [2]*Universidade Federal do Pará (UFPa), Belém Do Pará, Brazil,* [3]*AgResearch, Ruakura, New Zealand,* [4]*AgResearch, Palmerston North, New Zealand.*

The rumen microbiome has been associated with important livestock production traits. Breeding that targets the rumen microbiome composition, therefore, has potential to make further genetic gains in these traits. For this to be implemented, a low-cost and high-throughput measure is required that has prediction power across systems and time. Hess et al. (2020) developed a low-cost, high-throughput restriction enzyme reduced representation sequencing (RE-RRS) method for metagenomic sequencing of rumen microbiomes. The gold standard (GS) approach for preserving rumen samples, however, requires laborious and low-throughput processing steps before sequencing, limiting utility for large scale phenotyping. We investigate the performance of rumen microbial profiles from RE-RRS for trait prediction and the feasibility of using a TNES lysis buffer solution (Solution A) for preserving rumen samples before DNA extraction. Rumen samples from 3,139 sheep of different ages and on different diets was generated using RE-RRS. Different training sets were formed based on different combinations of ages and diets. To estimate prediction accuracies, 2 independent data sets of 150 different animals from the same flock sampled at 2 time points (PD1) and 93 animals from an entirely different flock (PD2) were generated using RE-RRS. Rumen samples were preserved using both the GS method and Solution A. Methane phenotypes in CH_4 g/d were measured immediately before rumen sampling and rumen microbiome profiles were generated using the reference-free approach by Hess et al. (2020). A linear mixed model with a microbial relationship matrix was used for prediction. Prediction accuracies ranged between 29 and 38% within the training sets, between 23 and 51% for PD1 and between 8 and 28% for PD2. The difference in prediction accuracies using Solution A compared with GS was 0.2% ± 2.7% for PD1 and 0.5% ± 3.4% for PD2. These results indicate that the rumen microbial profile can be used for trait prediction across flocks and suggests Solution A is a viable, low-cost preservative method for rapid high-quality microbial DNA extraction. This development could significantly advance the implementation of rumen microbiomes as a phenotype for breeding in ruminant livestock.

Key Words: metagenomics, restriction enzyme reduced representation sequencing, sample preservation, genomic prediction

W206 Mapping the livestock microbiome. M. Watson*, L. Glendinning, A. Warr, and J. Mattock, *The Roslin Institute, University of Edinburgh, Midlothian, Edinburgh, UK.*

The microbiome is the entire complement of microorganisms that exist in a given environment, including bacteria, archaea, microbial eukaryotes such as fungi and protozoa, and viruses. Despite their abundance and importance, the majority of microbial species remain undiscovered, and we lack the tools to study them. Genome assembly and binning of metagenomic data has become the dominant way of characterizing unknown and uncultured components of the microbiome, and recent studies have computationally isolated hundreds of thousands of bacterial and archaeal genomes from chickens, pigs, and ruminants. I will present our work in unraveling the livestock microbiome, including an analysis of currently available data sets, their strengths and weaknesses, discussion of bioinformatics tools for the reconstruction of genomes from metagenomes, a discussion of scale and potential solutions for producing more accurate metagenomic bins. I will also present evidence linking elements of the microbiome with traits of interest in livestock

Key Words: livestock, microbiome, traits

ISAG-FAO Genetic Diversity Workshop

W207 Donkey worldwide diversity based on control-region data and entire mitochondrial genomes. D. Bigi[1], N. Rambaldi Migliore[2], M. Milanesi[3,4], P. Zambonelli[1], R. Negrini[3], A. Verini-Supplizi[5], L. Liotta[6], F. Chegdani[7], S. Agha[8], A. Torroni[2], P. Ajmone-Marsan[3,9], A. Achilli[2], and L. Colli*[3,10], [1]*Dipartimento di Scienze e Tecnologie Agro-Alimentari (DISTAL), Alma Mater Studiorum University of Bologna, Bologna, BO, Italy,* [2]*Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani," University of Pavia, Pavia, PV, Italy,* [3]*Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti (DIANA), Università Cattolica del S. Cuore, Piacenza, PC, Italy,* [4]*Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, VT, Italy,* [5]*Dipartimento di Medicina Veterinaria, University of Perugia, Perugia, PG, Italy,* [6]*Dipartimento di Scienze Veterinarie, University of Messina, Messina, ME, Italy,* [7]*Department of Biology, Faculty of Sciences Aïn Chock, University HAssan II, Casablanca, Morocco,* [8]*Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt,* [9]*PRONUTRIGEN Centro Ricerca Nutrigenomica e proteomica, Università Cattolica del S. Cuore, Piacenza, PC, Italy,* [10]*BioDNA Centro di Ricerca sulla Biodiversità e sul DNA Antico, Università Cattolica del S. Cuore, Piacenza, PC, Italy.*

Donkeys (*Equus asinus*) have played a significant role in agriculture and transports as burden animals for millennia, until the growing diffusion of mechanization led to significant changes in these sectors. Consequently, the number of breeds and population sizes progressively decreased, particularly in developed countries, with a concurrent erosion of genetic diversity. In this study, we analyzed 1,350 donkey mitochondrial (mtDNA) control-region sequences and 164 complete mitogenomes to (1) evaluate worldwide diversity, (2) assess geographical trends of variation, and (3) revise the nomenclature of mtDNA haplogroups. Diversity indices, mismatch distribution and phylogenetic networks were computed using control-region data, while a maximum parsimony (MP) tree was built based on coding-region data of complete mtDNAs. The topology of the MP tree confirmed the occurrence of the 2 previously described major clades, i.e., clades 1 and 2, resulting from independent domestications in Northeastern Africa, which were respectively renamed as haplogroups A and B to harmonize the nomenclature with the standard adopted for other livestock species. Furthermore, we detected a new lineage, tentatively called pre-B, which radiates before the root of haplogroup B. Control-region data highlighted extensive variations of haplotype (h) and nucleotide diversities (n) between geographical areas (h = 0.403–0.922, n = 0.011–0.025), with the highest figures in Northeastern Africa (h = 0.922, n = 0.025), confirming it as the likely domestication center, an evidence further supported by mismatch distribution curves indicating that the population expansion in this area occurred earlier than in other regions of the world. Differences in regional phylogenetic networks pointed at the joint effects of demography, past human migrations and trade. Despite the strong decline of donkey populations worldwide, the sizable mtDNA variability and the identification of the new early radiating lineage further stress the need for an extensive characterization also of nuclear genome diversity to identify hotspots of variation and aid the conservation of donkey breeds worldwide.

Key Words: donkey, mitochondrial DNA, diversity, control region, mitogenome

W208 Estimation of inbreeding load and purging in animal conservation programs. N. Pérez-Pereira*[1], E. López-Cortegano[1,3], A. García-Dorado[2], and A. Caballero[1], [1]*Centro de Investigación Mariña, Universidade de Vigo, Vigo, Spain,* [2]*Universidad Complutense de Madrid,*

Madrid, Spain, [3]Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK.

The inbreeding load, i.e., the genomic load of partially recessive deleterious mutations in the heterozygote state, is assumed to be the main source of inbreeding depression in inbred populations and a key parameter in conservation genetics and animal breeding. In turn, genetic purging can mitigate the magnitude of inbreeding depression by removing deleterious mutations as the inbreeding load is exposed in homozygosis. Methods aimed at estimating the inbreeding load and the purging coefficient (which measures the magnitude of purging) in pedigreed populations have been developed assuming random mating and variable contributions from parents to progeny. However, in conservation and animal breeding programs it is common to manage matings and the contribution from parents to progeny to reduce the impact of inbreeding and to preserve genetic diversity, usually by equalizing contributions and avoiding inbred matings. Other strategies propose instead to carry out some degree of inbreeding to enhance purging. Thus, different mating systems may differ substantially in the intensity of purging. Using computer simulations, we tested the accuracy of the estimates of the inbreeding load and the purging coefficient, both obtained with the software PURGd, for pedigreed populations under equalization of contributions, partial full-sib mating and circular mating. Accurate estimates were obtained for each of these scenarios which, together with the corresponding predictions of the inbreeding coefficient and the variance of contributions, allowed the prediction of fitness over time to be obtained taking into account the effects of purging.

Key Words: inbreeding depression, inbreeding load, fitness, deleterious mutations, genetic purging

W209 Functional and population genomics of admixed trypanotolerant African cattle breeds. G. P. McHugo*[1], J. A. Ward[1], T. J. Hall[1], G. M. O'Gorman[2], E. W. Hill[1], and D. E. MacHugh[1,3], [1]*UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [2]*National Office of Animal Health Ltd., Enfield, UK,* [3]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Bos primigenius taurus (taurine) and *Bos primigenius indicus* (zebu) cattle diverged at least 500,000 years ago and, since that time, significant genomic differences have accumulated between them. Subsequent admixture in Africa has resulted in a complex mosaic of taurine-zebu ancestry, with most cattle breeds containing varying levels of admixture. Some African taurine populations have an important evolutionary adaptation known as trypanotolerance, a genetically determined tolerance to infection by trypanosome parasites (*Trypanosoma* spp.). These are transmitted by infected tsetse flies (*Glossina* spp.) and cause African animal trypanosomiasis (AAT) disease. ATT is one of the largest constraints to livestock production in sub-Saharan Africa and causes a financial burden of approximately $4.5 billion annually. Some West African taurine breeds, such as the N'Dama, are trypanotolerant; these cattle have an ability to control trypanosome parasite loads and to limit disease pathology compared with trypanosusceptible zebu breeds. However, zebu animals are generally larger, produce higher milk yields and are therefore favored by many producers. We have examined transcriptomics data generated from trypanosome infection studies with trypanotolerant and trypanosusceptible breeds to identify differentially expressed genes. Outputs from this experiment were then integrated with sub-chromosomal admixture results from local ancestry analysis of genome-wide high-density SNP data to explore the functional biology of differentialy introgressed genomic regions in admixed African cattle breeds.

Key Words: cattle and related species, functional genomics, integrative genomics, admixture, animal health

W210 Microbiota characterization of traditional cattle breeds. R. Gonzalez-Prendes[1], R. Gomez Exposito[2], T. Reilas[3], M. Makgahlela[4], J. Kananen[3], C. Ginja[5], D. Kugonza[6], N. Ghanem[7], H. Smidt[2], and R. Crooijmans*[1], [1]*Animal Breeding and Genomics Group, Wageningen University and Research, Wageningen, the Netherlands,* [2]*Micriobiology group, Wageningen University and Research, Wageningen, Wageningen, the Netherlands,* [3]*Natural Resources Institute Finland, Jokioinen, Finland,* [4]*Agricultural Research Council-Animal Production Institute, Pretoria, South Africa,* [5]*CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal,* [6]*Department of Agricultural Production, School of Agricultural Sciences, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda,* [7]*Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt.*

The Optibov project has a focus on the characterization of traditional and indigenous breeds to identify genomic variants associated with adaptation traits such as longevity and disease resistance. Understanding the genetic basis for these traits in well-adapted native cattle is useful for the improvement of highly-selected and less diverse commercial breeds. In this study we investigated the microbiota diversity and its composition in traditional breeds. Fecal microbiota was characterized using 16S ribosomal RNA sequencing in 200 animals of traditional cattle breeds, namely, Deep Red (24), Groninger White Headed (20), Meuse-Rhine-Yssel (23), Dutch Belted (23), Dutch Friesian (24) from the Netherlands; the Northern Finncattle (25) Eastern Finncattle (25), Western Finncattle (25) from Finland; and 10 commercial Holstein Friesian animals sampled in both countries. The PCR products of the V4 region of the 16S rRNA gene (~250 bp fragment) were amplified and sequenced using the Illumina NovaSeq platform. A total of 2,182 amplicon sequence variants (ASVs) were identified and significant differences were observed between breeds. At country level the diversity index were different between breeds only in Dutch breeds. Our results suggest that the breed may impact the microbiota diversity index and its composition although additional studies are necessary to discriminate among others effects as well as farm or diet. In addition, functional prediction of the identified ASVs are under analysis.

Key Words: microbiome, traditional cattle breeds, Optibov

W211 Towards a comprehensive horse Y-chromosomal tree: Signatures from local breeds and ancient DNA. E. Bozlak*[1,2], L. Radovic[1,2], D. Rigler[2], T. Kunieda[3], R. Juras[4], G. Cothran[4], and B. Wallner[2], [1]*Vienna Graduate School of Population Genetics, Vienna, Austria,* [2]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria,* [3]*Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Japan,* [4]*Department of Integrative Biosciences, College of Veterinary and Biomedical Sciences, Texas A&M University, College Station, TX, USA.*

The Y chromosome carries pivotal information about male-driven demography and is an indispensable tool for studying the historic development of domestic animals. Y haplotype patterns in modern-day domestic horses are characterized by the dominance of a recent 'Crown' clade, which is a consequence of extensive breeding with stallions of Oriental origin in the past 500 years. However, Northern European breeds, Asian horses, and presumably other populations in remote regions could have avoided recent Crown introgressions and retained autochthonous variation. Here we augmented the established modern horse Y-phylogeny with horses from remote regions around the globe. 82 samples were carefully selected from more than 2000 individuals by genotyping haplotype-defining mutations. We focused on horses that do not carry a 'Crown' haplotype. We performed target enriched resequencing (TES) of 5 Mb of the Y chromosome. NGS reads were mapped to the 6.4 Mb LipY764 reference, variants ascertained with GATK4, and filtered for single-copy regions in the reference and multiple quality parameters. Based on the remaining variants (>2200), a parsimony tree was created. Most of the TES samples represented unique haplotypes that were reported for the first time. This proves, that genotyping prior sequencing was effective to catch low frequent HTs in remote populations. The extended Y phylogeny supports a pronounced radiation 'Dom-West', encompassing most domestic horse Y

lineages, which substantiates previous findings. To investigate the origin and dissemination of the contemporary Y haplotypes, we combined the domestic horse Y phylogeny with data from a published set of whole-genome sequenced ancient males using the program PathPhynder. Data from ancient periods enable more accurate dating of branching points, disclose the lineages lost in recent times, and allow us to trace the origin of the present horses' lineages. The complemented Y-chromosomal phylogeny significantly reduces ascertainment bias when studying remote populations and it is the next step toward a better understanding of horse breeding via males.

Key Words: Y chromosome, horses and related species, phylogeny, resequencing, ancient DNA

W212 Researching on the fine-structure and admixture of the worldwide chicken population reveal connections between populations and important events in breeding history. Y. Guo*[1,3], J.-H. Ou[5], Y. Zan[5], Y. Wang[1], H. Li[4], C. Zhu[4], K. Chen[4], X. Zhou[3], X. Hu[1,2], and Ö. Carlborg[5], [1]*State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing, China,* [2]*National Engineering Laboratory for Animal Breeding, China Agricultural University, Beijing, China,* [3]*Beijing Advanced Innovation Center for Food Nutrition and Human Health, China Agricultural University, Beijing, China,* [4]*Jiangsu Institute of Poultry Science, Jiangsu Yangzhou, China,* [5]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.*

Here, we have evaluated the general genomic structure and diversity, and studied the divergence resulting from selection and historical admixture events for a collection of worldwide chicken breeds. In total 636 genomes (43 populations) were sequenced from chickens of American, Chinese, Indonesian and European origin. Evaluated populations included wild junglefowl, rural indigenous chickens, breeds that have been widely used to improve modern western poultry populations and current commercial stocks bred for efficient meat and egg production. In-depth characterizations of the genome structure and genomic relationships among these populations were performed, and population admixture events were investigated. In addition, the genomic architectures of several domestication traits and central documented events in the recent breeding history were explored. Our results provide detailed insights into the contributions from population admixture events described in the historical literature to the genomic variation in the domestic chicken. In particular, we find that the genomes of modern chicken stocks used for meat production both in eastern (Asia) and western (Europe/US) agriculture are dominated by contributions from heavy Asian breeds. Further, by exploring the link between genomic selective divergence and pigmentation, connections to functional genes feather coloring were confirmed.

Key Words: genomic structure, admixture, selection, chickens, Asian breeds

W213 Demographic history and genetic diversity of wild African harlequin quail (*Coturnix delegorguei*) populations of Kenya. S. Ogada[1], N. Otecko[2], G. Kennedy[1], J. Musina[3], B. Agwanda[3], V. Obanda[4], J. Lichoti[5], M.-S. Peng[2], Y.-P. Zhang[2], and S. Ommeh*[1], [1]*Institute For Biotechnology Research (IBR), Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya,* [2]*State Key Laboratory of Genetic Resources and Evolution, Yunnan Key Laboratory of Molecular Biology of Domestic Animals, Germplasm Bank of Wild Species, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China,* [3]*Department of Zoology, National Museums of Kenya, Nairobi, Kenya,* [4]*Department of Veterinary Services, Kenya Wildlife Service, Nairobi, Kenya,* [5]*State Department of Livestock, Ministry of Agriculture, Livestock, Fisheries and Irrigation, Nairobi, Kenya.*

The harvesting of wild African harlequin quails (*Coturnix delegorguei*) as bushmeat using traditional methods in Kenya has been ongoing for generations yet their genetic diversity and evolution history is largely unknown. In addition, over the years, there has been an uncontrolled introduction of several domestic Japanese quail breeds in Kenya and other East African countries, whose effect on the local quail populations is yet to be determined. In this study, the genetic variation and demographic history of wild African harlequin quails was assessed using a 347-bp mitochondrial DNA (mtDNA) control region fragment and genotyping by sequencing (GBS) data. Genetic diversity analyses revealed that the genetic variation in wild African harlequin quails was predominantly among individuals than populations. Demographic analyses indicated a signal of rapid expansion and the estimated time since population expansion was found to be 257,000 years ago, corresponding to around the Pliocene–Pleistocene boundary. A recent sharp bottleneck that was also observed exposed a decline in their effective population size number which raised concerns about their conservation status. These results provide the first account on the genetic diversity of wild African harlequin quails thereby providing a foundation useful in their biodiversity conservation.

Key Words: emerging poultry, genetic erosion, origins, sustainability

W214 Genetic relationships among Canarian, African, and European goats using SNPs. M. Macri*[1,2], A. Martínez[2], M. G. Luigi[3], J. Capote[4], A. Canales[1,2], M. Amills[3], J. V. Delgado[2], and M. R. Fresno[4], [1]*Animal Breeding Consulting, S.L, Cordoba, Cordoba, Spain,* [2]*Department of Genetics, University of Córdoba, Cordoba, Cordoba, Spain,* [3]*Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, University of Barcelona, Bellaterra, Barcelona, Spain,* [4]*ICIA, Canary Islands Institute for Agricultural Research, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain.*

Majorera, Palmera and Tinerfeña are 3 local goat breeds reared in the Canary Islands which are specialized in the production of milk to elaborate cheese. Several studies have shown that the founder population of Canarian goats had an African origin, with posterior admixture, particularly in the 20th century, from European goats. The goal of this research is to determine the genetic contributions of African and European goats to the Canarian gene pool by using a high-throughput genotyping approach. Blood samples from 72 Canarian goats (24 Majorera, 24 Palmera and 24 Tinerefeña) were analyzed using the GoatSNP50 BeadChip (Illumina) and compared with 654 goats from 30 different breeds investigated in the ADAPTmap Project (http://www.goatadaptmap.org/). The SNPs were filtered using the PLINK v 1.9 program, excluding SNPs with a MAF less than 0.05, missing genotype data higher than 0.01 and significant departure from HWE (P-value <0.001), retaining a total of 23,527 SNPs. The PLINK v1.9 program was used to calculate H_O, H_E and inbreeding coefficient (F_{IS}) as well as to perform principal component analysis (PCA). The PCA was visualized with the ggplot2 R package. Reynolds genetic distance and F_{ST} between pair of breeds were obtained with Arlequin v3.1. and a Neighbor-Net was visualized using the Splits-tree4 v4.14.4 software. In both the Neighbor-net tree and PCA, the Canarian goats were located closer to the Central and North African breeds than to the Spanish and remaining European ones, being the North African closer to the Canarian goats than the central African breeds. These results agree with the African origin of Canarian breeds that, combined with insularity, has contributed to create a unique gene pool. This study was funded by the RTA2014-00047-00-00 Project (INIA).

Key Words: goat breeds, SNPs, GoatSNP50 BeadChip, principal component analysis (PCA)

W215 The eastward dispersal of domestic goats and their introgression, population stratification, and genetic adaptation in East Asia. Y. Cai*, W. Fu, Z. Zheng, X. Liu, Y. Jiang, and X. Wang, *Northwest A&F University, Yangling, Shaanxi, China.*

Goat domestication began ~11,000 years ago from a mosaic of wild bezoar populations. Following domestication, goats dispersed throughout the world, along with human migration. In Asia, domestic goats were present in China more than 4,000 years ago. However, their migration route to East Asia and their local evolutionary history remain poorly understood.

Here, we sequenced 101 modern *Capra* genomes and 31 ancient Chinese goat genomes. Together with the publicly available genomic resources for modern and ancient goats, we revealed that the ancient Chinese goats descended from the eastern Fertile Crescent and began to migrate into China about 7,000 to 6,000 years ago. Compare with European and African goats, we found Asian goats have a specific higher level of introgression from markhor. Modern Chinese goats were divided into a northern and a southern group, coinciding with the most prominent climatic division in China. Northern Chinese goats have a lower level of introgression than other Asian goats. And, compared with northern Chinese goats, southern Chinese goats maintained a gene pool more similar to that of ancient Chinese goats. The gene flow from European goats into northern Chinese goats may contribute to this divergence. The 2 most divergent genes between northern and southern Chinese goats are both related to hair follicle development, which may play an important role in their environmental adaptation. And, the evolutionary trajectories of these genes were uncovered through ancient DNA. Our findings add to our understanding of the eastward dispersal of domestic goats and the specific evolutionary process of East Asian goats.

Key Words: introgression, ancient genomes, population genomics, environmental adaptation, genomic selection

Applied Genetics and Genomics in Other Species of Economic Importance Workshop

W216 Evaluation of population structure alpacas maintained in Poland and identification alpaca-llama hybrids based on microsatellite markers. A. Podbielska*[1], K. Piórkowska[1], and T. Szmatola[1,2], [1]*Department of Animal Molecular Biology, National Research Institute of Animal Production, Balice, Poland,* [2]*Center for Experimental and Innovative Medicine, University of Agriculture in Krakow, Kraków, Poland.*

The study aimed to characterize the alpaca population structure maintained in Poland using 17 microsatellite markers recommended by the International Society for Animal Genetics. The classification of llamas, alpacas, or hybrids based on phenotype is often difficult due to long-term hybridization and not keeping the species pure-bred. Although, their taxons were separated a long time ago into *Lama glama* and *Vicugna pacos*. Moreover, alpacas are frequently purchased without certificates of pedigree registration (price factor), and llama admixture is revealed in the following generations. According to our knowledge, the present study, for the first time, assesses the population structure of alpacas bred and maintained in Poland. The hair follicle and buccal swabs were taken from 234 animals. Among them were 216 alpacas, 15 llamas, 1 control llama-alpaca hybrid and 2 potential hybrids. Llama samples were collected as a control group. DNA was extracted using Sherlock AX by A&A Biotechnology following the suggested manufacturer protocol. Seventeen microsatellite markers were analyzed: *LCA5*, *LCA8*, *LCA19*, *LCA37*, *LCA56*, *LCA65*, *LCA66*, *LCA94*, *LCA99*, *LGU49*, *LGU50*, *YWLL44*, *YWLL29*, *YWLL36*, *YWLL40*, *YWLL43-X*, and *YWLL46*. The genetic structure of the studied populations was investigated using Structure 2.3.4 software with a burn-in period of 100,000 and 200,000 iterations fitting K from 1 to 4 with 10 runs for each K. The Structure Harvester was used to select the best K and to visualize the best K graphically Clumpak program was used. Obtained results showed that microsatellite markers can distinguish alpacas from llamas, while Structure software analysis indicated the level of admixture of one species in another. Moreover, it was observed that purebred alpacas are those for which $q \geq 0.98$; in turn, alpacas with an admixture of llama constituted 8.8% of the tested individuals, the first-generation hybrid had only 7.4% llama admixture, and the level of this admixture was due to random allele segregation.

Key Words: alpaca, hybrid, microsatellite markers, population structure

W217 Rate of rejection of first-degree relationships for assigning parent-offspring relationships and estimation of genotyping errors with a high-density array in pigs. L. Gomez-Raya*[1], E. Gomez Izquierdo[2], E. de Mercado[1], and W. M. Rauw[1], [1]*INIA-CSIC, Madrid, Spain,* [2]*ITACyL, Hontalbilla, Spain.*

A first-degree relationship shares about 50% of their genes. Individuals with a first-degree relationship cannot share the 2 alleles at a genetic marker; that is, they cannot be homozygotes for alternative alleles. Applying this concept to HD arrays typed in individuals with a mixture of first and other relationships may allow detection of first-degree relationship (parent-offspring or full-sibs). We define genome-wide rate of rejection of first-degree relationships as the rate at which 2 individuals typed for a large number of SNPs do not share at least one allele. Although first degree relationships would impede markers to be homozygotes for alternative alleles, in practice, genotyping errors occur. A model of mixture of 2 binomials with parameters of the rate of genotyping errors and a general rate of rejection is proposed. A solution is found via Expectation-Maximization algorithm and tested with a crossbred experiment of Iberian x Duroc together with an Affymetrix 600K array. There were 9 candidate sires and 55 dams that produced 214 burrows. We were able to establish paternity and maternity of 75 and 101 piglets, respectively. A lower bound of the genotyping error rate among autosomal SNPs was estimated in 0.1243%. There were 8,652 SNPs that were rejected in 6 or more truly first-degree relationship and were eliminated for further analysis. This study shows that animal experiments allow to establish or to verify first degree relationships as well as to estimate genotyping errors.

Key Words: swine, HD array, SNP, paternity testing, genotyping errors

W218 Molecular characterization and occurrence of variation within the promoter region of *CASK* gene in racing pigeons. M. Stefaniuk-Szmukier*[1], K. Piórkowska[2], K. Ropka-Molik[2], and A. Dybus[3], [1]*University of Agriculture in Kraków, Krakow, Poland,* [2]*National Research Institute of Animal Production, Balice, Poland,* [3]*West Pomeranian University of Technology, Szczecin, Poland.*

Sport pigeons have been selectively bred for superior athletic phenotypes and homing ability. Recently several studies investigated the molecular background of sport traits by the use of modern molecular techniques such as whole-genome and transcriptome sequencing. Several genes have been shown to be under positive selection such as *CASK*, *DRD4*, *AVPR1A*, and *MFSD2A*. The *CASK* gene encoding calcium/calmodulin-dependent serine protein kinase, member a MAGUK protein family. Primarily expressed in brain tissue where interact with presynaptic N-type voltage-dependent calcium channel in hippocampal neurons. However, further evidence described their appearance in cardiomyocytes regulates the voltage-gated sodium channels near or within the T-tubule systems. The aim of the research was to identify polymorphisms in 2 fragments of the *CASK* gene in sport pigeons, which might be related to racing performance traits. The primers for Sanger sequencing designed to span the first exon and upstream region (667 bp) and the second exon and its upstream region (430 bp) based on the NW_004973256.1 sequence. The analysis was performed on 150 sport pigeons and reveals 16 polymorphisms localized in upstream regions (8 in the promoter regions and 1 in the 5`UTR) and in both exons (5 missense variants, 1 codon stop, and 1 of 17bp insertion). The frequencies of investigated SNP are in Table1. Furthermore, detected polymorphisms: might have the ability to affect transcripts abundance and thus affect performance. More detailed research is

necessary to establish their potential association with flying performance and identified variation.

Table 1. Polymorphisms localized in upstream regions and in both exons of the *CASK* gene

CASK SNP	MAF	HET	HOM D	HOM R
g.2514619	0.110	0.222	0.778	0.000
2514653	0.110	0.222	0.778	0.000
2515028	0.042	0.071	0.923	0.006
2515029	0.081	0.148	0.845	0.006
2523738	0.063	0.126	0.874	0.000
2523794	0.287	0.427	0.500	0.073
2523861	0.070	0.126	0.868	0.007
2523937	0.040	0.079	0.921	0.000
2523995	0.320	0.430	0.490	0.079
2524017	0.255	0.377	0.556	0.066
2524043	0.424	0.530	0.311	0.159
2524083	0.192	0.291	0.662	0.046
2524088	0.287	0.413	0.507	0.080
INSERCJA	0.007	0.013	0.987	0.000
2524156	0.285	0.423	0.503	0.074
2524175	0.225	0.356	0.597	0.047

Key Words: racing pigeons, performance, *CASK*

Comparative and Functional Genomics Workshop

W219 Invited Workshop Presentation: The Functional Annotation of Animal Genomes Project: Progress and challenges for our continued global effort. P. W. Harrison*, *EMBL-European Bioinformatics Institute, Cambridge, UK.*

The Functional Annotation of Animal Genomes (FAANG) Project is a coordinated international effort to produce and collate high-quality functional annotation of animal genomes. It has a collaborative focus across multiple coordinated projects on the generation, annotation and presentation of FAIR open access and richly validated genotype and phenotype data sets. The data sets and accompanying supportive informatics infrastructure, that has been developed in recent years, advances the scope (Abstract W218)of comparative genomics and furthers the understanding of functional elements in these communities. The project provides terrestrial and aquatic animal agriculture communities powerful resources for supporting improvements to farmed animal production, disease resistance and genetic diversity. The FAANG Data Coordination Centre (DCC) at EMBL-EBI has been developing the core infrastructure to support the global community to create this rich genome to phenome resource. The focal point is the FAANG Data portal that hosts all of the rich data sets generated by the projects (http://data.faang.org). The EuroFAANG projects that focus upon cattle (BovReg), chicken and pigs (GENE-SWitCH) and Salmonids (AQUA-FAANG), collectively provide a focal point for European FAANG research and are supporting the continued development of the FAANG DCC infrastructure at EMBL-EBI. This talk focuses on the progress made within FAANG thus far, the main gaps in both data sets and infrastructure and the challenges community needs to address going forward. Some key infrastructure challenges include automated visualization, track hub enhancements, single-cell atlases, cross referencing to biorepositories, and maintaining FAIR data sets at increasing scale. EMBL-EBI will continue to tackle these challenges with the FAANG community to develop the coordination and Data Portal technological developments for continued improvement in functional annotation of animal genomes. FAANGs recent whitepaper outlines the key scientific challenges and opportunities that the community needs to address to more accurately use genotype to predict phenotype in the world's farmed animal species, both terrestrial and aquatic to improve farmed animal health, welfare, efficiency and diversification.

W220 Insights into translation through transfer RNA sequencing and ribosome profiling. A. Goldkamp* and D. Hagen, *Oklahoma State University, Stillwater, OK, USA.*

Differentially methylated regions (DMRs) have been associated with large offspring syndrome (LOS) in cattle. Some DMRs overlap transfer RNA (tRNA) gene clusters, potentially altering tRNA expression patterns uniquely by treatment group or tissue type. tRNAs are classified as adapter molecules, serving a key role in the translational machinery implementing genetic code. Variation in tRNA expression has been identified in several disease pathways suggesting an important role in the regulation of biological processes. tRNAs also serve as a source of small noncoding RNAs. To better understand the role of tRNA expression in LOS, total RNA was extracted from skeletal muscle and liver of 105-d fetuses and the tRNAs sequenced. This study detected expression of 474 and 487 bovine tRNA genes in skeletal muscle and liver, respectively, with the remainder being very lowly expressed or transcriptionally silent. We observed differential expression of tissue- and treatment-specific tRNA genes that could modulate translation during protein homeostasis or cellular stress. In addition, the most highly expressed isodecoders differed by treatment and tissue type with roughly half correlated with codon frequency. While the absence of certain isodecoders may be relieved by wobble base pairing, missing tRNA species could likely increase the likelihood of mistranslation or mRNA degradation. One of the greatest factors in determining translation fidelity and efficiency is codon usage bias. Sources of sequence variation, like codon usage, often drive differences in elongation rate and gene expression. Highly expressed genes are thought to be codon-biased to support efficient translation, in which the encoded codons correspond to highly abundant tRNAs. Further, synonymous SNPs were once considered to be silent due to the degeneracy of the genetic code. However, synonymous SNPs may disturb protein abundance and function through alterations in translational efficiency. To investigate the relationship between tRNA expression and translation, ribosome profiling was

used to capture transcripts bound by the ribosome. Following nuclease digestion, we performed high-throughput sequencing of ribosome bound fragments that are being actively translated at a single-nucleotide resolution.

Key Words: protein translation, tRNA, ribosome profiling

W221 Reference transcriptomes of porcine peripheral blood immune cells created through bulk and single-cell RNA sequencing. J. Herrera-Uribe[1], J. E. Wiarda[2,5], S. K. Sivasankaran[2,6], L. Daharsh[1], H. Liu[1], K. A. Byrne[2], T. P. L. Smith[3], J. K. Lunney[4], C. L. Loving[2], and C. K. Tuggle*[1], [1]*Iowa State University, Ames, IA, USA,* [2]*USDA-ARS-NADC, Ames, IA, USA,* [3]*USDA-ARS-MARC, Clay Center, NE, USA,* [4]*USDA-ARS-BARC, Beltsville, MD, USA,* [5]*Immunobiology Program Iowa State University, Ames, IA, USA,* [6]*Genome Informatics Facility Iowa State University, Ames, IA, USA.*

Two approaches to examine porcine immune cell function and annotate these functions in the porcine genome were taken. First, we used cell sorting to isolate 8 cell types from peripheral blood mononuclear cells (PBMCs), representing monocytes, NK cells and specific populations of T and B cells. Transcriptomes (deep RNA-seq) for each cell type were generated, that detected 10,974 genes; 210 were validated using NanoString. Gene expression enrichment analysis identified 1,885 to 3,591 significantly enriched genes (SEG, $q < 0.05$ and 2-fold above average expression) for all cell types. Comparison of gene expression indicated highly significant correlations (Spearman's rank $P < 2.2e-16$) between pig cells and corresponding human cells. Second, single-cell RNA-sequencing (scRNA) of PBMC populations was performed to more highly resolve PBMC population transcriptomes. Across 7 PBMC samples, 28,810 cells distributed across 36 clusters were classified into 13 general cell types including plasmacytoid dendritic cells (DC), conventional DCs, monocytes, >10 B-cell clusters, CD4 and CD8 ab T cells, NK cells, and 4 clusters of gd T cells. Signature gene sets for a human bulk RNA-seq data set were assessed for relative enrichment in genes expressed in pig cells, and substantial overlap in gene expression between specific pig and human PBMC populations was detected. Integration of pig scRNA with a public human scRNA data set provided further validation for similarity between human and pig. Overall, the data provides deep and well-validated transcriptomic data from sorted cell populations and the first single-cell atlas for porcine PBMCs, including highly resolved comparisons to human data sets. Work was supported by USDA-2018-67015-27501 and USDA-ARS CRIS project 5030-31320-004-00D. JH-U and JEW contributed equally; CLL and CKT contributed equally.

Key Words: pig, immune cells, RNA-seq transcriptome, single-cell RNA-seq, FAANG

W222 Detailed molecular and epigenetic characterization of pig IPECJ-2 and chicken SL-29 cell lines. J. de Vos*[1], R. Crooijmans[1], M. Derks[1], S. Kloet[2], M. Groenen[1], and O. Madsen[1], [1]*Animal Breeding and Genetics Group, Wageningen University and Research, Wageningen, the Netherlands,* [2]*Leids Universitair Medisch Centrum, Leiden, the Netherlands.*

The IPECJ2 cell line in pigs and the chicken SL-29 cell line are of interest for the animal genomics community because of the untransformed nature and wide use in functional studies of these cells. The IPECJ2 cell line in pigs from intestinal epithelial cells has been used to investigate e.g., intestinal transportation while the chicken SL-29 cell line, which is derived from embryonic untransformed fibroblast cells, has been used to provide insight into immune function. It is important to molecular characterize these cell lines to gain insight into possible molecular aberrations, and their effects on e.g., gene expression. Gene expression is regulated by interaction between enhancers, promoters, insulators, epigenetic marks and chromatin binding factors, often referred to as the functional genome. The aims of this research were 2-fold. First, a molecular and epigenetic characterization of the pig IPEC-J2 and chicken SL-29 cell lines and second providing a cell-line reference for the FAANG community. These aims were obtained through whole-genome sequencing (WGS), gene expression (RNA-seq), DNA methylation (WGBS and RRBS), chromatin accessibility (ATAC-seq) and ChIP-seq of 4 histone marks (H3K4me1, H3K4me3, H3K27ac, H3K27me3) and an insulator (CTCF). In both cell lines heteroploidy (aneuploidy) of various chromosomes was observed from WGS analysis. Furthermore, gene expression analyses showed higher gene expression for genes located on chromosomes with aneuploidy, in comparison to diploid chromosomes. Investigation into the chromatin accessibility identified promoters, enhancers and gene silencing regions. Using motif analyses of the identified promoters and enhancers, we identified transcription factors (TF) regulating possible corresponding genes. Insights into regulatory complexity of gene expression and chromatin accessibility was achieved by integrating histone marks and DNA methylation with expression values. These analyses showed that histone marks H3K4me3, H3K27ac and hypomethylation have a positive correlation to gene expression. This investigation into pig and chicken cell lines gives insight into the genome structure, and possibilities of further research using these cell lines as a reference within the FAANG community.

Key Words: Functional Annotation of Animal Genomes (FAANG), epigenomics, cell line, pig, chicken

W223 Genome-wide analysis of transcription start sites across *Bos indicus* tissues. M. Forutan*, E. Ross, L. Nguyen, and B. Hayes, *Queensland Alliance for Agriculture and Food Innovation, Brisbane, QLD, Australia.*

Transcriptional regulation is one of the most important features of gene expression. To figure out the exact mechanism of a gene, it is important to identify and evaluate its transcriptional start sites (TSSs), which are located at the beginning of the sequence. Transcription start sites act as an integration region for a wide range of molecular signals to control transcription and finally, expression levels. Previous studies have confirmed that most genes have an array of close TSSs instead of the expected single TSS, and the transcription of a gene may start from one of several TSSs, a phenomenon known as alternative transcriptional initiation (ATI). Cap analysis of gene expression (CAGE) has developed as one of the main high-throughput assays for studying TSSs and their expression. Sequencing short reads (or tags) from the 5′ end of full-length cDNA allows TSSs to be mapped and their expressions to be analyzed. To assess TSS expression and distribution across bovine tissues, CAGE-Seq (CAGE followed by sequencing) was performed on 9 tissues at adult stages, including liver, lung, kidney, thyroid, spleen, muscle, uterus, ovary, blood in *indicus* subspecies. The total number of TSSs expressed (in promoter) in 9 tissues from a single *Bos indicus* adult female was 48,473 (16,676). Interestingly, a noticeable proportion of the genes (24%) had divergent consensus TSS clusters, i.e., they had at least one TSS cluster not expressed in one or some tissues. When tissues were clustered based on their correlation between Shannon indexes of TSS diversity across adult tissues, they grouped mainly together into clusters reflecting their function. Our results highlight the high potential for differential transcriptional regulation across tissues.

Key Words: cap analysis of gene expression (CAGE), functional annotation, promoter, tissue-specific, transcription start site

AG2PI: Agricultural Genome to Phenome Initiative (AG2PI): Introduction and Community Building and Listening Workshop

W224 Agricultural Genome to Phenome Initiative: Introduction and community building and listening workshop. C. K. Tuggle*[1], J. Clarke[2], J. C. M. Dekkers[1], C. Lawrence-Dill[1], E. Lyons[3], B. Murdoch[4], P. S. Schnable[1], and D. Ertl[5], [1]*Iowa State University, Ames, IA, USA,* [2]*University of Nebraska–Lincoln, Lincoln, NE, USA,* [3]*University of Arizona, Tucson, AZ, USA,* [4]*University of Idaho, Moscow, ID, USA,* [5]*Iowa Corn Growers Association, Johnston, IA, USA.*

To achieve sustainable genetic improvements of agricultural species, the expertise of a broad community of agricultural researchers must be engaged from both crop and livestock communities. This includes integrative disciplines such as biology, statistics, as well as computer, data and engineering sciences. The objective of the Agricultural Genome to Phenome Initiative (AG2PI) is to assemble and prepare a transdisciplinary community to conduct AG2P research. To accomplish this, AG2PI seeks to engage a broad and diverse researcher community through Field Days, Conferences, Training workshops, and Seed grants. In this presentation we will provide an overview of AG2PI and highlight examples and opportunities to participate in AG2PI research. Furthermore, we welcome community input and will use these comments to improve and expand AG2PI activities. AG2PI operations are funded by USDA-NIFA award 2020–70412–32615.

Key Words: Agricultural Genome to Phenome Initiative (AG2PI)

Companion Animal Genetics and Genomics Workshop

W225 ROS_Cfam_1.0: A high-quality, *de novo* assembly of a male Labrador retriever. L. Eory, W. Zhang, D. Ozdemir, E. Clark, A. Archibald, and J. Schoenebeck*, *The Roslin Institute and Royal (Dick) School of Veterinary Studies, Midlothian, UK.*

The Kennel Club recognizes 218 dog breeds which are assigned to 7 groups based on animals' form and function. Phylogenetics indicate that clades roughly reflect breeds' group assignments, as expected since many group-level breeds share common ancestries. To date, dog genome assemblies are available for the boxer (working group), great Dane (working group), German shepherd (pastoral), basenji (hound) and Australian dingo. All but the basenji assembly were produced from female dogs. Here we describe our efforts to generate a high-quality genome assembly from a male Labrador retriever, a medium-sized member of the gundog group that traces its origins to Newfoundland, Canada. The Labrador retriever is one of the most recognizable breeds of dogs worldwide. Because of their trainability and disposition, this breed is not only popular among pet owners, but as well they are commonly used as service animals. Using FALCON-Unzip, we generated 1,161 primary contigs from PacBio Sequel long-read sequences (56.5x coverage, N50 = 9.1Mb). The primary haploid genome was 2.4Gb. Bionano optical mapping (214x raw coverage) was used to assembly 75 scaffolds which included 30 chromosome-level assemblies (scaffold N50 = 77Mb). Following identification and correction of misassemblies, further scaffolding was done using 56x Hi-C data which were processed using Dovetail HiRise. The remaining scaffolds and unplaced contigs were gap-filled and polished using Illumina short-read data. The final assembly contained 376 scaffolds with a scaffold N50 of 64Mb and total length of 2.4Gb. Mitochondrial sequence was assembled and added to the primary assembly separately. Designated "ROS_Cfam_1.0," our Labrador retriever assembly and annotation are available on popular archives including NCBI and Ensembl's Rapid Release site. Future efforts to improve the utility of ROS_Cfam_1.0 are underway, including deposition of CAGE-seq and Iso-seq annotation data as well as efforts to resolve diploid content.

Key Words: *de novo* assembly, dog, genomics, reference

W226 Genome-wide association studies identify novel quantitative trait loci for canine health traits. H. J. Huson*[1], D. M. Holle[2], A. Walker[1], N. Anclade[1], and K. M. Evans[2], [1]*Department of Animal Science, Cornell University, Ithaca, NY, USA,* [2]*The Seeing Eye Inc., Morristown, NJ, USA.*

Working dogs, particularly those trained as detection, guide, search and rescue, or law enforcement, undergo rigorous health evaluations to minimize the risk of inherited disease and increase a dog's productive life. To improve our understanding of hereditary abnormalities, 37 health traits were assessed in genome-wide association studies (GWAS). German Shepherds, Labrador retrievers, Golden retrievers, and Labrador by golden retriever crosses in a guide dog program were evaluated by veterinarians before 2 years of age for dental (13), ocular (8), dermatologic (7), orthopedic (2), gastrointestinal (4), connective tissue (1), cartilaginous (1), and muscular (1) abnormalities. Nine hundred ninety-one dogs were genotyped on the Illumina Canine HD array, providing 939 dogs with 122,966 single nucleotide polymorphisms for analysis after quality control filtering on call rate, minor allele frequency, number of alleles, and identity by descent. Genomic principal component analysis was conducted to validate individual breed designation and overall population structure. A single-locus mixed model (EMMAX) using a genomic relationship matrix as a fixed effect and an additive inheritance model was used for an across-breed GWAS for each trait. Twenty-three trait GWAS produced SNPs passing false discovery rate multiple testing correction and 18 trait GWAS produced SNPs passing Bonferroni multiple testing correction (P-value <0.05). Of the traits passing one or both multiple testing corrections, 9 were dental, 7 were ocular, 1 was orthopedic, 4 were dermatologic, 1 was muscular, and 1 was cartilaginous. Pseudo-heritability estimates ranged from 0 to 0.62 across the varying traits with a mean of 0.16 and median of 0.09. In all, 62% of the health traits showed significant genetic association and are being explored further for candidate genes and considered for fine mapping. The current GWAS highlighted SNPs potentially influencing traits in multiple breeds whereas future studies will seek breed-specific associations. These findings increase our knowledge of quantitative trait loci of health traits and provide a foundation for future genetic selection programs.

Key Words: working dogs, canine, GWAS, health

W227 *ABHD5* frameshift deletion in golden retrievers with ichthyosis. S. Kiener*1,2, D. J. Wiener[3], K. Hopke[4], A. B. Diesel[4], V. Jagannathan[1], E. A. Mauldin[5], M. L. Casal[5], and T. Leeb[1,2], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Dermfocus, University of Bern, Bern, Switzerland,* [3]*Department of Veterinary Pathobiology, Texas A&M College of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA,* [4]*Department of Small Animal Clinical*

Sciences, Texas A&M College of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA, [5]*University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA, USA.*

Ichthyoses are hereditary disorders caused by defects in the formation of the outermost layer of the epidermis, the stratum corneum. So far, 5 different genetically determined breed-specific forms of canine ichthyoses have been described. An insertion-deletion (indel) variant in *PNPLA1* causes a relatively common ichthyosis in golden retrievers. In this study, we investigated golden retrievers with fine and large scales adhering to the skin surface and exfoliating into the hair coat. Histopathological examinations showed lamellar, orthokeratotic hyperkeratosis and mildly hyperplastic epidermis that led to the diagnosis of non-epidermolytic ichthyosis. Despite the clinical and histopathological similarity with the *PNPLA1*-related ichthyosis, the affected golden retrievers of our investigation were not homozygous for the *PNPLA1* indel. Combined linkage analysis and homozygosity mapping in 14 cases and 30 nonaffected family members delimited a critical interval of ~12.7 Mb on chromosome 23. Whole-genome sequencing of one affected dog revealed a single protein-changing variant within this region that was not present in 796 control genomes. The identified variant, c.1006_1019del, is a 14-bp deletion in the *ABHD5* gene, leading to a frameshift and truncating the last 14 codons p.(Asp336Serfs*6). The variant showed perfect association to the ichthyosis phenotype in a golden retriever cohort of 14 cases and 470 controls. *ABHD5* encodes an acetyltransferase required for the synthesis of phosphatidic acid, the major intermediate in membrane and storage lipid biosynthesis. In humans, variants in *ABHD5* have been described to cause Chanarin-Dorfman syndrome, a neutral lipid storage disease with ichthyosis. The perfect genotype-phenotype association together with the knowledge on the effects of *ABHD5* variants in humans strongly suggest *ABHD5*:c.1006_1019del as candidate causative genetic variant for a new form of ichthyosis in golden retrievers.

Key Words: dog, dermatology, metabolism, animal model, precision medicine

W228 A genome-wide association study of hypertrophic cardiomyopathy susceptibility in cats. J. Raffle*, J. N. Matos, D. J. Connolly, V. L. Fuentes, and A. Psifidi, *Royal Veterinary College, London, UK.*

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease in humans and cats. In humans, thousands of causative HCM mutations have been identified mainly in cardiac sarcomeric genes; however, in 40% of human cases the cause remains unknown. In cats, only 5 causative mutations have been identified both in sarcomeric (*MYBPC3*, *MYH7*) and non sarcomeric genes (*ALMS1*, *TNNT2)*. In most cat breeds and domestic shorthair (DSH) cats HCM etiopathogenesis remains unknown. Until now only candidate gene approaches have been used to identify causative variants in cats with limited success. The aim of this study was to expand the scanning for genetic variants associated with feline HCM at genome-wide level. We performed a genome-wide association study (GWAS) for HCM-susceptibility (n = 200) across 4 cat breeds (Bengal, Norwegian forest cat, Sphynx and DSH) to identify novel genomic associations with HCM. Cat phenotyping was performed by echocardiography and genotyping using the feline 60K SNP Illumina Infinium iSelect DNA array. Quality control included minor allele frequency >0.01, call rate >95% and Hardy–Weinberg equilibrium ($P > 10^{-6}$). GWAS was performed with the GEMMA algorithm using a standard univariate linear mixed model in which breed, age and sex were fitted as fixed effects and the genomic relatedness matrix among individuals fitted as a polygenic random effect. Genome-wide significance and suggestive significant levels were set at $P < 0.05$ and one false discovery per genome scan, respectively, and a Bonferroni correction for multiple testing was applied. GWAS revealed the presence of 7 markers located on chromosomes A1, B2, B3, B4 and D4 associated with HCM at the suggestive significance level. One such marker located close to *CITE2* gene has been previously associated with cardiac disease in humans. The data highlighted numerous genomic associations with feline HCM, indicating that the genomic architecture of the disease is complex polygenic with similarities to human HCM. Future plans include validation in another cat population alongside whole-genome sequencing analysis (n = 16) to identify candidate causative variants for feline HCM.

Key Words: cat, hypertrophic cardiomyopathy (HCM), SNP marker, GWAS

W229 Canine Y chromosome features uncovered by long-read sequencing assembly and male dog phylogeny inferred from Y haplotype. W. Zhang*, L. Eory, E. Clark, A. Archibald, and J. Schoenebeck, *Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK.*

The Y chromosome is the most rapidly evolving nuclear chromosome, and its gene content and structural complexity varies across mammals, which bears a unique record of evolutionary history. The male-specific haplotype characterizes and calibrates Y-chromosome phylogeny, and the acquisition, loss, and amplification of male-specific genes have an impact on male biology such as development and fertility. This study assembled a male Labrador Retriever dog Y chromosome using PacBio, Bionano, Dovetail HiC, and Illumina sequencing data. Our assembly resolves the 5 Kb pseudoautosomal boundary region (PAB) and presents a nearly full-length male-specific Y region (MSY) compared with the previous study [1]. As a result, a total of 21 male-specific genes are annotated, 5 of which are multiple copies, and another 16 genes are single-copy genes. The self-similarity plot shows a 1.5 Mb length ampliconic region, which contains 7 single-copy genes. For the PAB, we observed the X-Y similarity decreased to ~80%, down from 95% similarity observed in the pseudoautosomal region (PAR). By comparing X-PAB and Y-PAB, we found 2 Y-specific SINE transposable element insertions that are conserved in the Canina subtribe. These 2 SINE elements are inserted in the first exon and first intron of the claudin 34 (*CLDN34*) gene. The SINE insertions in Y-*CLDN34* are predicted to cause loss of protein-coding regions. Using short-read resequencing data, we built a canine phylogeny based on a Y-chromosome haplotype. A series of single nucleotide variants (SNV) filtering steps were conducted, resulting in a total of 1,092 SNVs in 422 male samples. Our phylogenetic tree shows, with the exception of Arctic breeds and dogs of African origin, that modern dogs have a very close paternal ancestry. Moreover, 4 gray wolf samples are carriers of dog haplogroups, indicating ancient admixture events between dogs and wolves, or that the Y chromosome haplotype predated that split between wolves and dogs. The observation of wolves' placement on dogs' clades is consistent with the previous study [2]. Our study highlights the dynamic nature of the Y chromosome and provides a reference sequence for an improved understanding of *Canis* evolution and male fertility. [1] Li, Gang, et al. *Genome Research* 23.9 (2013): 1486–1495. [2] Oetjens, Matthew T., et al. BMC genomics 19.1 (2018): 1–9.

Key Words: modern dogs, Y chromosome, pseudoautosomal boundary, phylogeny

W230 More than a moggy: A population genetics analysis of the United Kingdom's non-pedigree cats. J. Irving McGrath*[1], W. Zhang[1], R. Hollar[2], A. Collings[3], R. Powell[4], R. Foale[5], N. Thurley[5], R. Campbell[5], R. Mellanby[1], D. Gunn Moore[1], J. Brockman[2], and J. Schoenebeck[2], [1]*Royal (Dick) School of Veterinary Studies and Roslin Institute, University of Edinburgh, Easter Bush Veterinary Campus, Midlothian, UK,* [2]*Hill's Pet Nutrition Centre, Topeka, KS, USA,* [3]*Idexx Laboratories, Wetherby, UK,* [4]*DragonVet Consulting Ltd., Hertfordshire, UK,* [5]*Dick White Referrals, Station Farm, Six Mile Bottom, Cambs., UK.*

The domestic cat is one of the most popular pets in the world. It is estimated that 89–92% of domestic cats in the UK are non-pedigree Domestic shorthair, Domestic longhair or Domestic semi-longhair cats. Despite their popularity, little is known of the UK cats' population struc-

ture and breeding dynamics. Using a custom designed single nucleotide variant (SNV) array, this study investigated the population genetics of 1,344 UK cats. Principal component analysis (PCA) and fastSTRUCTURE analysis verified that the UK's non-pedigree cats are random bred, rather than admixed, mix breed or crossbred. In contrast to pedigree cats, the linkage disequilibrium of random bred cats was least extensive and decayed rapidly. Autozygosity analysis showed the majority of random-bred cats had proportionally less of their genome in homozygosity by descent (HBD) segments compared with pedigree cats, and that these segments were older. Together, these findings suggest that these cats should be considered as breeds of their own and warrant the scientific focus traditionally reserved for recognized breeds. Unexpectedly, 19% of random bred cat genomes displayed a higher proportion of HBD segments associated with more recent inbreeding events. Therefore, while random bred cats as a whole are genetically diverse, they are not impervious to inbreeding and its health risks.

Key Words: cats and related species, population genomics, genotyping, population structure, breed/population identification

W231 New variant in *ADAMTS2* segregates with recessively inherited Ehlers-Danlos syndrome in a cat family. R. Simon*[1], S. Kiener[2,3], N. Thom[4], L. Schäfer[4], M. Roy[1], E. K. Schlohsarczyk[5], C. Herden[5], T. Leeb[2,3], and G. Lühken[1], [1]*Institute of Animal Breeding and Genetics, Justus Liebig University, Giessen, Germany,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [3]*Dermfocus, University of Bern, Bern, Switzerland,* [4]*Clinic for Small Animals, Justus Liebig University, Giessen, Germany,* [5]*Institute of Veterinary Pathology, Justus Liebig University, Giessen, Germany.*

The Ehlers-Danlos syndrome, known in humans as well as in different animal species, belongs to the group of heritable connective tissue disorders. Skin hyperextensibility, abnormal scarring, and poor wound healing are typical symptoms. Pathogenic variants in 20 different genes have been reported in different types of human EDS including genes associated with the synthesis of collagen. In domestic cats, only one gene, *COL5A1*, was reported to be involved in an autosomal-dominant form of EDS. As index case, a kitten with skin lesions suspicious for EDS was discovered in a cohort of European domestic shorthair cats free roaming on a farm. The kitten was found dead next to 2 unaffected littermates. Later, 3 additional affected kittens were observed in 2 subsequent litters. All 3 litters were probably sired by one unaffected tomcat. Crusted areas on the face were observed as a result of wound healing, as well as large areas of deep traumatic ulceration at friction sites. Their skin was thin and showed excessive tearability. A diaphragmatic hernia and a rectal prolapse were present in the first affected kitten. We sequenced the whole genome of the first affected kitten and mapped it against the FelCat9.0 reference genome. Filtering for homozygous variants absent in 48 other cats from diverse breeds revealed a frameshift variant in the *ADAMTS2* gene encoding a protease required for the correct processing of procollagen. Loss-of-function variants in *ADAMTS2* cause autosomal-recessively inherited EDS (dermatosparaxis or type VII C) in humans and dogs. The identified variant is predicted to truncate ~80% of the wildtype. PCR primers were designed for genotyping all available family members by Sanger sequencing. The mutant allele was absent or heterozygous in all nonaffected cats. The presumable tomcat and the 3 mothers were found to be heterozygous, which confirms the expected autosomal recessive inheritance of this EDS form. The 3 other affected kittens were homozygous for the mutant allele demonstrating perfect co-segregation of the phenotype with the genotype in the family. Due to the severity of the skin lesions, 2 affected kittens had to be euthanized shortly after the examination.

Key Words: cats and related species, genome sequencing, animal health, genetic disorder

Plenary IV

W232 Applying functional knowledge to accelerate animal genetic improvement. A. J. Chamberlain*[1], R. Xiang[1,2], I. M. MacLeod[1], M. Khansefid[1], C. P. Prowse-Wilkins[2], M. E. Goddard[1,2], and H. D. Daetwyler[1,3], [1]*Agriculture Victoria, Agribio, Centre for AgriBiosciences, Bundoora, Victoria, Australia,* [2]*Faculty of Veterinary and Agricultural Science, The University of Melbourne, Parkville, Victoria, Australia,* [3]*School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

Functional variants are more likely to be causal or closely linked to causal mutations for complex traits, therefore their SNP effects for genomic prediction are more robust across breeds and generations. We utilized various evolutionary and regulatory data, including expression and metabolite QTL, histone modification marks, selection signatures and variant annotations, to determine the contribution variants in these classes have on the genetic variance of 34 traits important to dairy cattle. Seventeen million variants from the 1000 bull genomes project were ranked according to their contribution to these traits and 300K informative variants selected. Bayesian genome mapping in multiple traits, reduced the set to 80K informative markers. After checking designability, 40K were included in a 50K custom panel. Genomic prediction accuracy using this custom 50K panel was compared with the standard bovine 50K as well as higher density panels for milk production traits in Holstein, Jersey, Reds and Crossbred animals. The customized panel improved or matched the prediction accuracy of other panels, including higher density panels with both GBLUP and Bayesian models, with the best results achieved in crossbred animals. The above prioritization utilized a limited number of functional sites identified via Chromatin immunoprecipitation (ChIP) sequencing, yet found that variants in ChIP-seq peaks had a high per variant heritability. ChIP sequencing for 4 histone modifications (H3K4Me1, H3K4Me3, H3K27ac and H3K27Me3) and one transcription factor (CTCF) in 6 tissues (heart, kidney, liver, lung, mammary and spleen) has since been performed on 2-3 lactating dairy cows. Peaks and chromatin states were called and then tested for enrichment of putative causal variants. Eleven causal variant datasets, including milk production trait QTL and expression QTL were tested. Peaks, as well as peaks correlated with gene expression and functional chromatin states were all found to have enrichment of putative causal variants, in particular milk production trait QTL. This ChIP-seq data as well as expression QTL have been used to again prioritize variants important to dairy traits.

Key Words: functional variants, genomic prediction

W233 Comparative analysis of single nucleotide polymorphisms and microsatellite markers for parentage verification and sire/dam allocation within equine Thoroughbred breed. P. Flynn*[1,2], R. Morrin-O'Donnell[1], R. Weld[1], J. Carlsson[2], P. Siddavatam[3], and K. Reddy[3], [1]*Weatherbys Scientific, Naas, Ireland,* [2]*University College Dublin, School of Biology and Environmental Science, Belfield, Dublin, Ireland,* [3]*Thermo Fisher Scientific, Austin, TX, USA.*

Short tandem repeats (STR), also known as microsatellite molecular markers, are currently used for parentage verification within equine. Transitioning from STR to single nucleotide polymorphism (SNP) molecular markers to perform equine parentage verification is becoming an ever more feasible prospect and key areas that merit exploration are ensuring maintenance of test accuracy and suitability of current genotyping technologies to support such a transition. We established a targeted equine genotyping by sequencing (EQ-GBS) panel of 562 SNPs, consisting of SNPs currently undergoing feasibility testing by International Society of Animal Genetics (ISAG) and an additional SNP panel to perform sire/dam allocation. A sample group of 309 thoroughbreds, inclusive of 55 previously parentage verified offspring/sire/dam cases, underwent SNP genotyping and availability of historic STR profiles allowed for comparative analysis of parentage verification and allocation accuracy between both SNP and STR panels. An average sample call rate of 97.2% was observed for EQ-GBS SNP panel when using medium grade DNA, an average minor allele frequency of 0.38 demonstrated SNP panel informativeness and a subset panel of 516 SNPs was identified as "Optimum Performing." Simulated offspring/sire/dam parentage verification resulted in positive separation values and no false positive cases (i.e., expected to fail parentage, but pass) for ISAG pilot and EQ-GBS SNP panels, in comparison to a zero-separation value and 28 false positive cases for STR panels. This study has proven GBS as a proficient technology to generate low-density SNP profiles for equines and provides insight into the value of using SNPs within equine parentage verification in comparison to STRs.

Key Words: equine, parentage, microsatellites, single nucleotide polymorphism, genotyping by sequencing

W234 Evaluation of the ISAG equine parentage testing SNP panel across multiple breeds. R. Bellone*[1,2], B. Till[1], A. Kallenberg[1], F. Avila[1], and R. Grahn[1], [1]*Veterinary Genetics Laboratory, University of California–Davis, Davis, CA, USA,* [2]*Department of Population Health and Reproduction, University of California–Davis, Davis, CA, USA.*

Equine parentage testing using microsatellite (mSAT) markers has been utilized since the 1990s to ensure the integrity of pedigrees. Advances in technologies and discovery of genetic variants for marker assisted selection has made the transition to SNPs for parentage analysis attractive. The ISAG equine parentage testing SNP panel, comprised initially of 154 markers, was developed based on 2 studies: one in the Thoroughbred (SNP 53 JPN System, n = 95), and the other using an across-breed approach (101 SNPs, n = 388 across 35 breeds). Analysis of the combined marker set has only included 3 ISAG comparison tests, with no further analysis reported. These ISAG comparison tests utilizing only 20 horses identified 7 problematic markers that were subsequently removed from the panel. It is not yet clear how accurately the current ISAG equine SNP panel of 147 markers will resolve parentage questions across breeds as compared with the established mSAT testing. The aim of this study is to evaluate the SNP panel in a relatively large sample set to (1) determine allele frequencies across breeds to identify potentially uninformative markers; and)2) determine the efficiency of this panel in parentage exclusion across multiple breeds in comparison to mSAT genotypes. We utilized the Ion GeneStudio S5 System to genotype 1,572 randomly selected samples across 45 breeds for 145 of the 147 SNPs. Ten markers failed the call rate threshold of 90% and were excluded from further analysis. The minor allele frequency (MAF) ranged from 0.0054 to 0.49 across all breeds. Evaluation of 6 breeds with more than 25 individuals (29 to 669 per breed) provided evidence that 20 markers are likely not informative for parentage (MAF < 0.3 in all 6 breeds). Furthermore, 12 of those markers had MAF <0.05 in at least 2 of the 6 breeds. Evaluation of this SNP panel for parentage exclusion in closely and distantly related individuals to samples included in this study and comparison to previously collected mSAT data is ongoing. These data support the need for additional markers and future studies will be aimed at identifying and evaluating more informative markers using a large cohort of horses from diverse breeds.

Key Words: horse, SNP, parentage

W235 Pioneer 100 Horse Health Project: A deep phenotypic and multiomic resource. C. Donnelly*[1], N. Cohen[2], G. Mulcahy[3], J. Manfredi[4], S. Valberg[5], E. Oberhaus[6], J. Morgan[7], E. Graham-Williams[8], K. Knickelbein[8], R. Bellone[1,9], N. Price[10,11], and C. Finno[1], [1]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,* [2]*Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA,* [3]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland,* [4]*Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA,* [5]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA,* [6]*School of Animal Sciences, Louisiana State University, Baton Rouge, LA, USA,* [7]*Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,* [8]*Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,* [9]*Veterinary Genetics Laboratory, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA,* [10]*Institute for Systems Biology, Seattle, WA, USA,* [11]*Onegevity Health, New York, NY, USA.*

Precision medicine is the future for the diagnosis, treatment and prevention of disease in animals as it is in humans. By leveraging extensive phenotypic data with genomic and multi-omic data, it has the potential to tailor diagnoses and therapies to individuals by embracing personal variation. In this way, it also allows for the characterization of health traits in an individual at detailed resolution. The Pioneer 100 Horse Health Project has been established as the first longitudinal equine precision medicine study. The aim of this project is to develop dense dynamic data clouds for individual horses maintained as a single population. Individual data clouds include deep phenotype, multiomic analyses and environmental data. A total of 108 horses (60 mares, 45 geldings and 3 stallions), aged 3–18 yr (median = 11 yr) and including a diverse set of breeds form the Horse Pioneer 100 cohort. High coverage whole-genome sequencing has been completed for all animals. Extensive prospective and retrospective phenotype data have been collected for each horse. Prospective phenotype data, paralleled with sample collection for high-throughput omic analysis for temporal data sets has been collected over a period of 2 years. Data set acquisition includes the plasma metabolome, proteome, fecal microbiota and conserved methylome. In addition to these analyzed samples the project has generated an extensive biorepository for use in future analyses. Data usage will be 2-fold: (1) multi-correlational analysis of the existing phenotype and multiomic data sets to precisely define equine health traits in the Pioneer 100 cohort and (2) an opportunity for researchers to evaluate additional specific phenotypes within this highly characterized equine cohort. Summarily, the product of this endeavor is to provide an enhanced phenotype resource for the equine genetics and genomics community.

Key Words: precision medicine, equine, systems biology

W236 Invited Workshop Presentation: Monitoring of fish and pathogens around aquaculture facilities through analysis of environmental DNA (eDNA) using an environmental sample processor. M. W. Jacobsen*[1], B. K. Hansen[1], A. Krolicka[2], D. Strand[2], T. Vrålstad[3], T. Baussant[3], and E. E. Nielsen[1], [1]*Danish Technical University, Section for Marine Living Resources, Silkeborg, Denmark,* [2]*Norwegian Research Centre AS (NORCE), Stavanger, Norway,* [3]*Norwegian Veterinary Institute, Oslo, Norway.*

Globally, disease accounts for large losses in aquaculture production due to mortality and economical loss associated with treatment. Pathogens may also spread to wild populations, either directly from farms or through escaped individuals, thereby posing significant problems for the infected populations. Despite these issues, preventing disease is difficult. This is due to a limited understanding of links between outbreaks and environmental factors, and that many traditional disease-screening approaches are limited to identification at late, highly infected stages preventing early treatment. One potential new screening method is through analysis of so-called environmental DNA (eDNA). Environmental DNA is defined as the genetic material, sampled directly from the environment, outside the living hosts (e.g., from faces, urine, scales). It can easily be collected via water filtration and DNA extraction. Due to fast degradation of DNA in aquatic environments, eDNA is a good proxy for the present living biodiversity, which can be analyzed using specific single species quantitative PCR (qPCR) methods, or metabarcoding approaches aimed at analyzing larger taxonomic groups. Here, we present the application of eDNA for detection and quantification of pathogens associated with fish farming, and for detection of escaped farmed individuals to the wild. The focus is on ongoing work using a so-called second-generation environmental sample processor (ESP) for analyzing eDNA around marine farms with Atlantic salmon or rainbow trout. The ESP is an autonomous instrument, which can collect, extract and analyze eDNA samples in situ, using qPCR, and store filters with DNA for analysis after deployment. The instrument is deployed at a specific site and operates for several months. While deployed, it is controlled by, and sends back, data to scientists on land, thus allowing results to be obtained in real time. These unique features make the ESP a good candidate for an early warning system for pathogen surveillance around fish farms, as well as a system for detection of escaped individuals from potentially invasive species. Analysis of the preserved filters using metabarcoding methods may further allow investigations of the impact of the farms to the full surrounding ecosystem.

W237 Pikeperch *Sander lucioperca* genome data: Basis for smart farming in aquaculture. T. Goldammer*[1,2], M. Verleih[1], R. M. Brunner[1], A. Rebl[1], J. A. Nguinkal[1], L. de los Ríos-Pérez[1], N. Schäfer[1], M. Stüeken[3], F. Swirplies[3], and D. Wittenburg[1], [1]*Fish Genetics Unit, Institute of Genome Biology and Statistics in Genomics Unit, Institute of Genetics and Biometry, Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*Molecular Biology and Fish Genetics, Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany,* [3]*Research Centre for Agriculture and Fisheries, State Research Center of Agriculture and Fisheries M-V, Rostock, Germany.*

The pikeperch (*Sander lucioperca* Linnaeus, 1758) is one of the new promising fish species for aquaculture in Europe. The economic viability of pikeperch aquaculture and the successful adaptation of pikeperch to different farming systems are partly determined by breeding programs. The knowledge of genetically determined breeding parameters and of family structures is an essential prerequisite for their success. From the perspective of genome biology, elucidation of the pikeperch genome, genes and their function and joint activity provide essential metadata for smart breeding approaches. This also includes the elucidation of genotypes and their correlation to phenotype as well as the identification of gene-based indicators for monitoring health, growth and well-being, etc. Our research conducted for this purpose resulted in the first near-complete elucidation of the pikeperch genome with a length of about 900 million nucleotides distributed over 24 chromosomes, the representation of 26,000 protein and non-protein encoding genes, the development of a genetic linkage map based on about one million nucleotide polymorphisms in the genome, and the development of gene-based BioChips to assess fish welfare under various conditions in pikeperch aquaculture (Nguinkal et al., Genes (Basel) 10, 2019; Swirplies et al., Aquaculture 501, 2019; de los Ríos-Pérez et al., Sci Rep 10, 2020; Schäfer et al., Fish Physiol Biochem 47, 2021). This knowledge is freely available and we will use it to contribute to the optimization of pikeperch aquaculture in Germany.

Key Words: pikeperch, genome, SNP-map, gene map, welfare markers

W238 A blue mussel chromosome-scale assembly and genomic resources for aquaculture, marine ecology, and evolution. T. Hori*[1,2], [1]*PEI Marine Sciences Organization, Charlottetown, PE, Canada,* [2]*Atlantic Aqua Farms, Charlottetown, PE, Canada.*

The blue mussel is commonly described as the *Mytilus* species complex, encompassing at least 3 putative species: *M. edulis*, *M. galloprovincialis* and *M. trossolus*. These 3 species occur on both sides of the Atlantic and hybridize in nature, and both *M. edulis* and *M. galloprovincialis* are important aquaculture species. They are also invasive species in many parts of the world. This project aimed at assembling a high-quality genome for *M. edulis* and develop tools that can be used in breeding, molecular ecology and evolution to address questions of both commercial and environmental perspectives. We used a combination of PacBio sequencing and Dovetail's Omni-C technology to generate an assembly with 14 long scaffolds containing 94% of the predicted length of the *M. edulis* genome (1.6 out of 1.6 Gb). Assembly statistics were total length 1.65 Gb, N50 = 116 Mb, L50 = 7 and, L90 = 13. BUSCO analysis showed 90.59% complete eukaryote BUSCOs identified. AB-*Initio* annotation using RNA-seq from mantle, gills, muscle and foot predicted 41,319 genes. Using GBS and shotgun sequencing, we sequenced 3 North American populations of *Mytilus* to characterize single nucleotide as well as structural variance. Population genetics analysis data will also be presented.

Key Words: blue mussel, aquaculture, genome assembly, SNPs, *Mytilus*

W239 An application of the MedFish SNP array: Determining population structure and genetic variability of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*). M. Saura*[1], A. Fernández[1], J. Fernández[1], R. Peiro-Pastor[1], C. Peñaloza[2], L. Bargelloni[3], T. Manousaki[4], C. Tsigenopoulos[4], and B. Villanueva[1], [1]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, CSIC), Madrid, Spain,* [2]*The Roslin Institute, University of Edinburgh, Midlothian, Scotland, UK,* [3]*University of Padova, Padova, Italy,* [4]*Hellenic Centre for Marine Research (HCMR), Heraklion, Crete, Greece.*

Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) are the most important marine fish species farmed in the Mediterranean. Understanding population structure and genetic diversity within and between wild and farmed populations is of paramount importance to develop optimal strategies for the conservation of wild populations and to achieve sustainable aquaculture production. In this study we used a new 60K SNP array recently developed for both species to determine patterns of population structure and genetic variability of seabream and seabass populations. A total of 50 populations from both species (23 wild and 27 farmed) were genotyped with the SNP array. Population structure was assessed through principal compo-

nent, phylogenetic and clustering analyses and F_{ST} fixation index. Genetic variability was assessed through the effective population size (N_e), estimated from linkage disequilibrium. Clustering methods revealed a clear differentiation between wild and farmed populations. Despite the little differentiation among wild populations, Atlantic/Mediterranean (seabream) and West/East Mediterranean (both species) patterns were detected. In general, N_e was large (>1,000) for wild and small (<100) for farmed populations of both species, with some exceptions. The low N_e estimated for some farmed populations highlight the need of applying measures to control the loss of genetic variability. The differentiation between wild and farmed populations suggests that special care must be taken to avoid escapees that could have undesirable genetic effects on native populations. To our knowledge, this is the first time that a population genetic analysis in these species has been carried out with such a high number of SNP markers.

Key Words: admixture, aquaculture, effective population size, population genomics, single nucleotide polymorphism (SNP)

W240 Signatures of selection and genomic diversity of muskellunge (*Esox masquinongy*) from 2 populations in North America. J. Chinchilla-Vargas*[1], J. R. Meerbeek[2], M. F. Rothschild[1], and F. Bertolini[3], [1]*Iowa State University, Ames, IA, USA,* [2]*Iowa Department of Natural Resources, Spirit Lake Fish Hatchery, Spirit Lake, IA, USA,* [3]*National Institute of Aquatic Resources, Technical University of Denmark, Lyngby, Denmark.*

Muskellunge (*Esox masquinongy*) is the largest and most prized game fish for anglers in North America. Despite its popularity, little is known about the species' genetic diversity in Iowa's propagation program. We used WGS from 12 brooding individuals from Iowa and publicly available RAD-seq of 625 individuals from Canada to study the genetic differences between populations, analyze signatures of selection and evaluate the levels of genetic diversity in both populations. Given that there is no reference genome available for muskellunge, reads were aligned to the genome of Pike (*Esox lucius*), a closely related species. Variant calling produced 7,886,471 biallelic variants for the Iowa population and 16,867 high-quality SNPs that overlap with the Canadian samples. The Ti/Tv values were 1.09 and 1.29 for samples from Iowa and Canada, respectively. PCA and Admixture analyses showed large genetic differences between the Canadian and Iowa populations. Window-based pooled heterozygosity found 6 highly heterozygous windows containing 244 genes in the Iowa population and Fst comparing the Iowa and Canadian populations found 14 windows with Fst values larger than 0.9 containing 641 genes. One enriched GO term (sensory perception of pain) was found through pooled heterozygosity analyzes. Although not significant, several enriched GO terms associated with growth and development were found through Fst analyses. Inbreeding calculated as FROH was 0.03 on average for the Iowa population and 0.32 on average for the Canadian samples. FROH results point toward Canadian inbreeding rate being higher than that of the Iowa population, presumably due to isolation of its subpopulations. This study was the first to document that brood stock muskellunge from Iowa showed marked genetic differences with the Canadian population. Additionally, despite genetic differentiation based on sex being observed, no major locus has been detected. Inbreeding does not seem to be an immediate concern for muskellunge in Iowa, but apparent isolation of subpopulations has caused levels of homozygosity to increase in the Canadian muskellunge population. Finally, these results prove the validity of using genomes of closely related species to perform genomic analyses when no reference genome assembly is available.

Key Words: muskellunge, fisheries, population genomics, sport fishing

W241 Influence of estimated breeding value for growth trait on spawning quality in gilthead seabream (*Sparus aurata*). C. Pérez-García*[1], Á. Lorenzo-Felipe[1], S. Ferosekhan[1], S. Leon-Bernabeu[1,2], M. Izquierdo[1], R. Ginés[1], J. M. Afonso[1], H. S. Shin[1], and M. J. Zamorano[1], [1]*Universidad de Las Palmas de Gran Canaria (ULPGC), Instituto Universitario de Acuicultura Sostenible y Ecosistemas Marinos (IU-ECOAQUA), Grupo de Investigación en Acuicultura (GIA), Telde, Spain,* [2]*Quanaria, Prolongación Bentejui, San Bartolomé de Tirajana, Las Palmas, Spain.*

Genetic factors have been poorly studied (Lorenzo-Felipe et al., 2021). The aim of this study is to study the spawning quality between different genetic line in terms of growth (high and low growth) in gilthead seabream. Broodstock were selected from the third generation of the PROGENSA Project (National Breeding Program of Spain). Two groups of broodstock were selected based on their EBV growth (high and low growth). The floating eggs were collected 6 d per week at 09:00 h from each mass spawning tank and egg number was counted under binocular stereoscope to estimate the oocyte yield, viable eggs, viability rate, and number of alive larvae according to Lorenzo-Felipe et al. (2021). The data were checked for normality and homocedasticity. For all traits, data where grouped in 12 fortnights throughout the spawning season: In this study, only where consider from 4th to 12th fortnights. When a normal distribution and/or homogeneity of variance was not achieved, data were subjected to the Kruskal–Wallis non-parametric test (Zar, 1984). In the present study, for oocyte yield, viable eggs, and viability rate, HG broodstock showed significantly lower values than LG broodstock in 4th and 6th fortnights. In the case of number of alive larvae, LG broodstock showed significant higher values than HG broodstock in 6th fortnight. These results are in concordance with Lorenzo-Felipe et al. (2021) who found that genetic factors are more determinant in the middle of the spawning season. On the other hand, the higher quality of LG broodstock than HG broodstock is related with genetic correlation between growth and deformity traits, previously described by Lee-Montero et al. (2015). Furthermore, the significant difference reported in oocyte yield, viable eggs and number of alive larvae, is in agreement with the results of Fernández-Palacios et al. (1995) and Lorenzo-Felipe et al. (2021) who proposed that oocyte yield and viable eggs explained the majority of variation of the spawning quality.

Key Words: fish, management, animal breeding

W242 Resistance of common carp to Cyprinid herpes virus-3: Individual survival is more affected by different genomic loci than family percent survival. M. Amir[1], J. Lighten[2], and L. David*[1], [1]*The Hebrew University of Jerusalem, Rehovot, Israel,* [2]*University of Exeter, Devon, UK.*

Common carp (*Cyprinus carpio*) is a major aquaculture species in both amount and distribution of production. Infectious diseases are damaging aquaculture significantly, impeding the further development of this sector. For common carp, widespread outbreaks of a disease caused by Cyprinid herpes virus-3 (CyHV-3) have been damaging production significantly. We have been breeding for CyHV-3 resistant strains and using our stocks to study disease resistance genetics. We and others reported that CyHV-3 resistance is a polygenic heritable trait and so far, only a few quantitative trait loci (QTLs) for CyHV-3 resistance were identified. QTLs were so far identified by comparing between fish that died and survived a disease challenge. Thus, these QTLs affect individual survival. Yet, in our hands, selecting fish as parents based only on if they survived a challenge (within-families variation) was inefficient in improving progeny resistance compared with selecting survived fish from families with higher family % survival (between-families variation). In this study, we compared genetic bases between individual survival and family % survival. We compared genotype frequencies of 57,000 SNPs, once between dead and survived individuals (individual survival), regardless of the family to which individuals belonged, and second between family % survival of individuals, regardless of whether individuals survived a challenge or died. We identified 4 QTLs for individual survival and 7 for family % survival. We validated QTLs using additional samples from independent individuals with various values of family % survival. In all QTLs, we

found genes related to immune system function, consistent with the transcriptomic differences between resistant and susceptible fish in response to infection that we previously published. Importantly, we found no overlap between QTLs affecting individual's survival and family % survival. Thus, our results enhance the understanding of polygenic disease resistance in fish and support the possibility that, at least in part, different genes affect individual survival and family % survival, both of which can help improving disease resistance.

Key Words: Koi herpes virus, disease resistance, fish immunity, aquaculture, genetic variation

W243 Omics study for viral hemorrhagic septicemia virus (VHSV) resistance in *Paralichthys olivaceus*. J. Shin*[1], S. H. Lee[1], W. J. Kim[2], J.-W. Park[3], D.-I. Lee[3], H. S. Jung[3], and J. Kim[3], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea,* [2]*East Sea Fisheries Research Institute, National Institute of Fisheries Science, Gangneung, Korea,* [3]*Fish Genetics and Breeding Research Center, National Institute of Fisheries Science, Geoje, Korea.*

Paralichthys olivaceus is one of the most cultivated fish in Korea. However, viral diseases in fish farms raised at high density are strongly contagious and have high mortality rate, which causes a large economic loss in the fishery industry. Viral hemorrhagic septicemia virus (VHSV) is well known as the most serious viral disease in fish. Therefore, selective breeding program would be useful to select fishes which have resistance in viral disease by using omics data. In this study, Omics analysis (GWAS, eQTL, and RNA-seq) were performed using 125 flounders' NGS data to identify genetic variants associated with VHSV resistance in *Paralichthys olivaceus*. Genome-wide association study (GWAS) and RNA-seq analysis were performed to discover VHSV resistance related genomic loci and differentially expressed transcripts (DETs). Finally, to integrate these 2 studies (GWAS and RNA-seq) eQTL analysis was performed to investigate genomics networking between genetic variants from noncoding region, which might affect gene expression, and the expression level of DETs that we have found in RNA-seq. As a result, loci on chromosome 23 was detected to be significant in GWAS. And then, 453 DETs are discovered in RNA-seq. Among them, 21 DETs are located on chromosome 23, and involved in various immune related pathways such as protein processing in endoplasmic reticulum, proteasome. In addition, the result of eQTL analysis shows that genotype change of 3 upstream and 2 intron variants directly affect the expression level of upregulated DETs located on chromosome 23. Also, 416 upstream, 249 intergenic, and 689 intron variants, located more than 1Mb from DETs, are discovered to secondarily affect the expression level of chromosome 23 located DETs. According to our findings, it was confirmed that loci on chromosome 23 are associated with VHSV resistance, and some variants on chromosome 23 directly affect the expression level of transcripts. In conclusion, these results would be useful for breeding VHSV resistance flounders by selecting the individuals according to the breeding value calculated by these loci information.

Key Words: *Paralichthys olivaceus*, viral hemorrhagic septicemia virus (VHSV) resistance, omics data analysis, selective breeding program, breeding value

W244 Genome editing to produce monosex and sterile fish for aquaculture. X. Lauth*[1], T. Umazume[1], S. Herbert[1], V. Williams[2], and J. Buchanan[1], [1]*Center for Aquaculture Technologies, San Diego, CA, USA,* [2]*The JEM Project, San Diego, CA, USA.*

The ability to mass-produce reproductively sterile fish for aquaculture will increase culture performance and environmental sustainability by preventing early sexual maturation and uncontrolled reproduction. While varied reproductive containment solutions have been proposed, none to date has proved fully effective, or has been widely adopted by the industry. Here, we describe strategies to generate, breed and mass-produce infertile fish. Our solutions rely on selected gene edits to create broodstock lines that only produce monosex, sterile populations of progeny. Thus, our design combines the benefit of sterility with sexually dimorphic performance traits in culture. These approaches were validated in tilapia but are transferrable to multiple species of fish. The edited broodstock can be propagated and incorporated into breeding programs. We identified and inactivated 12 genes in 2 evolutionarily conserved pathways, one governing sex differentiation and the other sex competency. We isolated null alleles of genes necessary for spermiogenesis and estrogen synthesis causing male sterility and masculinization, respectively. Double edited combinations for these genes produced all-male sterile populations. Likewise, we inactivated genes which caused females to develop atrophic ovaries arrested at a previtellogenic stage or string-like ovaries lacking oocytes. We further disrupted genes causing genetic males to sex reverse into females. Double edited combinations for these genes produced all-female, sterile populations. We successfully propagated and amplified the double edited lines via germ cell transplantation from a juvenile mutant donor into several germ cell free wildtype recipient embryos. In the resulting recipient broodstock chimera, the induced edits had no effect as the genes targeted are not expressed in germ cells. With this approach, we generated fertile broodstock that successfully mass-produced sterile, monosex populations. Finally, we tested the performance of all-male sterile tilapia in grow-out trials. Monthly average of daily body weight gain indicated that sterile tilapia grew 12% faster than their maturing siblings starting around the time of puberty.

Key Words: fish, genome editing, CRISPR-Cas9, fertility, aquaculture

W245 Thermal stress generates oxidative damage in liver and gills of red cusk-eel (*Genypterus chilensis*) juvenile. P. Dettleff*[1,2], R. Zuloaga[2], P. Gonzalez[2], M. Fuentes[2], J. Aedo[2], J. M. Estrada[3], A. Molina[2], and J. A. Valdes[2], [1]*Nucleus of Applied Research in Veterinary and Agronomic Sciences, Universidad de Las Americas, Santiago, Chile,* [2]*Laboratory of Molecular Biotechnology, Faculty of Life Sciences, Andres Bello University, Santiago, Chile,* [3]*Marine research center of Quintay, Andres Bello University, Quintay, Chile.*

The *Genypterus* genus contains native species of economic relevance with high potential for aquaculture diversification, including the red cusk-eel (*Genypterus chilensis*). Environmental factors such as temperature can generate stress in the native and commercial populations, affecting the performance of fish. The objective of this work was to study the effect of heat stress in red cusk-eel juveniles, determining the effect of this stressor in liver and gills. Red cusk-eel juveniles were collected from CIMARQ and separated into control and stress groups, with duplicated tanks. The groups were maintained at control temperature (14°C) or subjected to high-temperature stress (19°C) for 5 d. At the end of the experiment, fish were euthanized, sampling plasma, liver, and gills for cortisol level, oxidative damage evaluation (lipid peroxidation, protein carbonylation, and DNA damage), and gene expression evaluation through RNA-seq. High temperature produces a significant increase in cortisol levels in the stress group, generating oxidative damage in liver and gills. In liver, a significant increase in hepatic enzymes (ALT, AST, and AP) associated with thermal stress was observed, with a relevant modulation of gene expression with 3,354 differentially expressed genes, including enrichment terms associated with protein folding problems. Additionally, in gills, a relevant modulation of gene expression was observed in response to thermal stress, with 4,791 differentially expressed genes, with enriched terms related to unfolded proteins and DNA replication process, among others. This study showed that thermal stress can affect different key tissues generating damage and modulating the expression of relevant processes that can affect the performance of red cusk-eel, information that should be considered in a climate change world scenario. Funding: CONICYT FONDECYT Postdoctorado 3180283.

Key Words: fish, RNA-seq, aquaculture

W246 Reproductive performance of the sea urchin *Tripneustes gratilla* in first- and second-generation cultured cohorts. M. Brink-

Hull*[1,2], C. Rhode[1], M. D. Cyrus[2,3], B. M. Macey[2,3], J. du Plessis[1], K. L. Hull[1], and R. Roodt-Wilding[1], [1]*Stellenbosch University, Stellenbosch, Western Cape, South Africa,* [2]*University of Cape Town, Cape Town, Western Cape, South Africa,* [3]*Department of Forestry, Fisheries and the Environment, Cape Town, Western Cape, South Africa.*

Broadcast spawning animals, such as *Tripneustes gratilla*, display differential parental contributions in aquaculture environments, resulting in decreased genetic diversity and subsequent reduced adaptability, poor responses to artificial selection and diminished production output. This study aimed to assess genetic diversity, pedigree relationships and phenotypic performance of 2 first-generation (F_1) cultured cohorts (n = 50) established by combining eggs and sperm of wild broodstock (n = 12). Using 21 species-specific microsatellite markers, a decline in genetic diversity and differential parental contributions were observed, with a single female contributing to 70% of the first F1 cohort and a male contributing to 92% of the second F1 cohort. Various genetic- and biological factors, such as family-advantages, gonad and gamete quality, and feeding regimens used for broodstock conditioning, may drive reproductive competition. To assess this, F1 broodstock (n = 32) were conditioned on 4 diets [formulated feed with 20% *Ulva rigida*, fresh kelp (*Ecklonia maxima)*, fresh *U. rigida* and a mixture of the diets] for 4 mo, and a factorial breeding design was implemented. Larvae from broodstock fed kelp (n = 8) and a mixed diet (n = 8) survived for the duration of larval rearing (20 d) with similar growth rates throughout ($P > 0.05$; ANOVA). Three months post-settlement, parentage analysis revealed 26 of 32 possible parent pairs contributed to the F_2 generation (n = 364). No statistically significant differences between F_1 broodstock and F_2 offspring were observed for genetic diversity indices, likely due to equal parental contributions. Offspring phenotypic performance assessments revealed that body diameter was lowly heritable ($h^2 = 0.050 \pm 0.058$). However, offspring assigned to kelp-fed broodstock were significantly smaller (ave. diameter = 0.66 ± 0.07 cm) than juveniles assigned to broodstock fed a mixed diet (ave. diameter = 0.94 ± 0.10 cm) indicating that the maternal provisioning strategy of sea urchins may benefit future commercial production.

Key Words: animal breeding, aquaculture, microsatellite, parentage, quantitative genetics

Genetics of Immune Response and Disease Resistance Workshop

W247 Invited Workshop Presentation: Genome-wide association study of disease resilience traits from a natural polymicrobial disease challenge model in pigs identifies the importance of the MHC. J. Cheng[1], R. Fernando[1], H. Cheng[2], S. D. Kachman[3], K.-S. Lim[1], J. C. S. Harding[4], M. K. Dyck[5], F. Fortin[6], G. S. Plastow[5], PigGen Canada[7], and J. C. M. Dekkers*[1], [1]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [2]*Department of Animal Science, University of California, Davis, CA, USA,* [3]*Department of Statistics, University of Nebraska–Lincoln, Lincoln, NE, USA,* [4]*Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, SK, Canada,* [5]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [6]*Centre de Développement du Porc du Québec Inc., Québec City, QC, Canada,* [7]*PigGen Canada Research Consortium, Guelph, ON, Canada.*

To investigate the genetic basis of disease resilience, which is the ability of an animal to maintain performance under disease, a natural polymicrobial disease challenge model was established in which pigs are challenged in the late nursery phase by multiple pathogens. The objectives here were to identify genomic regions that are associated with disease resilience and to evaluate whether these regions are enriched for previously published quantitative trait loci (QTL), functional pathways, and physiological states based on gene set enrichment analyses. Multiple QTL were detected for all recorded performance and clinical disease traits. The MHC region was found to explain substantial genetic variance for multiple traits, including for growth rate in the challenge nursery (12.8%) and finisher (2.7%), for several clinical disease traits (up to 2.7%), and for several feeding and drinking traits (up to 4%). Further fine mapping identified 4 QTL in the MHC region for growth rate in the challenge nursery that spanned the class I, II, and III regions, with one SNP in the MHC class I region capturing the largest effects for growth rate and for multiple clinical disease traits. The MHC region was pleiotropic for growth rate in the challenge nursery and in the finisher, for treatment rate, and for mortality rate. Growth rate in the challenge nursery showed strong negative genetic correlations in the MHC region with treatment or mortality rates (−0.62 to −0.85) and a strong positive genetic correlation with growth rate in the finisher (0.79). Gene set enrichment analyses found genomic regions associated with resilience phenotypes to be enriched for previously identified disease susceptibility and immune capacity QTL, for genes that were differentially expressed following bacterial or virus infection and immune response, and for gene ontology terms related to immune and inflammatory response. The MHC and other QTL identified play an important role in host response to infectious diseases and can be incorporated in selection to improve disease resilience, in particular the identified SNP in the MHC class I region. Funding by Genome Canada, Genome Alberta, PigGen Canada, and USDA-NIFA grant #2017-67007-26144.

W248 Exploration of glucocorticoid and inflammatory responses in porcine PBMC to reveal mechanisms underlying the enhanced endotoxin sensitivity of $GR_{Ala610Val}$ pigs. E. Murani*, Z. Li, F. Hadlich, N. Trakooljul, S. Ponsuksili, and K. Wimmers, *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Glucocorticoid receptor (GR), a key component of the neuroendocrine stress response, mutually interacts with the immune system to maintain homeostasis during a pathogen challenge. We have shown previously that a gain-of-function mutation Ala610Val in the porcine GR enhances response and vulnerability to lipopolysaccharide (LPS)–induced endotoxemia. The $GR_{Ala610Val}$ mutation thus provides a unique opportunity to study the role of GR signaling in the regulation of the immune response in pigs. To this end, we treated peripheral blood mononuclear cells (PBMCs) for 2 h, separately or combined, with lipopolysaccharide (LPS), a potent activator of the inflammatory response, and dexamethasone (DEX), a synthetic glucocorticoid-based anti-inflammatory drug and selective GR agonist. Resultant transcriptional responses depending on treatment, genotype, and their interactions, were examined by mRNA sequencing. For each $GR_{Ala610Val}$ genotype 8 individual PBMC samples were used. Both, LPS and DEX, induced vast transcriptional changes. Based on their response profile, the regulated genes were allocated into 5 modules; one module each showing response to either stimulant, and 3 modules featuring antagonistic and synergistic interactions between GR and LPS responses, respectively. Our results suggest i.a. involvement of TNF, and for the first time Ca^{2+} signaling, in the LPS-induced glucocorticoid-resistance, which represents a serious complication during inflammation. On the other hand, we discovered also proinflammatory actions of GR such as induction of genes involved in LPS recognition, which helps to explain increased disease susceptibility caused by stress exposure. As expected, $GR_{Ala610Val}$ caused stronger LPS response, particularly of interferon-regulated genes. This is likely triggered by changes in the expression of LPS recognition genes associated with $GR_{Ala610Val}$. Our results provide knowledge base to

advance breeding and pharmacological interventions to improve immune responses in pigs.

Key Words: glucocorticoid receptor, inflammation, resilience, pig, transcriptome

W249 Genome-wide association study of thyroid hormone suppression following challenge with porcine reproductive and respiratory syndrome virus. A. Van Goor[1], A. Pasternak[2], M. Walugembe[3], N. Chehab[1], G. Hamonic[4], J. Dekkers[3], J. Harding*[4], and J. Lunney[1], [1]*USDA ARS BARC Animal Parasitic Diseases Laboratory, Beltsville, MD, USA,* [2]*Department of Animal Science, Purdue Univ., West Lafayette, IN, USA,* [3]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [4]*Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada.*

Porcine reproductive and respiratory syndrome virus (PRRSV2) causes respiratory disease in piglets and reproductive disease in sows. Piglet and fetal serum thyroid hormone (i.e., T3 and T4) levels decrease rapidly in response to PRRSV infection. However, the genetic control of T3/T4 homeostasis during infection is not completely understood. Our objective was to estimate genetic parameters and identify QTL for T3 and/or T4 levels of piglets and fetuses challenged with PRRSV2. Sera from 5-week old pigs (n = 1,792) at 11 d post inoculation (dpi) with PRRSV2 were assayed for T3 levels (piglet_T3). Sera from fetuses (n = 1,267) at 12 or 21 dpi from sows (n = 150) challenged with PRRSV2 in late gestation were assayed for T3 (fetal_T3) and T4 (fetal_T4) levels. Animals were genotyped using 60K or 650K SNPs panels. Heritabilities and pheno/genotypic correlations were estimated in ASREML, and genome-wide association studies were performed for each trait separately using JWAS. All 3 traits were low to moderately heritable (10–18%). Phenotypic and genotypic correlations of piglet_T3 levels to weight gain (0–42 dpi) were 0.26 ± 0.11 and 0.66 ± 0.13, respectively. Significant QTLs (n = 9) were identified for piglet_T3 on SSC3, 4, 5, 6, 7, 14, 15, and 17, collectively explaining 30% of the genetic variation (GV), with the largest identified on SSC5 explaining 15% of the GV alone. Significant QTLs (n = 3) were identified for fetal_T3 on SSC1 and SSC4, and collectively explained 10% of the GV. Significant QTLs (n = 5) were identified for fetal_T4 on SSC1, 6, 10, 13, and 15, and collectively explained 14% of the GV. Overlapping QTL for ≥2 traits were identified on SSC4, SSC6, and SSC15. Overall, thyroid hormone levels in PRRSV2 challenged animals were low to moderately heritable; a high genetic correlation was uncovered for piglet_T3 levels to weight gain; relatively few markers explained a large amount of the GV; and several pleiotropic QTL were identified. Collectively, our results suggest thyroid hormone levels maybe a promising biomarker for genetic improvement of resilience during PRRSV challenge.

Key Words: pigs and related species, genome-wide association, biomarker, disease resilience, animal health

W250 Molecular characterization of the serum amyloid A (SAA) mutation R90S in chicken hepatocellular carcinoma (LMH) cells. C. Falker-Gieske*[1], N. Paul[1], J. Gilthorpe[2], K. Gustmann[1], and J. Tetens[1], [1]*Department of Animal Sciences, Georg-August-University, Göttingen, Germany,* [2]*Department of Integrative Medical Biology, Umeå University, Umeå, Sweden.*

Brown layer chickens are susceptible to amyloid arthropathy (AA), which is a disease caused by bacterial infection that leads to serum amyloid A (SAA) deposition in knee joints of affected animals. White layer chickens are resistant to the disease. Hence, disease susceptibility clearly has a genetic component that has yet to be identified. By analyzing whole-genome sequencing data we discovered a missense variant in the SAA gene (rs739601959) in 41 out of 50 white layer alleles whereas brown layers are non-carriers of the allele. The variant leads to an amino acid (aa) exchange from arginine to serine at position 90 (R90S) in the SAA protein. To characterize the mutation, we created stable chicken hepatocellular carcinoma (LMH) cell lines that overexpress wildtype SAA (SAA-WT) and the R90S isoform (SAA-R90S). Intra- and extracellular SAA protein levels were significantly elevated in SAA-R90S cells (Western blot, $P = 0.0042$, $P = 0.0039$), although mRNA levels as determined by qPCR were similar in SAA-WT and SAA-R90S cells ($P = 0.052$). After separation of cell lysate preparations SAA-R90S accumulated in the detergent insoluble phase whereas no SAA-WT protein was detectable in the insoluble phase. This might indicate that SAA-R90S has a lower binding capacity to high density lipoprotein (HDL), which is known to promote SAA aggregation. To further characterize the impact of the SAA-R90S isoform on LMH cells we compared the transcriptomes of SAA-WT cells and SAA-R90S cells with cells transfected with empty vector plasmid. Preliminary analyses of the results suggest, that overexpression of SAA-WT induces a cell fate change with 21 transcription factors (TFs) differentially expressed (DE) in SAA-WT cells and only 6 TFs DE in SAA-R90S cells ($P < 0.05$, abs. LFC ≥1). Protein-protein-interaction and gene set enrichment analyses revealed a loss-of-function phenotype in SAA-R90S cells and shift toward the plasma membrane, the cell periphery, and the extracellular space. Furthermore, our data suggests that glycosylation of aa 90 in the SAA-R90S might affect SAA's function as an apolipoprotein, which could explain the resistance of white layer chickens to AA due to the lack of HDL associated SAA during the acute phase response to bacterial infection.

Key Words: serum amyloid A, amyloid arthropathy, disease resistance, acute phase response, cell model

W251 Ovine mastitis: Does early live nutrition influence immunity response in later life? C. Hervás-Rivero, R. Pelayo, B. Gutiérrez-Gil, C. Esteban-Blanco, H. Marina, J. Arranz, and A. Suárez-Vega*, *Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, León, Castilla y León, Spain.*

Nutritional status in early life is a critical determinant of immunity status. Specifically, these differences in immune response or immune system vulnerability can be more accused under stress situations, such as lactation. This study aimed to evaluate mammary gland transcriptomic changes after an intramammary lipopolysaccharide (LPS) infusion in lactating sheep subjected to a nutritional challenge at the prepuberal stage (3–5 mo of age). Briefly, 40 Assaf ewe-lambs born in the same period and the same flock were divided into 2 groups, 20 animals received a regular growth diet (control group), and the 20 others were fed with a low protein diet (challenge group; NC). The NC period lasted 2 mo and was performed during the allometric growth period of the mammary gland. At the last stage of their first lactation, all ewes, control, and NC were subjected to an intramammary LPS challenge. At the peak of temperature and somatic cell counts (+6 h post LPS inoculation), 19 milk samples (12 NC and 7 control) were collected to perform RNA-seq. A bioinformatic pipeline was applied to determine changes in expression between NC and control ewes. We identified 548 differentially expressed genes. Among the biological processes found to be affected by the nutritional challenge during the inflammation peak period, we identified processes such as *ribosome biogenesis*, *neutrophil-mediated immunity*, and *granulocyte activation*. Some immunity-related markers were found downregulated in the NC ewes; e.g., *cyclooxygenase 1* whose gene expression differences were associated with host immunity status in mice. In conclusion, this preliminary study suggests that, in sheep, a nutritional restriction at the prepuberal age can influence gene expression and biological pathways patterns triggered in response to an intramammary LPS infusion in later life.

Key Words: sheep and related species, RNA-seq, immune system, milk production, nutrigenomics

W252 The natural cytotoxicity receptor genes in the family *Felidae*. J. Bubenikova[1,2], J. Futas[1,2], J. Oppelt[2], M. Plasil[2], R. Vodicka[3], and P. Horin*[1,2], [1]*Department of Animal Genetics, University of Veterinary Sci-*

ences, Brno, Czech Republic, [2]Ceitec VETUNI, University of Veterinary Sciences, Brno, Czech Republic, [3]Zoo Prague, Prague, Czech Republic.

Natural killer cells (NKC) play important roles in immune responses. Various NKC receptors (NKR) mediate multiple NKC activities. No single conservative model of NKR genes has been observed in mammals. As NKC in cats seem to be different from those of other mammals, analyses of NKR of the Felidae may bring new information on NKR evolution in mammals. Natural cytotoxicity receptors (NCR) represent a group of activating receptors encoded by genes *NCR1*, *NCR2*, and *NCR3* expressed on immune cells. Their presence or functional characteristics may differ even in related species. The objective of this work was to characterize *NCR* genes in felids. Thirty-eight individuals of 15 felid species were analyzed. Based on bioinformatic analyses of the most recent genome assemblies and next-generation sequencing, potentially functional *NCR1*, *NCR2* and *NCR3* genes were found in all species analyzed. Similarities with the human *NCR* genes were 78%, 77% and 86%, respectively. Out of currently annotated mammalian *NCR* sequences, the most related were genes of families *Hyaenidae* and *Herpestidae*. Based on coding sequences (CDS), protein variants were inferred. Within the family, most interspecific differences in CDS were found in *NCR1* (25, 1–4 per species), followed by *NCR2* (23, 1–2 per species) and *NCR3* (16, 1–2 per species). As for protein variants, 22 different NCR1 amino acid sequences (1–3 per species) were found, 21 (1–2 per species) for NCR2 and 12 (1–2 per species) for NCR3. Some protein variants were shared among species. For *NCR1* and *NCR2*, their phylogenetic trees based on CDS reflected the current zoological taxonomy of felids. This finding was supported by selection analyses detecting neutral selection in both genes. Low variability of the *NCR3* CDS did not allow a robust phylogeny reconstruction. Selection analysis detected purifying selection for *NCR3*. *NCR1* showed most interspecific differences. A high level of within-species variation of *NCR1* was also found in a group of 221 domestic cats. As *NCR1* was associated with feline coronavirus shedding and considering differences in innate immunity observed among felid species, our data indicate a potential role of *NCR1* in the interspecific variability.

Key Words: cats and related species, immunogenomics, DNA sequencing, innate immunity, evolution, selection

W253 Systemic transcriptomic response of sheep and cattle to acute and chronic *Fasciola hepatica* infection. D. A. Niedziela*[1], A. Naranjo-Lucena[1], V. Molina-Hernández[2], J. A. Browne[3], Á. Martínez-Moreno[4], J. Pérez[2], D. E. MacHugh[3,5], and G. Mulcahy[1,5], [1]*UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland,* [2]*Department of Anatomy and Comparative Pathology and Toxicology, Faculty of Veterinary Medicine, University of Córdoba, Córdoba, Spain,* [3]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, Dublin, Ireland,* [4]*Parasitology section, Department of Animal Health, Faculty of Veterinary Medicine, University of Córdoba, Córdoba, Spain,* [5]*UCD Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland.*

The objective of this study was to investigate the transcriptomic response of ovine peripheral blood mononuclear cells (PBMC) to *Fasciola hepatica* infection, and to elucidate the differences between ovine and bovine PBMC responses. *F. hepatica* is a zoonotic trematode which leads to delayed growth and loss of productivity in cattle, while infection in sheep can have more severe effects, potentially leading to death. Previous transcriptomic analyses revealed upregulation of *TGFB1*, cell death and Toll-like receptor signaling, T-cell activation, and inhibition of nitric oxide production in macrophages in response to the parasite; however, differences between bovine and ovine responses are unexplored. Sixteen male Merino sheep were randomly assigned to infected or control groups (n = 8 per group) and orally infected with 120 *F. hepatica* metacercariae. Transcriptomic data were generated from PBMC at 0, 2, and 16 weeks post-infection (wpi), and analyzed for differentially expressed (DE) genes between infected and control animals at each time point (analysis 1), and for each group relative to time 0 (analysis 2). Analysis 2 was then compared with a similar study performed previously on bovine PBMC. A total of 453 DE genes were found at 2 wpi, and 2 DE genes at 16 wpi (FDR <0.1, analysis 1). Significantly overrepresented biological pathways at 2 wpi included *role of PKR in interferon induction and antiviral response*, *death receptor signaling* and *RIG-I-like receptor signaling*, which suggested that an activation of antiviral response and inhibition of cellular apoptosis was taking place. Comparison of analysis 2 with a bovine transcriptomic study revealed significantly overrepresented pathways in the acute phase in cattle, which were upregulated only in the chronic phase in sheep: *IL-10 signaling*, *Th2 pathway*, and *Th1 and Th2 activation*. The early anti-inflammatory response may be the cause of lack of clinical signs in the acute infection stage in cattle. These findings offer scope for "smart vaccination" strategies for this important livestock parasite.

Key Words: sheep, immunology, RNA-seq, infectious disease, One Health

W254 Bimodal haplotype distribution in bovine antibacterial toll-like receptors. K. Samaké*[1] and K. Novák[2], [1]*Charles University, Prague, Czech Republic,* [2]*Institute of Animal Science, Prague-Uhrineves, Czech Republic.*

The *TLR* genes coding for Toll-like receptors of antibacterial series, namely *TLR1*, *-2*, *-4*, *-5* and *-6*, were re-sequenced in Czech Simmental (Czech Red) cattle using HiSeq and PacBio technologies. Hybrid sequencing allowed to determine SNPs for individual genotyping with primer extension method. Haplotypes were established within the range of the designed PacBio amplicons, especially for *TLR2*. A more general statistical reconstruction of haplotypes from individual genotyping was carried out in parallel. The directly determined haplotypes from PacBio reads demonstrated randomly distributed frequencies of haplotypes in the amplicon 2 – 5 of *TLR2*, however, 15 haplotypes in amplicon 1 in the proximal part of the transcript formed 2 distinct groups. The results were consistent with the distribution of haplotypes obtained by statistical reconstruction in *TLR2*. Similarly, the bimodal distribution was detectable in *TLR5* and other *TLR*s. In all cases, the trend for bimodal distribution was expressed stronger in proximal regions of the transcripts. The clustering of *TLR* haplotypes has been reported earlier in a panel of world breeds (Fisher C.A. et al., Plos One 6:e27744, 2011; Bilgen N. et al., Diversity 8:23, 2016), however, the origin of this disequilibrium is still not documented. Alternating infectious agents are feasible (Bilgen et al., 2016) along with 2 different essential functions performed by a *TLR* gene or its product. An example of a dual function might be formation of 2 kinds of products differing in specificity. The association of the groups of haplotypes with the transcript proximal region suggests the selection target in the 5′- regulatory regions of the *TLR* genes, although functional interactions in the proximal part of the transcript cannot be excluded. The confirmation of the haplotype reconstruction data by targeted genotyping and new resequencing technologies is in progress in the set of bulls studied.

Key Words: cattle, innate immunity, toll-like receptors, haplotype diversity

W255 Variation in circulatory serum biomarkers in dairy heifers exposed to endotoxin indicate disparity in induced physiological responses. A. Sharma*, T. Sullivan, K. Lamers, and N. Karrow, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Hypothalamic–pituitary–adrenal axis is a major 'stress' axis that is activated upon microbial exposure and culminates the secretion of the blood glucocorticoid cortisol. Individual differences in cortisol levels indicate differences in the responsiveness of animals to aversive situations and their ability to manage immunological challenges. Genetic selection of animals for enhanced stress resilience could be a possible strategy to mitigate these differences. In the present study, 180 heifers (6–10 mo age) were immune challenged with endotoxin (lipopolysaccharide, 200 ng/kg) and blood was collected T0 prior challenge and 2 h (T2), 4 h (T4) post-challenge to assess the variation in serum circulatory biomarkers including cortisol, cytokines /chemokines and miRNAs levels. Cortisol levels significantly increased and differed among animals post endotoxin

challenge. Based on peak (T4) cortisol levels animals were categorized (n = 30/group) as high (HSR, >1050 pg/mL) and low (LSR, <250 pg/mL) stress responders. The HSRs showed a gradual increase in cortisol from T2 to T4, whereas in LSRs cortisol returned to basal levels from T2 to T4. Immune proteins were measured in a subset of animals (n = 8/group) using a panel of 10 cytokines/chemokines. Among these, cytokines (TNF-α, IL-10) and chemokines (CCL2, CCL3, CXCL10) were more significantly ($P < 0.05$) induced in HSR than LSR animals. A high and positive Pearson correlation (>0.8) was observed between cortisol and cytokines levels in the HSRs. Further, a panel of 384 bovine specific miRNAs was employed to determine the changes in circulatory miRNAs between HSR and LSR animals (n = 4/group). Six miRNAs were significantly ($P < 0.05$) differentially expressed between HSR and LSR groups with a fold change (FC) > 2 or < 2; miR-101 (FC > 2.08), miR-484 (FC > 3.21), miR-339a (FC > 3.08) and miR-494 (FC > 10.51) were upregulated, whereas miR-34a (−2.2) and miR-541 (−2.07) were downregulated. The variations in the induced immune response (cytokines, miRNAs) between the HSR and LSR groups indicate differences in individual abilities to cope with microbial stressors. Collectively, these results suggested the suitability of cortisol levels as a stress resilience trait and the assessed circulatory biomarkers also implicate differences in their immune regulation.

Key Words: immune response, cortisol, cytokines, miRNAs, stress resilience

W256 Transcriptomic analysis of host resistance to tick infestation with *Rhipicephalus microplus* in leukocytes of Brangus cattle. E. Mantilla Valdivieso*[1], E. Ross[1], A. Raza[1], B. Hayes[1], N. Jonsson[2], P. James[1], and A. Tabor[1], [1]*Queensland Alliance for Agriculture and Food Innovation, Queensland Alliance for Agriculture and Food Innovation, Brisbane, Queensland, Australia,* [2]*Institute of Biodiversity Animal Health and Comparative Medicine, Institute of Biodiversity Animal Health and Comparative Medicine, Glasgow, UK.*

Rhipicephalus microplus, also known as the cattle tick, is a blood-feeding ectoparasite that negatively impacts animal health and cattle production in tropical and subtropical regions worldwide. Control strategies to limit tick infestations include breeding cattle for tick resistance by maintaining a high *Bos indicus* genetic content in breeds. However, selection for tick resistance is still difficult due to reliance on phenotypic assessment by tick scoring methods which are not widely standardized. Therefore, development of predictive biomarkers of host resistance to ticks is a promising approach to increase accuracy of selection and accelerate the genetic gain in cattle. Previous literature suggests that host immunity is relevant in the expression of host resistance, however, the differences in the transcriptomic profiles of leukocytes of tick-resistant and tick-susceptible cattle have not yet been studied. In this study, 30 tick-naïve Brangus steers were exposed to a 12-week artificial tick infestation trial to differentiate the most resistant and most susceptible individuals. The number of developing adult ticks after an infestation cycle (21 d) was estimated with a tick scoring scale from 1 (<50 ticks = Highly Resistant) to 5 (>300 ticks = Highly Susceptible). Animals were subsequently classified as being the most resistant (n = 5) and the least resistant (n = 5) based on the mean tick score. Leukocyte-derived RNA was isolated from blood samples collected immediately before the first infestation (T0) and then at 3 weeks (T3) and 12 weeks (T12) post-initial infestation. RNA-seq was used to identify differentially expressed genes between tick-susceptible and tick-resistant cattle at each of the 3 observed time points. We identified 59, 11, and 32 significant differentially expressed genes (false discovery rate <0.05) between resistant and susceptible animals at time points T0, T3, and T12, respectively. These findings indicate a differential response between the cattle of divergent host resistance before and after repeated tick infestation which shows potential for elucidation of biomarkers of tick resistance that could be examined independently of the levels of tick exposure.

Key Words: RNA-seq, biomarker, animal health, cattle and related species

W257 Identification of loci associated with susceptibility to paratuberculosis in Holstein cattle using combinations of diagnostic tests and imputed whole-genome sequence data. M. Canive*[1], G. Badia-Bringué[1], O. González-Recio[2,3], A. Fernandez[2], P. Vázquez[1], J. Garrido[1], R. Juste[1], and M. Alonso-Hearn[1], [1]*Department of Animal Health, NEIKER-Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain,* [2]*Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, CSIC, Madrid, Spain,* [3]*Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.*

Bovine paratuberculosis (PTB) is a chronic inflammatory disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Genomic selection could enhance natural resistance to MAP infection and complement existing control strategies. The main objective of this study was to identify quantitative trait loci (QTL) associated with PTB susceptibility in Spanish Holstein cows (n = 983) using combinations of diagnostic tests and imputed whole-genome sequence (WGS) data. The infection status of these animals was defined by 3 diagnostic methods including ELISA for detection of humoral responses against MAP and culture and PCR detection of MAP in gut tissues. The 983 cows included in this study were genotyped with the Bovine MD SNP50 Bead Chip, and the corresponding genotypes were imputed to WGS using the 1,000 Bull genomes reference population. In total, 33.77 million SNP variants per animal were identified across the genome. Linear mixed models were used to calculate the heritability (h^2) estimates for each diagnostic test and test combinations. Next, we performed a case-control genome-wide association study (GWAS) using the imputed WGS data sets and the phenotypes and combinations of phenotypes with h^2 estimates >0.080. After performing the GWAS, the test combinations that showed SNPs with a significant association ($P_{FDR} \leq 0.05$), were the ELISA-tissue PCR-tissue culture, ELISA-tissue culture, and ELISA-tissue PCR. A total of 12 quantitative trait loci (QTLs) highly associated with MAP infection status were identified on the *Bos taurus* autosomes (BTA) 4, BTA5, BTA11, BTA12, BTA14, BTA23, BTA24, and BTA28, and some of these QTLs were linked to immune-modulating genes. The identified QTLs on BTA23 spanning from 18.81 to 22.95 Mb of the *Bos taurus* genome overlapped with several QTLs previously found to be associated with PTB, bovine tuberculosis, and mastitis infection. In summary, combining phenotypes and WGS improved the power for detecting genetic associations in Spanish Holstein cattle. The results from this study provide more clues regarding the molecular mechanisms underlying susceptibility to PTB and might be used to develop national genetic evaluations for PTB in Spain

Key Words: cattle, genome-wide association, diagnostics, imputation, animal health

W258 The host genetic underlying pathological outcomes to *Mycobacterium avium* subsp. *paratuberculosis* infection is governed by distinct genetic variants. M. Alonso-Hearn*[1], M. Canive[1], G. Badia-Bringué[1], O. González-Recio[2,3], A. Fernández[2,3], P. Vázquez[1], J. Garrido[1], and R. Juste[1], [1]*NEIKER-Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain,* [2]*Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, CSIC, Madrid, Spain,* [3]*Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.*

Bovine paratuberculosis (PTB) is a granulomatous enteritis caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). According to their severity and extension, PTB-associated histological lesions have been classified into the following groups; focal, multifocal, and diffuse. It is unknown whether these lesions represent sequential stages or divergent disease outcomes. In the current study, the associations between host genetics, host response, and pathology were explored by genotyping 813 Spanish Holstein cows with focal (n = 371), multifocal (n = 33), diffuse

(n = 33), and with undetected visible lesions (n = 373) in gut tissues and regional lymph nodes. DNA from peripheral blood samples of these animals was genotyped with the Bovine MD SNP50 Bead Chip, and the corresponding genotypes were imputed to whole-genome sequencing (WGS) data using the 1,000 Bull genomes reference population. A genome-wide association study (GWAS) was performed using the WGS data and the presence or absence of each type of histological lesion in a case-control approach. We identified 129 and 92 SNPs highly associated ($P \leq 5 \times 10^{-7}$) with the multifocal (heritability = 0.075) and the diffuse lesions (heritability = 0.189), respectively. Twelve and 9 distinct quantitative trait loci (QTLs) highly associated with the multifocal and diffuse lesions were identified, respectively. Some of the identified QTLs overlapped with QTLs previously associated with PTB, bovine tuberculosis, and mastitis infection. Pathway analysis with candidate genes overlapping the identified QTLs revealed a significant enrichment of the keratinization pathway and cholesterol metabolism in the animals with multifocal and diffuse lesions, respectively. While keratin variants may predispose cows to the development of multifocal lesions, cholesterol variants associate with the more severe lesions. Total plasma cholesterol in animals with diffuse lesions (0.080 μg/μL) was significantly lower when compared with cows with focal (0.126 μg/μL), multifocal (0.141 μg/μL) or with undetected lesions (0.129 μg/μL). Taken together, these findings suggest that the variation between the multifocal and diffuse lesions may be genetically determined and indicative of distinct host responses in genetically predisposed individuals.

Key Words: cattle, genome-wide association, imputation, infectious disease, animal health

W259 Alternative splicing modulates the immune response in peripheral blood and gut tissues of Holstein cattle naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*. G. Badia-Bringué*[1], M. Canive[1], J. Lavín[2], R. Casais[3], C. Blanco-Vázquez[3], and M. Alonso-Hearn[1], [1]*Department of Animal Health, NEIKER-Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain,* [2]*Department of Applied Mathematics, NEIKER- Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain,* [3]*SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Center of Animal Biotechnology, Deva, Asturias, Spain.*

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes significant losses to the dairy industry worldwide and has been associated with human diseases. Alternative splicing (AS) is an important mechanism of gene regulation that can influence pre-mRNA stability, structure, function and localization, with important physiological consequences. However, the role of AS in regulating the host response to MAP infection is still unclear. In the current study, differential AS was analyzed using RNA-seq data from peripheral blood (PB) and ileocecal valve (ICV) samples collected from Holstein cattle with undetectable lesions (n = 4) and with focal/multifocal (n = 7) or diffuse (n = 5) PTB-associated lesions in gut tissues. A multivariate analysis of AS events was performed with rMATS 4.1.1, which uses a likelihood-ratio test to calculate if the difference in the mean exon inclusion levels between 2 sample groups exceeds a given threshold (>5%). In PB samples of infected cows, several neutrophil degranulation pathway genes (*CD53*, *CLEC12A*, *CPNE1*, *ITGA2B*, *NCF1*, *SGSH*, *SIRPA*, *SLC11A1*, *TARM1*, *TMC6*) and clathrin-mediated endocytosis (*CLTA*, *DNM1*, *DNM2*, *EPS15L1*, *FCHO1*) showed significant AS perturbations. In ICV samples of infected cows, proteins with RNA-recognition and coiled-coil domains showed differential AS events when compared with control cows. Specifically, in the ICV from animals with focal lesions, several genes related to the innate immune response (*C2*, *C4A*, *CD46*, *CFH*, *CYLD*, *IRF3*, *PYCARD*, *TMEM173*, *TRIM38*) showed differences in splicing when compared with cows without lesions. Changes AS of several genes also correlated with changes in gene expression. In the ICV of animals with diffuse lesions, for instance, several components of the immune response (*BOLA*, *CLEC7A*, *PSBM10*, *IFI30*, *IRF5*, *ARID5A*, *IL7*) showed deregulation both in mRNA expression and AS pattern. Interestingly, AS in *BOLA*, *MHCI-A* and *BOLA-NCI* was found associated with PTB and several autoimmune diseases such as Type I diabetes mellitus, autoimmune thyroid disease, Kaposi sarcoma-associated herpesvirus infection, and Epstein-Barr virus infection.

Key Words: cattle, genome regulation, RNA-seq, autoimmunity, animal health

W260 Identification and validation of loci associated with facial eczema tolerance in New Zealand sheep. K. M. McRae*, S. J. Rowe, P. L. Johnson, and S. M. Clarke, *AgResearch Limited, Mosgiel, New Zealand.*

Facial eczema (FE) is a metabolic disease of great importance in ruminants in New Zealand. Ingestion of the mycotoxin sporidesmin leads to liver and bile duct damage, which can result in photosensitization and reduced production. In sheep, there is considerable genetic variation in tolerance to facial eczema, and a commercial testing program is available for ram breeders who aim to increase tolerance. The objective of this study was to utilize a data set of these phenotyped animals with high- and low-density genotypes to interrogate the sheep genome for regions associated with variability in tolerance to FE in New Zealand sheep. Two quantitative trait loci (QTL) on chromosomes 15 and 24 are reported, which explain 5% and 2% of the phenotypic variance in the response to FE, respectively. The QTL on chromosome 24 contains the β-globin locus, and mass spectrometry of hemoglobin from animals with differing genotypes at this locus indicated that the QTL is associated with different forms of adult β-globin. Haplotype A animals appeared to be more tolerant to FE, however, the overall frequency of the haplotype in genotyped animals was 0.5, indicating that the locus may be under balancing selection in the New Zealand sheep population. Hemoglobin haplotypes have previously been associated with variation in several health-related traits in sheep, and therefore warrant further investigation regards their role in tolerance to FE in sheep. This study highlights the power of using increased density genotyping for the identification of influential genomic regions, combined with subsequent inclusion on lower density genotyping platforms

Key Words: sheep, disease, facial eczema, hemoglobin, GWAS

Animal Epigenetics Posters

P100 Sestrin-3 regulates adipogenesis via the Smad3/miR-124 axis. W. Lin, J. Zhao, M. Yan, K. Yang, W. Wei, L. Zhang, and J. Chen*, *College of Animal Science, Nanjing Agricultural University, Nanjing, Jiangsu, China.*

Sestrin-3 (Sesn3), as part of the sestrin family that includes sestrin-1 and sestrin-2, has been shown to be involved in antioxidation, metabolic homeostasis, and the development of nonalcoholic steatohepatitis (NASH). However, the regulatory role of Sesn3 in adipogenesis needs further exploration. In this study, we showed that Sesn3 inhibited adipogenesis of porcine pre-adipocytes via the Smad3/miR-124 pathway. We used real-time PCR analysis of genes after transfecting porcine pre-adipocytes with either reconstructed vector or small interfering (si)RNA, and used chromatin immunoprecipitation (ChIP)-PCR to check binding of FoxO1 to the Sesn3 promoter and binding of Smad3 to the miR-124 promoter. Relative luciferase activities were checked by using the dual-luciferase reporter assay system. The regulatory roles of Sesn3 and miR-124 in pre-adipocytes was examined by O red oil staining and quantitation of

triglycerides. We analyzed both upstream and downstream of Sesn3 to determine the regulatory role it played in porcine pre-adipocyte adipogenesis; we found that Sesn3 suppressed pre-adipocyte adipogenesis, whereas FoxO1 promoted Sesn3 by binding to its promoter. In contrast, Sesn3 inhibited the Smad family, especially Smad3, thus influencing its promotional effect on miR-124. Furthermore, miR-124 inhibited adipogenesis by targeting the 3′ untranslated region (UTR) of *C/EBPα* and *GR*. In brief, Sesn3 inhibited the Smad3/miR124 signaling pathway; thus, it affected *C/EBPα* and *GR* to suppress adipogenesis of pre-adipocytes.

Key Words: pig, epigenetics, miR-124-3p, adipocytes

P101 Livestock methylomics: Systematic evaluation of DNA methylation profiling assays for industry. A. Caulton*[1,2], R. Brauning[1], K. G. Dodds[1], A. Hagani[3], J. Zoller[4], C. Couldrey[5], S. Horvath[3], and S. M. Clarke[1], [1]*AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand,* [2]*University of Otago, Dunedin, New Zealand,* [3]*Department of Human Genetics, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA,* [4]*Department of Biostatistics, Fielding School of Public Health, University of California Los Angeles, Los Angeles, CA, USA,* [5]*Livestock Improvement Corporation, Hamilton, New Zealand,* [6]*University of Idaho, Moscow, ID, USA.*

DNA methylation plays a fundamental role in the regulation of growth and development in mammals and serves as a biomarker of chronological age. While the importance of DNA methylation is well established, its potential for breeding applications within the livestock sector is yet to be realized. To successfully incorporate DNA methylation data into current genetic merit predictions, high throughput, robust and cost-effective assays should be employed. Here we compare 4 sequence-based approaches to determine genome-wide methylation signatures across 8 different tissue types in sheep (1) whole-genome bisulfite sequencing (WGBS); (2) reduced representation bisulfite sequencing (RRBS); (3) methylation sensitive restriction enzyme sequencing (MRE-seq) and; (4) direct detection of methylation with Nanopore single molecule sequencing technology. The concordance between the methodologies is evaluated and benchmarked against WGBS, the gold standard methylation profiling assay. The benefits, and disadvantages of each method are examined with emphasis on industry implementation. In addition to sequence-based approaches, we have used the custom mammalian methylation array "HorvathMammalMethyl40" to assay DNA methylation levels at approximately 37 thousand CpG sites across key livestock species. From this work we have constructed the first 'epigenetic clock' for domesticated goat, cattle, red and wapiti-breed deer, and composite-breed sheep. This livestock epigenetic clock uses only 214 CpG sites to estimate age (relative to the maximum lifespan of the species) with high accuracy across all 4 species ($r > 0.93$), using a single mathematical model. The applications of this livestock epigenetic clock could extend well beyond the scope of chronological age estimates. Many independent studies have demonstrated that a deviation between true age and clock derived molecular age is indicative of past and/or present health (including stress) status. There is, therefore, untapped potential to utilize epigenetic clocks in breeding programs as a predictor for age-related, production traits.

Key Words: methylation, epigenetic clock, livestock, epigenetics, ovine FAANG

P102 Maternal methionine supplementation alters alternative splicing and DNA methylation in bovine skeletal muscle. L. Liu* and F. Peñagaricano, *University of Wisconsin-Madison, Madison, WI, USA.*

The evaluation of alternative splicing, including differential isoform expression and differential exon usage, can provide some insights on the transcriptional changes that occur in response to environmental perturbations. Maternal nutrition is considered a major intrauterine regulator of fetal developmental programming. The objective of this study was to assess potential changes in splicing events in the longissimus dorsi muscle of beef calves gestated under control or methionine-rich diets. RNA sequencing and whole-genome bisulfite sequencing were used to evaluate muscle transcriptome and methylome, respectively. Alternative splicing patterns were significantly altered by maternal methionine supplementation. Most of the altered genes were directly implicated in muscle development, muscle physiology, ATP activities, RNA splicing and DNA methylation, among other functions. Interestingly, there was a significant association between DNA methylation and differential exon usage. Indeed, among the set of genes that showed differential exon usage, significant differences in methylation level were detected between significant and nonsignificant exons, and between contiguous and noncontiguous introns to significant exons. Overall, our findings provide evidence that a prenatal diet rich in methyl donors can significantly alter the offspring transcriptome, including changes in isoform expression and exon usage, and some of these transcriptomic changes are mediated by changes in DNA methylation.

Key Words: differential isoform expression, differential exon usage, fetal programming

P103 Micrococcal nuclease sequencing of pig sperm suggests a relationship between nucleosome retention and both semen quality and early embryo development. M. Gòdia[1], S. S. Hammoud[2], M. Naval-Sánchez[3], I. Ponte[4], J. E. Rodriguez-Gil[4], A. Sánchez[4,1], and A. Clop*[1,5], [1]*Centre for Research in Agricultural Genomics CRAG, Cerdanyola del Valles, Catalonia, Spain,* [2]*University of Michigan, Ann Arbor, MI, USA,* [3]*CSIRO, St Lucia, Brisbane, Australia,* [4]*Universitat Autonoma de Barcelona, Cerdanyola del Valles, Catalonia, Spain,* [5]*CSIC, Barcelona, Catalonia, Spain.*

In animals, the chromatin structure of the mature spermatozoon is ultra-compacted due to the replacement of histones by protamines during spermatogenesis. However, a small fraction of nucleosomes remains bound to DNA at specific sites of the genome and it has been linked to sperm biology and embryogenesis. The genomic characterization of nucleosome occupancy in the sperm chromatin could help identifying molecular markers for sperm quality and fertility traits. Nonetheless, these maps are not yet available for most livestock species, including swine. In this study, we performed micrococcal nuclease digestion followed by high-throughput sequencing on pig ejaculated spermatozoa and mapped the mono-nucleosomal and sub-nucleosomal chromatin fractions. We found 25,293 mono-nucleosomal and 4,239 sub-nucleosomal peaks covering 0.3% and 0.02% of the porcine genome, respectively. We detected positional conservation of the nucleosome-associated DNAs in sperm between human and pig. We also carried gene ontology analysis of the genes mapping nearby the mono-nucleosomal peaks and also searched for putative transcription factor binding motifs within the mono-nucleosomal peaks and found an enrichment for sperm function and embryo development-related processes. Remarkably, we detected enrichment for the canonical binding site of Znf263. In humans, this transcription factor has been suggested as a key regulator of the genes with paternal preferential expression during early embryo development. In addition, we also observed co-occupancy of the RNAs present in pig sperm and these RNAs related to sperm quality, with the mono-nucleosomal peaks. We also found a co-location trend between GWAS hits for semen quality in swine and the mono-nucleosomal sites. The results obtained in this study clearly indicate that there is a relationship between nucleosome positioning in sperm with sperm phenotypes and embryo development.

Key Words: pig, sperm, chromatin nucleosome, micrococcal nuclease

P104 Identifications of epigenetic regulation mechanism according to the growth of pig in abdominal fat tissue through multi-omics integration analysis. D.-Y. Kim* and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Republic of Korea.*

Fat is a major organ involved in the synthesis of new fatty acids (FA), FA circulation, and lipid metabolism. Various genetic studies have been conducted on pig fat, but understanding the growth and specific ad-

ipose tissue is insufficient. The purpose of this study is to investigate the epigenetic difference of abdominal fat according to the growth of pigs. Abdominal fat was collected at each time point of 10 and 26 weeks from crossbreeding F1 pigs between KNP and Yorkshire breeds, and then methylome and transcriptome data were produced using MBD-seq and RNA-seq. Differentially methylated genes (DMG) and differentially expressed genes (DEG) were identified as 2,251 and 5,768, respectively. DMG and DEG have been shown to be primarily involved in immune responses, such as the chemokine signaling pathway and the B cell receptor signaling pathway, and functions related to lipid metabolisms, such as the PPAR signaling pathway and fatty acid breakdown. When we investigated the effect of DNA methylation on gene expression through cis-regulation and trans-regulation analysis, cis-regulation related to immune responses and trans-regulation associated with fat metabolism were identified. Genes in these pathways are associated with immunity, lipolysis, and synthesis during growth, suggesting that they may be key biomarkers. Thus, we provide a systematic investigation of changes in the epigenetic regulation of porcine abdominal fat with aging to broaden our understanding of the regulatory mechanisms involved in fat metabolism and immune response growth.

Key Words: pigs and related species, epigenomics, functional genomics, genome sequencing, fat/lipid

P105 Epigenetic marks in the promoter of *GNAS* and *EBF3* are associated with meat tenderness in *Bos indicus*. M. M. de Souza[1,2], S. C. M. Niciura[1], M. I. P. Rocha[1,3], W. J. S. Diniz[1,4], J. J. Bruscadin[1,3], J. Afondo[1], P. S. N. de Oliveira[1], G. B. Mourão[5], A. Zerlotini[6], L. L. Coutinho[5], J. E. Koltes[2], and L. C. A. Regitano*[1], [1]*Embrapa Pecuária Sudeste, Empresa Brasileira de Pesquisa Agropecuária, São Carlos, São Paulo, Brazil,* [2]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [3]*Department of Genetics and Evolution, Federal University of São Carlos, São Carlos, São Paulo, Brazil,* [4]*Department of Animal Sciences, North Dakota State University, Fargo, ND, USA,* [5]*Department of Animal Science, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil,* [6]*Embrapa Informática Agropecuária, Empresa Brasileira de Pesquisa Agropecuária, Campinas, São Paulo, Brazil.*

Tenderness is a complex trait with economic value for the beef market. Understanding the genetics and epigenetics mechanisms underlying this trait may help improve the accuracy of breeding programs and deliver a better-quality product to the consumers. However, little is known about epigenetic effects in the muscle of *Bos taurus* and their implications in tenderness, and no study is available in *Bos indicus* so far. Therefore, we searched for differences in the methylation profile of *Bos indicus* muscle with extreme values for meat tenderness (tender = 6, tough = 6). For this, we analyzed reduced representation bisulfite sequencing (RRBS) and identified 123 differentially methylated CpGs (DMCs) and 42 regions (DMRs) ($P < 0.05$ and methylation difference >25%), although the global methylation profile had low variation in the population. Most of the DMR (70.73%) and DMC (83.72%) were hypermethylated in the tender group. Enrichment analysis of previously predicted target genes suggested that signal transduction pathways may be affected by differences in methylation between tender and tough meat, with G protein-coupled receptor signaling being a key pathway. Our analysis suggested that different methylation levels related to tenderness may regulate the expression of *GNAS* and *EBF3* (RNA-seq; Pearson correlation, $P < 0.05$). *GNAS* showed expression positively correlated with CpG methylation level in the DMC23 (r = 0.75) while *EBF3* expression was negatively correlated with DMC89 (r = −0.72), DMC90 (r = −0.75) and DMR40 (r = −0.81). These elements were in CpG islands, located in the promoter of the gene *EBF3* and intron 1 of *GNAS*, an alternative promoter of this gene. *GNAS* is known to have a complex imprinted status and is a member of the G protein-coupled receptor signaling pathways. This pathway and the gene *EBF3* function in muscle homeostasis, relaxation, and muscle cell-specificity. We present DMCs and DMRs that may be of interest to decipher the epigenetic mechanisms affecting tenderness. Further, *EBF3* and *GNAS* were identified as potential candidate genes associated with tenderness via methylation.

Key Words: reduced representation bisulfite sequencing (RRBS), cattle, Nelore, methylation, imprinting

P106 Characterization of the adipose tissue DNA methylation framework between male and female suckling lambs. A. Suarez-Vega, C. Esteban-Blanco, H. Marina, R. Pelayo, M. Alonso-Garcia, C. Hervas-Rivero, B. Gutierrez-Gil, and J.-J. Arranz*, *Universidad de León, León, Spain.*

Epigenetics is emerging as a cutting-edge area of research in livestock nutrition, genetics, and breeding. The epigenetic variation is due to epigenetic marks, including DNA methylation, chromatin remodeling, histone modification, long noncoding RNA, and microRNAs. For animal breeding, epigenetics may help in understanding the genetic architecture of complex traits. For example, in the sheep industry, the quantity and composition of adipose deposits influence the quality of lamb carcasses. Moreover, one determining factor influencing adipose deposit distribution, physiology, and cell signaling is sex. However, little is known about how DNA methylation marks in males and females can contribute to sex differences in adipose metabolism in livestock species. To explore the functional importance of genome-wide DNA methylation sex differences in adipose tissue of lambs, we proposed a systematic identification of the genomic DNA methylation patterns in perirenal fat tissue between 6 male and 6 female sucking lambs (~30 d of age) using high-throughput whole-genome bisulfite sequencing. Read mapping, and DNA methylation calling was performed using the Oar_rambouillet_v1.0 reference assembly. The average percentage of reads mapped to the reference genome was 79%. The pattern of methylated sites in males and females was very similar, with the majority of sites being CG sites (>95%), whereas some few CHH (~3%) and CHG (<1%) sites (C = cytosine; G = guanine; H = adenine, cytosine or thymine) were also found. The genomic regions with the highest methylation levels were 3′ UTRs, introns, repeated regions, and exons. Preliminary results on differential methylated regions between male and female lambs opened new insights on a potentially relevant role of sex in adipose epigenetic regulation. These results suggest that changes in methylation patterns could trigger fat deposition in both sexes.

Key Words: sheep and related species, epigenomics, fat/lipid, product quality

P107 Pig genome functional annotation enhances biological interpretations of complex traits and comparative epigenomics. Z. Pan*[1], Y. Yao[2], H. Yin[3], Z. Cai[4], Y. Wang[1], L. Bai[3], C. Kern[1], M. Halstead[1], K. Chanthavixay[1], N. Trakooljul[5], K. Wimmers[5], G. Sahana[4], G. Su[4], M. Sandø Lund[4], M. Fredholm[6], P. Karlskov-Mortensen[6], C. W. Ernst[7], P. Ross[1], C. K. Tuggle[8], L. Fang[2], and H. Zhou[1] [1]*Department of Animal Science, University of California, Davis, Davis, CA, USA,* [2]*MRC Human Genetics Unit at the Institute of Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh, UK,* [3]*Agricultural Genome Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China,* [4]*Center for Quantitative Genetics and Genomics, Faculty of Technical Sciences, Aarhus University, Tjele, Denmark,* [5]*Leibniz-Institute for Farm Animal Biology, Dummerstorf, Germany,* [6]*Animal Genetics, Bioinformatics and Breeding, Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark,* [7]*Department of Animal Science, Michigan State University, East Lansing, MI, USA,* [8]*Department of Animal Science, Iowa State University, Ames, IA, USA.*

The functional annotation of livestock genomes is crucial for understanding the molecular mechanisms that underpin complex traits of economic importance, adaptive evolution and comparative genomics. Here, we provide the most comprehensive catalog to date of regulatory elements in the pig (*Sus scrofa*) by integrating 223 epigenomic and transcriptomic data sets, representing 14 biologically important tissues. We systematically describe the dynamic epigenetic landscape across tis-

sues by functionally annotating 15 different chromatin states and defining their tissue-specific regulatory activities. We demonstrate that genomic variants associated with complex traits and adaptive evolution in pig are significantly enriched in active promoters and enhancers. Furthermore, we reveal distinct tissue-specific regulatory selection between Asian and European pig domestication processes. Compared with human and mouse epigenomes, we show that porcine regulatory elements are more conserved in DNA sequence, under both rapid and slow evolution, than those under neutral evolution across pig, mouse, and human. Finally, we provide novel biological insights on tissue-specific regulatory conservation and demonstrate that, depending on the traits, mouse or pig might be more appropriate biomedical models for different complex traits and diseases in humans through integrating comparative epigenomes with 47 human genome-wide association studies.

Key Words: pig, epigenome, comparative genomics, chromatin states, complex traits

P108 A *Bos indicus* epigenetic clock predicts age from tail hair. L. T. Nguyen*, M. Forutan, B. J. Hayes, and E. M. Ross, *Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Queensland, Australia.*

Methylation pattern changes associated with aging have been documented in a range of species including humans, mice, and dogs. Age-associated epigenetics markers have been used to generate algorithms that predict the individual's age based on methylation patterns, these predictions are referred to as epigenetic clocks. Here we aimed to derive the first epigenetic clock for indicine cattle. Indicine cattle are extensively grazed in low-input systems and often only mustered once a year, which results in very few or inaccurate birthdate records. Deriving an age prediction epigenetic clock for indicine cattle could increase the accuracy of recorded birthdates, which are often estimates based on size when mustered. We used minIONs (Oxford Nanopore Technologies) to sequence the genomes of 100 cattle with ages ranging from 5 d to 17 years. Methylation sites were called on 56 of the samples using fc5. Sites that were called in at <80% of animals or with a standard deviation <0.5 were removed from the analysis. A 5-fold cross-validation was then used to predict the age of each animal using BLUP (Best Linear Unbiased Prediction), where the relationship matrix was calculated from the methylation matrix and represented similarity of methylation patterns. A second relationship matrix was also calculated that contained genes associated with age in both humans and dogs identified from the literature. In all cases, the animals being predicted were removed from the reference population. The correlation between predicted age and actual age was 0.65 when genome-wide markers were used, and the limited panel of genes identified in human and dog epigenetic clocks. The mean absolute deviation (MAD) was 1 year for animals aged less than 3 years and 1.5 years for animals aged 3-10 years. This is the first reported epigenetic clock in cattle. Work is continuing to increase the accuracy of the clock. Accurate prediction of age will have implications for breed registrations (which require birthdates), herd management and genomic selections for economically important traits reliant on accurate ages such as growth rate and age at puberty.

Key Words: epigenetic clock, indicine cattle, best linear unbiased prediction, Oxford Nanopore Technology, age prediction

P109 ISO-seq data reveal allele-specific isoform expression. S. Bardoloi*, L. Nguyen, B. Engle, B. Hayes, and E. Ross, *University of Queensland, Brisbane, Queensland, Australia.*

Allele-specific expression (ASE) is the imbalance in transcription of paternal and maternal alleles at a locus. The few ASE studies using RNA sequencing data have been reported in cattle. Since RNA-seq produces short reads, much of the data is unable to be associated with a haplotype of origin, as it does not overlap a SNP. Follow in from this, the isoform that is being expressed by each haplotype has not been considered. We hypothesized that some of the ASE observed in transcriptomes was not due to different levels of expression between haplotypes, but rather that each haplotype was expressing a different isoform. Allele-specific isoform expression (ASIE) can only be identified when transcriptome data is able to be characterized both in terms of haplotype of origin, and isoform. To address this hypothesis, we conducted an ASIE analysis in Brahman cattle using data generated from isoform sequencing technology (ISO-seq, PacBio) of liver. A phased haplotype level assemble of the animal was first used to assign each ISO-seq read to the haplotype of origin. Isoforms were then identified in the same data using TAMA. For each gene, a contingency table of haplotype of origin (columns) and isoform (rows) was calculated. The values in the table were the number of ISO-seq reads for each isoform from each haplotype. A Fisher's exact test was used to test for independence between the isoform and the haplotype of origin. After filtering and correcting for multiple testing using a Bonferroni correction, 12 of the 36 genes showed a significant relationship between isoform and haplotype of origin. The significant genes are Proteosome subunit alpha 1, B. indicus complement factor H, Fibrinogen alpha chain, Glycoxalase 1, Aldo keto reductase, UDP glucuronosyltransferase, Uncharacterized, Aldolase, Acyl-CoA dehydrogenase, Proteosome subunit alpha 2, CXXC repeat containing interactor of PDZ3 domain (CRIPT). This is the first observation of ASIE reported in Brahman cattle. Further studies on more tissues and examining the relationship between ASIE and ASE are currently underway.

Animal Forensic Genetics Posters

P110 SNP marker combination for discrimination of Korean native chickens using a machine learning model. S. Cho*[1], D. Seo[1,2], M. Kim[2], E. Cho[3], P. Manjula[1], T. Kalhari[2], and J. Lee[1,2], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea,* [2]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea,* [3]*Department of Bio-big data, Chungnam National University, Daejeon, Republic of Korea.*

Korean native chicken (KNC) is a unique genetic resource in Korea and an important pure breed for the conservation and development of commercial native chicken. KNCs are classified into 5 lines according to their plumage colors. This study aims to develop an optimal SNP marker combination that can distinguish KNCs from other chicken breeds. The 600K high-density SNP chip data from 443 Korean native chicken purebred (NG: 81, NL: 70, NR: 115, NW: 90, NY: 87) were used and compared with the SYNBEED SNP data set. A total of 400 candidate markers for breed identification were selected by case-control genome-wide association test using PLINK 1.9 software from 544,830 quality-controlled SNPs. For the candidate markers selection, one SNP per block with an interval of 50 haploblocks was considered based on the linkage disequilibrium (LD) information. After filtering the candidate markers through the feature selection process by the random forest machine learning model, 40 SNP markers were finally selected as the minimum number of marker sets. To evaluate the discrimination power of selected markers, total data were divided into 70% for training and 30% for test data sets. A total of 8 machine learning models were tested to derive and evaluate their respective accuracy levels. As the results, all the tested models confirmed more than 99% of accuracies. In particular, 100% sensitivity was obtained in all machine learning models. Therefore, the selected SNP marker combi-

nation can provide an effective genomic identification tool for the KNC native chicken lines.

Key Words: breed identification, Korean native chicken, machine learning, single nucleotide polymorphisms

P111 Development of a 14-short tandem repeat panel for forensic DNA analysis of red fox. A. E. Hrebianchuk*[1], N. S. Parfionava[1], V. N. Lukashkova[1], S. A. Kotava[1], and I. S. Tsybovsky[2], [1]*Scientific and Practical Centre of the State Forensic Examination Committee of the Republic of Belarus, Minsk, Republic of Belarus,* [2]*Republican unitary service enterprise "BelJurZabespechenne", Minsk, Republic of Belarus.*

The study of samples of animal origin for forensic purposes of the scientific and practical center conducts in the investigation of the facts of illegal hunting, theft of domestic animals or animal abuse. The development of a test system for the DNA identification of individuals of the red fox was carried out on the basis of short tandem repeat (STR) loci developed for the red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*) and the domestic dog (*Canis lupus familiaris*). The sample included 242 foxes from all regions of the Republic of Belarus. As a result of statistical analysis, we formed a 14-locus panel, including 3 loci of species affiliation (internal control for differentiation of individuals of a dog, wolf, raccoon dog, which can be found on material evidence), 9 identifying loci and 2 markers for sex determination. In the analyzed STR loci, allelic diversity ranged from 5 to 21 alleles. In total, 111 alleles we were identified in this study. To calculate the efficiency of the panel for individual identification and paternity testing, we estimated the cumulative probabilities of parentage exclusion, when 1 parent is known (CPE1), when 2 parents are known (CPE2), the combined power of discrimination (CPD), and the combined probability of identity (CPID; theoretical). The cumulative probabilities of parentage exclusion CPE1and CPE2 for 14 loci averaged 0.888 and 0.932, respectively. The power of discrimination for each marker showed high values, and varied from 0.825 to 0.979, CPD values were near 1.0 for this panel. The theoretical estimates of CPID for 14 markers were7.95×10^{-13}. The test system is validated according to the SWGDAM protocol and tested on collection samples of red fox and on real forensic objects. The developed test system will be used in expert practice when investigating the facts of illegal hunting in the Republic of Belarus.

Key Words: dogs and related species, forensics, short tandem repeat (STR) profiling

P112 Genetic profiling of horses in forensic cases. A. Fornal*, K. Kowalska, T. Zabek, A. Piestrzynska-Kajtoch, and K. Ropka-Molik, *Department of Animal Molecular Biology, National Research Institute of Animal Production, Balice, Poland.*

DNA from a crime scene does not always have to come from a human. Animal DNA can also be evidence in a case. Forensic laboratories in human crime cases are highly specialized units, however, in cases involving animals, they may not have enough information, tools and experience to identify genetic evidence from animals. Horse pedigree testing and individual identification can be a valuable tool in forensics and in cases of mysterious horse death. In the presented cases, we used biological traces like hair follicles and blood stains (materials were collected at the crime scenes), and animal remains like macerated hoof found in the ground. Biological traces had various quality. We obtained DNA with different degree of degradation. The hoof was well-preserved, however, we received DNA only from the hoof wall but not from the solar surface of the hoof. DNA isolated from biological traces and the hoof was used for DNA profiling of horses (we used 17 microsatellite markers for routine equine genotyping). Determining the DNA profile of a horse from biological material from a crime scene can be difficult and is dependent mainly on the quality of the biological material collected. The degree of degradation of the biological material can impede testing. Statistically, for biological traces of horses in our lab, 77.08% of the core set markers and 83.72% of the additional set markers were obtained for all materials (regardless of material quality). Biological trace analysis is usually feasible and allows for at least a minimal set of microsatellite loci to establish a concordance with a comparison sample. The chances of success in obtaining a DNA profile from animal remains (like a hoof or tooth) largely depends on the degree of decomposition of the material tested. Isolation of DNA from such material as a hoof wall is sufficient. We have obtained a complete genetic profile from DNA isolated from hoof wall. Based on herd structure analyses, we successfully selected the parental pair of the missing offspring and identified the remains as missing foal. A study of the cases presented demonstrates that DNA profiling of horses can be a valuable tool in criminal cases and missing individual cases in wild or semi-wild horse herds.

Key Words: pedigree testing, horse

Applied Genetics and Genomics in Other Species of Economic Importance Posters

P113 Estimation of inbreeding load and purging in animal conservation programs. N. Pérez-Pereira*[1], E. López-Cortegano[1,3], A. García-Dorado[2], and A. Caballero[1], [1]*Centro de Investigación Mariña, Universidade de Vigo, Vigo, Spain,* [2]*Universidad Complutense de Madrid, Madrid, Spain,* [3]*Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK.*

The inbreeding load; that is, the genomic load of partially recessive deleterious mutations in the heterozygote state, is assumed to be the main source of inbreeding depression in inbred populations and a key parameter in conservation genetics and animal breeding. In turn, genetic purging can mitigate the magnitude of inbreeding depression by removing deleterious mutations as the inbreeding load is exposed in homozygosis. Methods aimed at estimating the inbreeding load and the purging coefficient (which measures the magnitude of purging) in pedigreed populations have been developed assuming random mating and variable contributions from parents to progeny. However, in conservation and animal breeding programs it is common to manage matings and the contribution from parents to progeny to reduce the impact of inbreeding and to preserve genetic diversity, usually by equalizing contributions and avoiding inbred matings. Other strategies propose instead to carry out some degree of inbreeding to enhance purging. Thus, different mating systems may differ substantially in the intensity of purging. Using computer simulations, we tested the accuracy of the estimates of the inbreeding load and the purging coefficient, both obtained with the software PURGd, for pedigreed populations under equalization of contributions, partial full-sib mating and circular mating. Accurate estimates were obtained for each of these scenarios which, together with the corresponding predictions of the inbreeding coefficient and the variance of contributions, allowed the prediction of fitness over time to be obtained considering the effects of purging.

Key Words: inbreeding depression, inbreeding load, fitness, deleterious mutations, genetic purging

P114 Common quail identification by mitochondrial and nuclear DNA analysis. L. Borreguero*, M. Hernandez, M. R. Maya, A. Trigo, T.

Mayoral, and J. A. Bouzada, *Laboratorio Central de Veterinaria, Algete, Madrid, Spain.*

Common quail (*Coturnix coturnix*) and Japanese quail (*Coturnix japonica*) are species difficult to differentiate by their phenotypic characteristics. Common quail is a protected migratory bird of European countries, whereas Japanese quail is a very valued poultry bird because of its high-quality meat and eggs. Restocking the common quail population with Japanese quail is forbidden in Spain because it may cause the disappearance of autochthonous quails. To prevent the widespread of Japanese quail and the disappearance of common quail in its natural ecosystem, it is important to stablish a method able to determine the genetic origin of each quail in restocking farms. Identification Department of Laboratorio Central de Veterinaria have developed a method based in mitochondrial and nuclear DNA analysis to differentiate *Coturnix coturnix* from *Coturnix japonica*. The method consists of extracting DNA from feathers of quails, analyzing mitochondrial DNA by FINS, and analyzing nuclear DNA by STR. Mitochondrial DNA was amplified using PH-H521 and DLOOPLONG-REV primers; and nuclear DNA was analyzed amplifying the following microsatellite markers: MCW118, MCW252, MCW280, UB0004, UB0005, GUJ01, GUJ17, GUJ28, GUJ44, GUJ57 and GUJ85. The results obtained after the analysis of 250 samples confirm the presence of samples proceeding form *Coturnix coturnix* animals, and helps us to determinate the presence of hybrid animals between *Coturnix coturnix* and *Coturnix japonica*. Following this method, we were able to classify each control sample in its corresponding group.

Key Words: *Coturnix*, species, FINS, microsatellite

P115 Evaluation of population structure alpacas maintained in Poland and identification of alpaca-llama hybrids based on microsatellite markers. A. Podbielska*[1], K. Piórkowska[1], and T. Szmatola[1,2], [1]*Department of Animal Molecular Biology, National Research Institute of Animal Production, Balice, Poland,* [2]*Center for Experimental and Innovative Medicine, University of Agriculture in Krakow, Kraków, Poland.*

The study aimed to characterize the alpaca population structure maintained in Poland using 17 microsatellite markers recommended by the International Society for Animal Genetics. The classification of llamas, alpacas, or hybrids based on phenotype is often difficult due to long-term hybridization and not keeping the species purebred. Although, their taxons were separated a long time ago into *Lama glama* and *Vicugna pacos*. Moreover, alpacas are frequently purchased without certificates of pedigree registration (price factor), and llama admixture is revealed in the following generations. According to our knowledge, the present study, for the first time, assesses the population structure of alpacas bred and maintained in Poland. The hair follicle and buccal swabs were taken from 234 animals. Among them were 216 alpacas, 15 llamas, 1 control llama-alpaca hybrid and 2 potential hybrids. Llama samples were collected as a control group. DNA was extracted using Sherlock AX by A&A Biotechnology following the suggested manufacturer protocol. Seventeen microsatellite markers were analyzed: *LCA5*, *LCA8*, *LCA19*, *LCA37*, *LCA56*, *LCA65*, *LCA66*, *LCA94*, *LCA99*, *LGU49*, *LGU50*, *YWLL44*, *YWLL29*, *YWLL36*, *YWLL40*, *YWLL43-X*, and *YWLL46*. The genetic structure of the studied populations was investigated using Structure 2.3.4 software with a burn-in period of 100,000 and 200,000 iterations fitting K from 1 to 4 with 10 runs for each K. The Structure Harvester was used to select the best K and to visualize the best K graphically Clumpak program was used. Obtained results showed that microsatellite markers can distinguish alpacas from llamas, while Structure software analysis indicated the level of admixture of one species in another. Moreover, it was observed that purebred alpacas are those for which $q \geq 0.98$; in turn, alpacas with an admixture of llama constituted 8.8% of the tested individuals, the first-generation hybrid had only 7.4% llama admixture, and the level of this admixture was due to random allele segregation.

Key Words: alpaca, hybrid, microsatellite markers, population structure

P116 Identification of polymorphism in the *MC1R* gene in Polish pastel foxes: Preliminary research. G. Smolucha*[1], A. Koseniuk[1], and P. Bielanski[2], [1]*Department of Animal Molecular Biology, National Research Institute of Animal Production, Balice, Poland,* [2]*Department of Small Livestock Breeding, National Research Institute of Animal Production, Balice, Poland.*

According to the available literature, there are about 35–45 different species of foxes in the world. This species occurs on most continents and inhabits various environments, thanks to which many common fox ecotypes have developed, differing mainly in the color of the coat, body size, and fur quality. The occurrence of foxes with a rare coat color is related to spontaneous, lasting for generations, mutations within a certain population occurring in a given area. The genetic basis of coat color in mammals is complex and it is believed to be influenced by more than 350 genes. Among those genes, the most studied is *MC1R* (The melanocortin 1 receptor gene), which encodes a membrane-bound receptor protein. *MC1R* plays a significant role in melanogenesis by controls eumelanin (brown/black pigment) and pheomelanin (red/yellow pigment production). The *MC1R* is highly polymorphic and genetic variations are important determinations of hair color in mammals. There has not been identified the genetic basis of the beige-colored variety called pastel Polish so far. The pastel color is genetically determined by the homozygous arrangement of 2 bb recessive genes. Heterozygotes - carriers of this gene [Bb] have the silver fox genotype and do not differ phenotypically from homozygotes [BB]. This work aimed to characterize a CDS (Coding DNA Sequence) of the *MC1R* gene and its mutations associated with coat color in pastel foxes. A total of 21 individuals including 17 pastel foxes, 2 platinum foxes, 2 foxes carrying pastel gene were studied in this preliminary research. To explore variation in pastel foxes a 954bp fragment of *MC1R* gene containing a single exon was analyzed using PCR, Sanger sequencing, and capillary electrophoresis. According to the reference sequence, KJ48905058 total of 7 SNPs were detected: c.124A>G, c.289A>G, c.312C>T, c.373T>C, c.633T>C, c.648G>C, c.745A>T. All identified mutations occur in pastel colored foxes and platinum foxes which suggests that the pastel color induced mutation is not located in the CDS of the *MC1R* gene. Those findings may be helpful in future studies of the genetic basis of pastel coat color in foxes.

Key Words: fox, SNP, MC1R, color, coat

P117 Genomic structure in a divergent selected mice population for birth weight variability. C. Ojeda-Marin*[1], K. Arias[1], L. El-Ouazizi[1], N. Formoso-Rafferty[2], J. P. Gutiérrez[1], and I. Cervantes[1], [1]*Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain,* [2]*Departamento de Producción Agraria, E.T.S.I.A.A.B, Universidad Politécnica de Madrid, Madrid, Spain.*

A divergent selection experiment for birth weight environmental variability in mice has been successfully performed during 25 generations. Selection for low variability has been shown to be beneficial for animal production, welfare and robustness related traits. The aim of this study was to analyze the genomic structure and the inbreeding of both divergent lines: high variability (H-line) and low variability (L-line), using genomic relationship. A total of 1,224 animals genotyped using the Affymetrix Mouse Diversity Genotyping Array were used (613 H-line and 611 L-line). After quality control and MAF lower than 0.05 in base population; 191,630 SNPs were kept. The genomic relationship matrix was decomposed in eigenvectors and molecular inbreeding was computed using different genomic matrices: Nejati-Javaremi allelic relationship matrix (FNEJ), the Li and Horvitz matrix based on excess of homozygosity (FL&H), 2 VanRaden (FVR1 and FVR2) and Yang (FYAN). The genealogical pedigree was computed using ENDOG software. Also, the molecular effective population based on multilocus genotypes was computed using Colony software. The 5 most important eigenvector explained 28% of the total variance. The first eigenvector explained 13% of the variability and the divergence between both lines. Meanwhile, the second eigen-

vector explained 11%, being highly related to the inbreeding trend. The pedigree inbreeding was 32% for H-line and 30% for L-line in the last generation. The correlation between molecular inbreeding and pedigree inbreeding ranged from 0.82 to 0.87 across generations, being the highest value for FYAN. This molecular inbreeding was 27% in both lines in the last generation. The molecular effective population size captured the mating system being 40 in H-line and 39 in L-line in average of 40 in H-line and 39 in L-line across the last 10 generations These values were close to those obtained via pedigree (37 H-line and 39 L-line). Molecular information faithfully matched the structure and the effective population sizes originated by the selection process.

Key Words: genomic structure, divergent selection, molecular inbreeding, effective population size

P118 Differential gene expression reveals functional differences between selection strategies and generations in early mass-reared black soldier fly colonies. K. L. Hull*, M. P. Greenwood, A. E. Bester-van der Merwe, and C. Rhode, *Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

The micro-evolutionary forces that shape genetic diversity during the initial phases of domestication have been well-studied in many plant and animal systems. However, the impact of these processes on gene expression variation remains an understudied area that may provide insight into the functional drivers of adaptation. This study aimed to assess whole-transcriptome dynamics associated with the early stages of domestication in the black soldier fly (BSF), *Hermetia illucens*. Differential gene expression was evaluated with respect to the impact of (i) 2 selection strategies [no selective pressure (NS); and selection for greater larval mass (SEL)], and (ii) generational time within the cultured environment (F2 vs F3). RNA-seq was conducted on fifth instar BSF larvae (n = 46), representing equal proportions of the NS (F2 = 10; F3 = 13) and SEL (F2 = 9; F3 = 14) groups. Reads were mapped against a publicly available BSF reference genome in a 2-step approach, with resulting count data undergoing normalization before differential gene expression analyses. Between selection strategies, 2,240 and 2,764 genes were up- and downregulated, respectively (FDR-corrected $P < 0.1$), while between generations, 1,544 and 3,354 genes were up- and downregulated, respectively (FDR-corrected $P < 0.1$). A principal components analysis revealed greater gene expression variability within the NS and F2 subgroups, while the SEL group clustered separately with lower levels of variation being observed, most likely as a consequence of artificial selection for larval mass. Patterns of differentially expressed genes revealed cuticle formation factors were primarily upregulated in NS individuals, while genes involved in innate immunity, heme binding, eye pigment biosynthesis, and protein modification were highly expressed in SEL groups. The upregulation of genes involved in innate immune responses, fatty acid metabolism and growth were associated with the transition from the F2 to F3 generation. This suggests that indirect selective pressures of the captive environment may drive temporal shifts in the genetic function of BSF colonies.

Key Words: animal domestication, bioinformatics, gene expression, RNA-seq, selection

Applied Genetics of Companion Animals Posters

P119 Development of highly informative SNP panel for parentage assessment in dogs. K. R. Gujjula*, H. Suren, A. Burrell, and S. Chadaram, *Thermo Fisher Scientific, Austin, TX, USA.*

AgriSeq targeted genotyping-by-sequencing (GBS) is successfully being used as a high-throughput, customizable and cost-effective genotyping solution in animal breeding, parentage testing, and genetic purity testing. Traditionally, parentage identification was performed with microsatellites markers, also known as short tandem repeats (STRs). Genetic testing with STRs is difficult in closely related sires due to limited allelic variation, these minor variations in allele sizes are difficult to capture. For parentage determination and identification of canines, we have developed the Applied Biosystems AgriSeq panel containing 394 markers, including 386 autosomal markers and 8 sex chromosome markers. These markers were contributed by groups in canine parentage testing (Neogen, Orivet, and Vetgenomics) and contain all 233 ISAG recommended parentage markers. To evaluate the information content of the panel, we combined the published genotype data from 7,381 unique dog samples. Further, we retained only those breeds which had more than 20 uniquely genotyped samples which resulted in 83 breed group representations. For each SNP marker, we calculated allele frequencies within a breed only if the "marker x breed" combination had at least 20 unique genotyped samples which resulted in confident allele frequency estimation. Per breed 290 markers, on average, met the allele frequency estimation criteria, the lowest being 237 markers and the maximum being 382 markers. The results show that per breed 134 markers, on average, had a MAF (minor allele frequency) ≥0.30. The mean MAF for markers per breed varied from 0.15 to 0.35, with an average of 0.26. Out of 83 breeds, 79 breeds had a mean MAF for markers above 0.20. Another observation for SNPs with a MAF ≥0.30 is that the minor allele nucleotide is often different among breeds. The panel also contains 8 sex determination markers, 4 out of 8 are XY-based markers and 4 are Y-based markers. The panel evaluation shows that the markers are highly informative for use in parentage testing and traceability. For Research Use Only. Not for use in diagnostic procedures.

Key Words: canine, AgriSeq, ISAG, parentage, genotyping by sequencing (GBS)

P120 Breed, trait, locus, and allele nomenclature standardization for the domestic cat. L. A. Lyons*, *College of Veterinary Medicine, University of Missouri, Columbia, MO, USA.*

The X-linked coloration in domestic cats, the *Orange* locus, represents one of the first loci assigned to a chromosome in the field of genetics. Considering new traits under novelty selection for cat breed development, overall cats have approximately 30 phenotypic loci, including 24 genes and 55 allelic variants. Early coloration and coat length variants for the loci, *A* (*Agouti*), *B* (*Brown*), *C* (*Color*), and *L* (*Long hair*), followed the nomenclature of mice coat color phenotypes, indeed demonstrating cat phenotypic variants in homologous genes for *ASIP*, *TYRP1*, *TYR*, and *FGF5*, respectively. However, some allelic variants have been incorrectly assigned. For example, *Hairless* (*Hr*) was assigned to the nude Sphynx phenotype as like the nude mouse, but Sphynx hairless is actually a variant in the gene *KRT71*. True *Hr* variants have been more recently identified for a novelty breed called Lykoi. In addition, cats have patterning loci that are not represented in mice, such as *LVRN* variants for the *Tabby* locus and *DKK4* variants for the *Ticked* locus. Therefore, a need for nomenclature standardization and reconciliation is required as cat breeders often use terms that are inconsistent with the scientific literature and representing non- homologous loci across species. Commercial testing services also should be using a standardized nomenclature so that researchers, testing laboratories and cat breeders can all be making the same interpretations regarding cat phenotypes. To support standardization of genetic nomenclature in the cat, a group of researchers focused on cat studies, as well as breeders and cat judges with scientific backgrounds, have formed a working group to define loci and allele nomenclature, as well as breed nomenclature, for the domestic cat. The committee is reviewing the scien-

tific literature, specifically of other mammals, and will be using standards set by other nomenclature committees to define cat loci and variant alleles. Novel nomenclature rules are being established in cats to consider alleles from different species, which occurs in popular hybrid cat breeds, such as the Bengal, which are crosses of Asian leopard cats (*Prionailurus bengalensis*) and domestic cats (*Felis silvestris catus*). A table of historical and standardized nomenclature will be presented for the domestic cat for phenotypic traits and blood type.

Key Words: *Felis*, feline, domestic cat, coat color, nomenclature

P121 Supplementation of the AgriSeq Canine SNP Parentage and ID Panel with additional ISAG and sex determination markers. A. Burrell*, K. Gujjula, H. Suren, and R. Conrad, *Thermo Fisher Scientific, Austin, TX, USA.*

Parentage testing and genomics-assisted breeding are critical aspects of successful veterinary management. Due to its highly accurate and reproducible results, targeted GBS is becoming an increasingly favored technology for SNP genotyping. With the utilization of next-generation sequencing, labs can test hundreds of samples across thousands of SNPs simultaneously in a simple high-throughput workflow starting from either extracted nucleic acid or crude lysis samples. The AgriSeq Canine SNP Parentage and ID Panel, released in 2019, is an amplicon-based next-generation sequencing panel for parentage determination in dogs. In 2020 ISAG finalized the list of 233 recommended markers for canine parentage determination. The final recommendation contained 5 autosomal and 3 sex chromosome markers not present on the original AgriSeq panel. The AgriSeq Canine SNP Parentage and ID Panel was quickly updated to include not only the 8 missing markers, but 5 additional sex determination markers to ensure robust and repeatable sex determination results. The final panel contains 392 SNPs, including 4 markers targeting both the X and Y chromosome and 4 markers targeting the Y chromosome exclusively, and 2 deletions. Utilizing the AgriSeq HTS Library Kit, a high-throughput targeted amplification and resequencing workflow, performance was validated on a panel of >100 samples. Libraries were sequenced on the Ion S5 using an Ion 540 chip with genotyping calling generated using the Torrent Variant Caller (TVC) plugin. Mean call rates, the percentage of markers on a panel generating a genotype call, was >98%. Concordance with orthogonal testing, including the Axiom Canine HD array and CE sequencing, was >99.4%. Sex determination accuracy was 100% for all samples tested and parentage determination was accurately assigned when testing the ISAG Comparison test samples. The data demonstrates the utility of the AgriSeq targeted GBS approach for canine parentage and sex determination applications. For Research Use Only. Not for use in diagnostic procedures.

Key Words: AgriSeq, parentage, genotyping by sequencing (GBS), next-generation sequencing (NGS), genotyping

Avian Genetics and Genomics Posters

P122 Genetic diversity and population structure of Myanmar native chickens using double digest restriction-site associated DNA sequencing (ddRAD-seq). S. L. Y. Mon*[1], M. Lwin[2], A. A. Maw[3], L. L. Htun[3], S. Bawm[3], K. Kawabe[4], Y. Nagano[5,1], A. J. Nagano[6], Y. Wada[5,1], S. Okamoto[1], and T. Shimogiri[1], [1]*The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan,* [2]*Livestock Breeding and Veterinary Department, Yangon, Myanmar,* [3]*University of Veterinary Science, Nay Pyi Taw, Myanmar,* [4]*Education Center, Kagoshima University, Kagoshima, Japan,* [5]*Faculty of Agriculture, Saga University, Saga, Japan,* [6]*Faculty of Agriculture, Ryukoku University, Otsu, Shiga, Japan.*

Myanmar native chickens are main protein source in Myanmar. The breeding progress must be promoted to meet the demand of the increasing human population. However, the genetic information on Myanmar native chickens is limited. Therefore, in this study, we investigated the genetic diversity and population structure of Myanmar native chickens using ddRAD-Seq. A total of 343 chicken and junglefowl genomic DNA samples consisting of 9 Myanmar native populations (n = 171), red junglefowls (n = 17), 7 Asian native populations (Bangladesh, Cambodia, China, Indonesia, Japan, Laos, Thailand) (n = 95), and 4 commercial chickens (Barred Plymouth Rock, broiler, layer, Rhode Island Red) (n = 60) were used. The ddRAD-Seq was conducted as described by Sakaguchi et al. (2015). Genomic DNAs were digested with *Bgl*II and *Eco*RI. Sequencing was done using HiseqX. The SNPs were called with Stacks v2.53, filtering SNPs over 80% matching samples, cut off 0.05% minor allele frequency. Genetic diversity indices such as expected (HE) and observed heterozygosity (HO) were calculated using Plink v1.07. Principal component analysis (PCA) was drawn by SNPRelate R package. Population structure was analyzed by Admixture v1.3. We used 20,993 filtered autosomal SNPs for analysis. The HE and HO of 21 chicken populations ranged from 0.144 and 0.145 in Japan (JP) to 0.277 and 0.261 in YGN. Those of Myanmar native populations ranged from 0.255 and 0.241 in FCN to 0.277 and 0.261 in YGN, showing higher genetic diversities. Two-dimensional plot of the first 2 PCs revealed that commercial and JP chickens were clearly differentiated from native chickens and red junglefowls. Population structure analysis suggested appropriate 8 clusters for 21 populations. Four commercial and JP populations formed the respective homogeneous clusters. The remaining 3 clusters were observed in native chickens and red junglefowls: The first cluster was homogenously grouped by red junglefowls and China, second by the fighting chickens. The third was distributed only in Myanmar native populations in varying proportions.

Key Words: animal breeding

P123 Annotation of full-length transcripts including alternative splicing from 19 chicken tissues using Oxford Nanopore long-read sequencing. D. Guan*[1], M. M. Halstead[1], A. D. Islas-Trejo[1], D. E. Goszczynski[1], H. H. Cheng[2], P. Ross[1], and H. Zhou[1], [1]*Department of Animal Science, University of California–Davis, Davis, CA, USA,* [2]*Avian Disease and Oncology Laboratory, USDA-ARS, East Lansing, MI, USA.*

Alternative splicing of transcripts is a major factor affecting phenotypic variability of farm animals. Thus, it is important to obtain a comprehensive annotation of transcript isoforms across tissues to enhance precise genetic improvement. In this study, we utilized Oxford Nanopore Technology (ONT) to identify and annotate full-length transcript and alternative splicing in diverse chicken tissues derived from 68 biological samples, comprising 19 tissues from adult males and females of a line 6 × line 7 F_1 cross. ONT sequencing from a single flow cell resulted in more than 19.4 million unique mapped reads with mean read length of 648 bases, and average quality of 18.2. Using the StringTie computational pipeline, we annotated 79,885 transcripts with mean length of 1,664 bases at 54,590 genetic loci, representing ~1.5 transcripts per locus. There were 48,464 multi-exon and 31,421 single-exon transcripts. Compared with the Ensembl database (GRCg6a version 102), we annotated 2.7- and 3.3-fold more transcripts and gene loci, respectively. The sensitivity and precision of our annotation were 38.8% and 14.5% higher at the transcript level, and 57.7% and 17.5% higher at the locus level, respectively. Among them, 14.6% (11,624) fully matched to the reference, 35.4% (28,276) partially matched to known genes, and 50% (39,985) annotated transcripts had no match. Further analyses are underway to identify tissue-specific novel isoforms and their respective biological functions in chickens. In summary, identification of transcript isoforms across diverse tissues has significantly

improved the annotation of the chicken genome, and provides important knowledge in connecting genotype to phenotype in livestock species.

Key Words: full-length transcript, alternative splicing, long-read sequencing, annotation, chicken

P124 Serum creatine kinase as a biomarker to predict wooden breast and white striping on live broilers. F. Kong, Z. He, J. Sun, R. Liu*, and J. Wen, *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

The present study aimed to find a blood marker for wooden breast (WB) and white striping (WS) prediction to assist genetic selection of fast-growing chickens. The experiments were carried out with 2 chicken flocks. The 150 male broilers of flock 1 and 100 male and female broilers of block 2 were slaughtered and measured. The breast fillets were assessed by combining subjective scoring and compression force at 28 (only flock 1) and 42-d-old. The enzyme activity in serum and breast tissue (only flock 1) of normal and moderate affected groups was tested. The results showed the compression force was significantly different between the normal and affected group at 28 and 42 d ($P < 0.001$), and it increased significantly with the rising of WB score and WS score. The serum creatine kinase (CK) value increased significantly with the compression force rising at 42-d-old ($P < 0.001$). The serum CK positively correlated with compression force ($r = 0.608$; $P < 0.001$) and could be used as biomarker to predict the degree of WB and WS on live birds. The association of serum CK and compression force is consistency between flock 1 and 2. In conclusion, our study indicated that compression force could be an objective indicator to differentiate breast fillets and serum CK could be a candidate biomarker to assist WB and WS prediction to assist genetic selection in fast-growing broilers breeding.

Key Words: creatine kinase, wooden breast, white striping, genetic selection, broiler

P125 Hypothalamic and ovarian transcriptome profiling reveals potential candidate genes in low and high egg production of White Muscovy ducks (*Cairina moschata*). S. Bello*, H. Xu, and Q. Nie, *South China Agricultural University, Guangzhou, Guangdong, China.*

In China, the low egg production rate is a major challenge to Muscovy duck farmers. Hypothalamus and ovary play essential roles in egg production of birds. However, there are few reports in these tissues to identify potential candidate genes responsible for egg production in White Muscovy ducks. A total of 1,537 laying ducks was raised; the egg production traits which include age at first egg (days), number of eggs at 300days and number of eggs at 59 weeks were recorded. Moreover, the 4 lowest (LP) and 4 highest producing (HP) were selected at 59 weeks of age, respectively. To understand the mechanism of egg laying regulation, we sequenced the hypothalamus and ovary transcriptome profiles in LP and HP using RNA-seq. The results showed that the number of eggs at 300 days and number of eggs at 59 weeks were significantly higher ($P < 0.001$) in HP than in LP ducks. In total, 106.98G clean bases were generated from 16 libraries with an average of 6.68G clean bases for each library. Further analysis identified 569 and 2,259 differentially expressed genes (DEGs) in the hypothalamus and ovary between LP and HP, respectively. The KEGG pathway enrichment analysis revealed 114 and 139 pathways in the hypothalamus and ovary, respectively which includes Calcium signaling pathway, ECM-receptor interaction, Focal adhesion, MAPK signaling pathway, Apoptosis and Apelin signaling pathways that are involved in egg production. Based on the GO terms and KEGG pathways results, 10 potential candidate genes (*P2RX1*, *LPAR2*, *ADORA1*, *FN1*, *AKT3*, *ADCY5*, *ADCY8*, *MAP3K8*, *PXN*, and *PTTG1*) were identified to be responsible for egg production. Further, protein-protein interaction was analyzed to show the relationship between these candidate genes. Therefore, this study provides useful information on transcriptome of hypothalamus and ovary of LP and HP Muscovy ducks

Key Words: egg production, transcriptome, gene expression, candidate genes, Muscovy duck

P126 Withdrawn

P127 Phylogenetic characterization of Yoruba ecotype and broiler chicken using 18s rRNA. D. I. Ibiwoye[1,2], F. E. Sola-Ojo[1], T. A. Adisa*[1], I. A. Abubakar[1], N. B. Afolabi-Balogun[3], C. A. Adeniyi[4], and A. O. Oni[3], [1]*University of Ilorin, Ilorin, Kwara, Nigeria,* [2]*Huazhong Agricultural University, Wuhan, China,* [3]*Fountain University, Osogbo, Osun, Nigeria,* [4]*Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China.*

Over the years, there have been intense importations of exotic breeds of chicken over indigenous chicken in Nigeria. This has caused inadequate information on the genetic background and diversity of local chicken in Nigeria which is now a major threat to the local breed which could lead to extinction of some important genetic resources which may be needed for the successful production of our local breed. To improve indigenous chicken productivity, genetic information as well as genetic variation among different breeds has to be identified to avoid depletion setting in and extinction of species. To prevent the total depletion of these indigenous chickens, their evolutionary pattern was studied. The 18s rRNA gene has been incriminated to be widely used in molecular analysis to reconstruct the evolutionary history of organisms, especially vertebrates because of their slow evolutionary rate, thus making it suitable for reconstruction of ancient divergences. This study was to evaluate the phylogenetic variation between yoruba and broiler their molecular characterization, their relationship with the gene DNA size using the18s rRNA small subunit gene which is an important marker for the random target PCR in environmental biodiversity screening. The component exons of the local and exotic chicken bases (5′ and 3′) were amplified. It was sequenced and aligned against each other and against NCBI outputs from published orthologs spanning Aves, Mammals, Chordata, and other vertebrates. Phylogenetic tree was constructed from the aligned sequences. Regulatory motifs from the aligned sequences were identified. The total numbers of nucleotide bases were gotten from the Genomics % GC content calculator and the guanine to cytosine content was obtained as 41.8 for broiler and 52.5 for Yoruba. Polymorphism was detected from the sequence result obtained from the breeds. Evolutionary relationship was observed between the local and exotic breed from the phylogenetic tree, and they were closer to the *Melopstittacus undulatus* (budgerigar), *Saccharomyces cerevisiae* (brewer's yeast) and *Drosophila melanogaster* (common fruit fly) in their ancestry line. This closeness shows that the breeds are related along their mothering line with use of the 18s rRNA gene.

Key Words: gene, 18s rRNA, polymorphism, chicken, evolutionary relationship

P128 Withdrawn

P129 Estimation of genetic diversity in Muscovy duck found in Kwara State, Nigeria, using cytochrome P450 family 2 subfamily U member 1 (*CYP2U1*) mitochondrial gene. F. E. Sola-Ojo[1], C. A. Adeola[2], O. A. Yusuf[1], and A. R. Adekoya*[1], [1]*University of Ilorin, Ilorin, Kwara State, Nigeria,* [2]*State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences., Kunming, China.*

Over the past years, different types of molecular markers have been used to advance the study of genetic diversity within and between Muscovy duck populations. The domestic Muscovy duck (*Cairina moschata*) is an economically important species that is characterized with considerable variation in their phenotypic characteristic; they are raised by duck lovers and known around the world for its unique meat taste and low-caloric

content. In this study, genetic diversity and phylogenetic relationship of 13 Muscovy duck populations found among duck farmers in some part of North Central Nigeria (Ilorin, Kwara State, Nigeria) notably Oke-Oyi (KEY), Adeita (ADE), Idofian (IDO) were sequenced using 747-bp long fragment of mtDNA spanning the Cytochrome P450 Family 2 Subfamily U polypeptide 1 (CYP2U1) genes. A total of 18 variable sites were reported with the polymorphic site consisting of substitutions with 17 singletons variables. The haplotype diversity of each sampling locations are ADE (0.833), KEY (0.714) and IDO (1.000) respectively and this shows close relationship among ADE and KEY and also how far IDO is to ADE and KEY. The population contributes 8 haplotypes, 0.00388 of nucleotide diversity per site (Pi), 18 total mutations with parsimony informative site of 1. The population study shows haplotype (gene) diversity of (71.8%) which indicates high genetic variation within population and a reflection that the gene polymorphisms and genetic diversity were high. This study shows the suitability of mtDNA *CYP2U1* gene in genetic diversity evaluation.

Key Words: Muscovy duck, haplotype diversity, *CYP2U1*, nucleotide diversity

P130 Proteome profile of chicken cecum in the response to *Salmonella* enteritidis inoculation. X. Miao, H. Li, L. Liu, L. Liu, Y. Zhao, and X. Li*, *Shandong Agricultural University, Tai'an, Shandong, China.*

Salmonella enterica serovar Enteritidis (SE) is a food-borne pathogen, which can cause great threat to human health through consumption of the contaminated poultry products. Chicken is the main host of SE. This research combines TMT labeling, high performance liquid chromatography (HPLC)and mass spectrometry-based proteome on cecum of the F1 cross of Guangxi Yao chicken and Jining Bairi chicken. The treated group was inoculated with 0.3 mL inoculum of 6.37×10^7 cfu/mL SE, and the control group was inoculated with 0.3mL PBS. A total of 6,117 proteins were identified in the current study, of which 4,995 proteins were expressed at least in one sample. There were 315 proteins significantly expressed based on threshold of $|\log_2 \text{fold change}| > 1.2$ and $P < 0.05$. Among those significantly expressed proteins, 166 proteins were upregulated, and 149 proteins were downregulated. Functional analysis was performed for those significantly expressed proteins based on Gene Ontology (GO), protein domains, KEGG pathways and subcellular localization. The upregulated proteins were mainly enriched in lipid transport, activation of immune response, defense response to bacterium, protein activation cascade in GO analysis. Protein domains were enriched in MHC class I α chain, alpha1 alpha2 domains, MHC class I-like antigen recognition-like. PPAR signaling pathway, Vascular smooth muscle contraction were enriched following SE inoculation. The downregulated proteins were mainly enriched in positive regulation of cytokine production, fatty acid synthase activity in GO analysis. The domain was enriched in Fatty acid synthase, domain 2, Phosphopantetheine binding ACP domain, Globin-like. Proteins were gathered in Fatty acid biosynthesis, Metabolic pathways, Retinol metabolism, Fatty acid metabolism in KEGG pathway analysis. The results herein will provide a crucial theoretical foundation to understand the molecular mechanism of response to SE inoculation in chicken.

Key Words: chicken, *Salmonella* Enteritidis, cecum, proteome

P131 Causative variants associated with oculocutaneous albinism genes in Yeonsan Ogye chicken. E. Cho*[1], M. Kim[2], P. Manjula[3], S. Cho[3], T. Kalhari[2], D. Seo[2,3], and J. Lee[1,2], [1]*Department of Bio-big data, Chungnam National University, Daejeon, Korea,* [2]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Korea,* [3]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea.*

The Yeonsan Ogye is a unique chicken breed in South Korea that has black feathers, skins, bones, and comb. In this population, an exceptional individual with an albinism phenotype was discovered. This study aims to search the causative mutations in this individual. Whole-genome sequences for one albinism (case) and 10 normal individuals (control) were obtained through next-generation sequencing (NGS). After the quality control and calibration steps, the data were aligned to the reference genome (*GRC6a*) as per the GATK best practices pipeline. After variant filtration and annotation by SnpEff, a total of 7,666,658 variants in the region of 38,202 genes were observed. Among the identified genes, only 6 genes, which associated with oculocutaneous albinism (OCA), namely, *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, and *LRMDA*, were initially investigated in this study. As a consequence of comparison with control, 280 causative variants associated with OCA genes were observed (*TYR*: 0, *OCA2*: 97, *TYRP1*: 110, *SLC45A2*: 57, *SLC24A5*: 27, and *LRMDA*: 49). These included mutations located in the intergenic (151), exon (4), intron (76), UTR (1), upstream (29), and downstream regions (19). In particular, the *SLC45A2* gene is harboring 2 missense mutations (c.62A > C, and c.1039C > A) and one frameshift mutation (c.459_460delGA). It is expected that the most influential causative mutation was the c.459_460delGA that changes lysine to threonine by the 2-base pair deletion located in exon 2 which leads to the responsible protein alteration. It warrants further validations to confirm this result through Sanger sequencing and gene editing in future studies.

Key Words: whole-genome sequence, oculocutaneous albinism, variant detection, Yeonsan Ogye chicken

P132 Withdrawn

P133 Withdrawn

P134 Transcriptomic analysis of the *Musculus complexus* in naked neck broiler chickens. A. C. Mott*, C. Blaschka, A. Mott, A. R. Sharifi, and J. Tetens, *Georg-August University, Göttingen, Lower Saxony, Germany.*

The locus for naked neck (*Na*) in chickens reduces feather coverage and leads to increased heat dissipation from the body surface resulting in better adaptability to hot conditions. However, the *Na* gene is linked to significantly lower hatchability due to an increased late embryonic mortality. This preliminary study analyses the transcriptome of the hatching muscle (*M. complexus)* and attempts to elucidate the reasons behind reduced hatching rates of *Na* chickens. This was carried out by the sampling of embryos at 20 d of embryonic development (ED20) from 12 chicken embryos (6 × wild-type and 6 × *Na/Na*). RNA was extracted from the *M. complexus* of each embryo using an RNeasy Plus Universal Mini Kit (Qiagen) and was sequenced using NovaSeq 6000 (Illumina) 2x50bp v1.5. Raw sequencing reads were processed for adapter removal, trimming and removal of low-quality reads. Differential expression (DE) between experimental groups was then analyzed using DESeq2 (V. 1.28.1), with volcano plots being created with R package EnhancedVolcano (V. 1.6.0). Gene set analyses were also conducted using R package clusterProfiler (V. 3.16.0). Protein interaction maps were created using STRING (V. 11.0). The results of DE analyses led to the discovery of 221 DE genes in the *M. complexus* of the experimental animals, with 51.1% downregulated in the *Na*/*Na* animals (LFC > 1, $P < 0.05$). Among those, 119 genes were of uncertain function (LOC symbols), with 80 were classified as uncharacterized. To identify potential functional protein clusters a protein interaction network analysis was performed on the top 20 up and downregulated genes. This analysis revealed 2 protein clusters: (1) FABP1, GAL8, and ORM1, and (2) CYP7A1 and AKR1D1. Through gene cluster analysis, several KEGG pathways were observed, steroid hormone biosynthesis, linoleic acid metabolism, retinol metabolism, arachidonic acid metabolism, and primary bile acid synthesis. As these pathways are essential in the development of the chicken embryo, this study indicates that the changes in regulation of these pathways could play a significant role in the increased late mortality of *Na* broilers.

Key Words: chicken, naked neck, hatchability, RNA-seq, transcriptome

P135 Research on the fine structure and admixture of the worldwide chicken population reveals connections between populations and important events in breeding history. Y. Guo*[1,3], J.-H. Ou[5], Y. Zan[5], Y. Wang[1], H. Li[4], C. Zhu[4], K. Chen[4], X. Zhou[3], X. Hu[1,2], and Ö. Carlborg[5], [1]*State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing, China,* [2]*National Engineering Laboratory for Animal Breeding, China Agricultural University, Beijing, China,* [3]*Beijing Advanced Innovation Center for Food Nutrition and Human Health, China Agricultural University, Beijing, China,* [4]*Jiangsu Institute of Poultry Science, Jiangsu Yangzhou, China,* [5]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.*

Here, we have evaluated the general genomic structure and diversity, and studied the divergence resulting from selection and historical admixture events for a collection of worldwide chicken breeds. In total, 636 genomes (43 populations) were sequenced from chickens of American, Chinese, Indonesian and European origin. Evaluated populations included wild junglefowl, rural indigenous chickens, breeds that have been widely used to improve modern western poultry populations and current commercial stocks bred for efficient meat and egg production. In-depth characterizations of the genome structure and genomic relationships among these populations were performed, and population admixture events were investigated. In addition, the genomic architectures of several domestication traits and central documented events in the recent breeding history were explored. Our results provide detailed insights into the contributions from population admixture events described in the historical literature to the genomic variation in the domestic chicken. In particular, we find that the genomes of modern chicken stocks used for meat production both in eastern (Asia) and western (Europe/US) agriculture are dominated by contributions from heavy Asian breeds. Further, by exploring the link between genomic selective divergence and pigmentation, connections to functional genes feather coloring were confirmed.

Key Words: genomic structure, admixture, selection, chickens, Asian breeds

P136 Nextflow Iso-seq (nf-isoseq) pipeline provides a first insight into the chicken transcript landscape. S. Guizard*, J. Smith, R. Kuo, K. Miedzinska, J. Smith, M. Davey, and M. Watson, *The Roslin Institute, Edinburgh, Scotland, UK.*

The EU H2020 Gene-Switch project is a collaborative endeavor that will produce new genome information to enable the characterization of genetic and epigenetic determinants of complex traits in the 2 monogastric species (chicken and pig) that are the primary sources of meat worldwide. The characterization of genome functional elements is being made through a wide diversity of analysis methods (RNA-seq, miRNA-Seq, ATAC-seq, WGBS, ChIP-seq, Iso-seq, Promoter Hi-C), on 7 tissues at 3 developmental stages (pig: 30, 70 d post-fertilization, new born and chicken: E8, E15, hatched). Functional annotation of the genome partly relies on RNA sequencing. Illumina short-read sequencing has been extensively used, as it is low cost, labor saving and rapid. However, short sequence lengths make isoform reconstruction challenging. This drawback can be overcome with long-read data. Iso-Seq, with circular cDNA sequencing, generates accurate full-length transcripts (<1% error). It allows direct identification of gene intron-exon structure and better isoform detection. This method has growing utility and has been used to re-annotate genomes but no standard pipeline has yet been released. We have thus developed the nf-isoseq pipeline, which enables insight into the chicken transcriptomic landscape. The nf-core is a community initiative working on the development of standard, portable and reproducible analysis pipelines. The pipeline generates CCS (circular consensus sequence), maps transcripts to the genome and reduces gene model redundancy with TAMA collapse. Thanks to the Nextflow language and containerization technologies, it can be easily run on a wide range of cluster configurations. We processed the Iso-seq data for each chicken sample to reveal the transcriptome for a given tissue at a given time point. Comparison of annotations gives a first look at transcriptional landscapes across tissues and the dynamic over time development. This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the grant agreement no. 817998.

Key Words: poultry and related species, bioinformatics, computational pipeline, transcriptome, meat production

P137 Genomic signatures of selection for egg production rate using whole-genome sequence in Hinaidori chickens. T. Goto*[1], S. Fukuda[2], K. Rikimaru[2], R. A. Lawal[3], J. Pool[4], and O. Hanotte[5,6], [1]*Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan,* [2]*Akita Prefectural Livestock Experiment Station, Akita, Japan,* [3]*The Jackson Laboratory, Bar Harbor, ME, USA,* [4]*University of Wisconsin-Madison, Madison, WI, USA,* [5]*University of Nottingham, Nottingham, UK,* [6]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

Phenotypic variation of egg production rate is regulated by multiple quantitative trait loci (QTLs) in chickens. Though several QTLs in Animal QTLdb affecting egg production are mostly found in commercial layers and European breeds, little is known about the genetic control of egg production rate in indigenous chicken breeds. After checking egg production rates from 671 hens in Hinaidori, a Japanese indigenous breed, we selected high egg production (65.7% in average; n = 8) and low egg production (25.8% in average; n = 8) populations. We newly collected Illumina whole-genome sequence data from Hinaidori (HNI, n = 16) and added publicly available red jungle fowl genome sequences (RJF, n = 29). Using the GATK Best Practices, 31,381,234 bi-allelic single nucleotide polymorphisms (SNPs) on chromosomes 1–33 (GRCg6a) were called. To detect signatures of selection, F_{ST} was calculated for 20-kb sliding windows (total 73,918 windows). In addition, the Population Branch Excess (PBE) statistic was calculated from 3 F_{ST} values among High-HNI, Low-HNI, and RJF to focus more directly on loci under positive selection in the focal population (High-NHI) only. We identified 87 windows (PBE >0.4) which show High-HNI-specific genetic differentiation. These windows define 24 candidate positively selected genome regions on chromosomes 1–4. These regions contain 15 protein-coding genes and 13 long noncoding RNA genes. These results support the use of population genomics approaches for the identification of candidate regions links to egg production in parallel to pedigree-based analysis.

Key Words: chicken, population genomics, egg production, genes, F_{ST}

P138 Taxonomy classification of Nigerian local turkey using 12S mitochondrial rRNA gene. D. I. Ibiwoye[1,2], F. E. Sola-Ojo[1], D. O. Aremu*[1], I. A. Abubakar[1], N. B. Afolabi-Balogun[3], C. A. Adeniyi[4], and A. O. Oni[3], [1]*University of Ilorin, Ilorin, Kwara, Nigeria,* [2]*Huazhong Agricultural University, Wuhan, China,* [3]*Fountain University, Osogbo, Osun, Nigeria,* [4]*Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China.*

Taxonomic classification play key roles in evolutionary history of animal species. They are commonly observed within several orders in wild birds. The local turkey (*Meleagaris gallopavo* f. *domestica*) is a common specie that is raised for human consumption and use. This study was conducted using 40 poults (20 males and 20 females of white and black lavenders) of 8 weeks age, blood samples were collected for DNA extraction, the component exons of the poults were amplified, it was sequenced and aligned against each other and against NCBI outputs from orthologs spanning Aves mammals, Chordata and other vertebrates. Results from the study shows some mutations in form of insertions and substitutions. The phylogenetic tree was constructed from the aligned sequences to show how closely related the local turkey with the insect and vertebrate sequences, the result shows that the male and female local turkey are closely related to *Gallus gallus*.

Key Words: taxonomic classification, 12S gene, turkey, phylogenetic tree, mutation

P139 Withdrawn

P140 Molecular characterization and occurrence of variation within the promoter region of the *CASK* gene in racing pigeons. M. Stefaniuk-Szmukier*[1], K. Piórkowska[2], K. Ropka-Molik[2], and A. Dybus[3], [1]*University of Agriculture in Kraków, Krakow, Poland,* [2]*National Research Institute of Animal Production, Balice, Poland,* [3]*West Pomeranian University of Technology, Szczecin, Poland.*

Sport pigeons have been selectively bred for superior athletic phenotypes and homing ability. Recently several studies investigated the molecular background of sport traits by the use of modern molecular techniques such as whole-genome and transcriptome sequencing. Several genes have been shown to be under positive selection such as *CASK*, *DRD4*, *AVPR1A*, and *MFSD2A.* The *CASK* gene, encoding calcium/calmodulin-dependent serine protein kinase, is a member of the MAGUK protein family. Primarily expressed in brain tissue where interact with presynaptic N-type voltage-dependent calcium channel in hippocampal neurons. However, further evidence described their appearance in cardiomyocytes regulates the voltage-gated sodium channels near or within the T-tubule systems. The aim of the research was to identify polymorphisms in 2 fragments of the CASK gene in sport pigeons, which might be related to racing performance traits. The primers for Sanger sequencing designed to span the first exon and upstream region (667 bp) and the second exon and its upstream region (430 bp) based on the NW_004973256.1 sequence. The analysis was performed on 150 sport pigeons and revealed 16 polymorphisms localized in upstream regions (8 in the promoter regions and 1 in the 5′ UTR) and in both exons (5 missense variants, 1 codon stop, and 1 of 17-bp insertion). The frequencies of investigated SNP are in Table 1. Furthermore, detected polymorphisms might have the ability to affect transcripts abundance and thus affect performance. More detailed research is necessary to establish their potential association with flying performance and identified variation.

Table 1. Polymorphisms localized in upstream regions and in both exons of the *CASK* gene

CASK SNP	MAF	HET	HOM D	HOM R
g.2514619	0.110	0.222	0.778	0.000
2514653	0.110	0.222	0.778	0.000
2515028	0.042	0.071	0.923	0.006
2515029	0.081	0.148	0.845	0.006
2523738	0.063	0.126	0.874	0.000
2523794	0.287	0.427	0.500	0.073
2523861	0.070	0.126	0.868	0.007
2523937	0.040	0.079	0.921	0.000
2523995	0.320	0.430	0.490	0.079
2524017	0.255	0.377	0.556	0.066
2524043	0.424	0.530	0.311	0.159
2524083	0.192	0.291	0.662	0.046
2524088	0.287	0.413	0.507	0.080
INSERCJA	0.007	0.013	0.987	0.000
2524156	0.285	0.423	0.503	0.074
2524175	0.225	0.356	0.597	0.047

Key Words: racing pigeons, performance, *CASK*

P141 Genetic diversity in Muscovy ducks (*Cairina moschata*) found in Baruten local government area of Kwara State, Nigeria. F. E. Sola-Ojo[1], C. A. Adeola[2], O. A. Yusuf[1], and O. E. Momoh*[1], [1]*University of Ilorin, Ilorin, Nigeria,* [2]*State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences., Kunming, China.*

Analysis of genetic diversity within and between population is important for continuous sustainability and development of livestock production. The Muscovy duck (*Cairina moschata*) is an important poultry species raised by most peasant farmers in Baruteen local government for egg and meat production. In this study, the genetic diversity and relationship between Muscovy duck populations in some selected location Bani, Okuta, Yembeleku and Ilesha Baruba were evaluated using mtDNA cytochrome P450 family 2 subfamily U polypeptide 1 (*CYP2U1*) genes. A total of 25 variable sites were reported with the polymorphic sites consisting of substitutions with 16 singletons variables. The nucleotide diversity per site (Pi) was 0.00608 which indicates low genetic diversity with a total number of 26 mutations. The results show there is a low genetic diversity between the Muscovy duck in Ilesha, Yembeleku, Okuta and few ducks from Bani with exception to BON20 and ILE23 which seems to be of far distant from the other Muscovy duck populations on the phylogenetic tree with a distance value of 125.55 and 57.60 respectively. This result is due to genetic intermixing within the Muscovy duck populations in the 4 locations in Baruteen local government which is as a result of close relationship within and between some locations, sales and gifting of Muscovy duck in among families who are duck lovers. This study also showed the genetic variations between the Muscovy duck studied and its relevance in prevention of inbreeding between and within Muscovy duck found in the area of study.

Key Words: Muscovy duck, diversity, *CYP2U1*, phylogenetic tree

P142 Withdrawn

P143 Bridge 60k SNP panel for the chicken genome-wide study. D. Seo*[1,2], S. Cho[1], D. Lee[1], M. Kim[1], P. Manjula[1], J. Shin[1], D. Lim[3], H. Choo[4], J. Cha[4], K. Kim[4], I.-S. Jeon[4], K.-T. Lee[3], B. Park[4], S. H. Lee[1,2], J. H. Lee[1,2], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, South Korea,* [2]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, South Korea,* [3]*Animal Genomics and Bioinformatics Division, National Institute of Animal Science, RDA, Wanju, South Korea,* [4]*Poultry Research Institute, National Institute of Animal Science, RDA, Pyeongchang, South Korea.*

The native chicken is a unique asset held by countries worldwide, and various types of research are needed to preserve genetic diversity and utilize their potential characteristics. In particular, Korean native chicken (KNC) is well known for its excellent meat quality and endemic adaptability. But there are limited scientific investigations for KNC, and less profitable than commercial broilers warrant unique genetic information for their rapid genetic improvement. This study developed the Bridge 60k SNP panel to find economic trait-related genetic markers and apply them to genomic selection. This SNP panel was designed to link Illumina 60k array and Affymetrix 600k array information by genomic imputation. Illumina 60k data can impute to Bridge 60k panel using 12,289 SNPs, and Bridge 60k panel was imputed to Affymetrix 600k array data using 37,076 SNPs. The average genotype accuracy of imputed SNPs was confirmed as 0.88~0.94 (r^2 is 0.76–0.80). In addition, 11,880 SNPs were included to detect trait-related candidate SNPs from the GWAS for meat quality and growth-related traits in KNC, a comparative study for the signature of selection between KNC with commercial chickens, and an economic trait-related marker obtained from the chicken QTLdb. Moreover, the utilization was expanded by adding 492 markers for the MHC variation study and the identification of KNCs. It is expected that the SNP panel developed in this study will be the best implement for finding candidate genes for economic traits and improve KNC with various characteristics by applying genomic selection to conventional chicken utilization.

Key Words: single nucleotide polymorphism, imputation, Korean native chicken, economic traits, genomic selection

P144 Study on differentially expressed genes in granular layer and theca layer of laying Silky Fowl and White Leghorn. Y. Tai*[1], X. Yang[1], D. Han[2], and X. Deng[1], [1]*Lab of Animal Genetic Resource and Molecular*

Breeding, China Agricultural University, Beijing, China, [2]College of Veterinary Medicine, China Agricultural University, Beijing, China.

Under similar genetic conditions and the same feeding conditions, the egg production of local breed Silky Fowl is significantly lower than that of the White Leghorn. Egg laying performance is related to follicle growth and development, and follicle development is closely related to the granulosa cells and the theca cells. Therefore, transcriptome sequencing was used to analyze differential expression genes of the follicular granular layer and the theca layer of the Silky Fowl and White Leghorn. Silky Fowl and White Leghorn were raised in the ranch of China Agricultural University. We choose 4 of each breed and collected the granular layer and theca layer for high-throughput sequencing. Compared with the White Leghorn, in hierarchical granular layer tissues, the Silky Fowl has 389 upregulated genes and 270 downregulated genes. Downregulated genes were mainly associated with cell division, cell cycle and hormone receptor while the upregulated genes were related to ribosomal function, lipid metabolism and protein synthesis. Compared with the White Leghorn, in hierarchical theca layer tissues, the Silky Fowl has 232 upregulated and 139 downregulated genes. Downregulated genes were mainly associated with cell proliferation, steroid hormone synthesis, and angiogenesis while the upregulated genes were related to melanin synthesis. Different expression genes in granular layer and theca layer were provided in our study for the first time, related to follicular development of different chicken breeds. It is helpful to improve the understanding of the molecular mechanism of low egg production of local chickens.

Key Words: chicken, granular layer, theca layer

P145 Genetic markers associated with live body weight and carcass weight of Korean native chicken using 50k SNP panel. M. Kim*[1], S. Cho[2], E. Cho[3], D. Seo[1,2], A. Jang[4], K. Kim[5], I. Jeon[5], J. Cha[5], B. Park[5], H. Choo[5], and J. Lee[1,2], [1]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Korea,* [2]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea,* [3]*Department of Bio-big data, Chungnam National University, Daejeon, Korea,* [4]*Department of Applied Animal Science, Kangwon National University, Chuncheon, Korea,* [5]*Poultry Research Institute, National Institute of Animal Science, RDA, Pyeongchang, Korea.*

Korean native chicken (KNC) is a unique genetic resource in Korea, being conserved as an important pure breed which has high meat quality. However, the growth rate of KNC is relatively slow compared with that of commercial broiler breeds. As a basic research to enhance the growth performance and carcass traits of KNC, this study was carried out with 350 birds from KNC R line to identify associated variants and candidate genes affecting carcass traits. The genome-wide association study (GWAS) for the live body weight (LBW) and carcass weight (CW) at 10 weeks was carried out using Illumina 50k SNP panel. The result indicated that one significant single nucleotide polymorphism (SNP) associated with both traits ($P < 0.05$) are on the chromosome 1. Also, the Manhattan plot indicated that several SNPs on the chromosome 4 showing close to the genome-wide significance level in both traits. Among 33 genes annotated in those selected SNPs, *SLIT2* was reported as a candidate gene that associated with meat production traits. Small sample size might affect for detecting significant SNPs. By increasing number of animals, the results presented here can provide useful information for genomic selection to improve the targeted traits in KNC population.

Key Words: genome-wide association study (GWAS), Korean native chicken, live body weight, carcass weight, single nucleotide polymorphism

P146 Genetic markers associated with meat quality traits of Korean native chicken using 50k SNP panel. M. Kim[1], S. Cho[2], E. Cho[3], D. Seo[1,2], A. Jang[4], K. Kim[5], I. Jeon[5], J. Cha[5], B. Park[5], H. Choo[5], and J. Lee*[1,2], [1]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Korea,* [2]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea,* [3]*Department of Bio-big data, Chungnam National University, Daejeon, Korea,* [4]*Department of Applied Animal Science, Kangwon National University, Chuncheon, Korea,* [5]*Poultry Research Institute, National Institute of Animal Science, RDA, Pyeongchang, Korea.*

Korean native chicken (KNC) is an important genetic resource in Korea that possesses unique meat quality characteristics such as meat flavor and texture. Mainly, the meat is rich in amino acids and nucleic acids that work as desirable taste compounds. To identify the single nucleotide polymorphisms (SNPs) and genes associated with the unique meat quality in KNC, a genome-wide association study (GWAS) was performed using Illumina 50k SNPs panel. The sample group consisted of 350 KNC R line birds, which improved for their meat quality. A total of 19 flavor-related compounds belong to 4 groups (nucleic acids, amino acids, peptides, and organic acids) in the breast meat slaughtered at 10 weeks of age were used. Only one trait in each group had significant trends. Six SNPs, significant ($P < 0.05$) for Inosine contents were identified on chromosome 5, the lowest SNP significance $-\log_{10}$ *P*-value among those 6 was 6.079292 while Bonferroni genome-wide significance $-\log_{10}$ *P*-value level was 6.05813. These SNPs were reported in the candidate genes, associated with carcass traits (*ASCL2*, *IGF2*, *TNNI2*, *INNT3*), and 22 unknown genes. For tyrosine contents, a single SNP reached 5% of genome-wide significance level was reported in chromosome 12 ($P < 0.05$). The SNP is identified in the *RAF1* candidate gene that is functionally related to tyrosine phosphorylation. Several SNPs reached suggestive genome-wide significance with Carnosine peptide contents were found on chromosomes 4 and 7. The top noted SNPs were likely near the *ACTR3* and *DPP10* genes. Likewise, lactate content had an increasing trend of association with SNP on the chromosome 3 under the significance level, where *SIM1* muscle differentiation gene is located. Continuing study with additional samples can provide useful information for genomic selection to improve the meat quality traits in the KNC population.

Key Words: genome-wide association study, Korean native chicken, meat quality trait, single nucleotide polymorphism

P147 A new chromosome-level turkey genome. C. P. Barros*[1], M. F. L. Derks[1], J. Mohr[2], B. J. Wood[2,3], M. C. A. M. Bink[4], and M. A. M. Groenen[1], [1]*Wageningen University and Research, Wageningen, the Netherlands,* [2]*Hybrid Turkeys, Kitchener, ON, Canada,* [3]*School of Veterinary Science, University of Queensland, Gatton, QLD, Australia,* [4]*Hendrix Genetics Research, Technology and Services, Boxmeer, the Netherlands.*

The domesticated turkey (*Meleagris gallopavo*) is a species of large agricultural importance, as the second largest contributor to world poultry meat production. A high-quality reference genome is essential for research as well as for the turkey breeding industry. Our aim was to create a new chromosome-level turkey genome with improved genome completeness and continuity, gene annotation, mapping of functional sequences and structural variation discovery. Adopting the trio-binning approach, we sequenced 3 individuals (2 parents and one F1), using PacBio long-read sequencing technology, to create a high-quality chromosome-level turkey assembly, as well as 2 high-quality chromosome-level parental haplotype assemblies. We produced a chromosome-level F1 assembly with a total length of 1,001,818,376 bp captured in 151 scaffolds. The assembly covers 35 complete chromosomes in a single scaffold (N50: 70,339,173 bp), which even outperforms the current Gal6 chicken genome in terms of continuity. In addition, we assessed the completeness using BUSCO, showing that we cover more than 96% of the genes in the avian and vertebrate sets. The new turkey assembly provides a significant improvement in terms of gene space and contig orientation and continuity compared with the previous turkey build. Comparative analysis revealed a large inversion on the Z chromosome. We used synteny to compare the Z chromosome in turkeys to other Galliformes species and confirmed that this inversion is unique to turkeys. We assessed structural variance in the F1 assembly and in the parental assemblies (which are of equally high quality). Although being highly similar, the parental haplotypes show a small number of

structural differences, such as a large duplication on chromosome 8. Most of the assembled turkey chromosomes (2n = 80) are microchromosomes. We found that microchromosomes have a higher gene density and level of expression than macro-chromosomes. We also observed statistically significant differences in the length of several repeat and gene features. Collectively, we present a new high-quality chromosome-level turkey genome, which will significantly contribute to turkey and avian genomics research and benefit the turkey breeding industry.

Key Words: poultry and related species, genome assembly, genome sequencing, comparative genomics, genome annotation

P148 Effects of exogenous insulin injection on serum biochemical indices and tissue metabolites of different breeds of chicken. P. N. Luo*, H. J. Wang, Z. Y. Wang, Y. S. Wang, C. C. Su, H. Y. Zhang, W. Chen, and Y. Q. Huang, *Henan Agricultural University, Zhengzhou, Henan, China.*

The aim of this study was to investigate the effects of exogenous insulin on serum biochemical indexes and tissue metabolites of broilers and layers. Ninety 24-d-old broilers and layers were fasted for 16 h. Each breed of chicken was randomly divided into 2 groups: The insulin group (INS) and the control group (CON) were received abdominal subcutaneous injection of 5 IU/kg insulin or the same dose of PBS solution, and chickens (n = 10) were sampled at 0, 120 and 240 min after injection in each group. One-way ANOVA was used for the comparison of serum biochemical indexes and tissue metabolites among different insulin treatment time in the same breed or different breeds with the same insulin treatment time. Independent-sample *t*-test was used to compare the tissue metabolites between INS and CON at the same time point after injection. The results were as follows: (1) After the injection of exogenous insulin, there are differences between broilers and layers in the contents of serum urea, glucose and insulin at 0 and 240 min ($P < 0.05$). Moreover, the serum urea and uric acid levels were significantly different between broilers and layers at 120 min ($P < 0.05$). (2) In broilers and layers, the contents of serum glucose and urea decreased gradually from 0 to 120 min of injection of exogenous insulin, while the contents of cholesterol and uric acid showed an increasing trend. (3) Compared with 0 min, exogenous insulin injection for 120 min significantly decreased the content of glucose and glycogen in liver of broilers and layers and significantly increased the insulin content of the liver of layers ($P < 0.05$). In the leg muscle, 120 min after exogenous insulin injection increased the glucose content of broilers significantly ($P < 0.05$). (4) Compared with PBS treatment for 120 min, glucose level significantly increased in the breast muscles of broilers by insulin stimulation ($P < 0.05$). In the leg muscles, only the most significant increase in glucose was found in layers ($P < 0.05$). These trials demonstrated that there were differences in serum biochemical indexes and tissue metabolites between insulin injection time and different breeds and insulin injection promoted the absorption of glucose in serum.

Key Words: broiler and layer, biochemical, metabolite

P149 Uncovering abundant missing genes in the chicken reference genome solves the avian gene depletion puzzle. M. Li*[1], N. Xu[1], P. Bian[1], X. Hu[2], Y. Jiang[1], and N. Yang[3], [1]*Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of Animal Science and Technology, Northwest A&F University, Yangling, China,* [2]*State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, China,* [3]*National Engineering Laboratory for Animal Breeding and Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Agriculture and Rural Affairs, China Agricultural University, Beijing, China.*

In contrast to the rich biodiversity of the avian clade, both the gene number and evolutionary rate of birds appear far lower than in mammals, which has engendered long-standing controversy. By performing 20 de novo genome assemblies for chickens worldwide, we identified ~1,300 novel protein-coding genes in 159 Mb nonredundant novel sequences compared with the reference genome (GRCg6a). In the novel sequences, tandem repeats and secondary structures such as G-quadruplexes are associated with low read depth, which has previously prevented their assembly. However, we found that most of the novel sequences and coding genes are shared across the panel of sequenced genomes. The novel genes are mainly located on sub-telomeric regions with a much-elevated substitution rate, which could date back to the common ancestor of birds. Our study provides a framework for constructing a comprehensive avian reference genome and suggests that the integrated evolutionary rate of birds is underestimated and the true gene number of birds is comparable to that of other tetrapods, hence resolving a long-standing puzzle regarding avian evolution.

Key Words: chicken, de novo assembly, DNA secondary structures, novel genes, substitution rate

P150 Dissecting the polygenic genetic architecture of growth using genotyping by low-coverage sequencing in a deep intercross of the Virginia body weight lines: Novel loci revealed by increased power and improved genome coverage. T. Rönneburg*[1], Y. Zan[2,1], C. Honaker[3], P. Siegel[3], and Ö. Carlborg[1], [1]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden,* [2]*Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Science, Umeå, Sweden,* [3]*Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.*

Dissecting the basis of quantitative traits is a central topic in genetics and highly polygenic traits are particularly challenging due to the power necessary to confidently identify loci with minor effects. Experimental crosses are valuable resources for mapping such traits, though genome-wide analyses generally target major loci using a F_2 population, with additional individuals only generated for replication and fine-mapping. This was the case in attempts to dissect the genetic basis of the long-term, divergent bidirectional selection responses for 56-d body weight in the Virginia chicken lines. An 18-generation intercross line was developed from the low and high selected lines. Dissecting the genetic architecture of the 9-fold difference in 56-d body weight after 40 generations of selection revealed that although selection acted on a highly polygenic genetic architecture, only a handful of the 20 reported candidate loci reached individual significance levels. Making use of current methods, this study was designed to dissect this highly polygenic genetic architecture further, re-genotyping all available samples genome-wide and performing an integrated QTL mapping analysis across chicken from generations F_2-F_{18}. A cost-efficient low-coverage sequencing-based approach was implemented to obtain high-confidence genotypes for > 3,300 individuals. In total, 12 genome-wide significant QTL and 10 additional suggestive QTL were mapped. Of these, only 3 reached genome-wide significance or suggestive thresholds in the earlier F_2-based genome-wide scan. Five of the significant and 4 of the suggestive QTL overlapped with the 20 candidate loci reported in the fine-mapping and replication analyses of the later generations in the cross. Novel QTLs were primarily mapped due to an overall increase in power, with minor contributions from increased genome coverage and improved marker information content. Significant and suggestive QTL are estimated to explain 60.7% of the difference between the parental lines, 3 times as much as previously reported, resulting in a more confident, comprehensive view of the individual loci that form the genetic basis of the highly polygenic, long-term selection responses for 56-d body weight in the Virginia chicken lines.

Key Words: poultry and related species, complex trait, genotyping, genome-wide association

P151 Identification of splicing isoforms of bovine *ACSF3* gene and protein structure prediction. W. He*, X. Fang, and R. Yang, *College of Animal Science, Jilin University, Changchun, Jilin Province, China.*

The characteristics of meat are not only related to the fat content, but also closely related to the fatty acid composition. Acyl-CoA synthetase family member 3 (*ACSF3*) gene is involved in the synthesis of fatty acid formation enzymes. ACSF3 enzyme is only found in mitochondria and participates in mitochondrial fatty acid synthesis. Alternative splicing is an important mechanism to control gene expression and produce protein diversity. This study mainly explores the different splicing isoforms of the bovine *ACSF3* gene mRNA and predicts the protein structure. The Trizol method was used to extract total RNA from various bovine tissues; Use reverse transcription kit for cDNA synthesis; PCR amplification was carried out with specific primers (5′→3′ primer 1F: TCTCAAGCAGTCTGATGAAT; primer 1R: CGCAAGTAGAGGTCCTTAT; primer 2F: TGACAGCTCTCATCATCAC; primer 2R: CGTCCTGGTACTCACTTG); After detection by agarose gel electrophoresis, the target fragment was cloned and sequenced. The sequencing results were compared with the known nucleotide sequence (NM_001035068.2) on GenBank, and Phyre2 was used to predict the protein structure of the amino acid sequences of the 3 splicing isoforms. In this experiment, 3 types of splice isomers of *ACSF3* (type I, type II, and type III) were obtained, and they were named ACSF3a, ACSF3b, and ACSF3c. Among them, ACSF3a is only found in bovine liver, lung, and kidney tissues, but not found in other tissues. ACSF3b and ACSF3c can be identified in the heart, spleen, and lungs. At the same time, the arrangement order and alternative splicing mode of the 3 in the genome were preliminarily determined. Type I *ACSF3* contains the first intron retention on the basis of the full length; type II and type III are deletions in the first exon, the first intron, and part of the second exon. In addition, the 3 types of splice isoforms contain different 5′ untranslated regions. Among them, type I *ACSF3* is a full-length *ACSF3* containing the first intron retention, and type II and type III *ACSF3* lack part of the N-terminal catalytic region. mRNA splicing is an important means of posttranscriptional regulation. The splicing sequence changes, which affects the stability of RNA and its translation efficiency, further changes the amino acid sequence, and changes the activity or certain functions of the protein.

Key Words: *ACSF3* gene, splice isoforms, fatty acid synthesis, cattle

P152 Evolution of inbreeding: A gaze into the history of 5 Italian beef cattle breeds. G. Rovelli*[1,2], M. Luigi-Sierra[2], D. Guan[2,3], F. Sbarra[4], A. Quaglia[4], M. Amills[2,5], and E. Lasagna[1], [1]*Department of Agricultural, Food and Environmental Sciences (DSA3), University of Perugia, Perugia, Perugia, Italy,* [2]*Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus Universitat Autonòma de Barcelona, Bellaterra, Barcelona, Spain,* [3]*Department of Animal Science, University of California, Davis, California, United States of America,* [4]*National Association of Italian Beef-Cattle Breeders (ANABIC), San Martino in Colle, Perugia, Italy,* [5]*Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

Inbreeding is the main consequence of mating individuals related through common ancestry, to a higher degree than the average relatedness of this population. Minimizing inbreeding is a key point in cattle breeding to avoid the phenotypic expression of detrimental alleles in the offspring as well as to ensure the maintenance of genetic diversity. In the current work, we aimed to characterize the historical evolution of inbreeding in 5 Italian beef cattle breeds (Chianina, 909; Marchigiana, 879; Romagnola, 904; Maremmana, 334; and Podolica, 555) between 2002 and 2019 by using both molecular and genealogical estimates of inbreeding coefficients. PLINK v1.9 software was used to compute inbreeding coefficient F_{hat2}, while Endog v4.8 was used to calculate the pedigree inbreeding coefficient (F_{ped}), defined as the probability that an individual has 2 "identical-by-descent" alleles. Arlequin software v3.5.2.2 was used to estimate within-population diversity, by calculating observed (*Ho*) and expected (*He*) heterozygosities, and historical trends in effective population size (N_e) were estimated with the SNeP software using default settings. The average estimates of *Ho*, *He*, F_{hat2}, and F_{ped} across years (2002–2019) fluctuated between 0.340 and 0.401, 0.348 and 0.392, –0.121 and 0.072, and 0.000 and 0.068, respectively. Despite the fact that we detected fluctuations in the magnitudes of F_{hat2} and F_{ped} during the last 20 years, these oscillations were not very important. Changes in homozygosity and inbreeding coefficients across time might be partly explained by the fact that the number of breeders, and particularly sires, is not constant across years. We also observed a slight augment of F_{hat2} in the 5 breeds during 2002–2019 increasing 0.01–0.02% per year, while F_{ped} remained quite stable, with an annual increase rate of 0.003–0.004%. The use of a high number of bulls combined with strategies implemented by National Association of Italian Beef Cattle Breeders (ANABIC) to minimize inbreeding might explain these results. Moreover, we detected a sustained decrease of the population effective size (N_e) of these 5 breeds; such results should be interpreted with caution due to the inherent difficulty of estimating N_e from SNPs data in a reliable manner.

Key Words: *Bos taurus*, genetic diversity, coancestry, effective population size, single nucleotide polymorphism

P153 Investigating the accuracy of imputing variants on chromosome X in admixed dairy cattle using the ARS-UCD1.2 assembly of the bovine genome. Y. Wang*[1,2], K. Tiplady[1,2], T. J. J. Johnson[2], C. Harland[2], M. Keehan[1,2], T. J. Lopdell[2], R. G. Sherlock[2], A. Wallace[2], B. Harris[2], M. D. Littlejohn[2], R. Spelman[2], D. Garrick[1], and C. Couldrey[2], [1]*AL Rae Centre for Genetics and Breeding, School of Agriculture, Massey University, Hamilton, Waikato, New Zealand,* [2]*Research and Development, Livestock Improvement Corporation, Hamilton, Waikato, New Zealand.*

Sequence level imputation in dairy cattle to date has mainly focused on autosomes. The aim of this study was to evaluate the imputation performance of chromosome X (chrX) from low-density to high-density (HD) genotypes and subsequently to sequence level. Three low-density genotyped study populations: 41,551 animals genotyped on GeneSeek GGP panels, 55,465 animals genotyped on an Illumina BovineSNP50 panel (50k), and 28,315 animals genotyped on a GeneSeek GGP50k panel were separately imputed to Illumina BovineHD level (777k) using a reference of 3,769 animals. Subsequently, these animals were imputed to sequence level using 1,298 sequenced animals as a reference. In addition, a 10-fold cross-validation experiment was conducted to evaluate the imputation performance of Beagle 5.1. ChrX was divided into the pseudoautosomal (PAR) and non-PAR regions. Imputation of these regions was performed separately using Beagle 5.1. Imputation for the non-PAR was also undertaken separately for males and females using Minimac 3. For HD imputation, Beagle 5.1 achieved an average dosage R-squared (DR2) of 0.96 for both non-PAR and PAR for all study populations. For the PAR, Minimac 3 achieved an allelic R-squared (AR2) of 0.95 for all 3 populations. In the non-PAR, Minimac 3 produced an average AR2 for females of 0.95 for all study populations, however, for males AR2 of 0.81 for 50k study, 0.74 for GGP study and GGP50k panels were returned. For sequence imputation, Beagle 5.1 achieved a DR2 of 0.76 for non-PAR region and 0.87 for PAR region, whereas Minimac 3 achieved an AR2 of 0.69 for male non-PAR region, 0.46 for female non-PAR region and 0.53 for PAR region. For the 10-fold cross-validation groups, the average genotype concordance is 0.99 for non-PAR and 0.98 for PAR region. The average genotype correlation is 0.98 for non-PAR and 0.97 for PAR. Beagle 5.1 is able to perform non-PAR imputation for males or females, whereas Minimac 3 cannot. Using information of both sons and the dams imputed simultaneously allows for improved imputation accuracy given that chrX of the son is

directly inherited from the dam and provides the perfect phase. The chrX variants at the imputed sequence level will facilitate future genome-wide association studies and genomic prediction.

Key Words: imputation, cattle, X chromosome, sequence variation

P154 Identification of the β-casein gene genotype in Simmental cattle. I. Radkowska[1], D. Rubis[2], and K. Ropka-Molik*[2], [1]*Department of Cattle Breeding, National Research Institute of Animal Production, Balice, Poland,* [2]*Department of Animal Molecular Biology, National Research Institute of Animal Production, Balice, Poland.*

The latest scientific discoveries relating to dietetics as well as new nutritional trends indicate the need for a deeper look at animal products, including milk, and protein content. The most common genetic variants of β-casein (CSN2) are A1 and A2. The mutation causing the differences in the β-casein protein content results from a single nucleotide polymorphism at codon 67 in exon 7 of the CSN2 gene (A2, proline and A1, histidine). It has been suggested that opioid peptides are released during digestion or processing of milk containing form A1, which may cause hypersensitivity or allergy. Therefore, there is a need to research and promote milk derived from cows with the preferred type of β-casein A2. To identify the β-casein gene, biological material (hair) from Simmental cattle from the herd of the Experimental Station of the Odrzechowa Institute was collected for research. The 768 heads were examined, including 391 dairy cows, 58 heifers, 293 female calves, and 26 bulls. The *CSN2* alleles were estimated using the allelic discrimination approach with TaqMan MGB probes labeled VIC and FAM (StepOne Real-Time PCR System). The results showed that 31.38% of the tested animals are A2A2 homozygotes, 53.78% are A1A2 heterozygotes and 14.84% are A1A1 homozygotes. In the group of cows and heifers, A2A2 animals are 31.40%, while A1A1 18.04%. Similarly, in the group of calves, the highest percentage was constituted by A1A2 heterozygotes (59.04%), followed by A2A2 homozygotes (31.40%), and A1A1 homozygotes (9.56%). In the bulls tested, 50% were A1A2, 30.77% were A2A2 and 19.23% were A1A1. The obtained results indicate a significant percentage of A2A2 animals in the studied Simmental herd and significant breeding potential, as evidenced by a significant share of A1A2 heterozygotes. Therefore, the performed analysis will make it possible to conduct breeding work toward A2 milk production and contribute to broadening the knowledge about the frequency of particular β-casein genotypes in the dairy herd of the Experimental Station of the Odrzechowa National Institute.

Key Words: β-casein, milk, cow, marker

P155 A high-throughput Applied Biosystems Axiom Bovine Genotyping array with 100,000 markers optimized for dairy evaluation. A. Pirani*, D. Oliver, C. Bertani, and M. Patil, *Thermo Fisher Scientific, Inc., Santa Clara, CA, USA.*

While the world population increases at an unprecedented rate, meeting the growing food needs continues to be a challenge. For more than a decade, the bovine dairy industry has employed the genetics of their cattle to improve production traits, such as milk yield and protein percentage. These methods have shown to be critical for the improvement of dairy cattle productivity. This breeding strategy is achieved by genotyping thousands of biallelic SNPs, interrogating loci well-distributed across the entire genome, potentially capturing all relevant quantitative trait loci (QTL). These genotypes are converted to genomic estimated breeding values (GEBVs) using a method called genomic selection (GS). The application requires the interrogation of a fixed set of markers rapidly over thousands of samples, so medium-density, 25,000 to 100,000 marker microarrays are an ideal fit. For genotyping dairy cattle, Thermo Fisher Scientific provides numerous Applied Biosystems Axiom microarrays measuring around 65,000 markers. These arrays, such as the Axiom Bovine Genotyping v3 Array includes 44,000 markers recognized by the Council on Dairy Cattle Breeding (CDCB). Recently, the CDCB released a list of 80,000 markers used for genetic evaluation. Thermo Fisher Scientific has developed a 100,000-marker microarray to interrogate all 80,000 CDCB relevant makers. In addition, this array includes markers for even genomic coverage, economically valuable trait-associated markers, sex-linked markers, microsatellite imputation markers, and parentage verification, such as the International Society for Animal Genetics (ISAG) 200 and ICAR 354 markers. This higher-density panel can also be useful in tracking undesirable genetic trends, such as inbreeding depression, to drive overall genetic improvement of dairy cattle in commercial breeding programs.

Key Words: cattle and related species, animal breeding, bioinformatics, microarray, genomic selection

P156 Withdrawn

P157 Withdrawn

P158 Genetics of base coat color variations and coat color patterns of South African Nguni cattle investigated using high-density SNP genotypes. L. Kunene[1], K. Hadebe[2], G. Mészáros[3], J. Sölkner[3], F. Muchadeyi*[2], and E. Dzomba[1], [1]*University of KwaZulu-Natal, Pietermaritzburg, South Africa,* [2]*Agricultural Research Council, Pretoria, South Africa,* [3]*University of Natural Resources and Life Sciences, Boku University, Vienna, Austria.*

Nguni cattle are a hardy Sanga-type breed with coats of diverse colors that have found a niche market in the leather industry. There are limited studies on the genetics of coat color patterns, and absence of such information hampers potential breeding and improvement initiatives. This study investigated the genetics of base coat color, color-sidedness and white forehead stripe in Nguni cattle using Illumina Bovine HD (770K) genotypes. Genome-wide association tests were conducted using 622,103 quality-controlled SNPs and the Efficient Mixed Model Association eXpedited method implemented in Golden Helix SNP Variation Suite. GWAS for base coat color (eumelanin vs pheomelanin) resulted into 4 indicative SNPs on BTA18 and a well-known gene, *MC1R*, that play a role in the melanogenesis and the *MAPK* signaling pathway, was observed within 1MB from the indicative SNPs. GWAS for color-sidedness resulted in 4 indicative SNPs, none of which was associated with the *KIT* candidate gene for color-sidedness. GWAS for the white forehead stripe resulted in 17 indicative SNPs on BTA6 all with weak correlations with the well-known *KIT* gene ($r^2 < 0.007$). Other than the *KIT* gene, 4 genes *MAPK10*, *EFNA5*, *PPP2R3C* and *PAK1* were found to be associated with the white forehead stripe and are part of the *MAPK*, adrenergic and Wnt signaling pathways that are synergistically associated with synthesis of melanin. Overall, our results prove prior knowledge of the role *MC1R* in base coat colors in cattle and suggested a different genetic mechanism for forehead stripe phenotypes in South African Nguni cattle.

Key Words: coat color, coat patterns, GWAS, Nguni cattle, SNP genotype

P159 Impact of genomic breed composition on production traits in crossbred dairy cattle. M. Jaafar*[1], B. Heins[2], C. Dechow[3], and H. Huson[1], [1]*Cornell University, Ithaca, NY, USA,* [2]*University of Minnesota, Morris, MN, USA,* [3]*Penn State University, University Park, PA, USA.*

Understanding breed composition (BC) gives us insight into population structure and breeding history, informs crossbreeding programs, and provides mechanisms to correct population stratification in across-breed genetic evaluations and genome-wide association. This study examined variation in BC using Illumina BovineSNP50 genotypes and correlated BC to key performance traits. Ancestry was localized to chromosomal regions to determine the relationship between ancestry and trait selection. The intent was to determine how breed selection in crossbred dairy cattle influences traits and to determine if specific ancestral haplotypes are selected for at key performance QTL. To this end, 2 rotational crossbred populations, Procross and Grazecross were assessed. Procross is a product of rotational crossbreeding of Viking Red (VKR), Holstein (HOL), and

Montbéliarde (MON) whereas Grazecross consists of Viking Red (VKR), Normande (NOR), and Jersey (JER). Both breeding programs capitalized on the positive effect of heterosis. Genomic breed composition was generated on 610 crossbred cattle incorporating genotypes of the respective purebred breeds for genomic ancestry estimation using Admixture. The average genome-based prediction estimates for Procross were 22% VKR, 45% MON, and 33% HOL while Grazecross were 21% VKR, 49% NOR, and 30% JER. BC were correlated with the performance traits of milk, fat, protein, and somatic cell score, by comparing ancestry of extreme high and low-performance groups. Analysis showed that both MON and HOL breed composition plays a significant role in higher milk and fat production in Procross while VKR and NOR are related to improved health performance in Grazecross. This inference was supported by the local ancestry analysis using RFMIX software. The results showed higher Procross milk production animals more commonly have HOL ancestry on BTA 14 where the DGAT gene resides, while Grazecross cattle more commonly have NOR ancestry on BTA 4, 6, and 10 at known health QTL. In conclusion, we believe that it is crucial to select and maintain a particular variation in breed composition to ensure optimal trait performance in crossbred cattle.

Key Words: genomic breed composition, crossbred dairy cattle, local ancestry, admixture, production traits

Companion Animal Genetics and Genomics Posters

P160 *PRKG2* splice site variant in Dogo Argentino dogs with dwarfism. G. Rudd Garces*[1,2], M. E. Turba[3], V. Jagannathan[1], F. Gentilini[4], and T. Leeb[1], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Institute of Veterinary Genetics, La Plata, Argentina,* [3]*Genefast, Forlì, Italy,* [4]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy.*

Dwarfism is a widely known phenotype that occurs in many species and may be due to genetic or environmental causes. In humans, variants in more than 20 genes related to dwarfism phenotypes have been described. Canine forms of inherited dwarfism have been reported in several dog breeds. In this study, we investigated a family of Dogo Argentino dogs, in which 2 dogs were affected by dwarfism. Radiographs in an affected dog revealed a decreased endochondral ossification of growth plates. The pedigree suggested monogenic autosomal recessive inheritance. Combined linkage and homozygosity mapping assigned the most likely position of a potential genetic defect to 34 genome segments totaling 125 Mb. The genome of an affected dog was sequenced and compared with 795 control genomes. Three private protein-changing variants were found in the linked and homozygous regions. Prioritization of these variants according to functional knowledge of the affected genes revealed a clear top candidate variant for the observed dwarfism. Genotypes at this variant, *PRKG2*:XM_022413533.1:c.1634+1G>T, were perfectly associated with the phenotype in the studied family of dogs. *PRKG2* encodes the protein kinase cGMP-dependent type 2. The identified variant is predicted to disrupt a splice donor site and therefore to cause a loss-of-function allele. *Prkg2* deficient mice and rats show longitudinal growth retardation. A *PRKG2* nonsense variant was reported to cause dwarfism in American Angus cattle. Together with the comparative data from other species, our data suggests *PRKG2*:c.1634+1G>T as a candidate causative variant for the observed dwarfism phenotype in Dogo Argentino dogs.

Key Words: dogs and related species, animal breeding, genome sequencing, growth and development, animal health

P161 *KLF7* gene is a risk factor for congenital deafness in Australian stumpy tail cattle dogs. S. Shan*[1], F. Xu[1], S. Sommerlad[2], J. M. Seddon[2], and B. Brenig[1], [1]*Institute of Veterinary Medicine, University of Goettingen, Göttingen, Germany,* [2]*School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia.*

Congenital deafness is prevalent in Australian stumpy tail cattle dogs (ASCD). However, no causative gene for ASCD deafness has been identified at present. In this study, we attempted to detect the causative factors by comparing affected and normal individuals using a genome-wide association study (GWAS) and whole-genome sequencing (WGS). Since ASCDs are classified as herding dogs according to their breed standard, additional herding dogs from public data were selected as controls for GWAS. Three bilateral deaf ASCDs, 43 herding dogs, and one unaffected ASCD were used, yielding 13 significantly associated loci on 6 chromosomes (CFA3, 8, 17, 23, 28, and 37). Among these loci, CFA37 was further analyzed because it contains more than half of the associated loci and the most significantly associated variant. To further map candidate variants, the same 3 affected ASCDs and one unaffected ASCD were used for WGS. WGS data were analyzed using autosomal recessive and dominant models, respectively, and compared with 722 canines of the public database to improve filtering efficiency. Only potentially functional variants were selected for further analysis and the deleterious effects of the missense variants were predicted. Finally, a variant in the Kruppel-like factor 7 (*KLF7*) gene (NC_006619.3: g.15562684G>A; XP_022270984.1: p.Leu173Phe) was considered as a candidate variant under the dominance model. *KLF7* plays key roles in the nervous system, and is reported to be involved in the development of the inner ear. Further genotyping of the *KLF7* variant was performed on an additional 55 ASCD samples (28 deaf and 27 normal hearing dogs) and this variant remained significantly associated with deafness in ASCD ($P = 0.014$). The odds ratio$_{AA} = 6.8$ implies that homozygous carriers are 6.8 times more likely to be deaf than wild-type. In addition, 19 heterozygous and 5 homozygous mutants were detected, containing 18 deaf dogs. This revealed a penetrance of 0.75, which is consistent with the previously reported deafness penetrance of 0.72. In summary, *KLF7* is a risk gene for the congenital deafness in ASCD.

Key Words: *KLF7*, Australian stumpy tail cattle dog, congenital deafness

P162 More than a moggy; A population genetics analysis of the United Kingdom's non-pedigree cats. J. Irving McGrath*[1], W. Zhang[1], R. Hollar[2], A. Collings[3], R. Powell[4], R. Foale[5], N. Thurley[5], R. Campbell[5], R. Mellanby[1], D. Gunn Moore[1], J. Brockman[2], and J. Schoenebeck[2], [1]*Royal (Dick) School of Veterinary Studies and Roslin Institute, University of Edinburgh, Easter Bush Veterinary Campus, Midlothian, UK,*

[2]Hill's Pet Nutrition Centre, Topeka, KS, USA, [3]Idexx Laboratories, Wetherby, UK,

[4]DragonVet Consulting Ltd., Hertfordshire, UK, [5]Dick White Referrals, Six Mile Bottom, Cambs, UK.

The domestic cat is one of the most popular pets in the world. It is estimated that 89–92% of domestic cats in the UK are non-pedigree domestic shorthair, domestic longhair or domestic semi-longhair cats. Despite their popularity, little is known of the UK cats' population structure and breeding dynamics. Using a custom-designed single nucleotide variant (SNV) array, this study investigated the population genetics of 1344 UK cats. Principal component analysis (PCA) and fastSTRUCTURE analysis verified that the UK's non-pedigree cats are random bred, rather than admixed, mix breed or crossbred. In contrast to pedigree cats, the linkage disequilibrium of random-bred cats was least extensive and decayed rapidly. Autozygosity analysis showed the majority of random-bred cats had proportionally less of their genome in homozygosity by descent (HBD) segments compared with pedigree cats, and that these segments

were older. Together, these findings suggest that these cats should be considered as breeds of their own and warrant the scientific focus traditionally reserved for recognized breeds. Unexpectedly, 19% of random-bred cat genomes displayed a higher proportion of HBD segments associated with more recent inbreeding events. Therefore, while random-bred cats as a whole are genetically diverse, they are not impervious to inbreeding and its health risks.

Key Words: cats and related species, population genomics, genotyping, population structure, breed/population identification

P163 Whole-genome sequencing analysis of a cat family with radial hemimelia. N. Bilgen*[1], M. Y. Akkurt[1], B. Çinar Kul[1], R. M. Buckley[2], L. A. Lyons[2], and Ö. S. Çildir[1], [1]*Faculty of Veterinary Medicine, Department of Genetics, Ankara University, Ankara, Turkey,* [2]*Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA.*

Hemimelia is a congenital condition characterized by complete or partial absence of one or more bones. If malformation is affecting radius bones it is called radial hemimelia (RH). Thumbs may also be affected by this malformation. In nature, cats with RH cannot survive due to the defect on their forelimb and the mother tends not to nurture defective kittens, thus the kittens die. Sporadic cases of RH from around the world in unrelated stray cats were reported. Although it has been suggested that RH in Siamese and domestic shorthair cats may be a hereditary trait, it has not been possible to determine neither the inheritance pattern nor the genetic background of the disease. In this study, 4 Siamese cats, from an extended family of 24 cats, consisting of father, mother, bilaterally affected female kitten, and unilaterally affected male kitten were subjected to genetic analysis. First detailed radiological examinations were performed, and swab samples were taken for DNA extraction. To determine the possible genetic drivers of the malformation, extracted DNAs were subjected to WGS using the Illumina HiSeq platform. The variant call files for the 99 Lives WGS and WES cats were imported into VarSeq. Ensembl annotation 101 and NCBI annotation 99 were used for annotation. 336 domestic cats representing various breeds and populations from the 99 Lives consortium were available for comparison to the 4 Siamese cats. Assuming an autosomal recessive condition, candidate variants were defined as heterozygous in the 2 parents and homozygous in the 2 affected offspring. All other cats in the 99 Lives data set were considered homozygous reference. A total of 2,508,262 SNPs were determined. Of them, 336,763 SNP mutations were identified as causing large effects in the severely affected kitten. After filtering based on inheritance and the condition being specific to the 4 cats, only 22 variants were identified as candidates for RH. Most variants (n = 16) clustered between 140 and 146 Mb on cat chromosome A1. Candidate mutations validated by Sanger sequencing is proceeding. This is the first study describing the genetic background and inheritance model of the RH in cats depending on pedigree. Study was supported by Ankara University Scientific Research Projects Coordination Unit (Project no: 18B0239001).

Key Words: cats and related species, candidate gene, genome-wide association, high-throughput sequencing (HTS), radial hemimelia

P164 Assessment of similarity between the canine m.2683G>A variant found in the *tRNA-Leu* (UUR) gene and the deleterious m.3243A>G variant in the human *TRNL1* gene in carcinogenesis. K. Kowal*, A. Tkaczyk-Wlizlo, and B. Slaska, *Institute of Biological Bases of Animal Production, Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Lublin, Poland.*

The human mitochondrial genome has 16,569 base pairs (bp) and contains 13 genes for polypeptides, 2 genes for rRNAs, and 22 genes for tRNAs, whereas canine mitochondrial DNA (mtDNA) contains the same genes, but is 16,727 bp long. Different modifications of mitochondrial functions have been observed in human and canine cancer biology, including an increase in mtDNA mutation, and alteration of antioxidant activity. Canine malignancies have been established as strong comparative models for human cancers due to their spontaneous development and frequency. However, there is no information about comparison of the mitochondrial genome of these 2 organisms carried out to find similarities. After NGS sequencing of entire mitochondrial genomes isolated from blood and tumor tissues of dogs with mammary gland tumors, we analyzed the sequence homology between the human and canine mtDNA genes. The highest degree of homology was observed in the tRNA genes: *TRNM* (97%), *TRNI* (93%), and *TRNL1* (84%). In the canine *tRNA-Leu* (UUR) gene, polymorphism m.2683G>A was observed in 66% of samples. The analysis of the secondary structure of human and canine tRNA-Leu (UUR) revealed that canine m.2683G>A corresponded with human m.3423A>G polymorphism in the DHU loop. Noteworthy, the tRNA Leu (UUR) molecule transports one of the most frequently used amino acids in the structure of mitochondrial proteins. In addition, the most common mutation (over 80% patients) associated with MELAS syndrome is the m.3243A>G mutation. The m.3243A>G alteration results in impaired mitochondrial translation and protein synthesis, including the mitochondrial electron transport chain complex subunits, leading to impaired mitochondrial energy production. Moreover, the region surrounding m.3243 is an etiologic hotspot for mutations in humans i.e., colon cancer, lung cancer, or renal cell oncocytoma. Thus, it cannot be excluded that the presence of the m.2683G>A polymorphism in canine tRNA Leu (UUR) might be linked with the carcinogenesis process in dogs. The research was financed by the NSC Poland, grant (2019/35/B/NZ5/00775).

Key Words: dogs, DNA sequencing, comparative genomics, polymorphism

P166 Identification of the causative mutation for hair length variation in Sapsarees, a Korean native dog breed, using genome-wide association analysis. M. Kang*[1], B. Ahn[1], S. Yook[1], Y. Lee[2], J. Kim[2], and C. Park[1], [1]*Konkuk University, Seoul, Republic of Korea,* [2]*Yeungnam University, Yeungnam, Republic of Korea.*

Sapsarees are a Korean native dog breed with medium to large body sizes and their long double coats are the most prominent feature of these breeds. However, during the breeding process of Sapsarees, individuals with short hairs throughout the body were identified. Interestingly, the short-haired dogs appear in paintings of the Chosun dynasty, the last imperial dynasty of Korea (year 1392–1910). The pedigree analysis showed that the phenotype follows recessive inheritance in Mendelian segregation. Sapsarees were at the brink of extinction in the 1960s and since then effort has been made to restore the breed population. To detect significant single nucleotide polymorphism (SNP) for the hair length phenotypes, a total of 61 and 53 samples with short and long-hairs, respectively, were genotyped using the Illumina 170K CanineHD bead chips. The single marker simple regression analysis was conducted using the GLIMMIX procedure. Eighteen markers from chromosomes 5, 7, 10, 13, and 23 were shown to be significant at the level of $P < 10^{-5}$ with the largest odds ratio (>260) on chromosome 13. SNPs of *OXR1*, *RSPO2*, and *PKHD1L1* were strongly associated with the hair phenotype. Further analysis to identify the causative mutation underlying the hair length phenotype is ongoing. In addition, the comparative analysis of the causative mutations among other East Asian dog breeds will be conducted. This study will help to establish accurate breed lineage of Sapsarees and also get a glimpse into the origin of hair length variations of East Asian dog breeds.

Key Words: dog, genetic identification, microarray, hair/keratin, breed standardization

P167 Whole-genome sequence-based analysis of genetic relationships among East Asian dog breeds including Korean native breeds Sapsaree, Donggyeongi, and Jindo. B. Ahn*[1], M. Kang[1], J. J. Kim[2], H. Jiang[3], and C. Park[1], [1]*Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul, South Korea,* [2]*School of Biotechnology,*

Yeungnam University, Gyeongsan, South Korea, [3]College of Animal Science, Jilin University, Changchun, China.

Exploring genome structure of modern dogs provides an insight into the history of their breed formation. Genomic DNA from 5 individuals each of Sapsaree, Jindo, Pekingese, Pug and Tibetan Mastiff were isolated and next-generation sequencing was carried out with 30X coverage in average. The reads were mapped to the canine reference genome (CanFam3.1) and further analyzed to detect genetic variants together with additional 94 genome sequences with > 10X genome coverage available in public databases including 5 additional East Asian breeds including Donggyeongi, Tibetan Terrier, Pug, Shih Tzu, and Shiba. As a result, a total of 15.0 million SNPs from 119 modern canids were detected and subjected to bioinformatic analyses including admixture, phylogenetic analyses based on hierarchical clustering, and allele sharing using f_4 statistics. The phylogenetic analysis showed independent clustering of each breed in the same branches, supporting the uniqueness in their genetic characteristics. The admixture analysis showed that the Korean native dog breeds shared genetic components of multiple East Asian breeds. Our results will help to understand the ancestry and genetic diversity of Korean native dog breeds in relation to other East Asian and Western dog breeds. We also summarized the genetic characteristics of Korean native dog breeds for the previously reported genetic variations or mutations responsible for genetic diseases or phenotypes in other breeds.

Key Words: Sapsaree, East Asian dogs, whole-genome sequencing, admixture, phylogenetic tree

P168 Recessive deleterious mutations in the *TPO* gene associated with familial thyroid follicular cell carcinoma in Dutch German longhaired pointers. Y. Yu*, H. Bovenhuis, Z. Wu, K. Laport, M. Groenen, and R. Crooijmans, *Wageningen University and Research, Animal Breeding and Genomics, Wageningen, the Netherlands.*

Familial thyroid cancer originating from follicular cells accounts for 5 to 15% of all the thyroid carcinoma cases in humans. We found thyroid follicular cell carcinomas in a large number of the Dutch German longhaired pointers (GLPs) with likely an autosomal recessive inheritance pattern. Here, we investigated the genetic causes of the disease using a combined approach of genome-wide association study and runs of homozygosity analysis based on 170k SNP array genotype data and whole-genome sequences. A region 0–5 Mb on chromosome 17 was identified to be associated with the disease. In the region, by the whole-genome sequences, we identified 2 deleterious mutations in the *TPO* gene, chr17:800788G>A (686F>V) and chr17:805276C>T (845T>M), which are highly associated with the disease. These 2 SNP were subsequently genotyped in 182 GLPs (55 affected and 127 unaffected) and the recessive genotypes had relative risks of 16.57 and 16.27, respectively. This study provides novel insight into the genetic causes underlying the familial thyroid follicular cell carcinoma and we were able to develop a genetic test to screen susceptible dogs.

Key Words: dog, thyroid carcinoma, mutation, *TPO*, GWAS

P169 Deletion of the *SELENOP* gene leads to CNS atrophy with cerebellar ataxia (CACA) in dogs. M. Christen*[1], S. Högler[2], M. Kleiter[3], M. Leschnik[3], C. Weber[4], D. Thaller[5], V. Jagannathan[1], and T. Leeb[1], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Unit of Laboratory Animal Pathology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria,* [3]*Department for Companion Animals and Horses, University of Veterinary Medicine Vienna, Vienna, Austria,* [4]*Laboklin GmbH & Co. KG, Laboratory for Clinical Diagnostics, Bad Kissingen, Germany,* [5]*Institute of Pathology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria.*

We investigated a hereditary cerebellar ataxia in Belgian Shepherd dogs. Affected dogs developed uncoordinated movements and intention tremor at 2 weeks of age. The severity of clinical signs was highly variable. Histopathology demonstrated atrophy of the CNS, particularly in the cerebellum. Combined linkage and homozygosity mapping in a family with 4 affected puppies delineated a 52 Mb critical interval. The comparison of whole-genome sequence data of one affected dog to 735 control genomes revealed a private homozygous structural variant in the critical interval, chr4:66,946,539_66,963,863del17,325. This deletion includes the entire protein-coding sequence of *SELENOP* and is predicted to result in complete absence of the encoded selenoprotein P required for selenium transport into the CNS. Genotypes at the deletion showed the expected co-segregation with the phenotype in the investigated family. Total selenium levels in the blood of homozygous mutant puppies of the investigated litter were reduced to about 30% of the value of a *wt/wt* littermate. Genotyping a cohort of >600 Belgian Shepherd dogs revealed an additional homozygous mutant dog. This dog also suffered from pronounced ataxia, but reached an age of 10 years. So far, no human patients or other domestic animals with *SELENOP* loss-of-function variants were reported. *Selenop*$^{-/-}$ knockout mice were reported to develop ataxia, but their histopathological changes were less severe than in the investigated dogs. Our results demonstrate that deletion of the *SELENOP* gene in dogs cause a defect in selenium transport associated with CNS atrophy and cerebellar ataxia (CACA). The affected dogs represent a valuable spontaneous animal model to gain further insights into the pathophysiological consequences of CNS selenium deficiency.

Key Words: *Canis lupus familiaris*, neurology, selenium, animal model, precision medicine

P170 *ABHD5* frameshift deletion in golden retrievers with ichthyosis. S. Kiener*[1,2], D. J. Wiener[3], K. Hopke[4], A. B. Diesel[4], V. Jagannathan[1], E. A. Mauldin[5], M. L. Casal[5], and T. Leeb[1,2], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Dermfocus, University of Bern, Bern, Switzerland,* [3]*Department of Veterinary Pathobiology, Texas A&M College of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA,* [4]*Department of Small Animal Clinical Sciences, Texas A&M College of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA,* [5]*University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA, USA.*

Ichthyoses are hereditary disorders caused by defects in the formation of the outermost layer of the epidermis, the stratum corneum. So far, 5 different genetically determined breed-specific forms of canine ichthyoses have been described. An insertion-deletion (indel) variant in *PNPLA1* causes a relatively common ichthyosis in golden retrievers. In this study, we investigated golden retrievers with fine and large scales adhering to the skin surface and exfoliating into the hair coat. Histopathological examinations showed lamellar, orthokeratotic hyperkeratosis and mildly hyperplastic epidermis that led to the diagnosis of non-epidermolytic ichthyosis. Despite the clinical and histopathological similarity with the *PNPLA1*-related ichthyosis, the affected golden retrievers of our investigation were not homozygous for the *PNPLA1* indel. Combined linkage analysis and homozygosity mapping in 14 cases and 30 nonaffected family members delimited a critical interval of ~12.7 Mb on chromosome 23. Whole-genome sequencing of one affected dog revealed a single protein-changing variant within this region that was not present in 796 control genomes. The identified variant, c.1006_1019del, is a 14 bp deletion in the *ABHD5* gene, leading to a frameshift and truncating the last 14 codons p.(Asp336Serfs*6). The variant showed perfect association to the ichthyosis phenotype in a golden retriever cohort of 14 cases and 470 controls. *ABHD5* encodes an acetyltransferase required for the synthesis of phosphatidic acid, the major intermediate in membrane and storage lipid biosynthesis. In humans, variants in *ABHD5* have been described to cause Chanarin-Dorfman syndrome, a neutral lipid storage disease with ichthyosis. The perfect genotype-phenotype association together with the knowledge on the effects of *ABHD5* variants in humans strongly suggest

ABHD5:c.1006_1019del as candidate causative genetic variant for a new form of ichthyosis in golden retrievers.

Key Words: dog, dermatology, metabolism, animal model, precision medicine

P171 Transcriptomic profile of peripheral whole blood reveals novel potential diagnostic gene biomarkers of degenerative joint disease (osteoarthritis) in German shepherd dogs. G. Rudd Garces, P. Peral Garcia, G. Padula, and G. Giovambattista*, *IGEVET – Instituto de Genética Veterinaria "Ing. Noel Dolout" UNLP-CONICET LA PLATA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina.*

Degenerative joint disease (osteoarthritis) is one of the most common chronic musculoskeletal diseases in human population. Susceptibility to osteoarthritis has been associated with more than 90 quantitative trait loci (QTL). Canine forms of degenerative joint disease are very similar to those in humans and represent a welfare problem in the dog world population. In this study, we investigated the transcriptomic profile of peripheral whole blood in German shepherd dogs with degenerative joint disease to identify biomarkers. The bulk RNA-seq experiment was performed in a cohort of 12 adult dogs, 5 affected and 7 unaffected. Radiographs in affected dogs revealed severe osteoarthritis in hip and elbow joints. The differential expression analysis showed 688 genes differentially expressed in comparison to the control group (P-value <0.05). This pool of genes was functional annotated for signaling pathways using PANTHER tools. The enriched genes were those associated with inflammation mediate by chemokine and cytokine signaling pathway. To gain further insights of the functional role of these genes in osteoarthritis, we selected those with fold change values less or equal to −2.0 and bigger or equal to 2.0. We found 8 genes highly upregulated and 9 genes strongly downregulated. Prioritization of these genes according to their functional knowledge revealed 2 clear top candidate biomarkers. The downregulated *OSCAR* gene encodes the osteoclast associated protein which is involved in the regulation of osteoclastogenesis and bone homeostasis. On the other hand, the upregulated *TRPM2* gene is involved in oxidative stress-induced cell death and inflammation processes. Our data suggests *OSCAR* and *TRPM2* as novel candidate biomarkers for degenerative joint disease in German shepherds, this may facilitate genetic testing to improve the diagnosis and clinical treatment.

Key Words: dogs and related species, animal breeding, RNA-seq, gene expression, animal health

P172 Genome-wide association study identifies a risk locus on CFA18 for congenital laryngeal paralysis in Alaskan sled dogs. S. Krishnamoorthy*[1], D. J. F. von Pfeil[2], B. J. Stanley[3], C. Griffitts[4], and H. J. Huson[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY, USA,* [2]*Small Animal Surgery Locum, PLLC, Dallas, TX, USA,* [3]*Department of Small Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA,* [4]The Travelling Vet LLC, CO, USA.

Congenital laryngeal paralysis (CLP) is the inability to move the laryngeal cartilages during inspiration. Loss of normal function of the larynx causes respiratory distress and reduced exercise ability. Alaskan sled dogs (ASD) are bred for athletic prowess and are used in sprint and long-distance racing competitions in North America. CLP has been documented as an inherited disease in several dog breeds and also suggested to exist in ASD, for which it is commonly diagnosed before 1 year of age and can drastically affect their racing ability and can even become life-threatening. The genetic abnormality causing this disease has not been previously explored in ASD. Therefore, in this study, we genotyped 20 cases and 198 control ASD's using Illumina's Canine HD chip and performed a linear mixed model based genome-wide association study (GWAS) to identify genomic regions with potential candidate genes associated with CLP. Post quality control for minor allele frequency, genotyping rate, and missingness rate, 120,296 SNPs remained for the association analysis. Significant Bonferroni-corrected genome association ($P < 4.15 \times 10^{7}$) to CLP was found on chromosome 18. Peaks of suggestive association ($P < 8.3 \times 10^{6}$) were also noted on chromosomes 4, 11, 23 and 31. The most significantly associated SNP, BICF2G630691235 ($P < 49.58 \times 10^{12}$) was located on CFA18. The other top significantly associated SNPs were BICF2G630691232, BICF2S23647655, BICF2P3270, BICF2P870496, and BICF2P1510533, all of which are located on CFA 18. The top 2 significantly associated SNPs (BICF2G630691235 and BICF2S23647655) were in strong linkage disequilibrium ($r^2 > 0.8$) and the LD block spanned a 3.2-kbp region comprising *EXT2* (exostosin glycosyltransferase 2). The LD block regions of the other 4 SNPs harbored tumor protein *TP53I11* (p53 inducible protein 11), *TSPAN18* (tetraspanin 18), and *SHANK3* (SH3 and multiple ankyrin repeat domains). Mutations in *EXT2* and *TP53I11* have been associated with skeletal and muscular developmental disorders and tumors, while mutations in *TSPAN18* and *SHANK3* have been implicated in neurological disorders. Further investigations are needed to understand the role of these putative candidate genes and to identify the causative mutations of CLP in ASD.

Key Words: dog, genotyping, GWAS, congenital laryngeal paralysis

P173 Description of breed ancestry and genetic health traits in Arctic sled dog breeds. J. Thorsrud* and H. Huson, *Cornell University, Ithaca, NY, USA.*

This study describes the presence and frequency of health traits among 3 populations of dogs traditionally used for sledding and explores their ancestry and breed composition as provided by the commercially available Embark dog DNA test. The 3 populations include the purebred Siberian Husky and the admixed populations of Alaskan sled dogs and Polar Huskies. While the Siberian Husky represents a well-established breed with extensive historical and health data, the Alaskan sled dog is less studied but has been the subject of nutritional, physiological, and genetic studies related to ancestry and performance. In contrast, the Polar Husky is a relatively obscure and rare group of dogs used for Arctic exploration with very little known information. The 3 populations were compared using Embark results, providing new insight into the health traits circulating within the populations and the potential ancestral linkage of the health traits between the sledding populations. Embark results are based upon 228,588 single nucleotide polymorphisms (SNPs) spanning the canine genome, characterized using a custom-designed Illumina BeadChip array. Specifically, mitochondrial (MT) and Y chromosome haplogroups and haplotypes were found, and breed composition was summarized for the 2 admixed populations. A genomic principal component analysis was also constructed using the genotype data to identify population structure within the sledding dogs. Genetic markers associated with alanine aminotransferase (ALT) activity, Alaskan husky encephalopathy, dilated cardiomyopathy, collie eye anomaly, degenerative myelopathy, ichthyosis, and factor VII deficiency were identified in the populations of sledding breeds. These results provide a preliminary description of genetic characteristics found in sledding breeds, improving the understanding and care of working sled dogs.

Key Words: dog, animal health, animal breeding, breed diversity

P174 Genomic DNA extraction from canine feces for genotyping and identification with targeted genotyping by sequencing (GBS) application. Q. Hoang, K. Kice, C. Carrasco, S. Chadaram*, and R. Conrad, *Thermo Fisher Scientific, Austin, TX, USA.*

The desire to use canine feces as a source of canine genomic DNA is growing due to local governments wanting to identify those pet owners that do no pick up after themselves in public spaces. Unfortunately, obtaining genomic DNA from canine feces that is high enough in quality and quantity to use in many genomic sequencing applications is often difficult and expensive. Feces is known to contain high amounts of PCR inhibitors that must be effectively removed from extractions. Additionally, canine DNA is found mostly on the surface of the fecal material and must be

swabbed from the surface. Finally, the high amount of bacterial DNA that is extracted from the feces makes the total percentage of canine DNA very low in fecal DNA extractions. As a result of these factors, sequencing data from canine feces give poor results that are often unusable. Here, we look at genomic DNA extracted by swabs using the MagMAX CORE Nucleic Acid Purification Kit as a method for obtaining DNA from canine feces that is suitable for sequencing applications. We compare DNA obtained from feces with DNA obtained from a saliva swab. We found that though the call rates are lower using feces, the calls for both sample types are the same. We conclude that the MagMAX CORE Nucleic Acid Purification Kit yields genomic DNA from canine feces that is suitable for identifying individual dogs from sequencing results.

Key Words: fecal DNA, canine genotyping, DNA extraction, identification, targeted genotyping by sequencing (GBS)

P175 Canine Y chromosome features uncovered by long-read sequencing assembly and male dog phylogeny inferred from Y haplotype. W. Zhang*, L. Eory, E. Clark, A. Archibald, and J. Schoenebeck, *Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK.*

The Y chromosome is the most rapidly evolving nuclear chromosome, and its gene content and structural complexity varies across mammals, which bears a unique record of evolutionary history. The male-specific haplotype characterizes and calibrates Y-chromosome phylogeny, and the acquisition, loss, and amplification of male-specific genes have an impact on male biology such as development and fertility. This study assembled a male Labrador retriever dog Y chromosome using PacBio, Bionano, Dovetail HiC, and Illumina sequencing data. Our assembly resolves the 5 Kb pseudoautosomal boundary region (PAB) and presents a nearly full-length male-specific Y region (MSY) compared with the previous study [1]. As a result, a total of 21 male-specific genes are annotated, 5 of which are multiple copies, and another 16 genes are single-copy genes. The self-similarity plot shows a 1.5-Mb length ampliconic region, which contains 7 single-copy genes. For the PAB, we observed the X-Y similarity decreased to ~80%, down from 95% similarity observed in the pseudoautosomal region (PAR). By comparing X-PAB and Y-PAB, we found 2 Y-specific SINE transposable element insertions that are conserved in the Canina subtribe. These 2 SINE elements are inserted in the first exon and first intron of the claudin 34 (*CLDN34*) gene. The SINE insertions in Y-*CLDN34* are predicted to cause loss of protein-coding regions. Using short-read resequencing data, we built a canine phylogeny based on a Y-chromosome haplotype. A series of single nucleotide variants (SNV) filtering steps were conducted, resulting in a total of 1,092 SNVs in 422 male samples. Our phylogenetic tree shows, with the exception of Arctic breeds and dogs of African origin, that modern dogs have a very close paternal ancestry. Moreover, 4 gray wolf samples are carriers of dog haplogroups, indicating ancient admixture events between dogs and wolves, or that the Y chromosome haplotype predated that split between wolves and dogs. The observation of wolves' placement on dogs' clades is consistent with the previous study [2]. Our study highlights the dynamic nature of the Y chromosome and provides a reference sequence for an improved understanding of *Canis* evolution and male fertility. [1] Li et al. *Genome Research* 23.9 (2013): 1486–1495. [2] Oetjens et al. *BMC Genomics* 19.1 (2018):1–9.

Key Words: modern dogs, Y chromosome, pseudoautosomal boundary, phylogeny

P176 Genes differential expression analysis in spontaneously occurring canine melanoma. S. Perga[1], C. Beltramo[1], F. Fruscione[1], I. Martini[1], F. Cavallo[2], F. Riccardo[2], P. Buracco[3], S. Iussich[3], E. Razzuoli[1], K. Varello[1], L. Maniscalco[1], E. Bozzetta[1], A. Ferrari[1], and P. Modesto*[1], [1]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, National Reference Center for the Veterinary and Comparative Oncology (CEROVEC), Genoa, Italy,* [2]*Department of Molecular Biotechnology and Health Sciences Molecular Biotechnology Center, Turi, Italy,* [3]*Department of Veterinary Science, Turin, Italy.*

Human and canine melanoma have common clinical, histologic making dogs a good model for comparative oncology. The identification of specific genes and a better understanding of the genetic landscape, signaling pathways and tumor–microenvironmental interactions involved in the cancer onset and progression is essential for the development of therapeutic strategies against this tumor in both species. In our study we investigated the differential expression of genes in spontaneously occurring canine melanoma and in paired normal tissue by targeted RNA-seq. Total RNA was extracted from 17 canine malignant melanoma (CMM) samples and from 5 paired normal tissues stored in RNAlater. To capture the greater genetic variability, we carried out gene expression analysis using 2 panels (Qiagen): Human Immuno-Oncology (HIO) and Mouse-Immuno-Oncology (MIO) and the Miseq platform (Illumina). The kits allow the detection of the expression profile of 990 genes involved in the immune response against tumors in humans and mice. The data were analyzed through the CLCbio Genomics Workbench (Qiagen) software using the C*anis lupus familiaris* genome as reference. Data analysis were carried out both comparing the biologic group (tumoral vs healty tissues) and comparing neoplastic tissue vs paired healthy tissue setting a FC ≥2 and $P \leq 0.05$. Using HICP 63 downregulated genes were detected, 13 of those were also downregulated comparing neoplastic sample vs paired healthy tissue. Eighteen genes were upregulated, 14 of those were also downregulated comparing neoplastic sample vs paired healthy tissue. Using the MICP, 35 downregulated genes were detected, only 4 of these were downregulated also comparing neoplastic sample vs paired healthy tissue. Twelve genes were upregulated in both type of analysis. Dogs displayed a greater genetic homology with humans than mice, moreover, the results have shown that the 2 kits are able to detect different genes. Most of these genes have specific cellular functions or belong to some enzymatic categories, some have been already described tu be correlated to human melanoma and confirm the validity of the dog as a model for the study of molecular aspects of human melanoma.

Key Words: dogs and related species, oncology, RNA-seq, gene expression, biomedical model

P177 A genome-wide association study of hypertrophic cardiomyopathy susceptibility in cats. J. Raffle*, J. N. Matos, D. J. Connolly, V. L. Fuentes, and A. Psifidi, *Royal Veterinary College, London, UK.*

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease in humans and cats. In humans, thousands of causative HCM mutations have been identified mainly in cardiac sarcomeric genes; however, in 40% of human cases the cause remains unknown. In cats, only 5 causative mutations have been identified both in sarcomeric (*MYBPC3*, *MYH7*) and non-sarcomeric genes (*ALMS1*, *TNNT2)*. In most cat breeds and domestic shorthair (DSH) cats HCM etiopathogenesis remains unknown. Until now only candidate gene approaches have been used to identify causative variants in cats with limited success. The aim of this study was to expand the scanning for genetic variants associated with feline HCM at genome-wide level. We performed a genome-wide association study (GWAS) for HCM-susceptibility (n = 200) across 4 cat breeds (Bengal, Norwegian Forest Cat, Sphynx and DSH) to identify novel genomic associations with HCM. Cat phenotyping was performed by echocardiography and genotyping using the feline 60K SNP Illumina Infinium iSelect DNA array. Quality control included minor allele frequency >0.01, call rate >95% and Hardy–Weinberg equilibrium ($P > 10^{-6}$). GWAS was performed with the GEMMA algorithm using a standard univariate linear mixed model in which breed, age and sex were fitted as fixed effects and the genomic relatedness matrix among individuals fitted as a polygenic random effect. Genome-wide significance and suggestive significant levels were set at $P < 0.05$ and one false discovery per genome scan, respectively, and a Bonferroni correction for multiple testing was applied. GWAS revealed the presence of 7 markers located on chromosomes A1,

B2, B3, B4 and D4 associated with HCM at the suggestive significance level. One such marker located close to *CITE2* gene has been previously associated with cardiac disease in humans. The data highlighted numerous genomic associations with feline HCM, indicating that the genomic architecture of the disease is complex polygenic with similarities to human HCM. Future plans include validation in another cat population alongside whole-genome sequencing analysis (n = 16) to identify candidate causative variants for feline HCM.

Key Words: cat, hypertrophic cardiomyopathy (HCM), SNP marker, GWAS

P178 New variant in *ADAMTS2* segregates with recessively inherited Ehlers-Danlos syndrome in a cat family. R. Simon*[1], S. Kiener[2,3], N. Thom[4], L. Schäfer[4], M. Roy[1], E. K. Schlohsarczyk[5], C. Herden[5], T. Leeb[2,3], and G. Lühken[1], [1]*Institute of Animal Breeding and Genetics, Justus Liebig University, Giessen, Germany,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [3]*Dermfocus, University of Bern, Bern, Switzerland,* [4]*Clinic for Small Animals, Justus Liebig University, Giessen, Germany,* [5]*Institute of Veterinary Pathology, Justus Liebig University, Giessen, Germany.*

The Ehlers-Danlos syndrome, known in humans as well as in different animal species, belongs to the group of heritable connective tissue disorders. Skin hyperextensibility, abnormal scarring, and poor wound healing are typical symptoms. Pathogenic variants in 20 different genes have been reported in different types of human EDS including genes associated with the synthesis of collagen. In domestic cats, only one gene, *COL5A1*, was reported to be involved in an autosomal-dominant form of EDS. As index case, a kitten with skin lesions suspicious for EDS was discovered in a cohort of European domestic shorthair cats free roaming on a farm. The kitten was found dead next to 2 unaffected littermates. Later, 3 additional affected kitten were observed in 2 subsequent litters. All 3 litters were probably sired by one unaffected tomcat. Crusted areas on the face were observed as a result of wound healing, as well as large areas of deep traumatic ulceration at friction sites. Their skin was thin and showed excessive tearability. A hernia diaphragmatica and a rectal prolapse were present in the first affected kitten. We sequenced the whole genome of the first affected kitten and mapped it against the FelCat9.0 reference genome. Filtering for homozygous variants absent in 48 other cats from diverse breeds revealed a frameshift variant in the *ADAMTS2* gene encoding a protease required for the correct processing of procollagen. Loss-of-function variants in *ADAMTS2* cause autosomal-recessively inherited EDS (dermatosparaxis or type VII C) in humans and dogs. The identified variant is predicted to truncate ~80% of the wild-type. PCR primers were designed for genotyping all available family members by Sanger sequencing. The mutant allele was absent or heterozygous in all nonaffected cats. The presumable tomcat and the 3 mothers were found to be heterozygous, which confirms the expected autosomal recessive inheritance of this EDS form. The 3 other affected kittens were homozygous for the mutant allele demonstrating perfect co-segregation of the phenotype with the genotype in the family. Due to the severity of the skin lesions, 2 affected kittens had to be euthanized shortly after the examination.

Key Words: cats and related species, genome sequencing, animal health, genetic disorder

P179 Genome-wide association studies identify novel quantitative trait loci for canine health traits. H. J. Huson*[1], D. M. Holle[2], A. Walker[1], N. Anclade[1], and K. M. Evans[2], [1]*Department of Animal Science, Cornell University, Ithaca, NY, USA,* [2]*The Seeing Eye Inc., Morristown, NJ, USA.*

Working dogs, particularly those trained as detection, guide, search and rescue, or law enforcement, undergo rigorous health evaluations to minimize the risk of inherited disease and increase a dog's productive life. To improve our understanding of hereditary abnormalities, 37 health traits were assessed in genome-wide association studies (GWAS). German shepherds, Labrador retrievers, golden retrievers, and Labrador by golden retriever crosses in a guide dog program were evaluated by veterinarians before 2 years of age for dental (13), ocular (8), dermatologic (7), orthopedic (2), gastrointestinal (4), connective tissue (1), cartilaginous (1), and muscular (1) abnormalities. Nine hundred ninety-one dogs were genotyped on the Illumina Canine HD array, providing 939 dogs with 122,966 single nucleotide polymorphisms for analysis after quality control filtering on call rate, minor allele frequency, number of alleles, and identity by descent. Genomic principal component analysis was conducted to validate individual breed designation and overall population structure. A single-locus mixed model (EMMAX) using a genomic relationship matrix as a fixed effect and an additive inheritance model was used for an across-breed GWAS for each trait. Twenty-three trait GWAS produced SNPs passing false discovery rate multiple testing correction and 18 trait GWAS produced SNPs passing Bonferroni multiple testing correction (P-value <0.05). Of the traits passing one or both multiple testing corrections, 9 were dental, 7 were ocular, 1 was orthopedic, 4 were dermatologic, 1 was muscular, and 1 was cartilaginous. Pseudo-heritability estimates ranged from 0 to 0.62 across the varying traits with a mean of 0.16 and median of 0.09. In all, 62% of the health traits showed significant genetic association and are being explored further for candidate genes and considered for fine-mapping. The current GWAS highlighted SNPs potentially influencing traits in multiple breeds whereas future studies will seek breed-specific associations. These findings increase our knowledge of quantitative trait loci of health traits and provide a foundation for future genetic selection programs.

Key Words: working dog, canine, GWAS, health

P180 ROS_Cfam_1.0: A high-quality, *de novo* assembly of a male Labrador retriever. L. Eory, W. Zhang, D. Ozdemir, E. Clark, A. Archibald, and J. Schoenebeck*, *The Roslin Institute and Royal (Dick) School of Veterinary Studies, Midlothian, UK.*

The Kennel Club recognizes 218 dog breeds which are assigned to 7 groups based on animals' form and function. Phylogenetics indicate that clades roughly reflect breeds' group assignments, as expected since many group-level breeds share common ancestries. To date, dog genome assemblies are available for the boxer (working group), great Dane (working group), German shepherd (pastoral), basenji (hound) and Australian dingo. All but the basenji assembly were produced from female dogs. Here we describe our efforts to generate a high-quality genome assembly from a male Labrador retriever, a medium-sized member of the gundog group that traces its origins to Newfoundland, Canada. The Labrador retriever is one of the most recognizable breeds of dogs worldwide. Because of their trainability and disposition, this breed is not only popular among pet owners, but as well they are commonly used as service animals. Using FALCON-Unzip, we generated 1,161 primary contigs from PacBio Sequel long-read sequences (56.5x coverage, N50 = 9.1Mb). The primary haploid genome was 2.4Gb. Bionano optical mapping (214x raw coverage) was used to assembly 75 scaffolds which included 30 chromosome-level assemblies (scaffold N50 = 77Mb). Following identification and correction of misassemblies, further scaffolding was done using 56x Hi-C data which were processed using Dovetail HiRise. The remaining scaffolds and unplaced contigs were gap-filled and polished using Illumina short-read data. The final assembly contained 376 scaffolds with a scaffold N50 of 64Mb and total length of 2.4Gb. Mitochondrial sequence was assembled and added to the primary assembly separately. Designated "ROS_Cfam_1.0," our Labrador retriever assembly and annotation are available on popular archives including NCBI and Ensembl's Rapid Release site. Future efforts to improve the utility of ROS_Cfam_1.0 are underway, including deposition of CAGE-seq and Iso-seq annotation data as well as efforts to resolve diploid content.

Key Words: *de novo* assembly, dog, genomics, reference

P181 Populational structure analyses of Brazilian Mastiff dog breed. F. de Andrade*, R. Nunes, D. Tyska, and J. Cobuci, *Grupo de Pesquisa MegaGen, Departamento de Zootecnia, UFRGS, Porto Alegre, RS, Brazil.*

Although Brazil has the second biggest pet market of the world, only 3 national dog breeds are recognized by "Federation Cinologique Internationale" (FCI) system, being Brazilian Mastiff the oldest one. It is a molossus breed, probably originated from Mastiffs and Bloodhounds breeds, brought to Brazil by colonizers. In 2020, this was the fourth large size breed with the highest record number in the official Brazilian FCI institution (CBKC), with 2,202 dogs. Besides, since the 80´s, a dissident breeder association also records dogs from this breed. Despite of the expressive breeding in the country, the structure of Brazilian Mastiff population has never been evaluated, and the genetic status of the breed is totally unknown. Therefore, the present work has the aim of investigating populational parameters of Brazilian Mastiff and, for that, data from 51,105 dogs were downloaded from dogfamily.com website, where there is pedigrees from dogs of both institutions (CBKC and the dissident one), with free access. Effective size was calculated through 1/2ΔF, being ΔF obtained by Gutierrez et al. (2019) method. Parameters as inbreeding and effective population size were obtained through CFC and POPREP software's. The existence of populational genetic differentiation was investigated through principal component analyses (PCA), using 'kmeans' algorithm from R package. Mean value of inbreeding in the sample was of 5.21%, with 19.2% of the dogs with F values above 10%, being 16 animals with values above 50%. From total sample, 63.9% of the dogs were endogamic. The peak of mean inbreeding was attained in 2007, slightly decreasing after this year. Population effective size (Ne) varied from 48 to 55 animals in the last 5 years. PCA detected 2 different clusters in the population (k = 2). The present results indicate a small population diversity, due to an expressive number of endogamic dogs, with an extremely reduced effective size. Urgent interventions are needed, to decreasing the genetic proximity of dogs to be bred, with the aim of contributing to the breed conservation.

Key Words: dog, animal breeding, population structure, breed diversity, conservation

Comparative and Functional Genomics Posters

P182 Detailed molecular and epigenetic characterization of pig IPECJ-2 and chicken SL-29 cell lines. J. de Vos*[1], R. Crooijmans[1], M. Derks[1], S. Kloet[2], M. Groenen[1], and O. Madsen[1], [1]*Animal Breeding and Genetics Group, Wageningen University and Research, Wageningen, the Netherlands,* [2]*Leids Universitair Medisch Centrum, Leiden, the Netherlands.*

The IPECJ2 cell line in pigs and the chicken SL-29 cell line are of interest for the animal genomics community because of the untransformed nature and wide use in functional studies of these cells. The IPECJ2 cell line in pigs from intestinal epithelial cells has been used to investigate e.g., intestinal transportation while the chicken SL-29 cell line, which is derived from embryonic untransformed fibroblast cells, has been used to provide insight into immune function. It is important to molecular characterize these cell lines to gain insight into possible molecular aberrations, and their effects on e.g., gene expression. Gene expression is regulated by interaction between enhancers, promoters, insulators, epigenetic marks and chromatin binding factors, often referred to as the functional genome. The aims of this research were 2-fold. First, a molecular and epigenetic characterization of the pig IPEC-J2 and chicken SL-29 cell lines and second providing a cell-line reference for the FAANG community. These aims were obtained through whole-genome sequencing (WGS), gene expression (RNA-seq), DNA methylation (WGBS and RRBS), chromatin accessibility (ATAC-seq) and ChIP-seq of 4 histone marks (H3K4me1, H3K4me3, H3K27ac, H3K27me3) and an insulator (CTCF). In both cell lines heteroploidy (aneuploidy) of various chromosomes was observed from WGS analysis. Furthermore, gene expression analyses showed higher gene expression for genes located on chromosomes with aneuploidy, in comparison to diploid chromosomes. Investigation into the chromatin accessibility identified promoters, enhancers and gene silencing regions. Using motif analyses of the identified promoters and enhancers, we identified transcription factors (TF) regulating possible corresponding genes. Insights into regulatory complexity of gene expression and chromatin accessibility was achieved by integrating histone marks and DNA methylation with expression values. These analyses showed that histone marks H3K4me3, H3K27ac and hypomethylation have a positive correlation to gene expression. This investigation into pig and chicken cell lines gives insight into the genome structure, and possibilities of further research using these cell lines as a reference within the FAANG community.

Key Words: Functional Annotation of Animal Genomes (FAANG), epigenomics, cell line, pig, chicken

P183 A comprehensive RNA editome reveals RNA editing sites affecting the function of *HSPA12B* in myogenesis via altering binding ability for *miRNA-181b*. A. A. Adetula*[1,2], X. Fan[1], Y. Zhang[1], Y. Yao[1], J. Yan[1], M. Chen[1], Y. Tang[1], Y. Liu[1], G. Yi[1], K. Li[1,2], and Z. Tang[1,2], [1]*Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China,* [2]*Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

One of the most prevalent forms of posttranscriptional RNA modification is the conversion of adenosine (A) nucleotides to inosine (I), mediated by the ADAR family of enzymes. RNA editing generates diversity in mammals and results in amino acid transitions and changes in gene expression. However, the extent to which RNA editing affect gene expression via modifying microRNA (miRNA) binding site remains unexplored. In this study, we systematically profiled the RNA editome across 10 tissues from Duroc and Luchuan pigs and identified RNA editing sites. A total of 171,909 editing sites were discovered, of which 4,552 were differentially edited sites across tissues between the 2 breeds. The RNA-edited gene sets were enriched in known developmental pathways essential for physiological function and organ development, such as TGF-β, PI3K-Akt, AMPK, and Wnt signaling pathways. Moreover, we found that RNA editing events at the miRNA binding sites in the 3′-UTR of *HSPA12B* mRNA could prevent the miRNA-mediated mRNA downregulation of HSPA12B in the muscle-derived satellite (MDS) cell, consistent with the results obtained from the Luchuan skeletal muscle. This study demonstrates the importance of RNA editing in regulating gene expression and offers a novel approach to understand the genetic mechanisms underlying phenotypic variation in animals.

Key Words: RNA editing, gene expression, *HSPA12B*, microRNA, skeletal muscle-derived satellite cell

P184 Update and some new features of the Animal rDNA database. J. Sochorová*[1], S. Garcia[2], F. Gálvez[3], R. Symonová[4], and A. Kovarík[1], [1]*Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic,* [2]*Institut Botànic de Barcelona (IBB-CSIC-ICUB), Barcelona, Catalonia, Spain,* [3]*Bioscripts - Centro de Investigación y Desarrollo de Recursos Científicos, Sevilla, Andalusia, Spain,* [4]*Research Institute for Limnology, Mondsee, Mondsee, Austria.*

Ribosomal DNA (rDNA) loci encoding 5S and 45S (18S-5.8S-26S) rRNA are important components of eukaryotic chromosomes varying both in numbers and locations. Accumulation of cytogenetic information

about rDNA loci has led us to generate an internet database (http://www.animalrdnadatabase.com/). Here we present its update and new analysis of some data. The collected data are based on in situ hybridization studies (mostly fluorescence-based (FISH)) now collected from >1,040 papers (increase 100% increase), and covering major groups of vertebrates (fish, amphibians, reptiles, birds and mammals) and invertebrates including arthropods, mollusks and annelids. We statistically analyzed the numbers and positions of rDNA loci on different types of chromosomes in more than 2,800 karyotypes (over 340 families). It appeared that fishes have the greatest variability in the number of rDNA loci (1 to 27 5S sites/ 1C and 1 to 27 45S sites/1C). rDNA loci can occur on any chromosome including sex chromosomes (X, Y, Z, and W), microchromosomes and supernumerary B chromosomes. The proportion of rDNA localization on autosomes and sex chromosomes was similar in both mammals and fish. In birds and reptiles, rDNA is often localized on microchromosomes (80% and 41% karyotypes respectively).

Key Words: rDNA, FISH, animal, in situ hybridization

P185 Reference transcriptomes of porcine peripheral blood immune cells created through bulk and single-cell RNA sequencing. J. Herrera-Uribe[1], J. E. Wiarda[2,5], S. K. Sivasankaran[2,6], L. Daharsh[1], H. Liu[1], K. A. Byrne[2], T. P. L. Smith[3], J. K. Lunney[4], C. L. Loving[2], and C. K. Tuggle*[1], [1]*Iowa State University, Ames, IA, USA,* [2]*USDA-ARS-NADC, Ames, IA, USA,* [3]*USDA-ARS-MARC, Clay Center, NE, USA,* [4]*USDA-ARS-BARC, Beltsville, MD, USA,* [5]*Immunobiology Program Iowa State University, Ames, IA, USA,* [6]*Genome Informatics Facility Iowa State University, Ames, IA, USA.*

Two approaches to examine porcine immune cell function and annotate these functions in the porcine genome were taken. First, we used cell sorting to isolate 8 cell types from peripheral blood mononuclear cells (PBMCs), representing monocytes, NK cells and specific populations of T and B cells. Transcriptomes (deep RNA-seq) for each cell type were generated, that detected 10,974 genes; 210 were validated using NanoString. Gene expression enrichment analysis identified 1,885 to 3,591 significantly enriched genes (SEG, $q < 0.05$ and 2-fold above average expression) for all cell types. Comparison of gene expression indicated highly significant correlations (Spearman's rank $P < 2.2e\text{-}16$) between pig cells and corresponding human cells. Second, single-cell RNA sequencing (scRNA) of PBMC populations was performed to more highly resolve PBMC population transcriptomes. Across 7 PBMC samples, 28,810 cells distributed across 36 clusters were classified into 13 general cell types including plasmacytoid dendritic cells (DC), conventional DCs, monocytes, >10 B cell clusters, CD4 and CD8 ab T cells, NK cells, and 4 clusters of gd T cells. Signature gene sets for a human bulk RNA-seq data set were assessed for relative enrichment in genes expressed in pig cells, and substantial overlap in gene expression between specific pig and human PBMC populations was detected. Integration of pig scRNA with a public human scRNA data set provided further validation for similarity between human and pig. Overall, the data provides deep and well-validated transcriptomic data from sorted cell populations and the first single-cell atlas for porcine PBMCs, including highly resolved comparisons to human data sets. Work was supported by USDA-2018-67015-27501 and USDA-ARS CRIS project 5030-31320-004-00D. JH-U and JEW contributed equally; CLL and CKT contributed equally.

Key Words: pig, immune cells, RNA-seq transcriptome, single-cell RNA-seq, FAANG

P186 Uncovering *TUG1* lncRNA-chromatin interaction sites in the bovine genome using ChIRP-seq. R. Bhushan*[1], D. Becker[1], C. Kühn[1], and R. Weikard1,2, [1]*1Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*Agricultural and Environmental Faculty, University of Rostock, Rostock, Germany.*

In the recent past, it has become evident that the genomes of many organisms are pervasively transcribed resulting in numerous long noncoding RNA (lncRNA) transcripts. These lncRNA transcripts act as molecular elements by interacting with DNA, RNA and proteins to regulate the epigenome and diverse fundamental biological processes. In human and mice, lncRNA-chromatin interactions have been shown to regulate crucial cellular mechanisms such as genomic imprinting, dosage compensation and developmental and pathological processes. In the bovine genome, the nature and mechanisms of lncRNA interactions with specific chromatin regions are unexplored so far. ChIRP-seq (chromatin isolation by RNA precipitation followed by DNA sequencing) can be applied to genome-wide map the lncRNA interactome. Here, we aimed to unravel the chromatin occupancy sites of the evolutionary conserved lncRNA TUG1 (Taurine Upregulated 1) in the bovine genome using ChIRP-seq in MDBK (Madin-Darby bovine kidney) cells and bovine liver tissue. Our analysis in MDBK cells revealed a total of 77 specific chromatin binding sites for TUG1 in different genomic regions, such as intronic (37.66%), intergenic (46.75%), intronic-exonic (11.69%) and exonic (3.90%). For example, we detected that TUG1 occupied specific sites in intronic regions of *FGF10*, *HDAC4*, *CNTN4*, *IGFBP3* and lncRNA (ENSBTAT00000083690). Some of these chromatin binding sites were also found in the liver tissue, but the intensity of the ChIRP signals was much lower compared with MDBK cells, which is most likely due to special challenges in carrying out the ChIRP protocol with tissue samples. Nevertheless, we found TUG1 interaction sites in intronic regions of *ABHD12B* and *DEGS1*, exonic regions of *CDC73* and *ZNF142* and upstream of the *PPARGC1A* promoter region in both, cattle liver and MDBK cells, suggesting that these sites are conserved across different bovine cell types and may be targeted by TUG1. In summary, our study experimentally deciphered TUG1 lncRNA-chromatin interaction sites in the bovine genome for the first time, contributing to the functional annotation of noncoding elements in the bovine genome. In the future, our studies will provide the basis for gaining insights into the cis/trans modes of TUG1 regulatory networks.

Key Words: cattle and related species, genome regulation, functional genomics, long noncoding RNA

P187 BovReg: A high-resolution functional annotation of the cattle genome using novel breeds/crosses. G. Costa Monteiro Moreira*[1], S. Dupont[1], D. Becker[2], M. Salavati[3], R. Clark[4], E. L. Clark[3], G. Plastow[5], C. Kühn[2,6], C. Charlier[1], and BovReg Consortium[6], [1]*Unit of Animal Genomics, GIGA Institute, University of Liège, Liège,Belgium,* [2]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany,* [3]*The Roslin Institute, University of Edinburgh, Edinburgh, UK,* [4]*Genetics Core, Edinburgh Clinical Research Facility, The University of Edinburgh, Edinburgh, UK,* [5]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada,* [6]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Transcriptome (mRNA, total RNA and small-RNA) and ChIP-seq assays (antibodies H3K4me3, H3K4me1, H3K27me3, H3K27ac and CTCF) were compiled in a comprehensive catalog of 129 tissue samples collected from 6 individuals of both sexes, different ages, kept in different environments and from 3 divergent breeds/crosses: Belgian dairy (Holstein), Canadian composite (Kinsella crossbred) and German beef/dairy cross (Charolais x Holstein). Libraries were built, and sequenced on the Illumina NovaSeq 6000 (150nt paired-end - mRNA and total RNA; 100nt/50nt single-end - small-RNA; 100nt paired-end - ChIP-seq). Preliminary data analyses for transcriptome assays were performed using Nextflow/nf-core pipelines (RNA-seq-1.4.2, smrnaseq-1.0.0) adopting a guided transcript assembly approach (Stringtie) for mRNA and total RNA. For ChIP-seq analysis, mapping (ARS-UCD1.2, 1000bulls), read processing and peak calling were performed using BowTie2, SAMtools and MACS2 software, respectively. mRNA and total RNA predicted transcripts were compared with the annotation from Ensembl v.102. All annotated loci (Ensembl v.102) with exon-overlapping transcripts were represented in the new annotation. Moreover, 14,893 genes had at least one potentially novel transcript model and 133,226 novel transcripts were

predicted. Overall, miRNAs were the predominant small-RNA class detected across the tissues (81.1% corresponded to miRNAs, 0.4% rRNA, 0.3% tRNA, 3.02% snoRNA, 0.93% snRNA, 14.25% either mitosRNA, unknown or artifacts). piRNAs were detected in testis, representing the majority of small-RNAs in adult testis and about 30% in neonate testis. Combining the ChIP-seq results from a subset of tissue samples, 73,739 putative active promoter and transcription start site (TSS) states (H3K4me3), 27,408 active enhancer states (H3K27ac and H3K4me1), 12,365 repressive states (H3K27me3) and 68,505 insulators bound by CTCF were detected. The data generated, combined with the new map of TSS already available (CAGE) and ATAC-seq, Hi-C, ChIRP, RRBS, lncRNA and circRNA which will be generated in collaboration with other partners in the BovReg consortium, will yield improved functional annotation of the cattle genome.

Key Words: cattle and related species, Functional Annotation of Animal Genomes (FAANG), functional assay, regulatory element

P188 Seasonal changes in the adipose transcriptomes in semi-domesticated reindeer (*Rangifer tarandus*). M. Weldenegodguad*[1,2], K. Pokharel[1], L. Niiranen[3], P. Soppela[4], I. Ammosov[5], M. Honkatukia[6], H. Lindeberg[1], J. Peippo[1,6], T. Reilas[1], N. Mazzullo[4], K. A. Mäkelä[3], T. Nyman[7], A. Tervahauta[2], K.-H. Herzig[8,9], J. Kantanen[1], [1]*Natural Resources Institute Finland (Luke), Jokioinen, Finland,* [2]*Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland,* [3]*Research Unit of Biomedicine, Faculty of Medicine, University of Oulu, Oulu, Finland,* [4]*Arctic Centre, University of Lapland, Rovaniemi, Finland,* [5]*Board of Agricultural Office of Eveno-Bytantaj Region, Batagay-Alyta, The Sakha Republic (Yakutia), Russia,* [6]*NordGen—Nordic Genetic Resource Center, Ås, Norway,* [7]*Department of Ecosystems in the Barents Region, Norwegian Institute of Bioeconomy Research, Svanvik, Norway,* [8]*Research Unit of Biomedicine, Medical Research Center, Faculty of Medicine, University of Oulu, Oulu, Finland,* [9]*Oulu University Hospital, Oulu, Finland.*

Adipose tissues play a vital role in regulating energy homeostasis and thermogenic activity in animals living in northern and Arctic environments by changing gene expressions in their tissues. Here, we conducted transcriptome profiling of 3 adipose tissues from different anatomical depots (metacarpal, perirenal and prescapular) from Finnish and Even reindeer (from Sakha, Russia) at 2 seasons (spring and winter) using RNA-seq technology. A total of 220.5 Gb of clean reads were generated using Illumina HiSeq platform. On average 36.5 million pair-ended clean reads were obtained for each sample, and a total of 16,362 genes were expressed in our data. Gene expression profiles in metacarpal bone marrow adipose tissue were distinct and clustered separately from perirenal and prescapular adipose tissues. Metacarpal adipose tissue seemed to play a key role in reindeer energy metabolism regulation in the spring, when the animals are in their poorest nutritional condition after winter. During spring, the genes associated with the immune system, such as cytokines, chemokines, interferons and interleukin receptors (e.g., *CCL2*, *CCL11*, *CXCL14*, *IGSF3*, *IGHM*, *IGLC7*, *JCHAIN*, and *IGSF10*), were upregulated in perirenal and prescapular adipose tissue, while genes involved in energy metabolism (e.g., *ACOT2*, *APOA1*, *ANGPTL1*, *ANGPTL8*, *ELOVL7*, *PFKFB1*, and *ST3GAL6*) were upregulated in metacarpal adipose tissue. Finnish reindeer revealed relatively higher number of significantly differentially expressed genes irrespective of the season than Even reindeer, possibly owing to climatic and management differences. In summary, the findings of this study revealed several adipose candidate genes potentially involved in immune response, fat deposition, energy metabolism, development, cell growth, and organogenesis. These results provide helpful information on the mechanisms by which reindeer adapt to harsh Arctic conditions.

Key Words: energy metabolism, immune process, metacarpal adipose tissue, perirenal adipose tissue, prescapular adipose tissue

P189 Genes related to chemotaxis of the immune system underlie ongoing indicine-taurine cattle domestication at copy number variation hotspots. V. H. da Silva*[1], L. Correia De Almeida Regitano[2], A. Zerlotini Neto[3], G. Barreto Mourão[1], and L. Lehmann Coutinho[1], [1]*Department of Animal Science, University of São Paulo (USP), Luiz de Queiroz College of Agriculture (ESALQ), Piracicaba, São Paulo, Brazil,* [2]*Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil,* [3]*Embrapa Informática Agropecuária, Campinas, São Paulo, Brazil.*

The domestication of aurochs (*Bos primigenius*) gave rise to different taurine (*Bos taurus*) and indicine (*Bos indicus*) breeds. The beef production is either based on taurine, indicine or even mixed breeds, but the choice of the best breed, for a specific production system, depends on different factors such as climate. Indicine breeds are more resistant to tropical parasites whereas taurine breeds are adapted to temperate environments. Genes underlying taurine-indicine domestication can be uncovered by the sequences that are dissimilar between their genomes, and among them are the copy number variation (CNV) hotspots. To extend the knowledge about the biological pathways associated with ongoing cattle domestication we (i) inferred the synteny between taurus and indicus-hybrid reference genomes, (ii) overlapped the regions of synteny break with polymorphic CNVs from a population of 765 Nelore (*Bos indicus*) animals and (iii) performed an enrichment analysis with the genes at the regions of overlap. We found 51 polymorphic CNV regions, i.e., hotspots located at regions of synteny break, encompassing 219 genes that are significantly enriched (FDR adjusted *P*-value <0.05) for 3 different immune-related KEGG pathways (S. aureus infection, NOD-like receptor and IL-17 signaling). Moreover, several gene ontology (GO) biological processes associated with chemotaxis, which is essential to immune system function and homeostasis, were significantly enriched (FDR adjusted *P*-value <0.01). Our results indicate that CNVs on genes associated with the immune system chemotaxis are linked to the current taurine-indicine differentiation and are still a relevant source of variation for a genomic selection for disease resilience, at least in widespread indicine breeds such as Nelore. FAPESP process number 20/00340-0.

Key Words: synteny, evolution, structural variation

P190 Liver RNA-seq expression analysis in cattle supplemented with rumen-protected choline. D. Hernández Maizón[1], P. Alvarez Cecco[1], H. Morales Durand[1], L. H. Olivera[1], M. E. Fernandez[1], P. Peral Garcia*[1], G. Giovambattista[1], and A. Rogberg-Muñoz[1,2], [1]*IGEVET – Instituto de Genética Veterinaria "Ing. Noel Dolout" UNLP-CONICET LA PLATA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina,* [2]*IMPA - Instituto de Mejoramiento y Producción Animal, Facultad de Agronomía (UBA-CONICET), Ciudad Autónoma de Buenos Aires, Argentina.*

Nutrition plays a major role in animal performance, while nutrigenomic studies how food affects gene expression. Several researches were done over feeding systems including diets, feedstuffs, essential nutrients, vitamins and cofactors. Within them, choline is a water-soluble essential nutrient that serves as methyl donor and cofactor; as a precursor of acetylcholine, phospholipids and betaina; required for growth, neural development and fat metabolism. Choline is de novo synthesized but a dietary intake is required, but in ruminants has been used as rumen-protected choline (RPC) as it is easily degraded in rumen. Despite its demonstrated metabolic effect, little is known about the regulation of gene expression in the liver of choline supplement in beef cattle. The objective of this study was to evaluate the differential gene expression and enriched pathways when adding RPC to the diet of fattening beef cattle. A total of 11 Braford steers were fed during 85 d with a formulated diet (89% DM, 15.1% CP, 31% NDF; 5.6% EE, 43.3% starch, 2.9 Mcal ME/kg DM), whereas 6 of them receive 6 g/animal/day of RPC. At slaughter, a liver sample was taken, preserved in RNA-later, and whole expression NGS sequencing was performed. Quality control of sequences, alignment to reference genome (UMD3.1) and raw count matrix data was conducted using FASTQC, hisat2, samtools and featureCounts softwares. Differential expression analysis was carried out by edgeR and DESeq2, R packages. DAViD, FAANG

and Panther tools were used to get the Gene Ontology (GO) enriched terms. Results showed 945 genes with significantly modified expression ($P < 0.05$), and 13 of them remained after Holm-Bonferroni correction. The GO analysis identified terms related to the enhancement of immunity (innate immunity, T-cell activation and B cell receptor signaling pathway), cellular transportation (exosome, scavenger receptor activity and LDL transport), protein binding and collagen trimer. This agreed with several studies that showed an increment of immune response when choline is supplemented, and support the reported hepatic protection. Finally, could introduce information about the mechanism behind the liver fat reduction and enhanced milk production observed in dairy cattle.

Key Words: liver, choline, RNA-seq, expression, cattle

P191 Influence of fetal weight on liver transcriptome in purebred and crossbred Iberian pig fetuses. Y. Núñez*[1], C. García Contreras[1], M. Vázquez Gómez[3], S. Astiz[1], R. Benítez[1], A. Heras Molina[1], B. Isabel[2], A. Rey[2], A. González Bulnes[1], and C. Óvilo[1], [1]*INIA CSIC, Madrid, Spain,* [2]*UCM, Madrid, Spain,* [3]*Sorbonne Université, Paris, France.*

Iberian is a local pig breed, traditionally produced in extensive systems, subjected to scarce selection and less efficient than commercial breeds, but highly valued for its high-quality products. This breed is also characterized by low uterine capacity and high variability in birth weight within the same litter, which is accentuated when the mother's nutrition is deficient. The objective of this study was to evaluate how the body weight of the fetuses of undernourished sows, belonging to the same litter and to 2 different genotypes, affected the liver transcriptome. Pure Iberian sows were inseminated with heterospermic semen from Iberian and Large White males. Samples were obtained from 51 purebred and crossbred fetuses at d 77 gestation. The weight of the fetuses was employed to select 32 individuals phenotypically divergent for body weight, to perform liver RNA-seq analyses (16 from each genotype and from each sex). Transcriptome differences between fetuses with high and low weight were analyzed within each genotype. A great effect of weight was observed in purebred fetuses [747 differentially expressed genes (DEGs) with $q < 0.05$], but, in contrast, negligible effect was observed in crossbred fetuses (2 DEGs). The functional analysis of DEGs in purebred fetuses (high *vs* low weight) showed the activation in the high weight group of functions related to cellular movement, cell-to-cell signaling and interaction, immune cell trafficking and inflammatory response while functions related with organismal survival and apoptosis were increased in the low weight group. Potential upstream regulators were identified in both groups, with cytokines such as IL4, IL5 or PRL being activated in the high weight group, while PPARα, a regulator activated under conditions of energy deprivation, was activated in the low weight group. Transcriptome differences between low and high weight fetuses confirmed that low weight Iberian animals had signals of compromised viability from very early stages while in crossbred fetuses absence of transcriptomic differences between the small and large fetuses may suggest a more advanced and balanced developmental and metabolic status in this genotype.

Key Words: transcriptome, fetuses, pig, weight, liver

P192 Expression of taste receptor genes in growing Iberian and Duroc pigs. R. Benitez*[1], R. Peiro[1], Y. Nunez[1], F. Garcia[1], E. de Mercado[3], E. Gomez-Izquierdo[3], J. Garcia-Casco[1], and C. Lopez-Bote[2], [1]*INIA-CSIC, Madrid, Spain,* [2]*Faculty of Veterinary Medicine, UCM, Madrid, Spain,* [3]*Pig Test Center ITACYL, Hontalbilla, Segovia, Spain.*

Taste receptor genes are expressed in sensory cells located in the tongue taste buds. In pigs, these genes comprise 2 families: Tas1r (sweet and umami taste perception) and Tas2r (bitter taste perception). Besides, fatty acids receptors are involved in fat taste perception. Taste perception is related to feed intake and may differ between breeds showing differences in appetite. We analyzed the expression of a panel of 10 taste receptor genes by qPCR in the circumvallate papillae of growing Iberian and Duroc pigs fed diets with different energy sources. We employed 30 Iberian and 19 Duroc males kept under identical management conditions except the nutritional treatment (HO diet with 6% high oleic sunflower oil and CH diet with carbohydrates), killed after 47 d of treatment, with 51.2 kg of average LW. Differences in gene expression between breeds were observed for all analyzed genes. *TAS1R1*, *TAS1R2*, *TAS1R3*, *TAS2R4*, *TAS2R38*, *TAS2R39*, *GPR84*, and *CD36*, were overexpressed in Duroc pigs and *GPR40* gene showed a similar trend. Only *GPR120* gene was overexpressed in the Iberian pigs. Diet effect was small with only *TAS1R3* gene being overexpressed in HO diet. In Iberians, *TAS1R* and *TAS2R* gene families were positively correlated among them and negatively correlated with *GPR40* and *CD36* genes. However, in the Duroc pigs, the opposite occurred. Correlations between gene expression and main phenotypic traits differed between breeds and between diets within each breed. In Iberian pigs, *TAS1R1* expression correlated to IMF content in *Biceps femoris*. In Duroc pigs, *GPR40* expression was negatively correlated to feed intake. SNP detection was performed from *Biceps femoris* muscle RNA-seq data obtained from the same animals, with 7,512 common variants being detected in the 2 breeds. Moreover, in the studied genes we detected 3 SNPs and 1 INDEL located in *TAS1R3* and *CD36* genes, respectively, specific for Iberian pigs and 1 Duroc-specific SNP located at *CD36* gene. Also, 1 common INDEL was identified at *CD36* gene. The taste receptor genes characterization could contribute to improve the knowledge on the genetic basis of voluntary feed intake and other relevant traits.

Key Words: taste receptors, gene expression, taste buds, appetite, pig

P193 Investigation of bovine leukemia virus (BLV) proviral DNA integration in cattle genome. M. Polat*[1,2], S. Saito[2], K. Hosomichi[3], and Y. Aida[1,2], [1]*The University of Tokyo, Tokyo, Japan,* [2]*RIKEN, Saitama, Japan,* [3]*Kanazawa University, Ishikawa, Japan.*

Bovine leukemia virus (BLV) is the etiological agent of B-cell leukemia/lymphoma in cattle. BLV infects cattle worldwide, and cause huge economic lost. BLV integrates into host genome and remains in cellular genome as provirus. The mechanism behind the BLV-induced leukemia is still unclear and it is believed that BLV integration has potential role in leukemia progression. The aim of this study is to investigate BLV integration to understand of oncogenic mechanisms of BLV. For this purpose, we choose monoclonal originated 2 B-cell lines (Ku-1 and Ku-17) derived from BLV-infected lymphoma cattle and one clinical leukemic cattle. BLV integration into host genome is investigated by high throughput next-generation sequencing (NGS). DNA library were constructed using KAPA Hyper Plus Library Preparation Kit. BLV LTR specific biotinylated probes were used to enrich libraries and sequenced using Miseq Reagent kit V3 (600 cycle). NGS paired-end sequences were mapped and aligned to BLV and host genome separately. Sequences of 40 to 300 bp were obtained centering the viral integration site (IS). BLV proviral ISs were detected in the Chr1 and the intron of *CHEK2* gene in Chr17 of leukemic cattle. *CHEK*2 is a tumor suppressor gene involved in DNA repair, apoptosis and linked to cancers. We successfully found that BLV was integrated into intron of *RPTOR* gene of Chr19 of KU-1 cell line and upstream of *ATG5* gene in Chr9 of KU-17 cell lines. *RPTOR* gene evolves in mTOR pathway and signaling in cancer. In addition, *ATG3* plays important role in autophagy, apoptosis and cell cycle arrest. Proviral ISs were further confirmed by both inverse PCR and standard PCR, plus Sanger sequencing. Defective provirus in KU-1 and whole BLV proviral sequences in Ku17 were also confirmed and obtained by long PCR. Current study provides comprehensive information about the proviral structure and the viral integration in tested sample. BLV integration were detected in the intron of *CHEK2*, *RPTOR* and *ATG* genes which are playing kay roles in DNA repair, apoptosis, cell signaling and autophagy. Detailed analysis concerned the biological impact of BLV into host genome, especially on nearby genes will be carried out.

Key Words: bovine leukemia virus, next-generation sequencing, viral integration, PCR

P194 Expression profiles of porcine parathyroid glands altered by pre- and postnatal dietary phosphorus supply. M. Oster[1], H. Reyer[1], C. Gerlinger[1], N. Trakooljul[1], J. Keiler[2], S. Ponsuksili[1], P. Wolf[3], and K. Wimmers*[1,3], [1]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*Institute of Anatomy, Rostock University Medical Center, Rostock, Germany,* [3]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany.*

Limited mineral phosphorus (P) resources and P emissions from animal husbandry require an improvement of endogenous P utilization. However, a continuous supply of P is essential for life. The parathyroid gland (PTG) is one key factor in the hormonal regulation of mineral homeostasis, but its specific molecular pathways in response to environmental factors and intrinsic hormonal responses are not fully understood. Therefore, gene expression profiles will contribute to disentangle the functional properties and specific responsiveness of PTGs at the molecular level. Accordingly, the study aims to establish holistic expression patterns of porcine PTGs triggered by variable dietary P supply. To test the hypothesis that a sparse maternal P supply during gestation and lactation can program the P utilization in the progeny, sows were fed standard iso-energetic diets with recommended (n = 4), reduced (n = 5), or higher P content (n = 5) throughout gestation (115 d) and lactation (28 d). At weaning of the respective piglets (d 28), 3 male and 3 female offspring per litter were randomly assigned to one of 3 diet groups with correspondingly different P content, resulting in a total of 9 experimental groups at the offspring level. At slaughter (d 120), PTGs were collected for mRNA-Seq expression profiling (n = 63). Expression analysis revealed 12,752 transcripts, with parathyroid secretory protein 1 (CHGA) and parathyroid hormone (PTH) by far the most abundant. Variation in maternal P supply resulted in 36 responsive genes (FDR ≤0.05) related to immunity, cell integrity and intermediary metabolism. Variable P supply after weaning resulted in significantly altered expression of 1,671 genes (FDR ≤0.05) enriched in 17 signaling pathways covering a range of downstream signaling cascades for PTH expression, stability and secretion, and collagen abundance, which may influence the paracrine interaction of PTH regulation. The study revealed comprehensive lists of transcripts of porcine PTG modulated by variable P supply, reflecting acute and long-term responses with relevance to bone integrity and mineral utilization efficiency.

Key Words: phosphorus, transcriptomics, mineral homeostasis, nutritional programming

P195 Insights into translation through transfer RNA sequencing and ribosome profiling. A. Goldkamp* and D. Hagen, *Oklahoma State University, Stillwater, OK, USA.*

Differentially methylated regions (DMRs) have been associated with large offspring syndrome (LOS) in cattle. Some DMRs overlap transfer RNA (tRNA) gene clusters, potentially altering tRNA expression patterns uniquely by treatment group or tissue type. tRNAs are classified as adapter molecules, serving a key role in the translational machinery implementing genetic code. Variation in tRNA expression has been identified in several disease pathways suggesting an important role in the regulation of biological processes. tRNAs also serve as a source of small noncoding RNAs. To better understand the role of tRNA expression in LOS, total RNA was extracted from skeletal muscle and liver of 105-d fetuses and the tRNAs sequenced. This study detected expression of 474 and 487 bovine tRNA genes in skeletal muscle and liver, respectively, with the remainder being very lowly expressed or transcriptionally silent. We observed differential expression of tissue- and treatment-specific tRNA genes that could modulate translation during protein homeostasis or cellular stress. In addition, the most highly expressed isodecoders differed by treatment and tissue type with roughly half correlated with codon frequency. While the absence of certain isodecoders may be relieved by wobble base pairing, missing tRNA species could likely increase the likelihood of mistranslation or mRNA degradation. One of the greatest factors in determining translation fidelity and efficiency is codon usage bias. Sources of sequence variation, like codon usage, often drive differences in elongation rate and gene expression. Highly expressed genes are thought to be codon-biased to support efficient translation, in which the encoded codons correspond to highly abundant tRNAs. Further, synonymous SNPs were once considered to be silent due to the degeneracy of the genetic code. However, synonymous SNPs may disturb protein abundance and function through alterations in translational efficiency. To investigate the relationship between tRNA expression and translation, ribosome profiling was used to capture transcripts bound by the ribosome. Following nuclease digestion, we performed high-throughput sequencing of ribosome bound fragments that are being actively translated at a single nucleotide resolution.

Key Words: protein translation, transfer RNA, ribosome profiling

P196 FAANGMine genomic data mining warehouse: 2021 update. C. Elsik*, A. Walsh, and D. Triant, *University of Missouri, Columbia, MO, USA.*

FAANGMine (http://faangmine.org) is a genomic data mining warehouse for domesticated animal species, including species of interest to the Functional Annotation of Animal Genomes (FAANG) Consortium. FAANGMine provides simple and sophisticated search tools to enable researchers without scripting skills to create and export customized annotation data sets merged with their own research data for use in downstream analyses. FAANGMine contains genes and genomes of cat, chicken, cow, dog, goat, horse, pig, sheep and water buffalo. Human, mouse and rat genes are also included to facilitate comparison to model organisms. The newest FAANGMine release (v1.2) includes human diseases and associated genes from Online Mendelian Inheritance in Man (OMIM), which can be related to animal genes using orthology. FAANGMine v1.2 also contains RNA-seq-based gene expression levels that have been computed for all species and equine histone modification marks generated by the members of the FAANG Consortium, along with sample and experiment metadata to enable flexible data mining. The Genomic Regions Search tool, which executes queries based on genome coordinates, now allows users to select ChIP-seq analyses to retrieve tissue-specific histone marks along with other genome features in specific regions. FAANGMine continues to integrate genes and genomes (RefSeq and Ensembl) with proteins (UniProt), protein families and domains (InterPro), orthologs and paralogs (EnsemblCompara, OrthoDB), pathways (KEGG, Reactome), interactions (BioGRID, IntAct), Gene Ontology (GO), QTL (AnimalQTLdb), variation (Ensembl) and publications (PubMed).

Key Words: multispecies, functional genomics, genome annotation, bioinformatics tools, data mining

P197 Online Mendelian Inheritance in Animals (OMIA): Standardized vocabularies for breeds and traits. I. Tammen[1], N. Vasilevsky[2], C. A. Park[3], Z. Hu[3], M. Haendel[4], and F. W. Nicholas*[1], [1]*Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, Australia,* [2]*Oregon Clinical and Translational Research Institute, Department of Medical Informatics and Clinical Epidemiology, Oregon Health and Science University, Portland, OR, USA,* [3]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [4]*Center for Health AI, University of Colorado Anschutz Medical Campus, Aurora, CO, USA.*

Online Mendelian Inheritance in Animals (OMIA, https://omia.org) provides up-to-date summary information on all known harmful and beneficial variants in animals, together with background information on all known inherited disorders and non-disease traits, which are called 'phenes'. OMIA curation focuses on phenes with confirmed and suspected Mendelian modes of inheritance. Several phenes caused by somatic mutations, chromosomal abnormalities, genetic modifications, or phenes with unknown or complex modes of inheritance are also included. As OMIA developed over the past 25 years, breed and phene names were entered as published. Sometimes phene names were altered to reflect similarities

to phenes in the hyperlinked database Online Mendelian Inheritance in Man (OMIM; https://omim.org). The ever-increasing interconnectedness of the internet has highlighted the need for OMIA to adopt standardized vocabularies that will enable OMIA to be reciprocally hyperlinked with relevant internet resources. The most powerful of these vocabularies are ontologies such as the Mondo Disease Ontology developed as part of the Monarch Initiative (https://mondo.monarchinitiative.org/). Starting with breeds, and using the existing Livestock Breed Ontology (https://www.animalgenome.org/bioinfo/projects/lbo/) as a model, we are using the Ontology Development Kit (https://ontology-development-kit.readthedocs.io/en/latest/) to combine existing breed lists of all OMIA species, thereby creating a Universal Breed Ontology that will be made available for general use. As a first step toward an OMIA Phene Ontology, we are enhancing OMIA's existing reciprocal links with OMIM. Because OMIM phenes are already incorporated into the Mondo Disease Ontology, we can then use the enhanced OMIA/OMIM links to introduce the Mondo standardized phene vocabulary into OMIA. Subsequent efforts will involve incorporating OMIA phenes that do not have human homologs, using (where possible) existing vocabularies such as those in the VeNom codes (http://venomcoding.org/). These efforts will help to standardize OMIA vocabularies, and will improve interconnectivity between databases for unambiguous information links.

Key Words: databases/repositories, bioinformatics tools, nomenclature, breed standardization

P198 Genome-wide analysis of transcription start sites across *Bos indicus* tissues. M. Forutan*, E. Ross, L. Nguyen, and B. Hayes, *Queensland Alliance for Agriculture and Food Innovation, Brisbane QLD, Australia.*

Transcriptional regulation is one of the most important features of gene expression. To figure out the exact mechanism of a gene, it is important to identify and evaluate its transcriptional start sites (TSSs), which are located at the beginning of the sequence. Transcription start sites act as an integration region for a wide range of molecular signals to control transcription and finally, expression levels. Previous studies have confirmed that most genes have an array of close TSSs instead of the expected single TSS, and the transcription of a gene may start from one of several TSSs, a phenomenon known as alternative transcriptional initiation (ATI). Cap analysis of gene expression (CAGE) has developed as one of the main high-throughput assays for studying TSSs and their expression. Sequencing short reads (or tags) from the 5′ end of full-length cDNA allows TSSs to be mapped and their expressions to be analyzed. To assess TSS expression and distribution across bovine tissues, CAGE-Seq (CAGE followed by sequencing) was performed on 9 tissues at adult stages, including liver, lung, kidney, thyroid, spleen, muscle, uterus, ovary, blood in *indicus* sub-species. The total number of TSSs expressed (in promoter) in 9 tissues from a single *Bos indicus* adult female was 48,473 (16,676). Interestingly, a noticeable proportion of the genes (24%) had divergent consensus TSS clusters, i.e., they had at least one TSS cluster not expressed in one or some tissues. When tissues were clustered based on their correlation between Shannon indexes of TSS diversity across adult tissues, they grouped mainly together into clusters reflecting their function. Our results highlight the high potential for differential transcriptional regulation across tissues.

Key Words: cap analysis of gene expression (CAGE), functional annotation, promoter, tissue-specific, transcription start site

P199 AgriSum Toolkit plugin 2.0: Enabling multi-species panel analysis for AgriSeq. H. Suren*[1], S. Daly[2], and K. R. Gujjula[1], [1]*Thermo Fisher Scientific, Austin, TX, USA,* [2]*Thermo Fisher Scientific, Lissieu, France.*

The AgriSum Toolkit (AST) is a data summarization and visualization plugin for the Torrent Suite Software (TSS). AST was primarily developed for analyzing targeted genotyping-by-sequencing (tGBS) solutions for AgriSeq. ATS provides overall panel, markers and samples summary metrics with visualizations. ATS also reports the actual genotype alleles, which can be filtered and exported in multiple formats (Matrix, TOP/BOTTOM, and ISAG) compatible for downstream analyses. In the AST plugin 2.0, we have added a new feature which enables our customers to analyze multi species panel easily. Conventionally, only one single report was created for the entire panel aggregating all the metrics. Aggregated summary metrics for multi species panel were often confusing customers due to confounding effect (e.g., a very poor performing sub panel dragging the overall average metrics significantly down). In multi species report, key performance metrics are calculated and reported explicitly for each individual sub panel within the run. Customer can navigate back and forth accessing key performance metrics and data files for each sub panel as well as the overall panel within the report with ease. AgriSum Toolkit 2.0 enables our customers to genotype multi species samples on the same chip. Thereby increasing the multiplexing capabilities without worrying about interpretation of the sequencing data. AST is publicly available and can be downloaded via Thermo Fisher Cloud and installed on TSS at no additional cost. For Research Use Only. Not for use in diagnostic procedures.

Key Words: AgriSeq, genotyping-by-sequencing (GBS), Ion S5, next-generation sequencing (NGS), genotyping

P200 Systematic discovery and integration of functional formation in genome-wide analysis of cattle traits. R. Xiang*[1,2], E. Breen[2], I. MacLeod[2], A. Chamberlain[2], C. Prowse-Wilkins[1,2], H. Daetwyler[2,3], and M. Goddard[1,2], [1]*Faculty of Veterinary and Agricultural Science, The University of Melbourne, Parkville, Victoria, Australia,* [2]*Agriculture Victoria, AgriBio, Centre for AgriBiosciences, Bundoora, Victoria, Australia,* [3]*School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

Emerging evidence shows that variants with causal roles in biology can be used to improve genomic fine-mapping and prediction. Such improvement can be expected to maintain across multiple traits and breeds. While it is difficult to find causal mutations, our recent work shows that by integrating pleiotropic, functional and evolutionary information with advanced statistical techniques such as Bayesian methods, variant clustering and local genomic breeding value (local gEBV), potentially causal variants can be systematically prioritized genome-wide. To demonstrate the merit of prioritized variants, we combine them with standard Illumina SNP chip panels for further analysis. With data of more than hundreds of thousands of cattle from Australia and overseas, we show that using variants with functional importance significantly increase genomic mapping precision and prediction accuracy of multiple cattle traits, comparing to using the standard chip panels alone. In this presentation, I will detail the systematic approaches to 1) discover potentially causal variants using functional information and 2) use potentially causal variants together with SNP chip panels to improve genome-wide mapping and prediction of cattle complex traits.

Key Words: functional genomics, evolutionary genomics, fine-mapping, genomic prediction

P201 The Georgian mountain cow breed Khevsurian population exhibits specific single nucleotide polymorphisms across the mitochondrial genome that distinguish it from global cattle populations. G. Basiladze[1], L. Tabatadze[1], E. Khmaladze[2], and M. Kotetishvili*[1], [1]*Scientific-Research Center of Agriculture, Tbilisi, Georgia,* [2]*Richard Lugar Center for Public Health Research, National Center for Disease Control and Public Health, Tbilisi, Georgia.*

A great majority of studies on the cow population genetics have been generally focused on the mitochondrial DNA (mtDNA) highly variable segment within the D-loop, covering ≤697 bps. For deciphering the Georgian mountain cow Khevsurian population, we designed and used the primers for the PCR amplification and sequencing of the 810 bp region of the mtDNA spanning over the D-loop end. A total of 20 maternally unrelated cattle individuals, representing the Georgian cow Khevsurian population, were selected for the phylogenetic analysis. MtDNAs were extracted from hairs of these individuals using the tissue and hair extraction kit (Promega). The following primers were used for the PCR amplification and sequencing of the targeted 810 bp region of the mtDNA in the cow individuals: F5 (CCAACAAACTAGGAGGAGTA) and R5 (CGCGGCATGGTAATTAAG). ClustalX was applied for aligning the DNA sequences obtained from the above experiments, and for the ones available for *Bos taurus* in the nucleotide database of the National Center for Biotechnology Information (NCBI). MEGA X, employing the maximum likelihood (ML) method, was used for the Khevsurian cow population phylogenetic analysis, including cattle global populations. In the phylogenetic analysis, using the nucleotide sequences of the above-selected mtDNA region, we could identify the previously unknown haplotypes exhibiting collectively the following single nucleotide substitutions (SNPs): T→C, C→A, C→G, and A→G (occurring at 15840, 15854, 15902, and 15986 nucleotide positions according to the mitochondrial genome coordinates of *B. taurus* [V00654] available in the NCBI nucleotide database). In addition, these and all the other haplotypes of the Khevsurian cow population were found to also carry C→A and C→G nucleotide substitutions (SNPs coordinates: 15794 and 15796 respectively), distinguishing them from the rest of the cattle populations globally, and resulting in a separate genetic cluster as resolved by the MEGA-generated ML tree. We suggest that the above SNPs can appear to be at least the Khevsurian cow population-specific, although, a large number of cattle individuals from Georgia need yet to be examined to prove this hypothesis.

Key Words: cow, population, polymorphisms, mitochondrial DNA

P202 Molecular characterization of swine leukocyte antigen (SLA) gene diversity in European farmed pigs. S. E. Hammer*[1], T. Duckova[1], S. Groiss[1], M. Stadler[1], M. Jensen-Wearn[2], W. T. Golde[3], U. Gimsa[4], and A. Saalmueller[1], [1]*University of Veterinary Medicine Vienna, Vienna, Austria,* [2]*Swedish University of Agricultural Sciences, Uppsala, Sweden,* [3]*Moredun Research Institute, Edinburgh, Scotland, UK,* [4]*Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.*

In Europe, swine represent economically important farm animals and furthermore have become a preferred preclinical large animal model for biomedical studies, transplantation, and regenerative medicine research. The need for typing of the swine leukocyte antigen (SLA) is increasing with the expanded use of pigs as models for human diseases and organ-transplantation experiments, their use in infection studies, and for design of veterinary vaccines. In this study, we characterized the SLA class I (SLA-1, SLA-2, SLA-3) and class II (DRB1, DQB1, DQA) genes of 549 farmed pigs representing 9 commercial pig lines by low-resolution SLA haplotyping. In total, 50 class I and 37 class II haplotypes were identified in the studied cohort. The most common SLA class I haplotypes Lr-04.0 (SLA-1*04XX-SLA-3*04XX(04:04)-SLA-2*04XX) and Lr-32.0 (SLA-1*07XX-SLA-3*04XX(04:04)-SLA-2*02XX) occurred at frequencies of 11.02 and 8.20%, respectively. For SLA class II, the most prevalent haplotypes Lr-0.15b (DRB1*04XX(04:05/04:06)-DQB1*02XX(02:02)-DQA-*02XX) and Lr-0.12 (DRB1*06XX-DQB1*07XX-DQA*01XX) occurred at frequencies of 14.37 and 12.46%, respectively. Meanwhile, our lab contributed to several vaccine correlation studies (e.g., PRRSV, CSFV, FMDV, swine influenza A virus) elucidating the immunodominance in the T-cell response with antigen-specificity dependent on certain SLA-I and SLA-II haplotypes. Moreover, these SLA-immune response correlations could facilitate tailored vaccine development, as SLA-I Lr-04.0 and Lr-32.0 as well as SLA-II Lr-0.15b and Lr-0.12 are highly abundant haplotypes in European farmed pigs.

Key Words: *Sus scrofa*, sequence-specific primers PCR, polymorphism, swine leukocyte antigen (SLA)

P203 Development of a comprehensive high-resolution typing method for SLA-3, an MHC classical class I gene of pigs, using genomic DNA PCR and direct sequencing. S. Youk*[1], M. T. Le[1], M. Kang[1], B. Ahn[1], M. Choi[1], C. Ho[2], and C. Park[1], [1]*Konkuk University, Seoul, Republic of Korea,* [2]*Gift of Hope Organ and Tissue Donor Network, Itasca, IL, USA.*

We developed a high-resolution and comprehensive typing method for swine leukocyte antigen (SLA)-3, a MHC classical class I gene using the direct sequencing of locus-specific genomic PCR. We successfully typed 292 individuals consisting of 9 pure breeds, 1 cross bred, and 6 cell lines. A total of 21 SLA-3 alleles were identified among which 4 were novel alleles. The allelic diversity of SLA-3 was lower than that of previously reported other class I genes, SLA-1 and -2. The number of observed SLA-3 alleles was larger in Landrace and Yorkshire than other breeds. SLA*0401 was identified from 7 out of 9 breeds and was the most widely distributed alleles across breeds. The typing method reported in this study completes our efforts to develop high-resolution typing methods for major SLA molecules and allows the combined analysis of major SLA genes from field samples which is important to understand the relationship between adaptive immune responses against pathogens and the immunogenetic makeup of an individual.

Key Words: SLA-3, MHC class I, swine, locus-specific amplification, direct sequencing

P204 Application of MHC sequencing to vaccine development: Proteome-wide analysis of zoonotic bacterium *Coxiella burnetii* for conserved T-cell epitopes presented by multiple host species. L. M. Wright Piel[1], C. J. Durfee[1], and S. N. White*[1,2], [1]*USDA-ARS Animal Disease Research, Pullman, WA, USA,* [2]*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA,* [3]*Center for Reproductive Biology, Washington State University, Pullman, WA, USA.*

Immunoinformatic methods can leverage both host and pathogen genetic variation, but most proteome-wide T-cell epitope studies have focused on viral applications [11]. The technology is maturing for leveraging MHC sequences from a wide variety of host species and analysis of the bacterial proteome requires greatly increased calculations (by multiple orders of magnitude). *Coxiella burnetii* is a globally distributed (except New Zealand) gram-negative bacterium responsible for human Q fever and coxiellosis in ruminant livestock. Previous vaccines with whole cell inactivated bacteria can confer protection but can also produce reactogenic immune responses. A protective vaccine is required that does not cause excessive immune reactions. T-cell immunity plays a critical role in *C. burnetii* control, since either $CD8^+$ or $CD4^+$ T cells can empower clearance. We sought to identify *C. burnetii* epitopes that can interact with a range of major histocompatibility complex (MHC) alleles from multiple relevant host species (including human, mouse, and cattle). We screened

1,815 proteins from the reference Nine Mile phase I (RSA 493) *C. burnetii* assembly and we removed 402 proteins due to a lack of interisolate conservation. We eliminated an additional 391 proteins due to the presence of host homology to avoid potential autoimmune responses. We analyzed the 1,022 remaining proteins for ability to produce peptides that bind MHCI or MHCII. MHCI and MHCII predicted epitopes were filtered and compared between species yielding 777 MHCI epitopes and 453 MHCII epitopes. There were 31 epitopes with overlap between MHCI and MHCII across host species. Of these, there were 9 epitopes within epitope-dense proteins containing ≥ 5 total epitopes. Overall, 55 proteins contained high scoring T-cell epitopes. Besides Com1, most proteins were novel compared with previously interrogated vaccine candidates. These data contain the first proteome-wide assessment of *C. burnetii* peptide epitopes. Furthermore, we captured a range of hosts for this zoonotic pathogen through the inclusion of human, mouse, and bovine data, demonstrating opportunities for widely useful *C. burnetii* vaccine construction. This work identified new vaccine targets and enhanced opportunities for selecting T-cell epitopes in vaccine design.

Key Words: *Coxiella burnetii*, T-cell epitope, proteome-wide, cross-species

P205 Sequencing of LEI0258 marker reveals populations' specific alleles and new repeat motif patterns. P. Manjula*[1], T. Kalhari[2], S. Cho[1], M. Kim[2], E. Cho[3], and J. Lee[1,2], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea,* [2]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Republic of Korea,* [3]*Department of Bio-big Data, Chungnam National University, Daejeon, Republic of Korea.*

Chicken MHC-*B* diversity can be assessed using MHC-linked microsatellite markers including LEI0258. LEI0258 is a variable number tandem repeat (VNTR) marker characterized by 2-repeat sequences of 13 bp and 12 bp. Sequence information of LEI0258 alleles exemplifies the repeat motif variations, upstream and downstream insertion/deletion, and SNPs (single nucleotide polymorphisms). This method provides a clearer interpretation for the particular VNTR marker while implicitly indicating the MHC allele diversity in chicken breeds around the world. In this study, we analyzed a total of 604 LEI0258 sequences from Asian, African, and Americas and commercial chicken populations. As the allele size (bp) is mainly determined by its R13 and R12 copy number variations, the repeat motif combination patterns that correspond to each allele size and their additional polymorphisms were evaluated. A total of 86 allele sizes (182 – 552 bp) were reported. Asian and African chicken possess a higher number of alleles, numerically 61 and 57 than that of Americas and commercial breeds. Eighteen shared alleles, and varied private alleles were identified. Accordingly, 46 repeat motif combinations were reported in 86 allele sizes, including 14 novel combinations in Asian, 2 in the Americas, and one in African chickens. This number was evidently higher than the previously reported, and greatly supports the extreme polymorphism of the LEI0258 marker. In 26 alleles, these combinations consisted of a single copy of R13 with different R12 repeats (2 to 28), and remained alleles consist of varied copies of R13 and R12 repeats. Moreover, the results indicate that the same allele size can occur with different combinations due to the homoplasy. Additional allele variations were observed as different fragment sizes that possess the same repeat combination due to the occurrence of various indels and SNPs in the upstream and downstream of VNTR. As per the results, the loss or gain of VNTR and additional polymorphisms that collectively determine the allele variations for LEI0258, claim higher MHC diversity in Asian and African chickens.

Key Words: LEI0258, variable number tandem repeat (VNTR), MHC, allele diversity

P206 Expression of genes related with immunomodulation and immunogenicity of equine mesenchymal stem cells: Influence of major histocompatibility complex. A. Cequier*[1], S. Fuente[1,2], A. Vitoria[1,2], A. Romero[1,2], F. Vázquez[1,2], C. Rodellar[1], and L. Barrachina[1,2], [1]*Laboratorio de Genética Bioquímica LAGENBIO (Universidad de Zaragoza), Instituto Agroalimentario de Aragón– IA2 (Universidad de Zaragoza-CITA); Instituto de Investigación Sanitaria de Aragón (IIS), Zaragoza, Spain,* [2]*Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, Zaragoza, Spain.*

MSCs can be used for therapy due to their immunomodulatory properties and the horse is a suitable animal model to develop them. Allogeneic application presents several advantages over autologous therapy, but MSC immunogenicity should be considered. The immune response to equine allogeneic MSCs can be influenced by donor-receptor MHC-matching/mismatching and MHC-expression level, which can be modified by inflammation. The aim of this study was to analyze the gene expression of molecules related to the immunoregulatory and immunogenic profiles of equine MSCs, basal (MSC-B) and proinflammatory primed (MSC-P), after their coculture in vitro with lymphocytes from either autologous or allogeneic MHC-matched/mismatched animals. MHC-haplotypes were determined by analyzing 10 intra-MHC microsatellites regions associated with Equine Leukocyte Antigens. MSCs from 3 MHC-homozygous horses were exposed to activated lymphocytes from autologous and allogeneic horses for 3 d. Afterward, MSC gene expression was assessed by RT-qPCR for immunomodulatory (vascular cell adhesion molecule 1, VCAM-1; cyclooxygenase 2, COX-2; indoleamine 2–3-dioxygenase, IDO; inducible nitric oxide synthase, iNOS; interleukin 6, IL-6) and immunogenicity (MHC-I, MHC-II, CD40, CD80) genes. Gene expression of both immunomodulatory and immunogenicity-related genes was higher in all MSC-P combinations over MSC-B. Specifically, IL-6 was significantly upregulated in MSC-P exposed to both MHC-matched and mismatched lymphocytes, whereas only MSC-P exposed to MHC-mismatched lymphocytes also overexpressed VCAM-1, MHC-I and CD40. These results suggest that inflammation increases both the immunogenic and immunomodulatory MSC profiles. Importantly, the cellular response in a MHC-mismatched setting would promote a further upregulation of these genes. Further studies are needed to clarify how these changes may be translated in vivo into an immune recognition with clinical implications. The MHC-matching between donor and recipient is closely related to the immune recognition of allogeneic MSCs and thus, to the effectiveness and safety of the therapy.

Key Words: horses and related species, cell biology, immunology, microsatellite, qPCR

P207 Evaluation of polymorphisms in *BLB2* gene in Korean Ogye chicken using next-generation sequencing data. T. Kalhari*[1], P. Manjula[2], S. Cho[2], M. Kim[1], E. Cho[3], and J. Lee[1,2], [1]*Department of Bio-AI Convergence, Chungnam National University, Daejeon, Korea,* [2]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea,* [3]*Department of Bio-big data, Chungnam National University, Daejeon, Korea.*

The chicken MHC region contains highly complex and polymorphic genes that are involved in adaptive immune responses. Its competency primarily depends on the ability to identify specific pathogens. Consequently, the exon 2 of *BLB2* gene located at the MHC-B class II region, heavily responsible for the formation of Peptide-Binding Regions (PBRs) of class II glycoproteins which detects such pathogens becomes crucial. In this study, the Korean Ogye chickens, a national treasure in South Korea, were evaluated for the polymorphisms in exon 2 of the *BLB2* gene. The Ogye genome was sequenced using Illumina Next-Generation Sequencing followed by variant calling, utilizing NGS GATK best practices pipeline. Next, the targeted exon of *BLB2* was sequenced using Sanger sequencing, to confirm the in silico obtained variants in the same population. As per the NGS data, a total of 20 variants were called for the entire exon 2 (270 bp) of *BLB2*, including 15 missense, 2 frameshift, 1 synonymous, 1 conservative inframe insertion, and 1 disruptive inframe deletion. Focusing on the confirmation of derived variants, they were compared with the Sanger sequencing data and all most all the variants were well

confirmed. This characterizes a higher polymorphism in Ogye and this fairly manifests that especially these SNPs might involve in attributing unique immunity in Korean Ogye chicken. However, further studies are warranted to analyze the selection on variants and potential structure alterations in PBRs.

Key Words: next-generation sequencing (NGS), Sanger sequencing, Ogye, MHC, variants

P208 Association of bovine leukemia virus-induced lymphoma with BoLA-DRB3 polymorphisms at the DNA, amino acid, and binding pocket property levels. C.-W. Lo*[1], S.-N. Takeshima[2,3], K. Okada[4], E. Saitou[5], T. Fujita[6], Y. Matsumoto[1], S. Wada[7], H. Inoko[8], and Y. Aida[1,2], [1]*Laboratory of Global Infectious Diseases Control Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan,* [2]*Viral Infectious Diseases Unit, RIKEN, Saitama, Japan,* [3]*Department of Food and Nutrition, Jumonji University, Saitama, Japan,* [4]*Iwate University, Iwate, Japan,* [5]*Hyogo Prefectural Awaji Meat Inspection Center, Hyogo, Japan,* [6]*Livestock Research Institute of Oita Prefectural Agriculture, Forestry and Fisheries, Research Center, Oita, Japan,* [7]*Photonics Control Technology Team, RIKEN Center for Advanced Photonics, Saitama, Japan,* [8]*Genome Analysis Division, GenoDive Pharma Inc., Kanagawa, Japan.*

Bovine leukemia virus (BLV) causes enzootic bovine leucosis, a malignant B-cell lymphoma in cattle. The DNA sequence polymorphisms of bovine leukocyte antigen (BoLA)-*DRB3* have exhibited a correlation with BLV-induced lymphoma in Holstein cows. However, little is known about the relationship between BLV-induced lymphoma and DRB3 at the amino acid and structural diversity levels. Here, we aim to comprehensively analyze the correlation between BLV-induced lymphoma and DRB3 at DNA, amino acid, and binding pocket property levels. Genomic DNAs from 106 BLV-infected but clinically normal Japanese Black cattle and 227 BLV-infected Japanese Black cattle with lymphoma which were collected from a nationwide survey in Japan were used in this study. BLV infection was determined using enzyme-linked immunosorbent assays targeting BLV-gp51. *BoLA-DRB3* genotyping was based on PCR-sequence-based typing method. BoLA-DRB3 molecules 3D structures and electrostatic surface potential modeling were analyzed based on the crystal structure of HLA-DRB1. Association study based on Fisher's exact test was performed by comparing the allele, genotype, or amino acid frequencies between asymptomatic and lymphoma cows. The results were penalized with the Bonferroni correction procedure to correct for false positive rate. In allele level, among 382 alleles, *DRB3*011:01* was identified as a resistance allele, whereas *DRB3*005:02* and *DRB3*016:01* were susceptibility alleles. Amino acid association studies showed that positions 9, 11, 13, 26, 30, 47, 57, 70, 71, 74, 78, and 86 were associated with lymphoma susceptibility. Structure and electrostatic charge modeling further indicated that binding pocket 9 of resistance DRB3 was positively charged. In contrast, alleles susceptible to lymphoma were neutrally charged. Altogether, this is the first association study of *BoLA-DRB3* polymorphisms with BLV-induced lymphoma in Japanese Black cattle. In addition, our results further contribute to understanding the mechanisms regarding how *BoLA-DRB3* polymorphisms mediate susceptibility to BLV-induced lymphoma.

Key Words: bovine leukemia virus, lymphoma, *BoLA-DRB3*, peptide-binding pockets, association study

P209 The IPD-MHC database: Novel tools for the study of the major histocompatibility complex. G. Maccari*[1,2], J. Robinson[2,3], J. A. Hammond[1], and S. G. E. Marsh[2,3], [1]*The Pirbright Institute, Pirbright, Woking, Surrey, UK,* [2]*Anthony Nolan Research Institute, Royal Free Campus, London, UK,* [3]*UCL Cancer Institute, Royal Free Campus, London, UK.*

The IPD-MHC Database provides a centralized resource for the collection and analysis of sequences found within the major histocompatibility complex (MHC) of non-human species. Overseen by the Comparative MHC Nomenclature Committee, it is therefore the primary source of data for the study of the non-human MHC. Since 2015 the IPD-MHC project has been through a structured program of improvement, aimed at expanding the content and improving the utility of the database in line with improved sequencing methods and community demand. As a result, the IPD-MHC database has been redesigned to provide a unified resource for the inter- and intra- species comparison of genomic and nongenomic data from different taxonomic groups. This work has been performed in synergy with the Comparative MHC Nomenclature Committee to draft a unified and improved set of guidelines for the allele nomenclature to cover MHC variation at genomic level. The project has grown in both content and impact, now hosting 95 different species and almost 12,000 alleles. The increasing availability of high-quality, manually curated data spurred the need for advanced tools for the analysis and interpretation of allele variation. This included the redesign of existing tools to provide new pathways to access and consume the expanded volume of data. The IPD project has recently introduced a centralized API allowing the programmatic interrogation of the databases, and a more sophisticated extrapolation of the available data. A primer design and virtual PCR tool has recently been integrated, providing a resource for the *ad-hoc* design of inter- or intra-species, locus-specific or allele-specific probes. As a consequence of the improving the bioinformatic framework, additional species-specific metadata is hosted, including a taxonomic-specific haplotype section. Structural information from homology models will be included for class I MHC alleles, providing insight on the overall structure and the specific impact of variation, including the ability to allow users to analyse and compare peptide-binding pockets. With the latest improvements and expansions, we hope that the future of the IPD-MHC database has been secured by increasing the utility of the high-quality data being hosted.

Key Words: bioinformatics, MHC, comparative genomics, databases/repositories, polymorphism

P210 Characterization of the functional and transcriptional variation of cattle MHC class I alleles. J. C. Schwartz*[1], G. Maccari[1,2], D. Heimeier[1], and J. A. Hammond[1], [1]*The Pirbright Institute, Guildford, UK,* [2]*Anthony Nolan Research Institute, London, UK.*

The major histocompatibility complex (MHC) class I region of cattle is both highly polymorphic and, unlike many species, highly variable in gene content between haplotypes. Historically, cattle MHC class I alleles were phylogenetically grouped based on sequence similarity in the more conserved 3′ end of the coding sequence, which formed the basis of cattle MHC class I nomenclature. We have annotated 5 complete cattle MHC class I haplotypes from the recent cattle and yak genome assemblies and compared them to a previous assembly of an A14 haplotype. Of the 5 likely pseudogenes previously described in the class I region, we found that one is putatively functional in all haplotypes and transcription was confirmed using allele-specific expression analysis of transcriptomic data. Two further previously identified pseudogenes are also putatively functional in some haplotypes, but transcription has yet to be confirmed. Based on full gene sequences as well as 3′ coding sequence, we identified subgroups of BoLA-3 and BoLA-6 that represent distinct genetic loci. Transcriptomic analysis reveals that certain allele groups within all loci appear to be consistently weakly expressed compared with others, which may reflect functional characteristics and a promiscuous affinity for peptides as evidenced in other species. These observations will help to inform further studies into how MHC class I region variability influences T cell and NK cell functions in cattle.

Key Words: cattle, yak, recombination, pseudogenes, BoLA

P211 The importance of annotations (reference genome and parent gene) for the study of circRNAs. A. Robic*[1], T. Faraut[1], C. Cerutti[1], J. Demars[1], and C. Kühn[2,3], [1]*GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet-Tolosan, France,* [2]*Institute Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [3]*Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany.*

Circular transcripts can be of several types, although the majority of circular RNAs (circRNAs) are generated at the expense of a linear transcript as backsplicing competes with linear splicing. Many pipelines have been developed to identify circRNA in RNA-seq data sets depleted for ribosomal RNA with the core principle of identifying reads including a circular junction. However, sporadic circularization events should be excluded from circRNA lists for a good compromise between exhaustive identification of circRNAs and false positive data. In our study, we considered 2 widely used detection pipelines (CIRCexplorer2 (CE2) and CIRI2) as well as an in-house approach, and applied them on bovine, porcine and ovine data sets to understand the differences in their circRNA output lists. Substantial differences in results reflect the alternative circRNA detection strategies: CE2 only retains exonic and intronic lariat circRNAs compatible with an annotated gene, while CIRI2 retains exonic circRNAs due to requiring a junction of 2 putative exons with canonical splicing site signals. We show that considering only an intersection of circRNA output from these 2 pipelines is not the final compromise but only an option, which still requires applying a threshold to discard sporadic circulation events. All pipelines provide a list of circRNAs with an associated gene name, but some pipelines proceed to a comprehensive identification of the parent gene (CE2), while others (CIRI2) only propose a gene name based on raw mapping coordinates. We showed that a poor reference genome assembly in a given region can lead to the detection of artifactual circRNAs (possibly detected by CIRI2). In addition, we demonstrated that circRNAs can also originate from incompletely annotated regions (possibly detected by CIRI2) and that all types of genes can produce circRNAs even RNA genes (still incompletely annotated in livestock species). In the 3 species considered in this study, and with the current state of knowledge of the respective reference genomes and gene annotation, we suggest working with only properly annotated circRNAs.

Key Words: circular RNA, annotation, livestock species, noncoding RNA, FAANG

P212 A comparison of copy number variant discovery in New Zealand sheep when using different genotyping platforms. A. Hess*, H. Baird, R. Brauning, and S. Clarke, *AgResearch Ltd., Mosgiel, Otago, New Zealand.*

In recent years, the important role that copy number variants (CNVs) in economically important traits and adaptation to the environment has become clearer. A common method for identifying CNV regions (CNVRs) is to utilize SNP genotyping array information or sequencing. The power to detect CNVRs is dependent on genotype density or sequencing coverage and using different approaches may result in the discovery of different CNVRs. Furthermore, CNVs typically have low levels of linkage disequilibrium with neighboring SNPs, so utilizing neighboring SNP information typically has poor performance when attempting to impute the copy number of the CNV of interest. New methods to capture this information quickly and accurately are needed capture CNV genotypes and incorporate CNV information with the aim of improving the accuracy of breeding value prediction. The goal of this study was to compare 4 different approaches for CNV discovery and CNV genotyping in New Zealand sheep: the OvineHD BeadChip array (600K SNPs), genotyping-by-sequencing, Illumina whole-genome short-read sequencing, and Oxford Nanopore Technologies long-read sequencing. We will present a comparison of the CNVs captured both on a population and individual level using these different methods and propose methods to more completely capture CNV information so that it may be used at the industry level.

Key Words: sheep, copy number variants, sequencing, genotyping

P213 Annotation of transcription start sites in the bovine genome reveals novel breed-specific complexity. M. Salavati*[1], R. Clark[2], D. Becker[3], C. Kühn[3,4], G. Plastow[5], G. Costa Monteiro Moreira[6], C. Charlier[6,7], E. L. Clark[1], and BovReg Consortium[4], [1]*The Roslin Institute, University of Edinburgh, Edinburgh, UK,* [2]*Genetics Core, Edinburgh Clinical Research Facility, The University of Edinburgh, Edinburgh, UK,* [3]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany,* [4]*Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [5]*Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada,* [6]*Unit of Animal Genomics, GIGA Institute, University of Liège, Liège, Belgium,* [7]*Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.*

Mapping of transcription start sites (TSS) in multiple tissues is a key first step in understanding transcript regulation and diversity and how these might influence phenotypic plasticity in cattle. TSS mapping can provide information about complex promotor activity, pervasive transcription and tissue-specific promotor usage. Data from multiple tissues and nonreference breeds or crosses will increase our understanding of TSS usage and complexity, further improving the annotation of the bovine genome (ARS-UCD1.2). Using cap analysis gene expression (CAGE) sequencing of 105 samples (from 24 different tissues) from dairy (Holstein, n = 43), composite beef (KC-composite, n = 31) and beef/dairy cross (Charolais x Holstein, n = 31) breeds, we aimed to capture additional TSS complexity in cattle. CAGE libraries were prepared, sequenced and mapped to ARS-UCD1.2_Btau5.0.1Y (1000bulls run 9). The mapped reads were analyzed using the CAGEfightR package to generate uni-directional (TSS) and bidirectional (TSS-Enhancer) clusters. We identified more than 4.5 million putative TSS and 57,412 TSS-Enhancer clusters in total across all samples. Tissue-specific analysis captured, on average per tissue (±SE), 253,852 ± 24,713 TSS clusters, 41.6% of which were novel. On average per tissue 12,138 ± 889 TSS-Enhancer clusters were captured, of which 27.6% were novel. The greatest number of clusters were observed in lung (TSS) and spleen (TSS-Enhancer). Breed-specific analysis revealed differences in TSS complexity between the breeds and crosses analyzed. The highest number of breed-specific TSS were detected in the KC-composite (3,102) followed by 1,152 in Holstein and 1,092 in Charolais x Holstein. The same pattern was observed in the TSS-Enhancer clusters (419 in KC-composite, 286 in Charolais x Holstein and 202 in Holstein). These differences indicate that in this study the total number of TSS observed was greater in crossbred relative to purebred cattle. The data we have generated will provide a breed- and tissue-enriched map of TSS that combined with RNA-seq and small RNA-seq, generated by BovReg partners, will create a new high-resolution transcriptional map for the bovine genome.

Key Words: CAGE-Seq, regulatory element, transcriptome, cattle and related species, Functional Annotation of Animal Genomes (FAANG)

P214 Functional annotation of the bovine genome. H. Zhou*[1], X. Xu[1], H. Beiki[8], S. Corum[9], K. M. Davenport[7], X. Han[4], G. Wang[2], H. Wang[4], Y. Xing[2], X. Zhang[4], Y. Zhang[4], C. Kern[5], C. Kern[1], P. Lyu[3], W. Ma[5], J. J. Michal[4], C. A. Gill[2], H. Jiang[3], Z. Jiang[4], W. Liu[5], S. D. McKay[6], J. Medrano[1], B. M. Murdoch[7], J. M. Reecy[8], G. Rincon[9], M. Rijnkels[2], T. P. L. Smith[10], and P. J. Ross[1] [1]*University of California- Davis, Davis, CA, USA,* [2]*Texas A&M AgriLife Research, College Station, TX, USA,* [3]*Virginia Polytechnic Institute and State University, Blacksburg, VA, USA,* [4]*Wash-*

ington State University, Pullman, WA, USA, [5]Pennsylvania State University, State College, PA, USA, [6]University of Vermont, Burlington, VT, USA, [7]University of Idaho, Moscow, ID, USA, [8]Iowa State University, Ames, IA, USA, [9]Zoetis Inc., Kalamazoo, MI, USA, [10]USDA-ARS-USMARC, Clay Center, NE, USA.

The goal of the functional annotation of the bovine genome project is to generate high-quality transcript and chromatin status data sets from a comprehensive set of cattle tissues and cells (33 adult tissues, 10 fetal tissues, and 4 cell types) to discover and annotate the functional elements in both coding and noncoding regions of the bovine genome, including enhancers, promoters, insulators, and small and large RNA transcripts. Many tissues were collected from Hereford cattle closely related to L1 Dominette, the cow from which the reference bovine genome was sequenced, with some others collected from Holstein and Angus cattle. Transcriptomic data, including RNA-seq, miRNA-seq, and Whole Transcriptome Termini Site Sequencing (WTTS-seq) were generated. Current data sets for these tissues include 97 WTTS-seq libraries with a total of 536M sequenced reads, 123 RNA-seq libraries with 4.6B reads, and 123 miRNA-seq libraries with 1.9B reads. Analysis of these data has provided a comprehensive characterization of the expressed regions of the genome as well as accurate comparisons of differential gene expression across multiple tissues and cell types that will be harnessed for the identification of regulatory elements active in the bovine genome. Chromatin state data sets generated so far include profiling DNA methylation using whole-genome bisulfite sequencing (WGBS) in 71 adult tissues, with 24B sequenced reads. Also, ChIP-seq assays are underway for profiling 7 different chromatin marks including H3K27me3, H3K4me3, H3K27ac, H3K4me1, H3K36me3, H3K9me3, and CTCF. So far, 250 libraries have been sequenced representing 46 different tissues and 5.7B mapped reads. Finally, a total of 104 ATAC-seq libraries representing 2 biological replicates for 30 male adult tissues, 5 adult female reproductive tissues and 9 male fetal tissues from the Hereford L1 Dominette lineage, as well as for Holstein mammary gland tissue at 4 pregnancy and lactation stages, and 4 different primary cell preparations were sequenced and the data analysis is currently underway. The "omic" experimental data generated by this USDA funded project will be systematically analyzed, and data sets and findings will be made publicly available through database and genome information centers.

Key Words: bovine functional annotation, ChIP-seq, ATAC-seq, WTTS-seq, WGBS

P215 Performance of variant pathogenicity prediction methods in veterinary species. N. Tate*, K. Mahoney, N. Wanner, S. Durward-Akhurst, N. Gocker, J. Mickelson, S. Friedenberg, M. McCue, and E. Furrow, *University of Minnesota, College of Veterinary Medicine, St. Paul, MN, USA.*

A key challenge in sequencing studies is variant prioritization. Many in silico tools exist to predict pathogenicity of missense variants but are not validated for analysis of veterinary data sets. This study evaluates the performance of pathogenicity prediction programs in canine and equine data sets. MutPred2, PANTHER, PhD-SNP, PolyPhen2, Provean, SIFT, and SNPs&GO were chosen for their 1) ability to analyze data from any animal species, 2) performance with human data, and 3) public availability. The canine data set comprised 89 pathogenic and 232 benign variants, and the equine data set comprised 40 pathogenic and 160 benign variants. Pathogenic variants included missense variants listed as "likely causal variants" in Online Mendelian Inheritance in Animals and those recently published (PubMed search). Benign missense variants included those present at ~0.5 allele frequency in consortium databases (canine: 250 dogs, 44 breeds; equine: 534 horses, 44 breeds). Accuracy, Matthews correlation coefficient, precision, specificity, and sensitivity were calculated for each program. Overall, programs performed similarly between species. SNPs&GO was the best performing with high specificity (88–90%) and accuracy (85–86%). MutPred2 also had high specificity (81–87%) and accuracy (80–81%). PolyPhen2 had the highest sensitivity (90–92%). Panther had the lowest accuracy (65–67%) and many (36–42%) "unclassified" predictions. Only 77% (dogs) to 89% (horses) of the pathogenic variants were called as such by 3 or more programs. We are developing a classification tree using a combination of these programs. This data informs the usage of in silico prediction tools in veterinary genetics.

Key Words: multispecies, computational biology, variant prioritization

P216 AQUA-FAANG: Genome functional annotation of the 6 major European farmed fish species. D. J. Macqueen*[1], S. Lien[2], and AQUA-FAANG Consortium[3], [1]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, Scotland, UK,* [2]*Centre for Integrative Genetics (CIGENE), Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway,* [3]*AQUA-FAANG Consortium, Europe.*

High-quality reference genome sequences can be readily generated using modern sequencing technologies, but alone are rarely sufficient to identify the basis for complex biological traits. For many scientific and commercial reasons, we need to understand how phenotypes are being shaped by functional and regulatory features within farmed animal genomes, including aquaculture species. European aquaculture produces 3 million tonnes of fish (worth > 9 billion Euros) annually and employs around 50,000 people. AQUA-FAANG is a Horizon 2020 funded project that aims to promote sustainable growth within this sector by creating a major up-step in our ability to exploit the genomic basis for complex traits in the 6 major farmed fish species in Europe. Alongside BovReg and GENE-SWitCH, AQUA-FAANG is one of 3 consortia funded under the same Horizon 2020 call, which are formally linked by the EuroFAANG initiative. AQUA-FAANG is annotating functional and regulatory regions within the genomes of the target species using sequencing assays defined by the FAANG initiative; RNA-seq, ATAC-seq, and ChIP-seq. The work includes standardized biological samples representing a common panel of tissues at juvenile and sexually mature stages (BodyMaps), multiple key landmarks in embryogenesis (from zygotic genome activation to post-Pharyngula stage) (DevMaps) and immune tissues/cells stimulated with viral and bacterial PAMPs to mimic infection. AQUA-FAANG is also exploring novel approaches to understand the genomic basis for disease resistance, including single-cell transcriptomics and genome editing. A major goal of the project is to predict the genetic basis for disease resistance traits using genome functional annotation, identifying and prioritizing genetic variants responsible for resistance to problematic diseases in fish aquaculture, and developing tools to support uptake of functional genomic data for selective breeding in European aquaculture - done in partnership with industry. The project includes a major comparative objective to reveal the evolutionary conservation of functional and regulatory elements in farmed fish genomes. We are currently 24 mo into a 54-mo project, with approximately 2,000 libraries sequenced to date across the major functional annotation maps. This talk will provide an overview of the AQUA-FAANG project and latest status toward achieving our ambitious objectives.

Key Words: farmed fish, FAANG, genome, functional annotation, H2020 project

P217 Bovine genome annotation using integration of multi-omics data. H. Beiki[1], C. Gill[2], H. Jiang[3], W. Liu[5], Z. Jiang[4], S. McKay[6], B. M. Murdoch[7], J. Koltes[1], M. Rijnkels[2], T. P. L. Smith[8], P. Ross[9], H. Zhou[9], and J. Reecy*[1], [1]*Iowa State University, Ames, IA, USA,* [2]*Texas A&M University, College Station, TX, USA,* [3]*Virginia Tech University, Blacksburg, VA, USA,* [4]*Washington State University, Pullman, WA, USA,* [5]*Penn State University, State College, PA, USA,* [6]*University of Vermont, Burlington, VT, USA,* [7]*University of Idaho, Moscow, ID, USA,* [8]*US Meat Animal Re-*

search Center, Clay Center, NE, USA, [9]University of California–Davis, Davis, CA USA.

The diversity of RNA and miRNA transcripts among 47 different bovine tissues/cell types was assessed. Poly(A) selected RNA-seq and miRNA-seq data were generated from tissues of Hereford cattle closely related to Dominette L1, the individual represented in the reference bovine genome. A total of approximately 4.1 trillion RNA-seq reads and 1.9 billion miRNA-seq reads were collected, with a minimum of 27.5 million (M) RNA-seq and 4.2 M miRNA-seq reads from each tissue (average 87.8 M ± 49.7 M and 26.6 M ± 13.3 M, respectively). A total number of 171,985 unique transcripts (50% protein-coding) and 35,150 unique genes (64% protein-coding) were identified across tissues. A total of 159,033 transcripts (92% of predicted transcripts) were structurally validated by independent data sets such as Pacific Biosciences single-molecule long-read isoform sequencing, Oxford Nanopore Technologies sequencing, *denovo* assembled transcripts from RNA-seq, Ensembl and NCBI gene sets. In addition, all transcripts were supported by extensive independent data from different technologies such as WTTS (Transcriptome Termini Site Sequencing), RAMPAGE (RNA Annotation and Mapping of Promoters for the Analysis of Gene Expression), several different types of histone modification data (H3K4me3, H3K4me1, H3K27ac and CTFC) and ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing). A large proportion of transcripts (69%) were novel, although mostly produced by known protein-coding genes (87%), while 13% (9% of all transcripts) corresponded to novel genes. The median number of transcripts per known gene (tpg) was 4, which was higher than that was observed in either the Ensembl (1.5 tpg) or NCBI (2.3 tpg) annotated gene sets. Our new bovine genome annotation extended more than 11,000 known gene borders (5′ end extension, 3′ end extension, or both) compared with EBI or NCBI annotations. Furthermore, we detected 12,698 novel genes (80% noncoding), which are not reported in current bovine genome annotations. Most these genes were structurally validated by independent data (85%) or were replicated in multiple tissues (96%). These validated results show significant improvement over current bovine genome annotations.

Key Words: bovine, genome annotation, functional genomics

P218 The Ovine Functional Annotation of Animal Genomes project. B. M. Murdoch*[1,6], K. M. Davenport[1], M. Salavati[2], E. Clark[2], A. Archibald[2], A. T. Massa[3], M. R. Mousel[4,5], M. K. Herndon[3], S. N. White[3,4,6], K. C. Worley[7], S. Bhattarai[8], S. D. McKay[8], B. Dalrymple[9], J. Kijas[10], A. Caulton[11], S. Clarke[11], R. Brauning[11], T. Hadfield[12], T. P. L. Smith[13], and N. E. Cockett[12], [1]*Department of Animal, Veterinary, and Food Science, University of Idaho, Moscow, ID, USA,* [2]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, Scotland, UK, [3]*Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA,* [4]*USDA, ARS, Animal Disease Research Unit, Pullman, WA, USA,* [5]*Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, USA,* [6]*Center for Reproductive Biology, Washington State University, Pullman, WA, USA,* [7]*Baylor College of Medicine-Human Genome Sequencing Center, Houston, TX, USA,* [8]*University of Vermont, Burlington, VT, USA, 9University of Western Australia, Crawley, Western Australia, Australia,* [10]*CSIRO Agricultural Flagship, St. Lucia, Brisbane, Australia,* [11]*AgResearch, Hamilton, New Zealand,* [12]*Utah State University, Logan, UT, USA,* [13]*USDA, ARS, U.S. Meat Animal Research Center (USMARC), Clay Center, NE, USA.*

Annotation of regulatory and transcribed elements is important in understanding complex phenotypes related to production and health in livestock species. The Ovine Functional Annotation of Animal Genomes (FAANG) Project aims to characterize transcriptional regulatory elements across the sheep genome. Approximately 100 tissues were collected from the Rambouillet ewe, Benz 2616, used to assemble the ovine reference genome ARS-UI_Ramb v2.0. Functional assays, including sequencing of messenger RNA (mRNA-seq), microRNA (miRNA-seq), and full-length RNA transcripts (Iso-seq), cap analysis gene expression (CAGE), chromatin immunoprecipitation with sequencing (ChIP-seq), assay for transposase-accessible chromatin with sequencing (ATAC-seq), whole-genome bisulfite sequencing (WGBS) and reduced representation bisulfite sequencing (RRBS) were performed on a subset of these tissues. Fourteen chromatin states depicting promoters, enhancers (active, poised, and repressed), and accessible chromatin across the genome were defined with ChromHMM using ChIP-seq and ATAC-seq data and compared across tissues. These chromatin states in combination with DNA methylation, transcription start site identification, and RNA expression provide a very high resolution annotation of the expressed and regulatory regions of the ovine genome. Characterizing regulatory elements in sheep will provide a valuable resource to facilitate a deeper understanding of how gene regulation control influences complex traits in this globally important livestock species.

P219 Chromatin accessibility and regulatory vocabulary in indicine cattle. P. Alexandre*[1], M. Naval-Sánchez[1,2], M. Menzies[1], L. Nguyen[3], L. Porto-Neto[1], M. Fortes[4], and A. Reverter[1], [1]*CSIRO Agriculture and Food, Queensland, QLD, Australia,* [2]*Institute for Molecular Bioscience, The University of Queensland, Queensland, QLD, Australia,* [3]*Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Queensland, QLD, Australia,* [4]*School of Chemistry and Molecular Biosciences, The University of Queensland, Queensland, QLD, Australia.*

The nonuniform topological organization of nucleosomes across the genome reflects a dynamic process that controls chromatin accessibility, particularly at regulatory loci, ultimately influencing cell differentiation and response to the environment. In indicine cattle (*Bos indicus*), identifying regulatory elements in open chromatin regions across different tissues can shed light on the regulation of health and production outcomes, with specific relevance to adaptation to tropical environments. In this study, we generated open-chromatin profiles for liver, muscle, and hypothalamus of 3 Brahman heifers through ATAC-seq (Assay of Transposase-Accessible Chromatin sequencing). Briefly, ATAC-seq data processing and alignment were completed using the Harvard pipeline (https://informatics.fas.harvard.edu/atac-seq-guidelines.html) and MACS2 v2.1.1. was used to call peaks from merged bam files per tissue. Peaks were compared between tissues using bedtools v. 2.29.2 to define tissue-specificity. To identify enriched transcription factor binding sites and master regulators in each tissue, peaks were converted to human coordinates and motif discovery was performed using i-*cis*Target. To validate the relationships between the master regulator and its predicted targets at transcriptional level, previously described RNA-seq data of liver from the same animals was used to build a co-expression network using the Partial Correlation and Information Theory (PCIT) algorithm. We identified HNF1, MEF2, and NFYA as candidate master regulators of the epigenomic profile in liver, muscle, and hypothalamus, respectively. Integration with transcriptomic data in liver allowed us to validate direct targets of HNF1, a master regulator of hepatocyte differentiation, with 22% of precited target genes also presenting significant co-expression. Our findings provide insights into the identification and analysis of regulatory elements in non-model organisms and the evolution of regulatory elements within indicine cattle subspecies. Ongoing research aims at comparing our results with *Bos taurus* data, aiming at uncovering the functional genomic basis of climatic adaptation in beef cattle.

Key Words: cattle and related species, genome annotation, ATAC-seq, regulatory element, climatic adaptation

P220 Local farm animal populations as a potential reservoir for SARS-Cov-2 infections. M. Zorc, A. Tansek, T. Bevec, and P. Dovc*,

University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia.

Recently, the outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) threatened the global health and economy. Some reports confirmed the possibility of transmission of SARS-CoV-2 from humans to animals and vice versa. Mutations reported in the SARS-CoV-2 spike protein, as well as genetic variation in the host receptor, may affect the binding affinity of SARS-CoV-2. Experimental and in silico evidence suggests that human and gorilla ACE2 receptors have the highest, whereas mouse and rat ACE2 receptors have the lowest binding affinities for the SARS-CoV-2 spike protein binding. We propose a systematic screening approach for potential animal hosts for SARS-CoV-2 infection, to predict the potential risk of spreading SARS-CoV-2 infection in local autochthonous farm animal breeds. We collected bovine (NP_001019673), ovine (XP_011961657) and human (NP_001358344) ACE2 orthologs from NCBI, aligned sequences and identified residues present in the interface of hACE2 within 4 Å of the spike receptor binding domain. We analyzed the genetic variability of the identified regions using Ensembl. In cattle, 2 variants in critical binding sites were identified (Lys353 of hACE2) (X:g.127927692A>T Lys > Met X:g.127927693G>T Lys > Asn in ARS-UCD1.2.) In sheep, a variant (X:g.13027932C>T, Arg340Gln) was identified near viral binding hotspot residues (Asn330, Lys353). As the amino acid positions, critical for binding, are affected, this indicates that inter- and intraspecies variation in the target proteins may have a role in determining the potential risk for SARS-CoV-2 infection in different animal species/breeds.

Key Words: SARS-CoV-2, local farm animal breeds, ACE2, binding prediction

P221 Withdrawn

P222 The Bovine Pangenome Consortium. B. D. Rosen*[1], D. M. Bickhart[2], T. P. L. Smith[3], D. Boichard[4], G. A. Brockmann[5], A. J. Chamberlain[6], C. Couldrey[7], H. D. Daetwyler[6], A. Djikeng[8], C. Drögemüller[9], S. Elzaki[5], R. K. Gandham[10], D. Hagen[11], O. Hanotte[12], M. P. Heaton[3], Y. Jiang[13], Z. Jiang[14], D. Larkin[15], G. Liu[1], W. Y. Low[16], P. Ajmone Marsan[17], B. M. Murdoch[18], F. C. Muchadeyi[19], J. Mwacharo[20], H. L. Neibergs[14], H. Pausch[21], S. Demyda-Peyrás[22], J. Prendergast[23], P. J. Ross[24], R. D. Schnabel[25], J. Sölkner[26], A. Soudre[27], A. Tijjani[12], J. L. Williams[17], and Bovine Pangenome Consortium[28], [1]*USDA ARS AGIL, Beltsville, MD, USA,* [2]*USDA ARS DFRC, Madison, WI, USA,* [3]*USDA ARS MARC, Clay Center, NE, USA,* [4]*INRAE Animal Genetics and Integrative Biology, Jouy-en-Josas, France,* [5]*Humboldt-Universität zu Berlin, Berlin, Germany,* [6]*Agriculture Victoria, Melbourne, Victoria, AU,* [7]*LIC, Hamilton, New Zealand,* [8]*Centre for Tropical Livestock Genetics and Health, Midlothian, Scotland, UK,* [9]*University of Bern, Bern, Switzerland,* [10]*National Institute of Animal Biotechnology, Hyderabad, India,* [11]*Oklahoma State University, Stillwater, OK, USA,* [12]*International Livestock Research Institute, Addis Ababa, Ethiopia,* [13]*Northwest A&F University, Yangling, China,* [14]*Washington State University, Pullman, WA, USA,* [15]*Royal Veterinary College, University of London, London, UK,* [16]*The University of Adelaide, Adelaide, South Australia, Australia,* [17]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [18]*University of Idaho, Moscow, ID, USA,* [19]*Agricultural Research Council, South Africa, Pretoria, South Africa,* [20]*Scotland's Rural College, Midlothian, Scotland, UK,* [21]*ETH Zürich, Zürich, Switzerland,* [22]*Universidad de Córdoba, Córdoba, Spain,* [23]*The Roslin Institute, Midlothian, Scotland, UK,* [24]*STgenetics, Navasota, TX, USA,* [25]*University of Missouri, Columbia, MO, USA,* [26]*University of Natural Resources and Life Sciences, Vienna, Austria,* [27]*Université Norbert ZONGO, Koudougou, Burkina Faso,* [28]*Bovine Pangenome Consortium.*

Cattle are thought to have been domesticated over 10,000 years ago in 2 independent events, giving rise to the taurine (*Bos taurus taurus*) and indicine (*Bos taurus indicus*) sub-species. Their spread across the world through human migration, subsequent selection for multiple purposes, and adaptation to varying local conditions has written a complex history into their genomes. This story is further complicated by interspersed instances of genetic bottlenecks and hybridization with wild relatives. As such, a single linear reference genome is insufficient to fully describe and interrogate the extent of genetic variation in cattle. We have launched a global Bovine Pangenome Consortium (BPC) to generate reference-quality genomes of cattle breeds and closely related wild species from the *Bos* genus. Our goal is to capture the breadth of global diversity, including underrepresented cattle breeds, and integrate it into a single pangenome reference. The BPC includes over 60 members representing 40 institutions in 20 countries. We have already released multiple breed-specific and wild-relative reference assemblies using the latest DNA sequencing technologies and genome assembly tools. We are refining methods for incorporating these assemblies into a single reference and developing workflows for data utilization and visualization. This work will significantly increase the global cattle genomics community's ability to accurately identify and select for health and production traits in target populations around the world.

Key Words: cattle, genome assembly, pangenome, global diversity

P223 An improved, high-quality ovine reference genome to facilitate functional annotation of gene regulatory elements. K. M. Davenport*[1], D. M. Bickhart[2], K. C. Worley[3], S. C. Murali[3], N. E. Cockett[4], M. P. Heaton[5], T. P. L. Smith[5], B. M. Murdoch[1], and B. D. Rosen[6], [1]*Department of Animal, Veterinary, and Food Sciences, University of Idaho, Moscow, ID, USA,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI, USA,* [3]*Baylor College of Medicine, Houston, TX, USA,* [4]*Utah State University, Logan, UT, USA,* [5]*US Meat Animal Research Center, USDA-ARS, Clay Center, NE, USA,* [6]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD, USA.*

The domestic sheep is an important agricultural species raised for food and fiber across the world. A high-quality reference genome for this species allows for precise functional annotation of genetic regulatory elements. Further, better quality reference genomes lead to improved mappability of a variety of sequence data and facilitate the discovery of genetic mechanisms influencing biological traits in sheep. The rapid advances in genome assembly algorithms and emergence of increasingly long sequence read length provide the opportunity for an improved de novo assembly of the sheep reference genome. Tissue was collected postmortem from an adult Rambouillet ewe (Benz 2616) selected by USDA-ARS for the Ovine Functional Annotation of Animal Genomes project. Nanopore sequence (50x coverage) generated from lung tissue was combined with publicly available short-read Illumina (55x coverage) and long-read PacBio (75x coverage) sequence and assembled with canu v1.9. Assembled contigs were scaffolded with Salsa v2.2 using Hi-C data, followed by gap filling with PBsuite v15.8.24 and polishing with Nanopolish v0.12.5. Duplicates were removed with PurgeDups v1.0.1 and chromosomes were oriented by identification of centromeres and telomeres with RepeatMasker. Final polishing was performed with 2 rounds of a pipeline consisting of Freebayes v1.3.1 to call variants, Merfin to validate them, and BCFtools to generate the consensus fasta. The ARS-UI_Ramb_v2.0 assembly has improved continuity (contig N50 of 43.18 Mb) compared with Oar_rambouillet_v1.0 (contig N50 of 2.57 Mb) and Oar_v4.0 (contig N50 of 0.15 Mb). In addition, the ARS-UI_Ramb_v2.0 assembly has reduced contig L50, fewer total number of scaffolds, greater mappability, and fewer insertions and deletions identified by RNA-seq data than other ovine assemblies. This sheep reference assembly provides a basis for regulatory element annotation and offers a resource for the greater scientific community to facilitate further research in sheep and comparative genomics.

Key Words: sheep and related species, genome assembly, genome sequencing

P224 Developmental validation of an equine parentage testing kit producing letter and number alleles with 20 markers. S. Zeinali*[1,2], F. Rahiminejhad[1,3], and H. Samiee[3], [1]*Genetek Biopharma GmbH, Berlin, Germany,* [2]*Kawsar Biotechnology Co., Tehran, Iran,* [3]*Kawsar Human Genetic Research Center, Tehran, Iran.*

DNA-based horse parentage and identification tests have been in practice for more than 2 decades. Short tandem repeat markers have become the norm in the community. International Society for Animal Genetics (ISAG) has recommended at least 12 markers and comparisons tests include 17 core loci plus sex determination markers. More than 200 horse mane samples were tested for validation study either using StockMarks from Thermofisher Scientific (MA, USA) or GT Equine from Genetek Biopharma GmbH (Belin, Germany). When GT Equine kit was used, one mane shaft per-PCR was directly used. For StockMarks, DNA extracted from mane samples was used. For DNA sequencing we used extracted DNA as above. Multiplex PCR were performed according to GT Equine user manuals and allele calling was aided by following the instruction laid out in the kit's user manual. Since GT Equine kit provides allelic ladder and bin sets, allele calling was done more easily in comparison to the StockMarks kit, where a conversion table is needed. Since GT Equine has allelic ladder and analysis assistant for GeneMapperIDx and GeneMarker, allele calling as letters or numbers was easily done. We compared the results from GT Equine, StockMarks, and sequencing. We observed that whenever a marker was heterozygote with StockMark, GT Equine was also heterozygote. However, in some cases when the GT Equine kit showed heterozygosity, StockMark would show homozygosity. This was more prominent for HMS3 and ASB23 markers. We checked this observation on Horse Comparison Test 2020-2021 samples. GT Equine and sequencing showed heterozygote for 2 markers (HMS3 and ASB23) but StockMarks showed homozygote for HMS3 and ASB23 in 30% and 20% of cases, respectively for the above samples. Since GT Equine has 20 markers, we could only validate kit results with Sanger sequencing. We did not see any discrepancy. It is noteworthy to say that due to stutter bands, sequencing interpretation was not easy. The 2 extra polymorphic tetra nucleotide markers (i.e. KBC51 and KBC71) included in the kit, are advantageous to the kit and we hope that ISAG will adapt these markers in the future. We conclude that GT Equine kit is a superior kit for horse profiling and parentage testing due to its extra markers, allelic ladder, and option of using direct mane samples. The kit makes molecular testing an easy job comparable to kits used for human identification.

Key Words: equine, alleles, StockMark, Genetek, HMS3

P225 Evaluation of SNP markers for parentage testing in the draft horse population. T. Ishige*[1], M. Kikuchi[1], H. Kakoi[1], K.-I. Hirora[1], A. Ohnuma[1], T. Tozaki[1], Y. Hirosawa[2], S. Tanaka[2], and S.-I. Nagata[1], [1]*Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan,* [2]*National Livestock Breeding Center Tokachi Station, Otofuke, Hokkaido, Japan.*

Single nucleotide polymorphism (SNP) genotyping has emerged as a desirable alternative to microsatellite typing. The provisional International Society for Animal Genetics (ISAG) panel with 147 SNPs is currently proposed for horse parentage testing. It is important to validate whether this panel is suitable for parentage testing in horse breeds worldwide. Although the purebred draft horses, Breton and Percheron, are raised in Japan, there is no knowledge regarding the SNP variations in these horses. The aim of this study was to evaluate the SNP markers for parentage testing in draft horse populations and determine the efficiency of the ISAG SNP panel. Genomic DNA was extracted from the peripheral blood of 72 randomly selected horses belonging to the 2 breeds—Breton (n = 36) and Percheron (n = 36). Genotyping-by-sequencing (GBS) libraries were generated using the AgriSeq Custom Equine Parentage and ID Plus Traits and Disorders Panel with 605 makers. GBS libraries were genotyped using the ION 540 Chef kit and Ion 540 chip. Samples with a call rate of more than 90% were applied to the present analysis. Through sample analysis, the SNPs showing the following qualities were filtered: (1) coverage less than 10 × and (2) call rate lower than 90%. Based on the allelic frequencies of the remaining SNPs, the expected heterozygosity (He) and probability of exclusion (PE) of the respective SNPs were calculated. In this analysis, the averaged coverage of all SNPs was 588.2 ×. The call rate of all SNPs for the ISAG panel in Breton and Percheron was > 0.971 and > 0.972, respectively. Thirty-five Breton and 36 Percheron horses were subjected to the analysis with all SNPs of the ISAG panel, except for one Breton with call rate < 0.68. The averaged He of the Breton and Percheron populations were 0.387 and 0.378, respectively. The combined PE1 (given 2 parents and one offspring; exclude their relationship) and PE2 (given one parent and one offspring; exclude their relationship) in both populations were >0.9999999999 and > 0.99999, respectively. Thus, it was found that the proposed ISAG panel has enough power for parentage testing in the draft horses in Japan.

Key Words: horses and related species, single nucleotide polymorphism (SNP), genetic marker, parentage

P226 Withdrawn

P227 Comparative analysis of single nucleotide polymorphisms and microsatellite markers for parentage verification and sire/dam allocation within equine Thoroughbred breed. P. Flynn*[1,2], R. Morrin-O'Donnell[1], R. Weld[1], J. Carlsson[2], P. Siddavatam[3], and K. Reddy[3], [1]*Weatherbys Scientific, Naas, Ireland,* [2]*University College Dublin, School of Biology and Environmental Science, Belfield, Dublin, Ireland,* [3]*Thermo Fisher Scientific, Austin, TX, USA.*

Short tandem repeat (STR), also known as microsatellite molecular markers are currently used for parentage verification within equine. Transitioning from STR to single nucleotide polymorphism (SNP) molecular markers to perform equine parentage verification is becoming an ever more feasible prospect and key areas that merit exploration are ensuring maintenance of test accuracy and suitability of current genotyping technologies to support such a transition. We established a targeted equine genotyping-by-sequencing (EQ-GBS) panel of 562 SNPs, consisting of SNPs currently undergoing feasibility testing by International Society of Animal Genetics (ISAG) and an additional SNP panel to perform sire/dam allocation. A sample group of 309 Thoroughbreds, inclusive of 55 previously parentage verified offspring/sire/dam cases, underwent SNP genotyping and availability of historic STR profiles allowed for comparative analysis of parentage verification and allocation accuracy between both SNP and STR panels. An average sample call rate of 97.2% was observed for EQ-GBS SNP panel when using medium grade DNA, an average minor allele frequency of 0.38 demonstrated SNP panel informativeness and a subset panel of 516 SNPs was identified as "Optimum Performing." Simulated offspring/sire/dam parentage verification resulted in positive separation values and no false positive cases (i.e., expected to fail parentage, but pass) for ISAG pilot and EQ-GBS SNP panels - in comparison to a zero separation value and 28 false positive cases for STR panels. This study has proven GBS as a proficient technology to generate low-density SNP profiles for equines and provides insight into the value of using SNPs within equine parentage verification in comparison to STRs.

Key Words: equine, parentage, microsatellites, single nucleotide polymorphism, genotyping-by-sequencing

P228 Withdrawn

P229 Thermal stress generates oxidative damage in liver and gills of red cusk-eel (*Genypterus chilensis*) juvenile. P. Dettleff*[1,2], R. Zuloaga[2], P. Gonzalez[2], M. Fuentes[2], J. Aedo[2], J. M. Estrada[3], A. Molina[2], and J. A. Valdes[2], [1]*Nucleus of Applied Research in Veterinary and Agronomic Sciences, Universidad de Las Americas, Santiago, Chile,* [2]*Laboratory of Molecular Biotechnology, Faculty of Life Sciences, Andres Bello University, Santiago, Chile,* [3]*Marine research center of Quintay, Andres Bello University, Quintay, Chile.*

The *Genypterus* genus contains native species of economic relevance with high potential for aquaculture diversification, including the red cusk-eel (*Genypterus chilensis*). Environmental factors such as temperature can generate stress in the native and commercial populations, affecting the performance of fish. The objective of this work was to study the effect of heat stress in red cusk-eel juveniles, determining the effect of this stressor in liver and gills. Red cusk-eel juveniles were collected from CIMARQ and separated into control and stress groups, with duplicated tanks. The groups were maintained at control temperature (14°C) or subjected to high-temperature stress (19°C) for 5 d. At the end of the experiment, fish were euthanized, sampling plasma, liver, and gills for cortisol level, oxidative damage evaluation (lipid peroxidation, protein carbonylation, and DNA damage), and gene expression evaluation through RNA-seq. High temperature produces a significant increase in cortisol levels in the stress group, generating oxidative damage in liver and gills. In liver, a significant increase in hepatic enzymes (ALT, AST, and AP) associated with thermal stress was observed, with a relevant modulation of gene expression with 3,354 differentially expressed genes, including enrichment terms associated with protein folding problems. Additionally, in gills, a relevant modulation of gene expression was observed in response to thermal stress, with 4,791 differentially expressed genes, with enriched terms related to unfolded proteins and DNA replication process, among others. This study showed that thermal stress can affect different key tissues generating damage and modulating the expression of relevant processes that can affect the performance of red cusk-eel, information that should be considered in a climate change world scenario. Funding: CONICYT FONDECYT Postdoctorado 3180283.

Key Words: fish, RNA-seq, aquaculture

P230 Pikeperch *Sander lucioperca* genome data: Basis for smart farming in aquaculture. T. Goldammer*[1,2], M. Verleih[1], R. M. Brunner[1], A. Rebl[1], J. A. Nguinkal[1], L. de los Ríos-Pérez[1], N. Schäfer[1], M. Stüeken[3], F. Swirplies[3], and D. Wittenburg[1], [1]*Fish Genetics Unit, Institute of Genome Biology and Statistics in Genomics Unit, Institute of Genetics and Biometry, Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*Molecular Biology and Fish Genetics, Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany,* [3]*Research Centre for Agriculture and Fisheries, State Research Center of Agriculture and Fisheries M-V, Rostock, Germany.*

The pikeperch (*Sander lucioperca* Linnaeus, 1758) is one of the new promising fish species for aquaculture in Europe. The economic viability of pikeperch aquaculture and the successful adaptation of pikeperch to different farming systems are partly determined by breeding programs. The knowledge of genetically determined breeding parameters and of family structures is an essential prerequisite for their success. From the perspective of genome biology, elucidation of the pikeperch genome, genes and their function and joint activity provide essential metadata for smart breeding approaches. This also includes the elucidation of genotypes and their correlation to phenotype as well as the identification of gene-based indicators for monitoring health, growth and well-being, etc. Our research conducted for this purpose resulted in the first near-complete elucidation of the pikeperch genome with a length of about 900 million nucleotides distributed over 24 chromosomes, the representation of 26,000 protein and non-protein encoding genes, the development of a genetic linkage map based on about one million nucleotide polymorphisms in the genome, and the development of gene-based BioChips to assess fish welfare under various conditions in pikeperch aquaculture (Nguinkal et al., Genes (Basel) 10, 2019; Swirplies et al., Aquaculture 501, 2019; de los Ríos-Pérez et al., SciRep 10, 2020; Schäfer et al., FishPhysiolBiochem 47, 2021). This knowledge is freely available and we will use it to contribute to the optimization of pikeperch aquaculture in Germany.

Key Words: pikeperch, genome, SNP-map, gene map, welfare markers

P231 Signatures of selection and genomic diversity of Muskellunge (*Esox masquinongy*) from 2 populations in North America. J. Chinchilla-Vargas*[1], J. R. Meerbeek[2], M. F. Rothschild[1], and F. Bertolini[3], [1]*Iowa State University, Ames, IA, USA,* [2]*Iowa Department of Natural Resources, Spirit Lake Fish Hatchery, Spirit Lake, IA, USA,* [3]*National Institute of Aquatic Resources, Technical University of Denmark, Lyngby, Denmark.*

Muskellunge (*Esox masquinongy*) is the largest and most prized game fish for anglers in North America. Despite its popularity, little is known about the species' genetic diversity in Iowa's propagation program. We used WGS from 12 brooding individuals from Iowa and publicly available RAD-seq of 625 individuals from Canada to study the genetic differences between populations, analyze signatures of selection and evaluate the levels of genetic diversity in both populations. Given that there is no reference genome available for muskellunge, reads were aligned to the genome of Pike (*Esox lucius*), a closely related species. Variant calling produced 7,886,471 biallelic variants for the Iowa population and 16,867 high-quality SNPs that overlap with the Canadian samples. The Ti/Tv values were 1.09 and 1.29 for samples from Iowa and Canada, respectively. PCA and Admixture analyses showed large genetic differences between the Canadian and Iowa populations. Window-based pooled heterozygosity found 6 highly heterozygous windows containing 244 genes in the Iowa population and Fst comparing the Iowa and Canadian populations found 14 windows with Fst values larger than 0.9 containing 641 genes. One enriched GO term (sensory perception of pain) was found through pooled heterozygosity analyzes. Although not significant, several enriched GO terms associated to growth and development were found through Fst analyses. Inbreeding calculated as Froh was 0.03 on average for the Iowa population and 0.32 on average for the Canadian samples. Froh results point toward Canadian inbreeding rate being higher than that of the Iowa population, presumably due to isolation of its subpopulations. This study was the first to document that brood stock muskellunge from Iowa showed marked genetic differences with the Canadian population. Additionally, despite genetic differentiation based on sex being observed, no major locus has been detected. Inbreeding does not seem to be an immediate concern for muskellunge in Iowa, but apparent isolation of subpopulations has caused levels of homozygosity to increase in the Canadian muskellunge population. Finally, these results prove the validity of using genomes of closely related species to perform genomic analyses when no reference genome assembly is available.

Key Words: muskellunge, fisheries, population genomics, sport fishing

P232 Optimization of induced ovulation and spawning of *Clariid* catfish *Heterobranchus bidorsalis* (Geoffroy-Saint-Hilaire, 1809) using synthetic hormone. W. Olaniyi*, *Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.*

Successful induced spawning of *Clariid* catfish *Heterobranchus bidorsalis* has been challenged by hormonal manipulations. In this study, induced spawning of *H. bidorsalis* was optimized for artificial propagation through application of varying hormonal dosage. A single dose (mL) of the synthetic hormone, Ovaprim, per kg body weight (bw) at 0.5, 0.75, 1 and 1.25 were administered on the broodstock. Results showed that ovula-

tion was achieved within the latency period of 14 ± 1 h at 28.5 ± 0.5°C. Mean spawned eggs for doses of 1 mL/kg (56.77 ± 8.92 g) and 1.25 mL/kg (59.93 ± 3.26 g) gave significant production compared with 0.5 mL/kg (5.07 ± 2.29 g) and 0.75 mL/kg (9.13 ± 1.10 g) ($P < 0.05$). Percentages of fertility (72.33 ± 2.52, 79.33 ± 4.04, 84 ± 4.58, 82 ± 4.36); hatchability (43.47 ± 4.77, 61.73 ± 10.02, 70.4 ± 5.51, 68.57 ± 12.78) and survival (47.37 ± 3.16, 54.07 ± 7.74, 64.67 ± 4.63, 62.4 ± 2.82) for each test dose of 0.5, 0.75, 1, 1.25 mL/kg bw respectively, were recorded at 28.5 ± 0.5°C. The higher doses of 1 and 1.25 mL/kg bw showed significant effective dosage compared with lowest dose of 0.5 mL/kg ($P < 0.05$), while 0.75 mL/kg was not significant ($P > 0.05$) with respect to others. It is noteworthy that unique hatching occurred at 21 h in all the treatments. More importantly, the documented movie of unique characteristic mode of hatching in *H. bidorsalis* as revealed in this study plays significant effect on its hatchability and survival (https://youtu.be/HX-HjXZ7ImbM). Also, the level of fecundity revealed reproductive dysfunction in *H. bidorsalis* and recommends higher dose of 1 mL/kg bw for optimal and effective induction using synthetic hormone Ovaprim.

Key Words: spawning, *Clariid* catfish, *Heterobranchus bidorsalis*, hormone, hatching

P233 Resistance of common carp to Cyprinid herpes virus-3: Individual survival is more affected by different genomic loci than family percent survival. M. Amir[1], J. Lighten[2], and L. David*[1], [1]*The Hebrew University of Jerusalem, Rehovot, Israel,* [2]*University of Exeter, Devon, UK.*

Common carp (*Cyprinus carpio*) is a major aquaculture species in both amount and distribution of production. Infectious diseases are damaging aquaculture significantly, impeding the further development of this sector. For common carp, widespread outbreaks of a disease caused by Cyprinid herpes virus-3 (CyHV-3) have been damaging production significantly. We have been breeding for CyHV-3 resistant strains and using our stocks to study disease resistance genetics. We and others reported that CyHV-3 resistance is a polygenic heritable trait and so far, only a few quantitative trait loci (QTLs) for CyHV-3 resistance were identified. QTLs were so far identified by comparing between fish that died and survived a disease challenge. Thus, these QTLs affect individual survival. Yet, in our hands, selecting fish as parents based only on if they survived a challenge (within-families variation) was inefficient in improving progeny resistance compared with selecting survived fish from families with higher family % survival (between-families variation). In this study, we compared genetic bases between individual survival and family % survival. We compared genotype frequencies of 57,000 SNPs, once between dead and survived individuals (individual survival), regardless of the family to which individuals belonged, and second between family's % survival of individuals, regardless of whether individuals survived a challenge or died. We identified 4 QTLs for individual survival and 7 for family's % survival. We validated QTLs using additional samples from independent individuals with various values of family % survival. In all QTLs, we found genes related to immune system function, consistent with the transcriptomic differences between resistant and susceptible fish in response to infection that we previously published. Importantly, we found no overlap between QTLs affecting individual's survival and family's % survival. Thus, our results enhance the understanding of polygenic disease resistance in fish and support the possibility that, at least in part, different genes affect individual's survival and family's % survival, both of which can help improving disease resistance.

Key Words: Koi herpes virus, disease resistance, fish immunity, aquaculture, genetic variation

P234 Reproductive performance of the sea urchin *Tripneustes gratilla* in first- and second-generation cultured cohorts. M. Brink-Hull*[1,2], C. Rhode[1], M. D. Cyrus[2,3], B. M. Macey[2,3], J. du Plessis[1], K. L. Hull[1], and R. Roodt-Wilding[1], [1]*Stellenbosch University, Stellenbosch, Western Cape, South Africa,* [2]*University of Cape Town, Cape Town, Western Cape, South Africa,* [3]*Department of Forestry, Fisheries and the Environment, Cape Town, Western Cape, South Africa.*

Broadcast spawning animals, such as *Tripneustes gratilla*, display differential parental contributions in aquaculture environments, resulting in decreased genetic diversity and subsequent reduced adaptability, poor responses to artificial selection and diminished production output. This study aimed to assess genetic diversity, pedigree relationships and phenotypic performance of 2 first-generation (F1) cultured cohorts (n = 50) established by combining eggs and sperm of wild broodstock (n = 12). Using 21 species-specific microsatellite markers, a decline in genetic diversity and differential parental contributions were observed, with a single female contributing to 70% of the first F1 cohort and a male contributing to 92% of the second F_1 cohort. Various genetic- and biological factors, such as family-advantages, gonad and gamete quality, and feeding regimens used for broodstock conditioning, may drive reproductive competition. To assess this, F1 broodstock (n = 32) were conditioned on 4 diets [formulated feed with 20% *Ulva rigida*, fresh kelp (*Ecklonia maxima)*, fresh *U. rigida* and a mixture of the diets] for 4 mo, and a factorial breeding design was implemented. Larvae from broodstock fed kelp (n = 8) and a mixed diet (n = 8) survived for the duration of larval rearing (20 d) with similar growth rates throughout ($P > 0.05$; ANOVA). Three months post-settlement, parentage analysis revealed 26 of 32 possible parent pairs contributed to the F_2 generation (n = 364). No statistically significant differences between F_1 broodstock and F_2 offspring were observed for genetic diversity indices, likely due to equal parental contributions. Offspring phenotypic performance assessments revealed that body diameter was lowly heritable ($h^2 = 0.050 \pm 0.058$). However, offspring assigned to kelp-fed broodstock were significantly smaller (ave. diameter = 0.66 ± 0.07 cm) than juveniles assigned to broodstock fed a mixed diet (ave. diameter = 0.94 ± 0.10 cm) indicating that the maternal provisioning strategy of sea urchins may benefit future commercial production.

Key Words: animal breeding, aquaculture, microsatellite, parentage, quantitative genetics

P235 An application of the MedFish SNP array: Determining population structure and genetic variability of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*). M. Saura*[1], A. Fernández[1], J. Fernández[1], R. Peiro-Pastor[1], C. Peñaloza[2], L. Bargelloni[3], T. Manousaki[4], C. Tsigenopoulos[4], and B. Villanueva[1], [1]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, CSIC), Madrid, Spain,* [2]*The Roslin Institute, University of Edinburgh, Midlothian, Scotland, UK,* [3]*University of Padova, Padova, Italy,* [4]*Hellenic Centre for Marine Research (HCMR), Heraklion, Crete, Greece.*

Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) are the most important marine fish species farmed in the Mediterranean. Understanding population structure and genetic diversity within and between wild and farmed populations is of paramount importance to develop optimal strategies for the conservation of wild populations and to achieve sustainable aquaculture production. In this study we used a new 60K SNP array recently developed for both species to determine patterns of population structure and genetic variability of seabream and seabass populations. A total of 50 populations from both species (23 wild and 27 farmed) were genotyped with the SNP array. Population structure was assessed through principal component, phylogenetic and clustering analyses and F_{ST} fixation index. Genetic variability was assessed through the effective population size (N_e), estimated from linkage disequilibrium. Clustering methods revealed a clear differentiation between wild and farmed populations. Despite the little differentiation among wild populations, Atlantic/Mediterranean (seabream) and West/East Mediterranean (both species) patterns were

detected. In general, N_e was large (>1,000) for wild and small (<100) for farmed populations of both species, with some exceptions. The low N_e estimated for some farmed populations highlight the need of applying measures to control the loss of genetic variability. The differentiation between wild and farmed populations suggests that special care must be taken to avoid escapees that could have undesirable genetic effects on native populations. To our knowledge, this is the first time that a population genetic analysis in these species has been carried out with such a high number of SNP markers.

Key Words: admixture, aquaculture, effective population size, population genomics, single nucleotide polymorphism (SNP)

P236 A blue mussel chromosome-scale assembly and genomic resources for aquaculture, marine ecology and evolution. T. Hori*[1,2], [1]*PEI Marine Sciences Organization, Charlottetown, PE, Canada,* [2]*Atlantic Aqua Farms, Charlottetown, PE, Canada.*

The blue mussel is commonly described as the *Mytilus* species complex, encompassing at least 3 putative species: *M. edulis*, *M. galloprovincialis* and *M. trossolus*. These 3 species occur on both sides of the Atlantic and hybridize in nature, and both *M. edulis* and *M. galloprovincialis* are important aquaculture species. They are also invasive species in many parts of the world. This project aimed at assembling a high-quality genome for *M. edulis* and develop tools that can be used in breeding, molecular ecology and evolution to address questions of both commercial and environmental perspectives. We used a combination of PacBio sequencing and Dovetail's Omni-C technology to generate an assembly with 14 long scaffolds containing 94% of the predicted length of the *M. edulis* genome (1.6 out of 1.6 Gb). Assembly statistics were total length 1.65 Gb, N50 = 116 Mb, L50 = 7 and, L90 = 13. BUSCO analysis showed 90.59% complete eukaryote BUSCOs identified. AB-*Initio* annotation using RNA-seq from mantle, gills, muscle and foot predicted 41,319 genes. Using GBS and shotgun sequencing, we sequenced 3 North American populations of *Mytilus* to characterize single nucleotide as well as structural variance. Population genetics analysis data will also be presented.

Key Words: blue mussel, aquaculture, genome assembly, SNPs, *Mytilus*

P237 Omics study for viral hemorrhagic septicemia virus resistance in *Paralichthys olivaceus*. J. Shin*[1], S. H. Lee[1], W. J. Kim[2], J.-W. Park[3], D.-I. Lee[3], H. S. Jung[3], and J. Kim[3], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea,* [2]*East Sea Fisheries Research Institute, National Institute of Fisheries Science, Gangneung, Korea,* [3]*Fish Genetics and Breeding Research Center, National Institute of Fisheries Science, Geoje, Korea.*

Paralichthys olivaceus is one of the most cultivated fish in Korea. However, viral diseases in fish farms raised at high density are strongly contagious and have high mortality rate, which causes a large economic loss in the fishery industry. Viral hemorrhagic septicemia virus (VHSV) is well known as the most serious viral disease in fish. Therefore, selective breeding program would be useful to select fishes which have resistance in viral disease by using omics data. In this study, omics analysis (GWAS, eQTL, and RNA-seq) were performed using 125 flounders' NGS data to identify genetic variants associated with VHSV resistance in *Paralichthys olivaceus*. Genome-wide association study (GWAS) and RNA-seq analysis were performed to discover VHSV resistance related genomic loci and differentially expressed transcripts (DETs). Finally, to integrate these 2 studies (GWAS and RNA-seq) eQTL analysis was performed to investigate genomics networking between genetic variants from noncoding region, which might affect gene expression, and the expression level of DETs that we have found in RNA-seq. As a result, loci on chromosome 23 was detected to be significant in GWAS. And then, 453 DETs are discovered in RNA-seq. Among them, 21 DETs are located on chromosome 23, and involved in various immune-related pathways such as protein processing in endoplasmic reticulum, proteasome. In addition, the result of eQTL analysis shows that genotype change of 3 upstream and 2 intron variants directly affect the expression level of upregulated DETs located on chromosome 23. Also, 416 upstream, 249 intergenic, and 689 intron variants, located more than 1Mb from DETs, are discovered to secondarily affect the expression level of chromosome 23 located DETs. According to our findings, it was confirmed that loci on chromosome 23 are associated with VHSV resistance, and some variants on chromosome 23 directly affect the expression level of transcripts. In conclusion, these results would be useful for breeding VHSV resistance flounders by selecting the individuals according to the breeding value calculated by these loci information.

Key Words: *Paralichthys olivaceus*, VHSV resistance, omics data analysis, selective breeding program, breeding value

P238 Influence of estimated breeding value for growth trait on spawning quality in gilthead seabream (*Sparus aurata*). C. Pérez-García*[1], Á. Lorenzo-Felipe[1], S. Ferosekhan[1], S. Leon-Bernabeu[1,2], M. Izquierdo[1], R. Ginés[1], J. M. Afonso[1], H. S. Shin[1], and M. J. Zamorano[1], [1]*Universidad de Las Palmas de Gran Canaria (ULPGC), Instituto Universitario de Acuicultura Sostenible y Ecosistemas Marinos (IU-ECOAQUA), Grupo de Investigación en Acuicultura (GIA), Telde, Spain,* [2]*QUANARIA. C/ Prolongación Bentejui, San Bartolomé de Tirajana, Las Palmas, Spain.*

Genetic factors has been poorly studied (Lorenzo-Felipe et al., 2021). The aim of this study is to study the spawning quality between different genetic line in terms of growth (high and low growth) in gilthead seabream. Broodstock were selected from the third generation of the PROGENSA Project (National Breeding Program of Spain). Two groups of broodstock were selected based on their EBV growth (high and low growth). Evaluation of spawning quality The floating eggs were collected 6 d per week at 09:00 h from each mass spawning tank and egg number was counted under binocular stereoscope to estimate the *oocyte yield*, *viable eggs*, *viability rate*, *number of alive larvae* according to Lorenzo-Felipe et al. (2021). The data were checked for normality and homocedasticity. For all traits, data where grouped in 12 fortnights throughout the spawning season: In this study, only where consider from 4th to 12th fortnights. When a normal distribution and/or homogeneity of variance was not achieved, data were subjected to the Kruskal–Wallis nonparametric test (Zar, 1984). In the present study, for *oocyte yield*, *viable eggs* and *viability rate*, HG broodstock showed significant lower values than LG broodstock in 4th and 6th fortnights. In the case of *number of alive larvae*, LG broodstock showed significant higher values than HG broodstock in 6th fortnight. These results are in concordance with Lorenzo-Felipe et al. (2021) who found that genetic factors are more determinant in the middle of the spawning season. On the other hand, the higher quality of LG broodstock than HG broodstock is related with genetic correlation between growth and deformity traits, previously described by Lee-Montero et al. (2015). Furthermore, the significant difference reported in *oocyte yield*, *viable eggs* and *number of alive larvae*, is in agreement with the results of Fernández-Palacios et al. (1995) and Lorenzo-Felipe et al. (2021) who proposed that *oocyte yield* and *viable eggs* explaining the majority of variation of the spawning quality.

Key Words: fish, management, animal breeding

P239 Genome editing to produce monosex and sterile fish for aquaculture. X. Lauth*[1], T. Umazume[1], S. Herbert[1], V. Williams[2], and J. Buchanan[1], [1]*Center for Aquaculture Technologies, San Diego, CA, USA,* [2]*The JEM Project, San Diego, CA, USA.*

The ability to mass-produce reproductively sterile fish for aquaculture will increase culture performance and environmental sustainability by preventing early sexual maturation and uncontrolled reproduction. While varied reproductive containment solutions have been proposed, none to date has proved fully effective, or has been widely adopted by the industry. Here, we describe strategies to generate, breed and mass-produce infertile fish. Our solutions rely on selected gene edits to create broodstock

lines that only produce monosex, sterile populations of progeny. Thus, our design combines the benefit of sterility with sexually dimorphic performance traits in culture. These approaches were validated in tilapia but are transferrable to multiple species of fish. The edited broodstock can be propagated and incorporated into breeding programs. We identified and inactivated 12 genes in 2 evolutionarily conserved pathways, one governing sex differentiation and the other sex competency. We isolated null alleles of genes necessary for spermiogenesis and estrogen synthesis causing male sterility and masculinization, respectively. Double edited combinations for these genes produced all-male sterile populations. Likewise, we inactivated genes which caused females to develop atrophic ovaries arrested at a previtellogenic stage or string-like ovaries lacking oocytes. We further disrupted genes causing genetic males to sex reverse into females. Double edited combinations for these genes produced all-female, sterile populations. We successfully propagated and amplified the double edited lines via germ cell transplantation from a juvenile mutant donor into several germ cell free wild-type recipient embryos. In the resulting recipient broodstock chimera, the induced edits had no effect as the genes targeted are not expressed in germ cells. With this approach, we generated fertile broodstock that successfully mass-produced sterile, monosex populations. Finally, we tested the performance of all-male sterile tilapia in grow-out trials. Monthly average of daily body weight gain indicated that sterile tilapia grew 12% faster than their maturing siblings starting around the time of puberty.

Key Words: fish, genome editing, CRISPR-Cas9, fertility, aquaculture

Genetics of Immune Response and Disease Resistance Posters

P240 Withdrawn

P241 Whole blood transcriptome analysis in sheep affected with caseous lymphadenitis. J. Kyselová*[1], J. Marková[2], Z. Sztankóová[1], L. Tichý[1,3], M. Mušková[1], S. Šlosárková[2], and B. Bartošová[2], [1]*Intitute of Animal Science, Prague, Czech Republic,* [2]*Veterinary Research Institute, Brno, Czech Republic,* [3]*Czech University of Life Sciences, Prague, Czech Republic.*

Caseous lymphadenitis (CLA) is a chronic disease caused by the bacteria *Corynebacterium pseudotuberculosis* (*Cps*), which causes great economic losses in the small ruminant industry. To obtain detailed information about pathogen and host genome interaction in diseased and more resistant sheep, the gene expression was analyzed by the RNA-seq method. The blood samples were obtained both from *Cps* serologically positive (3) and negative ewes (3) considered resistant and from the health controls (3) of another herd. Trimmed reads were mapped to ovine transcriptome reference, and data were divided into 3 groups according to the health status and used for differential expression analysis employing the DESeq2. The transcripts with log fold change >1.5 or < −1.5 and $P <$ 0.05 were considered significantly differentially expressed. Genes were interpreted through the Gene Ontology system using the PANTHER classification. Gene Annotation and Pathway Mapping were provided using the KEGG database. Of the approximately 32 million sequences analyzed per sample, 55–58% of reads were unambiguously mapped to the reference. On average of 26 796 transcripts were detected, representing 63% of all known sheep transcripts. The transcripts were assigned to 12 295 genes. Resistant ewes from the affected herd had 245 differentially expressed genes (DEG), and diseased ones had 195 DEG compared with control females. Ewes infected with the *Cps* showed increased regulation of 57 biological processes, mainly cell cycle, and meiosis, segregation of chromosomes and organelles, including control of these processes. Differentially expressed genes were classified into 54 different metabolic and signaling biochemical pathways affected by *Cps* infection and CLA. Among them, up to 21 involved pathways can be considered to control the innate immune response. However, the intensive metabolism of both sick and more resistant ewes exposed to infectious pressure is evidenced by the fact that we did not find any significantly enriched biological processes or cellular structures in which there was lower gene expression than healthy individuals.

Key Words: genome sequencing, immunogenomics, infectious disease

P242 The host genetic underlying pathological outcomes to *Mycobacterium avium* subsp. *paratuberculosis* infection is governed by distinct genetic variants. M. Alonso-Hearn*[1], M. Canive[1], G. Badia-Bringué[1], O. González-Recio[2,3], A. Fernández[2,3], P. Vázquez[1], J. Garrido[1], and R. Juste[1], [1]*NEIKER- Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain,* [2]*Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, CSIC, Madrid, Spain,* [3]*Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.*

Bovine paratuberculosis (PTB) is a granulomatous enteritis caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). According to their severity and extension, PTB-associated histological lesions have been classified into the following groups; focal, multifocal, and diffuse. It is unknown whether these lesions represent sequential stages or divergent disease outcomes. In the current study, the associations between host genetics, host response, and pathology were explored by genotyping 813 Spanish Holstein cows with focal (n = 371), multifocal (n = 33), diffuse (n = 33), and with undetected visible lesions (n = 373) in gut tissues and regional lymph nodes. DNA from peripheral blood samples of these animals was genotyped with the Bovine MD SNP50 Bead Chip, and the corresponding genotypes were imputed to whole-genome sequencing (WGS) data using the 1,000 Bull genomes reference population. A genome-wide association study (GWAS) was performed using the WGS data and the presence or absence of each type of histological lesion in a case-control approach. We identified 129 and 92 SNPs highly associated ($P \leq 5 \times 10^{-7}$) with the multifocal (heritability = 0.075) and the diffuse lesions (heritability = 0.189), respectively. Twelve and 9 distinct quantitative trait loci (QTLs) highly associated with the multifocal and diffuse lesions were identified, respectively. Some of the identified QTLs overlapped with QTLs previously associated with PTB, bovine tuberculosis, and mastitis infection. Pathway analysis with candidate genes overlapping the identified QTLs revealed a significant enrichment of the keratinization pathway and cholesterol metabolism in the animals with multifocal and diffuse lesions, respectively. While keratin variants may predispose cows to the development of multifocal lesions, cholesterol variants associate with the more severe lesions. Total plasma cholesterol in animals with diffuse lesions (0.080 μg/μL) was significantly lower when compared with cows with focal (0.126 μg/μL), multifocal (0.141 μg/μL) or with undetected lesions (0.129 μg/μL). Taken together, these findings suggest that the variation between the multifocal and diffuse lesions may be genetically determined and indicative of distinct host responses in genetically predisposed individuals.

Key Words: cattle, genome-wide association, imputation, infectious disease, animal health

P243 Novel insight into mechanism of lnc-FUT3as regulating *Escherichia coli* F18 bacterial diarrhea in weaned piglets. H. Fan*[1], Z. Wu[1], J. Jin[1], X. Xu[1], S. Gao[2], S. Wu[1,3], and W. Bao[1,3], [1]*College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China,* [2]*College of Veterinary Medicine, Yangzhou University, Yangzhou,*

Jiangsu, China, [3]*Joint International Research Laboratory of Agriculture and Agri-Product Safety, Yangzhou University, Yangzhou, Jiangsu, China.*

Postweaning diarrhea in piglets is mainly caused by *Escherichia coli* F18. Long noncoding RNAs (lncRNAs) have emerged as critical players in pathogen infection, but their role in host anti-*E. coli* F18 infection remains unknown. Here, using RNA sequencing, we identified an antisense lncRNA termed *FUT3as* as a host regulator related to *E. coli* F18 infection. Downregulation of *FUT3as* expression contributed to the enhancement of *E. coli* F18 resistance in IPEC-J2 cells. *FUT3as* upregulated *FUT3* (fucosyltransferase 3) expression via H4K16ac modification to promote the binding of Sp1 to the *FUT3* promoter. In addition, the *FUT3as*/miR-212 axis could act as a competing endogenous RNA (ceRNA) to affect the expression of its target gene *FUT3*. Functional analysis demonstrated that *FUT3* is a promising new target for combating *E. coli* F18 infection in weaned piglets. Interestingly, *FUT3as* could enhance *E. coli* F18 susceptibility by activating glycosphingolipid biosynthesis signaling and toll-like receptor (TLR) signaling. In summary, we have identified a lncRNA-based glycosphingolipid biosynthesis/TLR signaling regulatory circuit that modulates *E. coli* F18 susceptibility in piglets via histone H4 modifications or ceRNA mechanism. Our results provide insights into the mechanism of lncRNA regulation of *E. coli* F18 infection in weaned piglets and provide theoretical guidance to solve the problem of molecular breeding to resist bacterial diarrhea in pigs.

Key Words: pig, lncRNA, *Escherichia coli* F18, susceptibility

P244 Variation in circulatory serum biomarkers in dairy heifers exposed to endotoxin indicate disparity in induced physiological responses. A. Sharma*, T. Sullivan, K. Lamers, and N. Karrow, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The hypothalamic–pituitary–adrenal axis is a major 'stress' axis that is activated upon microbial exposure and culminates the secretion of the blood glucocorticoid cortisol. Individual differences in cortisol levels indicate differences in the responsiveness of animals to aversive situations and their ability to manage immunological challenges. Genetic selection of animals for enhanced stress resilience could be a possible strategy to mitigate these differences. In the present study, 180 heifers (6–10 mo age) were immune challenged with endotoxin (lipopolysaccharide, 200 ng/kg) and blood was collected T0 prior challenge and 2h(T2), 4h(T4) post-challenge to assess the variation in serum circulatory biomarkers including cortisol, cytokines /chemokines and miRNAs levels. Cortisol levels significantly increased and differed among animals post endotoxin challenge. Based on peak (T4) cortisol levels animals were categorized (n = 30/group) as high (HSR, >1050 pg/mLL) and low (LSR, <250 pg/mL) stress responders. The HSRs showed a gradual increase in cortisol from T2 to T4, whereas in LSRs cortisol returned to basal levels from T2 to T4. Immune proteins were measured in a subset of animals (n = 8/group) using a panel of 10 cytokines/chemokines. Among these, cytokines (TNF-à, IL-10) and chemokines (CCL2, CCL3, CXCL10) were more significantly ($P < 0.05$) induced in HSR than LSR animals. A high and positive Pearson correlation (>0.8) was observed between cortisol and cytokines levels in the HSRs. Further, a panel of 384 bovine specific miRNAs was employed to determine the changes in circulatory miRNAs between HSR and LSR animals (n = 4/group). Six miRNAs were significantly ($P < 0.05$) differentially expressed between HSR and LSR groups with a fold change (FC) >2 or <2; miR-101 (FC >2.08), miR-484 (FC >3.21), miR-339a (FC >3.08) and miR-494 (FC >10.51) were upregulated, whereas miR-34a (−2.2) and miR-541 (−2.07) were downregulated. The variations in the induced immune response (cytokines, miRNAs) between the HSR and LSR groups indicate differences in individual abilities to cope with microbial stressors. Collectively, these results suggested the suitability of cortisol levels as a stress resilience trait and the assessed circulatory biomarkers also implicate differences in their immune regulation.

Key Words: immune response, cortisol, cytokines, miRNAs, stress resilience

P245 Differential expression of cow immune response genes in blood in response to phytochemicals. B. Mulakala[2], M. Worku*[1], and H. Ismail[1], [1]*North Carolina A&T State University, Greensboro, NC, USA,* [2]*University of Vermont, Vermont, VT, USA.*

The concept of immunomodulation has been gaining significance worldwide because of the indispensable role in maintaining a disease-free state. The immunomodulatory characteristics of plant-phytochemicals have gathered the attention of researchers; in fact including the phytochemicals in diets suggested many health benefits for a farm animal. However, excessive research on the immunomodulatory effects of phytochemicals may aid in developing novel immunomodulatory agents. The objective of this study was to evaluate the effect of phytochemicals on cow innate and adaptive responses. Blood was collected from (n = 3) clinically healthy Holstein-Friesian cows from the North Carolina A&T State University Dairy Unit. One milliliter of whole blood from 3 cows was treated individually with locust bean gum (Sigma-Aldrich St. Louis, MO), Modified Citrus Pectin (MCP) (PectaSol-C) or maintained in PBS, incubated at 37°C for 30 min. Total RNA was extracted, reverse transcribed, and real-time PCR was carried out using RT2 Profiler Human Innate and Adaptive Immune Responses PCR Array containing 84 genes, as recommended by the manufacturer (Qiagen). Livak method was used to calculate transcript abundance and fold change (FC > 2 considered significant). Exposure to locust gum or MCP expressed only 40 (4 upregulated, 17 downregulated), 33 (25 upregulated, 2 downregulated) genes, respectively. The highest upregulated and downregulated genes to the exposure locust gum or MCP was (MX1 and RAG1), (TNF and MAPK8) respectively. The significance of the phytochemicals identified targets needs further evaluation for cow health and homeostasis.

Key Words: phytochemicals, cow blood, innate and adaptive immune response genes

P246 Identification of loci associated with susceptibility to paratuberculosis in Holstein cattle using combinations of diagnostic tests and imputed whole-genome sequence data. M. Canive*[1], G. Badia-Bringué[1], O. González-Recio[2,3], A. Fernandez[2], P. Vázquez[1], J. Garrido[1], R. Juste[1], and M. Alonso-Hearn[1], [1]*Department of Animal Health, NEIKER-Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain,* [2]*Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, CSIC, Madrid, Spain,* [3]*Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.*

Bovine paratuberculosis (PTB) is a chronic inflammatory disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Genomic selection could enhance natural resistance to MAP infection and complement existing control strategies. The main objective of this study was to identify quantitative trait loci (QTL) associated with PTB susceptibility in Spanish Holstein cows (n = 983) using combinations of diagnostic tests and imputed whole-genome sequence (WGS) data. The infection status of these animals was defined by 3 diagnostic methods including ELISA for detection of humoral responses against MAP and culture and PCR detection of MAP in gut tissues. The 983 cows included in this study were genotyped with the Bovine MD SNP50 Bead Chip, and the corresponding genotypes were imputed to WGS using the 1,000 Bull genomes reference population. In total, 33.77 million SNP variants per animal were identified across the genome. Linear mixed models were used to calculate the heritability (h^2) estimates for each diagnostic test and test combinations. Next, we performed a case-control genome-wide association study (GWAS) using the imputed WGS data sets and the phenotypes and combinations of phenotypes with h^2 estimates >0.080. After performing the GWAS, the test combinations that showed SNPs with a significant association ($P_{FDR} \leq 0.05$), were the ELISA-tissue PCR-tissue culture, ELISA-tissue culture, and ELISA-tissue PCR. A total of 12 quantitative trait loci (QTLs) highly associated with MAP infection status were identified

on the *Bos taurus* autosomes (BTA) 4, BTA5, BTA11, BTA12, BTA14, BTA23, BTA24, and BTA28, and some of these QTLs were linked to immune-modulating genes. The identified QTLs on BTA23 spanning from 18.81 to 22.95 Mb of the *Bos taurus* genome overlapped with several QTLs previously found to be associated with PTB, bovine tuberculosis, and mastitis infection. In summary, combining phenotypes and WGS improved the power for detecting genetic associations in Spanish Holstein cattle. The results from this study provide more clues regarding the molecular mechanisms underlying susceptibility to PTB and might be used to develop national genetic evaluations for PTB in Spain

Key Words: cattle, genome-wide association, diagnostics, imputation, animal health

P247 Systemic transcriptomic response of sheep and cattle to acute and chronic *Fasciola hepatica* infection. D. A. Niedziela*[1], A. Naranjo-Lucena[1], V. Molina-Hernández[2], J. A. Browne[3], Á. Martínez-Moreno[4], J. Pérez[2], D. E. MacHugh[3,5], and G. Mulcahy[1,5], [1]*UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland,* [2]*Department of Anatomy and Comparative Pathology and Toxicology, Faculty of Veterinary Medicine, University of Córdoba, Córdoba, Spain,* [3]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, Dublin, Ireland,* [4]*Parasitology section, Department of Animal Health, Faculty of Veterinary Medicine, University of Córdoba, Córdoba, Spain,* [5]*UCD Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland.*

The objective of this study was to investigate the transcriptomic response of ovine peripheral blood mononuclear cells (PBMC) to *Fasciola hepatica* infection, and to elucidate the differences between ovine and bovine PBMC responses. *F. hepatica* is a zoonotic trematode which leads to delayed growth and loss of productivity in cattle, while infection in sheep can have more severe effects, potentially leading to death. Previous transcriptomic analyses revealed upregulation of *TGFB1*, cell death and Toll-like receptor signaling, T-cell activation, and inhibition of nitric oxide production in macrophages in response to the parasite; however, differences between bovine and ovine responses are unexplored. Sixteen male Merino sheep were randomly assigned to infected or control groups (n = 8 per group) and orally infected with 120 *F. hepatica* metacercariae. Transcriptomic data were generated from PBMC at 0, 2, and 16 weeks post-infection (wpi), and analyzed for differentially expressed (DE) genes between infected and control animals at each time point (analysis 1), and for each group relative to time 0 (analysis 2). Analysis 2 was then compared with a similar study performed previously on bovine PBMC. A total of 453 DE genes were found at 2 wpi, and 2 DE genes at 16 wpi (FDR <0.1, analysis 1). Significantly overrepresented biological pathways at 2 wpi included *role of PKR in interferon induction and antiviral response*, *death receptor signaling* and *RIG-I-like receptor signaling*, which suggested that an activation of antiviral response and inhibition of cellular apoptosis was taking place. Comparison of analysis 2 with a bovine transcriptomic study revealed significantly overrepresented pathways in the acute phase in cattle, which were upregulated only in the chronic phase in sheep: *IL-10 signaling*, *Th2 pathway*, and *Th1 and Th2 activation*. The early anti-inflammatory response may be the cause of lack of clinical signs in the acute infection stage in cattle. These findings offer scope for "smart vaccination" strategies for this important livestock parasite.

Key Words: sheep, immunology, RNA-seq, infectious disease, One Health

P248 Alternative splicing modulates the immune response in peripheral blood and gut tissues of Holstein cattle naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*. G. Badia-Bringué*[1], M. Canive[1], J. Lavín[2], R. Casais[3], C. Blanco-Vázquez[3], and M. Alonso-Hearn[1], [1]*Department of Animal Health, NEIKER-Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain,* [2]*Department of Applied Mathematics, NEIKER-Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain,* [3]*SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Center of Animal Biotechnology, Deva, Asturias, Spain.*

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes significant losses to the dairy industry worldwide and has been associated with human diseases. Alternative splicing (AS) is an important mechanism of gene regulation that can influence pre-mRNA stability, structure, function and localization, with important physiological consequences. However, the role of AS in regulating the host response to MAP infection is still unclear. In the current study, differential AS was analyzed using RNA-seq data from peripheral blood (PB) and ileocecal valve (ICV) samples collected from Holstein cattle with undetectable lesions (n = 4) and with focal/multifocal (n = 7) or diffuse (n = 5) PTB-associated lesions in gut tissues. A multivariate analysis of AS events was performed with rMATS 4.1.1, which uses a likelihood-ratio test to calculate if the difference in the mean exon inclusion levels between 2 sample groups exceeds a given threshold (>5%). In PB samples of infected cows, several neutrophil degranulation pathway genes (*CD53*, *CLEC12A*, *CPNE1*, *ITGA2B*, *NCF1*, *SGSH*, *SIRPA*, *SLC11A1*, *TARM1*, *TMC6*) and clathrin-mediated endocytosis (*CLTA*, *DNM1*, *DNM2*, *EPS15L1*, *FCHO1*) showed significant AS perturbations. In ICV samples of infected cows, proteins with RNA-recognition and coiled-coil domains showed differential AS events when compared with control cows. Specifically, in the ICV from animals with focal lesions, several genes related to the innate immune response (*C2*, *C4A*, *CD46*, *CFH*, *CYLD*, *IRF3*, *PYCARD*, *TMEM173*, *TRIM38*) showed differences in splicing when compared with cows without lesions. Changes AS of several genes also correlated with changes in gene expression. In the ICV of animals with diffuse lesions, for instance, several components of the immune response (*BOLA*, *CLEC7A*, *PSBM10*, *IFI30*, *IRF5*, *ARID5A*, *IL7*) showed deregulation both in mRNA expression and AS pattern. Interestingly, AS in *BOLA*, *MHCI-A* and *BOLA-NCI* was found associated with PTB and several autoimmune diseases such as type I diabetes mellitus, autoimmune thyroid disease, Kaposi sarcoma-associated herpesvirus infection, and Epstein-Barr virus infection.

Key Words: cattle, genome regulation, RNA-seq, autoimmunity, animal health

P249 Functional and population genomics of admixed trypanotolerant African cattle breeds. G. P. McHugo*[1], J. A. Ward[1], T. J. Hall[1], G. M. O'Gorman[2], E. W. Hill[1], and D. E. MacHugh[1,3], [1]*UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [2]*National Office of Animal Health Ltd., Enfield, UK,* [3]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Bos primigenius taurus (taurine) and *Bos primigenius indicus* (zebu) cattle diverged at least 500,000 years ago and, since that time, significant genomic differences have accumulated between them. Subsequent admixture in Africa has resulted in a complex mosaic of taurine-zebu ancestry, with most cattle breeds containing varying levels of admixture. Some African taurine populations have an important evolutionary adaptation known as trypanotolerance, a genetically determined tolerance to infection by trypanosome parasites (*Trypanosoma* spp.). These are transmitted by infected tsetse flies (*Glossina* spp.) and cause African animal trypanosomiasis (AAT) disease. ATT is one of the largest constraints to livestock production in sub-Saharan Africa and causes a financial burden of approximately $4.5 billion annually. Some West African taurine breeds, such as the N'Dama, are trypanotolerant; these cattle have an ability to control trypanosome parasite loads and to limit disease pathology compared with trypanosusceptible zebu breeds. However, zebu animals are generally larger, produce higher milk yields and are therefore favored by many producers. We have examined transcriptomics data generated from trypanosome infection studies with trypanotolerant and trypanosusceptible breeds to identify differentially expressed genes. Outputs from this experiment were then integrated with sub-chromosomal admixture results from local ancestry analysis of genome-wide high-density SNP data to ex-

plore the functional biology of differentially introgressed genomic regions in admixed African cattle breeds.

Key Words: cattle and related species, functional genomics, integrative genomics, admixture, animal health

P250 Integrative and comparative genomic analyses of mammalian macrophage responses to intracellular mycobacterial pathogens. T. J. Hall*[1], G. P. McHugo[1], M. P. Mullen[2], J. A. Ward[1], K. E. Killick[1,7], S. C. Ring[3], D. P. Berry[4], J. A. Browne[1], S. V. Gordon[5,6], and D. E. MacHugh[1,6], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, Belfield, Dublin, Ireland,* [2]*Bioscience Research Institute, Athlone Institute of Technology, Athlone, Westmeath, Ireland,* [3]*Irish Cattle Breeding Federation, Bandon, Cork, Ireland,* [4]*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Cork, Ireland,* [5]*UCD School of Veterinary Medicine, Belfield, Dublin, Ireland,* [6]*UCD Conway Institute of Biomolecular and Biomedical Research, Belfield, Dublin, Ireland,* [7]*Genuity Science, Loughlinstown, Dublin, Ireland.*

Mycobacterium tuberculosis, the causative agent of human tuberculosis (hTB), is one of the oldest and most successful pathogens and is currently classed as the thirteenth leading cause of death worldwide. *Mycobacterium bovis*, a very close evolutionary relative of *M. tuberculosis*, causes bovine tuberculosis (bTB) and is one of the most damaging infectious diseases to cattle agriculture, particularly in developing countries. Previous studies have shown that the pathogenesis of bTB disease is comparable to hTB disease, and that the bovine and human alveolar macrophage (bAM and hAM) transcriptomes are extensively reprogrammed in response to infection with these intracellular mycobacterial pathogens. However, although *M. bovis* and *M. tuberculosis* share 99.5% identity at the genome level, the innate immune responses to these pathogens can be characteristically different in the same mammalian host. In this study, a multi-omic integrative approach was applied to encompass functional genomics and GWAS data sets across the 2 primary hosts (*Bos taurus* and *Homo sapiens*) and both pathogens (*M. bovis* and *M. tuberculosis*). Four different experimental infection groups were used, each with parallel non-infected control cells: 1) bAM infected with *M. bovis*, 2) bAM infected with *M. tuberculosis*, 3) hAM infected with *M. tuberculosis*, and 4) human monocyte-derived macrophages (hMDM) infected with *M. tuberculosis*. RNA-seq data from these experiments 24 h post-infection (24 hpi) was analyzed using 3 separate computational pipelines: 1) differentially expressed genes, 2) differential gene expression interaction networks, and 3) combined pathway analysis. The results of these analyses were then integrated with high-resolution bovine and human GWAS data sets to detect novel QTLs for resistance to mycobacterial infection and resilience to disease. Results from this study revealed common and unique response macrophage pathways for both pathogens and identified 32 genes (12 bovine and 20 human) significantly enriched for SNPs associated with disease resistance, the majority of which encode key components

Key Words: integrative genomics, network analysis, functional genomics, cattle and related species, infectious disease

P251 Molecular characterization of the serum amyloid A (SAA) mutation R90S in chicken hepatocellular carcinoma (LMH) cells. C. Falker-Gieske*[1], N. Paul[1], J. Gilthorpe[2], K. Gustmann[1], and J. Tetens[1], [1]*Department of Animal Sciences, Georg-August-University, Göttingen, Germany,* [2]*Department of Integrative Medical Biology, Umeå University, Umeå, Sweden.*

Brown layer chickens are susceptible to amyloid arthropathy (AA), which is a disease caused by bacterial infection that leads to serum amyloid A (SAA) deposition in knee joints of affected animals. White layer chickens are resistant to the disease. Hence, disease susceptibility clearly has a genetic component that has yet to be identified. By analyzing whole-genome sequencing data we discovered a missense variant in the SAA gene (rs739601959) in 41 out of 50 white layer alleles whereas brown layers are non-carriers of the allele. The variant leads to an amino acid (aa) exchange from arginine to serine at position 90 (R90S) in the SAA protein. To characterize the mutation, we created stable chicken hepatocellular carcinoma (LMH) cell lines that overexpress wild-type SAA (SAA-WT) and the R90S isoform (SAA-R90S). Intra- and extracellular SAA protein levels were significantly elevated in SAA-R90S cells (western blot, $P = 0.0042$, $P = 0.0039$), although mRNA levels as determined by qPCR were similar in SAA-WT and SAA-R90S cells ($P = 0.052$). After separation of cell lysate preparations SAA-R90S accumulated in the detergent insoluble phase whereas no SAA-WT protein was detectable in the insoluble phase. This might indicate that SAA-R90S has a lower binding capacity to high-density lipoprotein (HDL), which is known to promote SAA aggregation. To further characterize the impact of the SAA-R90S isoform on LMH cells we compared the transcriptomes of SAA-WT cells and SAA-R90S cells with cells transfected with empty vector plasmid. Preliminary analyses of the results suggest, that overexpression of SAA-WT induces a cell fate change with 21 transcription factors (TFs) differentially expressed (DE) in SAA-WT cells and only 6 TFs DE in SAA-R90S cells ($P < 0.05$, abs. LFC ≥ 1). Protein-protein-interaction and gene set enrichment analyses revealed a loss-of-function phenotype in SAA-R90S cells and shift toward the plasma membrane, the cell periphery, and the extracellular space. Furthermore, our data suggests that glycosylation of aa 90 in the SAA-R90S might affect SAA's function as an apoliporotein, which could explain the resistance of white layer chickens to AA due to the lack of HDL associated SAA during the acute phase response to bacterial infection.

Key Words: serum amyloid A, amyloid arthropathy, disease resistance, acute phase response, cell model

P252 Whole-genome screening for resilience against porcine reproductive and respiratory syndrome virus outbreaks in breeding sows. M. Laplana, R. Ros-Freixedes, J. Estany, L. Fraile, and R. Pena*, *Departament de Ciència Animal, Universitat de Lleida – AGROTECNIO-CERCA Centre, Lleida, Spain.*

The porcine reproductive and respiratory syndrome virus (PRRSV) causes serious health and productivity problems both in growing pigs and sows. It is known that there is a genetic component in the resilience of reproductive traits, such as the abortion rate and the number of liveborn piglets and total lost piglets (mummified and stillborns) at farrowing, of sows facing a PRRSV outbreak. Using data from a farm of 305 Landrace x Large White sows where a PRRSV outbreak occurred, we investigated the stability of reproductive performance (SRP) of the sows as the number of losses during the PRRSV outbreak minus the mean losses per labor in an endemic situation. Sows were classified as "stable" if SRP = < 1 piglet or "sensitive" if SRP > 1 piglet. Extreme animals were considered divergent for resilience to the PRRSV outbreak. With the aim to identify genetic variants at whole-genome level that influence the SRP against PRRSV infection, 48 sows with extreme phenotypes ("stable," n = 22; "sensitive," n = 26) for SRP were sequenced at low coverage (7X). More than 14.5 million polymorphic variants were detected. A GWAS using a genomic relationship matrix to correct for population structure identified 13 genomic regions on 11 chromosomes that contained 44 variants ($P < 10^{-6}$) associated with the "stable" phenotype. A preliminary analysis of the 304 genes located in the ± 1Mb regions of the relevant variants highlighted the relationship of these genes with molecular functions such as binding to RAGE receptors, inflammatory processes, embryonic development, angiogenesis and cell migration or binding to growth factors. This result suggests the existence of SRP-related genetic variability. These findings will be tested in the entire population and in a population with different genetic background. The identification of genetic markers can be useful to identify resilient sows that can successfully cope with a PRRSV outbreak without compromising their health status or productivity.

Key Words: resilience, infection, sow, robustness, abortion

P253 The natural cytotoxicity receptor (NCR) genes in the family *Felidae*. J. Bubenikova[1,2], J. Futas[1,2], J. Oppelt[2], M. Plasil[2], R. Vodicka[3], and P. Horin*[1,2], [1]*Department of Animal Genetics, University of Veterinary Sciences, Brno, Czech Republic,* [2]*Ceitec VETUNI, University of Veterinary Sciences, Brno, Czech Republic,* [3]*Zoo Prague, Prague, Czech Republic.*

Natural killer cells (NKC) play important roles in immune responses. Various NKC receptors (NKR) mediate multiple NKC activities. No single conservative model of NKR genes has been observed in mammals. As NKC in cats seem to be different from those of other mammals, analyses of NKR of the Felidae may bring new information on NKR evolution in mammals. Natural cytotoxicity receptors (NCR) represent a group of activating receptors encoded by genes *NCR1*, *NCR2*, and *NCR3* expressed on immune cells. Their presence or functional characteristics may differ even in related species. The objective of this work was to characterize *NCR* genes in felids. Thirty-eight individuals of 15 felid species were analyzed. Based on bioinformatic analyses of the most recent genome assemblies and next-generation sequencing, potentially functional *NCR1*, *NCR2* and *NCR3* genes were found in all species analyzed. Similarities with the human *NCR* genes were 78%, 77% and 86%, respectively. Out of currently annotated mammalian *NCR* sequences, the most related were genes of families *Hyaenidae* and *Herpestidae*. Based on coding sequences (CDS), protein variants were inferred. Within the family, most interspecific differences in CDS were found in *NCR1* (25, 1–4 per species), followed by *NCR2* (23, 1–2 per species) and *NCR3* (16, 1–2 per species). As for protein variants, 22 different NCR1 amino acid sequences (1–3 per species) were found, 21 (1–2 per species) for NCR2 and 12 (1–2 per species) for NCR3. Some protein variants were shared among species. For *NCR1* and *NCR2*, their phylogenetic trees based on CDS reflected the current zoological taxonomy of felids. This finding was supported by selection analyses detecting neutral selection in both genes. Low variability of the *NCR3* CDS did not allow a robust phylogeny reconstruction. Selection analysis detected purifying selection for *NCR3*. *NCR1* showed most interspecific differences. A high level of within-species variation of *NCR1* was also found in a group of 221 domestic cats. As *NCR1* was associated with feline coronavirus shedding and considering differences in innate immunity observed among felid species, our data indicate a potential role of *NCR1* in the interspecific variability.

Key Words: cats and related species, immunogenomics, DNA sequencing, innate immunity, evolution, selection

P254 *DEL-1* gene is associated with increased weaning fecal egg counts in Katahdin sheep. G. Becker*[1], J. Burke[2], R. Lewis[3], J. Miller[4], J. Morgan[5], D. Notter[6], and B. Murdoch[1], [1]*Department of Animal, Veterinary and Food Sciences, University of Idaho, Moscow, ID, USA,* [2]*USDA, ARS, Dale Bumpers Small Farms Research Center, Booneville, AR, USA,* [3]*Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE, USA,* [4]*Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA,* [5]*Round Mountain Consulting, Fayetteville, AR, USA,* [6]*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA, USA.*

Gastrointestinal nematodes (GIN) pose a severe threat to sheep industries worldwide. Parasitic nematodes are abundant and widely dispersed in the environment, and their capacity to rapidly evolve has made common classes of anthelmintic drugs ineffective. Severity of GIN infection can range from subclinical or low pathogenicity to lethal anemia and/or diarrhea. With limited treatment options due to anthelmintic resistance, gaining a better understanding of the genetic mechanisms responsible for the host animals' response to GIN infection is critical in the development of strategies to mitigate disease severity. A genome-wide association study (GWAS) was conducted with cube root transformed fecal egg count data collected at weaning (69 +/ 11 d of age). A total of 600 DNA samples were genotyped on the high-density Illumina 600 K SNP BeadChip; 583 samples and 505,914 single nucleotide polymorphisms (SNPs) were carried through to analyses. The GWAS was conducted using Efficient Mixed-Model Association eXpedited (EMMAX) with a recessive inheritance model, with flock, birth litter size, and birth month as fixed effects. The genome-wide significance threshold was determined by permutation testing and defined as P-values <1.0e-06. Four SNPs located on chromosome 5 were significantly associated with the gene EGF-like repeats and discoidin domains 3 (*EDIL3*), also known as *DEL-1*. This gene expresses a cognate protein that influences leukocyte-endothelial adhesion and inflammation in humans and mice, identifying this gene as a potential candidate for similar roles in Katahdin sheep. This study lays the foundation for further research of *DEL-1* toward understanding genetic mechanisms of susceptibility, and suggests these SNPs can contribute to genetic strategies for improving parasite resistance in sheep.

Key Words: sheep and related species, genome-wide association, genotyping, candidate gene, genomic selection

P255 Genome-wide association study of thyroid hormone suppression following challenge with porcine reproductive and respiratory syndrome virus. A. Van Goor[1], A. Pasternak[2], M. Walugembe[3], N. Chehab[1], G. Hamonic[4], J. Dekkers[3], J. Harding*[4], and J. Lunney[1], [1]*USDA ARS BARC Animal Parasitic Diseases Laboratory, Beltsville, MD, USA,* [2]*Department of Animal Science, Purdue Univ., West Lafayette, IN, USA,* [3]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [4]*Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada.*

Porcine reproductive and respiratory syndrome virus (PRRSV2) causes respiratory disease in piglets and reproductive disease in sows. Piglet and fetal serum thyroid hormone (i.e., T3 and T4) levels decrease rapidly in response to PRRSV infection. However, the genetic control of T3/T4 homeostasis during infection is not completely understood. Our objective was to estimate genetic parameters and identify QTL for T3 and/or T4 levels of piglets and fetuses challenged with PRRSV2. Sera from 5-week old pigs (n = 1,792) at 11 d post inoculation (dpi) with PRRSV2 were assayed for T3 levels (piglet_T3). Sera from fetuses (n = 1,267) at 12 or 21 dpi from sows (n = 150) challenged with PRRSV2 in late gestation were assayed for T3 (fetal_T3) and T4 (fetal_T4) levels. Animals were genotyped using 60K or 650K SNPs panels. Heritabilities and pheno/genotypic correlations were estimated in ASREML, and genome-wide association studies were performed for each trait separately using JWAS. All 3 traits were low to moderately heritable (10–18%). Phenotypic and genotypic correlations of piglet_T3 levels to weight gain (0–42 dpi) were 0.26 ± 0.11 and 0.66 ± 0.13, respectively. Significant QTLs (n = 9) were identified for piglet_T3 on SSC3, 4, 5, 6, 7, 14, 15, and 17, collectively explaining 30% of the genetic variation (GV), with the largest identified on SSC5 explaining 15% of the GV alone. Significant QTLs (n = 3) were identified for fetal_T3 on SSC1 and SSC4, and collectively explained 10% of the GV. Significant QTLs (n = 5) were identified for fetal_T4 on SSC1, 6, 10, 13, and 15, and collectively explained 14% of the GV. Overlapping QTL for ≥2 traits were identified on SSC4, SSC6, and SSC15. Overall, thyroid hormone levels in PRRSV2 challenged animals were low to moderately heritable; a high genetic correlation was uncovered for piglet_T3 levels to weight gain; relatively few markers explained a large amount of the GV; and several pleiotropic QTL were identified. Collectively, our results suggest thyroid hormone levels maybe a promising biomarker for genetic improvement of resilience during PRRSV challenge.

Key Words: pigs and related species, genome-wide association, biomarker, disease resilience, animal health

P256 Withdrawn

P257 Integrated network based on mRNAs and long noncoding RNAs of porcine reproductive and respiratory syndrome virus-infected multiple tissues revealed the early host responses. B. Lim* and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of*

Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Republic of Korea.

Porcine reproductive and respiratory syndrome (PRRS), by PRRS virus (PRRSV) infection, is known to occur negative effects on piglet mortality and productivity in the porcine industry worldwide. To date, candidate genes and loci for the host's PRRSV resistance were identified by GWAS and RNA-seq. However, the functions of lncRNAs and interactions with protein-coding genes under PRRSV infection have still not been well understood. Therefore, we identified novel lncRNAs in 3 PRRSV-infected tissues (lungs, bronchial lymph nodes, and tonsil) data and investigated interactions among mRNAs and lncRNAs. Thirty-four pigs were intramuscularly challenged with JA142 strain and killed at 3, 10, 21, 28, and 35 d post infection (dpi). The serum samples were used for the measurement of viremia and antibodies. Differentially expressed mRNAs and lncRNAs were identified in each dpi based on 0 dpi, and co-expression networks were constructed. Networks were reconstructed to eliminate the vast majority of indirect interactions typically inferred by pairwise analysis. We focused on the network that strongly related to phenotypes, consisting 144 mRNAs and 15 lncRNAs. The network showed a 3 dpi-specific expression pattern, and functional enrichment based on mRNA-lncRNA interactions indicated viral host responses. We identified that lncRNAs are interacted with the main mRNAs in the early host responses. Further studies to validate lncRNAs and their functions are needed.

Key Words: immunogenomics, RNA-seq, noncoding RNA, network analysis, innate immunity

P258 Ovine mastitis: Does early-life nutrition influence immunity response in later life? C. Hervás-Rivero, R. Pelayo, B. Gutiérrez-Gil, C. Esteban-Blanco, H. Marina, J. Arranz, and A. Suárez-Vega*, *Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, León, Castilla y León, Spain.*

Nutritional status in early life is a critical determinant of immunity status. Specifically, these differences in immune response or immune system vulnerability can be more accused under stress situations, such as lactation. This study aimed to evaluate mammary gland transcriptomic changes after an intramammary lipopolysaccharide (LPS) infusion in lactating sheep subjected to a nutritional challenge at the prepuberal stage (3–5 mo of age). Briefly, 40 Assaf ewe-lambs born in the same period and the same flock were divided into 2 groups, 20 animals received a regular growth diet (control group), and the 20 others were fed with a low protein diet (challenge group; NC). The NC period lasted 2 mo and was performed during the allometric growth period of the mammary gland. At the last stage of their first lactation, all ewes, control, and NC were subjected to an intramammary LPS challenge. At the peak of temperature and somatic cell counts (+6 h post LPS inoculation), 19 milk samples (12 NC and 7 control) were collected to perform RNA-seq. A bioinformatic pipeline was applied to determine changes in expression between NC and control ewes. We identified 548 differentially expressed genes. Among the biological processes found to be affected by the nutritional challenge during the inflammation peak period, we identified processes such as *ribosome biogenesis*, *neutrophil-mediated immunity*, and *granulocyte activation*. Some immunity-related markers were found downregulated in the NC ewes; e.g., *cyclooxygenase 1* whose gene expression differences were associated with host immunity status in mice. In conclusion, this preliminary study suggests that, in sheep, a nutritional restriction at the prepuberal age can influence gene expression and biological pathways patterns triggered in response to an intramammary LPS infusion in later life.

Key Words: sheep and related species, RNA-seq, immune system, milk production, nutrigenomics

P259 Association of variants in innate immune genes *TLR4* and *TLR5* with reproductive and milk utility traits in Czech Simmental cattle. K. Novák*[1], K. Samaké[2], T. Valcíková[3], and M. Bjelka[4], [1]*Institute of Animal Science, Prague-Uhríneves, Czech Republic,* [2]*Charles University, Prague, Czech Republic,* [3]*Czech University of Life Sciences, Prague, Czech Republic,* [4]*Breeding Company CHD Impuls, Bohdalec, Czech Republic.*

The bovine genes *TLR4* and *TLR5*, which encode antibacterial toll-like receptors of the innate immune system, were screened for polymorphisms in Czech Red Pied (Czech Simmental) cattle to identify variants associated with reproduction, udder health and milk production traits, namely, milk fat percentage, fat yield, protein percentage and protein yield, total milk yield, somatic cell score, udder health index, milkability, lactation persistency, incidence of cystic ovaries, early reproductive disorders, calving ease, maternal calving ease, production longevity, and calf vitality index. Gene variants were discovered by hybrid resequencing using HiSeq X-Ten and PacBio technologies and then individually genotyped. Associations between 7 polymorphisms of each gene with phenotypic traits were found in 18 combinations using one-way ANOVA with subsequent Benjamini-Hochberg tests. Both the *TLR4* variants rs8193060 and rs8193072 (9422T>C and 10310T>G in reference AC000135.1, respectively) and the *TLR5* variants ss73689429, rs55617187 and rs55617288 (545T>C, 3714C>T and 4626T>C in EU006635, respectively) were associated with increased incidence of cystic ovaries and a general index of early reproductive disorders. Variants 610C>T (rs43578094) with 9422T>C of *TLR4* and 1736C>T (ss73689443) of *TLR5* were associated with calving ease. In addition, 9422T>C and 7999A>G (rs43578100) in *TLR4* were associated with production longevity. The association of 610C>T with calf vitality index might reflect calfhood infections. By contrast to *TLR4*, 488C>G, 1736C>T and 3891C>T in *TLR5* were associated with milk production traits. The discrepancy in the predicted impacts on protein function points at the role of haplotypes. Positional matches with known QTLs for calving ease, namely, #43837 on chromosome 8 and #48258 on chromosome 16, endorse the causative roles of *TLR4* and *TLR5*, respectively. The *TLR* polymorphism effect on female reproduction traits can also be mediated by non-immune functions of TLRs in myometrial signaling, consistently with the known role of TLRs in model species.

Key Words: cattle, genotyping, innate immunity, animal health, calving

P260 Bimodal haplotype distribution in bovine antibacterial toll-like receptors. K. Samaké*[1] and K. Novák[2], [1]*Charles University, Prague, Czech Republic,* [2]*Institute of Animal Science, Prague-Uhrineves, Czech Republic.*

The *TLR* genes coding for Toll-like receptors of antibacterial series, namely *TLR1*, *-2*, *-4*, *-5* and *-6*, were resequenced in Czech Simmental (Czech Red) cattle using HiSeq and PacBio technologies. Hybrid sequencing allowed to determine SNPs for individual genotyping with primer extension method. Haplotypes were established within the range of the designed PacBio amplicons, especially for *TLR2*. A more general statistical reconstruction of haplotypes from individual genotyping was carried out in parallel. The directly determined haplotypes from PacBio reads demonstrated randomly distributed frequencies of haplotypes in the amplicon 2 – 5 of *TLR2*, however, 15 haplotypes in amplicon 1 in the proximal part of the transcript formed 2 distinct groups. The results were consistent with the distribution of haplotypes obtained by statistical reconstruction in *TLR2*. Similarly, the bimodal distribution was detectable in *TLR5* and other *TLR*s. In all cases, the trend for bimodal distribution was expressed stronger in proximal regions of the transcripts. The clustering of *TLR* haplotypes has been reported earlier in a panel of world breeds (Fisher et al., Plos One 6:e27744, 2011; Bilgen et al., Diversity 8:23, 2016), however, the origin of this disequilibrium is still not documented. Alternating infectious agents are feasible (Bilgen et al., 2016) along with 2 different essential functions performed by a *TLR* gene or its product. An example of a dual function might be formation of 2 kinds of products differing in specificity. The association of the groups of haplotypes with the transcript proximal region suggests the selection target in the 5′- regulatory regions of the *TLR* genes, although functional interactions in the proximal part of the transcript cannot be excluded. The confirmation of the haplotype

reconstruction data by targeted genotyping and new resequencing technologies is in progress in the set of bulls studied.

Key Words: cattle, innate immunity, toll-like receptors, haplotype diversity

P261 Breed-associated risk for developing clinical leishmaniasis in dogs: Preliminary results. C. Sanz*, J. Sarquis, J. Martínez, and G. Miró, *Animal Health Department, Veterinary Faculty, Complutense University of Madrid, Madrid, Spain.*

Canine leishmaniosis (CanL) is caused by obligate intracellular parasites of the genus *Leishmania* and transmitted by blood-sucking phlebotomine sand flies. The outcome of the infection results from multiple host and parasite factors, and their interactions. In this sense, dog breed may be an important risk factor influencing susceptibility to CanL, although genetic research and knowledge gaps remain on this theme. The aim of this work was to investigate the effect of breed as a risk factor associated with the development of clinical CanL. We performed a retrospective study of dogs that were presented with clinical CanL (n = 365) to the Consultant of Infectious Diseases at the Complutense Veterinary Teaching Hospital (CVTH) between 2015 and 2019. Breed population percentages were compared with those of patients attended to CVTH during the same period of time for reasons other than CanL (n = 19,783). The odd ratio (OR) and the statistical significance were calculated for those breeds with more than 25 individuals and at least 2 cases of CanL, using mixed-breed dogs as control group. In addition, we evaluated the potential association between breeds with high risk to develop clinical CanL and their habitat (outdoor vs indoor), and the clinical stage they displayed, ranging from mild disease (stage I) to very severe disease (stage IV), based on LeishVet guidelines. These analyses resulted in the identification of 19 breeds at high risk (OR > 1; $P < 0.05$) for developing clinical CanL, with a predominance of molosser breeds, such as Boxer, Great Dane, Spanish Mastiff, Rottweiler and Pitbull Terrier. Although we did not find statistically significant differences regarding the clinical stage, we observed that Pitbull Terrier dogs tended to develop more severe forms of the disease (clinical stages III-IV). Spanish Mastiff was the only breed that showed a significant association ($P < 0.05$) with an outdoors habitat. This fact may indicate that Spanish Mastiff dogs could be more predisposed to acquire the infection as they are more exposed to the vector. In contrast, our results suggest that Yorkshire Terrier dogs are at low risk for developing clinical CanL (OR < 1; $P < 0.05$), probably because they tend to live indoors and are less exposed to the vector. We can thus conclude that breed may play a relevant role in the epidemiology of CanL and further research is needed to fully understand its influence on the pathophysiology of the disease.

Key Words: *Leishmania infantum*, infection, breed, susceptibility

P262 Identification of and validation of loci associated with facial eczema tolerance in New Zealand sheep. K. M. McRae*, S. J. Rowe, P. L. Johnson, and S. M. Clarke, *AgResearch Limited, Mosgiel, New Zealand.*

Facial eczema (FE) is a metabolic disease of great importance in ruminants in New Zealand. Ingestion of the mycotoxin sporidesmin leads to liver and bile duct damage, which can result in photosensitization and reduced production. In sheep, there is considerable genetic variation in tolerance to facial eczema, and a commercial testing program is available for ram breeders who aim to increase tolerance. The objective of this study was to utilize a data set of these phenotyped animals with high- and low-density genotypes to interrogate the sheep genome for regions associated with variability in tolerance to FE in New Zealand sheep. Two quantitative trait loci (QTL) on chromosomes 15 and 24 are reported, which explain 5% and 2% of the phenotypic variance in the response to FE, respectively. The QTL on chromosome 24 contains the β-globin locus, and mass spectrometry of hemoglobin from animals with differing genotypes at this locus indicated that the QTL is associated with different forms of adult β-globin. Haplotype A animals appeared to be more tolerant to FE, however, the overall frequency of the haplotype in genotyped animals was 0.5, indicating that the locus may be under balancing selection in the New Zealand sheep population. Hemoglobin haplotypes have previously been associated with variation in several health-related traits in sheep, and therefore warrant further investigation regards their role in tolerance to FE in sheep. This study highlights the power of using increased density genotyping for the identification of influential genomic regions, combined with subsequent inclusion on lower density genotyping platforms

Key Words: sheep, disease, facial eczema, hemoglobin, GWAS

P263 Transcriptomic analysis of host resistance to tick infestation with *Rhipicephalus microplus* in leukocytes of Brangus cattle. E. Mantilla Valdivieso*[1], E. Ross[1], A. Raza[1], B. Hayes[1], N. Jonsson[2], P. James[1], and A. Tabor[1], [1]*Queensland Alliance for Agriculture and Food Innovation, Queensland Alliance for Agriculture and Food Innovation, Brisbane, Queensland, Australia,* [2]*Institute of Biodiversity Animal Health and Comparative Medicine, Institute of Biodiversity Animal Health and Comparative Medicine, Glasgow, UK.*

Rhipicephalus microplus, also known as the cattle tick, is a blood-feeding ectoparasite that negatively impacts animal health and cattle production in tropical and subtropical regions worldwide. Control strategies to limit tick infestations include breeding cattle for tick resistance by maintaining a high *Bos indicus* genetic content in breeds. However, selection for tick resistance is still difficult due to reliance on phenotypic assessment by tick scoring methods which are not widely standardized. Therefore, development of predictive biomarkers of host resistance to ticks is a promising approach to increase accuracy of selection and accelerate the genetic gain in cattle. Previous literature suggests that host immunity is relevant in the expression of host resistance, however, the differences in the transcriptomic profiles of leukocytes of tick-resistant and tick-susceptible cattle have not yet been studied. In this study, 30 tick-naïve Brangus steers were exposed to a 12-week artificial tick infestation trial to differentiate the most resistant and most susceptible individuals. The number of developing adult ticks after an infestation cycle (21 d) was estimated with a tick scoring scale from 1 (<50 ticks = Highly Resistant) to 5 (>300 ticks = Highly Susceptible). Animals were subsequently classified as being the most resistant (n = 5) and the least resistant (n = 5) based on the mean tick score. Leukocyte-derived RNA was isolated from blood samples collected immediately before the first infestation (T0) and then at 3 weeks (T3) and 12 weeks (T12) post-initial infestation. RNA-seq was used to identify differentially expressed genes between tick-susceptible and tick-resistant cattle at each of the 3 observed time points. We identified 59, 11, and 32 significant differentially expressed genes (false discovery rate <0.05) between resistant and susceptible animals at time points T0, T3, and T12, respectively. These findings indicate a differential response between the cattle of divergent host resistance before and after repeated tick infestation which shows potential for elucidation of biomarkers of tick resistance that could be examined independently of the levels of tick exposure.

Key Words: RNA-seq, biomarkers, animal health, cattle and related species

P264 Study on genetic distance according to Mongolian breeds through microsatellite markers analysis. J. An*[1], T. Khaliunaa[1], O. Baatartsogt[2], J. Yun[1], G. H. Lee[3], Y. H. Lee[1], J. Seong[4], and H. S. Kong[4], [1]*Major in Applied Biotechnology, The Graduate School of Hankyong National University, Anseong, Korea,* [2]*Department of Biotechnology, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia,* [3]*Department of Animal Life and Environment Science, The Graduate School of Hankyong National University, Anseong, Korea,* [4]*Gyeonggi Regional Research Center, Hankyong National University, Anseong, Korea.*

The Mongolian horse is one of the most ancient breeds in the world. The 3 Mongolian domestic breeds (Undurshil horse, Gobi-shankh horse and Darkhad horse) are currently recognized in Mongolia as morphologically distinct. However, the genetic distance of these breeds has not been investigated using microsatellite (MS) markers. In this study, the genetic distance of Mongolian breeds using MS markers and 173 Mongolian horses belong to 3 different breeds (Darkhad horse, Undurshil horse, Gobi-shankh horse). The number of alleles of each marker ranged from 6 (HTG4, HTG7) to 22 (ASB2). In the case of H_{Exp}, the minimum value was 0.56 (HTG4) and the maximum value was 0.877 (ASB17) and in the case of H_{Obs}, the minimum value was 0.527 (HTG4) and the maximum value was 0.85 (VHL20). In the case of the PIC value, the minimum value was 0.517 (HTG4) and the maximum value was 0.855 (ASB17). As a result of the genetic distance analysis for each breed, the genetic distance between DKH and SHL was the closest at 0.1156, and the genetic distance between DKH and GCH was the farthest at 0.2703. As a result of analyzing principal coordinates analysis (PCoA) using GeneALEx, 3 breeds of DKH, SHL, and GCH were clearly distinguished, and analyzing factorial correspondence analysis (FCA) using Genetix, 3 breeds were also clearly separated. Finally, these results considered that these MS markers will be used to aid the conservation, traceability, and future improved ability to of horse population in Mongolia.

Key Words: Mongolian horse, microsatellite markers, genetic distance, principal coordinates analysis (PCoA), factorial correspondence analysis (FCA)

P265 Rare and common variant discovery by whole-genome sequencing of 101 Thoroughbred racehorses. T. Tozaki*, A. Ohnuma, M. Kikuchi, T. Ishige, H. Kakoi, K.-I. Hirora, and S.-I. Nagata, *Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan.*

The Thoroughbred is formed by crossing Arab breeds and British native horses and is currently used in horseracing worldwide. In this study, we constructed a genome-wide variant database from 101 unrelated Thoroughbred horses born in Japan, or born in the USA, the UK, Ireland, or France and then imported to Japan. whole-genome sequencing (WGS) data were obtained using Illumina paired-end (150 bp) sequencing technology with 36.8-fold coverage on average (range 29.5–54.2). WGS of 101 Thoroughbred racehorses revealed 11,570,312 and 692,756 SNVs from autosomal (1–31) and X chromosomes, respectively, in a total of 12,263,068 SNVs, and that around 11% of these are rare variants. Individual horses had a maximum of 25,554 rare variants; several of these were functional variants, such as nonsynonymous substitutions, start-gained, start-lost, stop-gained, and stop-lost variants, suggesting that rare functional variants affecting protein functions reflect individual phenotypes. In humans, rare variants are known to play a key role in many complex diseases, and rare variants in horses may also play a key role in Thoroughbred disease and/or racing performance, which could explain the missing heritability. The Equine Genetics and Thoroughbred Parentage Testing Standardization Committee of ISAG is investigating a move from STRs to SNVs as markers for determining parentage in Thoroughbreds. In the present study, we identified many SNVs as common variants and found duplicated regions in the horse genome and multiple mapped regions of sequence reads. Therefore, candidate SNVs for parentage verification should be selected from the common variants and exclude SNVs detected at these duplicated regions. The SNV database obtained in the present study can be used to confirm this. Currently, the generation and use of genetically modified racehorses is banned by the ISBC and the IFHA in horseracing. In the present study, we targeted only Thoroughbreds and determined the extent of Thoroughbred genomic diversity among the population of racehorses. Our findings will be useful as baseline information for gene-doping tests that use whole-genome and targeted resequencing.

Key Words: horse, whole-genome sequencing, Thoroughbred, rare variant, gene doping

P266 Improving the horse Y chromosome reference one step at a time. C. Castañeda*, B. Davis, A. Hillhouse, M. Jevit, and T. Raudsepp, *Texas A&M University, College Station, TX, USA.*

In 2018, we produced a 9.5-Mb annotated reference for the male-specific region of the horse Y (eMSY). Among the 52 characterized eMSY genes, 15 have multiple copies and are potentially prone for copy number variation (CNV). Despite a high quality, the eMSY reference contains structurally complex areas (ampliconic and palindromic sequences), which assembly requires improvement through cutting-edge long-read sequencing-based approaches. A high-quality eMSY reference allows to accurately pin-point and study male fertility genes and their natural variations (SNPs, CNVs) in male horse populations. Here we focus on improving the multicopy portion of eMSY (mcY) by (1) resequencing all BAC clones corresponding to mcY by Nanopore technology, and (2) determining the absolute copy number (CN) of 7 multicopy genes in 47 Thoroughbred males using digital-droplet PCR (ddPCR). Thoroughbreds were chosen to minimize breed CN variation since the eMSY reference is also based on a Thoroughbred (*Bravo*). High molecular weight DNA was isolated from 50 BACs (47 in mcY and 3 flanking mcY) using Roche HighPure Plasmid kit. Each BAC was sequenced to over 100X coverage using the MinIon Flongle. Raw reads were cleaned from *E. coli* and vector contaminates with BBDuk before de novo assembly of individual BACs with Canu. The assembly was aided with CN data for mcY genes. Forty-four BACs were successfully assembled with an average de novo assembly size of 170,134bp per BAC. Assemblies of 6 BACs remained highly fragmented and these clones were resequenced. The assembly of mcY with multiple alignment tools such as MAUVE, lamassemble and MAFFT is ongoing, and supported by pairwise fluorescence in situ hybridization of mcY BACs and CN analysis. The latter indicates that the current mcY region is over-assembled because all CN estimates, including those of the reference Thoroughbred *Bravo*, are lower than in the assembly. However, *SRY* (CN = 1) and *RBMY* (CN = 2) show the expected CN in Thoroughbreds and validate the accuracy of ddPCR to determine CN. The improved eMSY assembly is expected to increase the utility of horse reference genome for the study of equine biology and traits of interest.

Key Words: horse, Y chromosome, copy number, Nanopore MinIon

P267 Identification of putative lethal variants using whole-genome sequence data from various horse breeds. P. Reich*, C. Falker-Gieske, and J. Tetens, *Department of Animal Sciences, Georg-August-University Göttingen, Göttingen, Germany.*

Recessive mutations that are lethal in an early stage of embryonic development can be present in a population at rather high frequencies but never occur in the homozygous state in live animals. Unlike in other approaches to identify causal variants for genetic defects, their detection does not require any phenotypic data from affected animals but only genomic data. The purpose of this study was to use existing genomic data of a variety of horse breeds to characterize the genomic variation present in

different horse populations and to identify putative deleterious mutations. Publicly available whole-genome sequence data from 317 horses of 38 breeds was mapped to the horse reference genome EquCab3.0. A total of 24,540,424 single nucleotide polymorphisms (SNPs), 2,248,828 insertions and 2,776,484 deletions could be identified. On average, one variant was called every 81 bases and 17,323,823 (58.6%) of the variants were known. The transition-to-transversion ratio for SNPs was 1.98. To detect putative lethal mutations, variant effect prediction was performed for the identified variants using SnpEff. Thereby, 31,326 variants were predicted to have a high impact on protein-coding sequences, 8,691 of which were present in the heterozygous state in at least 2 horses but were not found in the homozygous state. Those variants were annotated with 16,820 effects having a high impact on a total of 3,701 genes. The comparison of those affected genes with several genes previously reported to be essential in various cell lines showed an overlap of 305 genes, which were further classified based on KEGG pathways. For example, high impact variants in 6 genes (*BARD1*, *BRCA1*, *PALB2*, *POLD1*, *RBBP8*, *XRCC3*), which are involved in homologous recombination were found. The detection of lethal variants in genes affecting embryonic development could allow for the development of genetic tests for those mutations and thereby help to improve fertility rates in domestic horse populations.

Key Words: horses and related species, bioinformatics, genetic disorder

P268 Genomic data reveals a serious underestimation of pedigree inbreeding levels in Polo Argentino horses. F. Azcona*[1], A. Molina[3], P. Peral-García[1], and S. Demyda-Peyrás[2,4], [1]*IGEVET-CONICET-UNLP, La Plata, La Plata, Buenos Aires, Argentina,* [2]*Departamento de Producción Animal, Facultad de Veterinaria, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina,* [3]*Departamento de Genética, Universidad de Córdoba, Córdoba, España,* [4]*CONICET-CCT La Plata, La Plata, Buenos Aires, Argentina.*

The Argentine Polo Horse (APH) is a novel breed created 40 years ago in Argentina by breeding selected native horses based on sports performance with Thoroughbreds to increase speed and reactiveness. Despite closing mating, our previous reports, based on pedigree data, do not show significant increases in inbreeding. Hereby, we aim to validate these findings using genomic data. To this end, we genotyped 49 APH using the Illumina Equine GGP array (71,805 SNPs) and determined the molecular inbreeding values (F_{ROH}) using ROH. The analysis included total F_{ROH} (using all the information available) and F_{ROH} theoretically produced during the last 3, 6, and 9 generations (minimum ROH length of 16.67Mb, 8.33Mb, and 5.55Mb respectively); and the ancient inbreeding (originated before the last 9 generations). Finally, results were compared with pedigree-based inbreeding values obtained using 112,000 birth records (F_{PED}). Average pedigree inbreeding was low (F_{PED} = 0.1%, with 23 of 49 individuals showing a F_{PED} = 0), even the acceptable pedigree completeness observed (4.4 EGC; 11.1 G_{MAX}). On the contrary, F_{ROH} values were notably higher, averaging 14.71%. Correlations between PED and SNP inbreeding values were moderate, but the analysis per generation showed that only 26.68% was explained by mating produced during the last 9 generations, and only 5.55% during the last 3 (Table 1). On the contrary, 63% of inbreeding detected is explained by ancient inbreeding ($F_{ROHANC)}$, which was not correlated with F_{PED}. Although the analysis of the robust APHpedigree revealed low inbreeding values, genomic data indicate a completely different situation. All the animals demonstrated increased levels of inbreeding (F_{ROH} ranged from 11.43% to 19.67%) mostly due to an ancient basis. These results can be explained by the strong influence of Thoroughbred, which is a very old and selected breed with a high inbreeding load, was probably inherited by the APC, without being noticed. Overall, we demonstrated that that genetic characterization using pedigree records is a valuable resource to guide breeding decisions, but it can underestimate inbreeding values. Therefore, we believe that genomic analysis are necessary to obtain a more realistic idea of the genetic variability in breeds with relatively new studbooks, but even more if they were influenced by inbred breeds such as TB

Key Words: horses and related species, breed/population identification, inbreeding, single nucleotide polymorphism (SNP), homozygosity

P269 Genomic improvement of the horse X chromosome and characterization of the pseudoautosomal boundary. M. Jevit*[1], B. Davis[1], C. Casanteda[1], D. Miller[2], and T. Raudsepp[1], [1]*Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA,* [2]*Cornell University, Ithaca, NY, USA.*

The horse reference genome was improved with the release of *EquCab3* and the first Y chromosome reference. The most complex sequences, such as those in the sex chromosomes, are still unresolved. These include large amplicons, repeats, and the pseudoautosomal boundary (PAB). We initiated a comprehensive study specifically to improve the assembly of the horse sex chromosomes. To refine the assembly of complex regions, we are utilizing 3 new technologies: trio-binning, Hi-C and Bionano optical mapping. Trio-binning uses long-read sequences from F1 interspecific hybrids and short reads from parent species. High molecular weight blood DNA was extracted from a female hinny and sequenced on 2 PacBio Sequel cells. Paired-end short reads (150bp) for horse (*Twilight*) and donkey (*Willy*) were obtained from SRA. These sequences as well as the hinny long reads were assembled with trio-binning function of the Canu assembler program. The initial assembly was scaffolded with Hi-C data as well as a Bionano optical maps one of a Thoroughbred stallion (*Bravo*). The resulting assembly is 2.4 Gb in total separated into 162 scaffolds. Our initial goal was to use these assembly to better define the PAB - the end of the pseudoautosomal region (PAR) where X-Y recombination stops. Despite the evolutionary and biological importance of the PAB, the region has been characterized at molecular level in only a few species, and is not well-defined in horse. Previously, we identified and Sanger sequenced 4 BAC clones - 2 spanning PAB-X and PAB-Y. To identify the PAB of the horse, BAC sequences were aligned to the Y assembly, EquCab3 and a 42 Mb-size contig from trio-binning assembly which corresponds to the short arm of the X. We identified a region on both X and Y where X-Y homology drops from over 97% (PAR) to almost zero, indicative of the PAB. This region corresponds to the location of the *XKR3Y* gene in the Y but is not well-annotated in the X. We identified a duplication and an inversion in EquCab3 which was not consistent with the corresponding region in the X-BACs, or the new 42 Mb Xp contig, suggesting a mis-assembly in EquCab3. We believe that these approaches combined will also resolve other complex portions in the horse sex chromosomes.

Key Words: trio-binning, HiC, sex chromosomes, Bionano, pseudoautosomal boundary

P270 Gene expression of chondrogenic markers to assess the differentiation of equine mesenchymal stem cells in different 3D systems. A. Cequier[1], A. Romero[1,2], A. Vitoria[1,2], F. Vázquez[1,2], P. Zaragoza[1], C. Rodellar[1], and L. Barrachina*[1,2], [1]*Laboratorio de Genética Bioquímica LAGENBIO (Universidad de Zaragoza), Instituto Agroalimentario de Aragón– IA2, Universidad de Zaragoza-CITA; Instituto de Investigación Sanitaria de Aragón (IIS), Zaragoza, Spain,* [2]*Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, Zaragoza, Spain.*

Mesenchymal stem cells (MSCs) can be used to treat several diseases in animals and people. The horse is particularly valuable as translational model for cartilage repair. The ability of MSCs to differentiate into chondrocytes has granted them an increasing interest for this purpose. However, obtaining quality cartilage in vitro remains challenging because MSC differentiation requires specific environmental signals. A 3D configuration is usually provided by pelleting cells, but nutrients may not reach the center of the pellet and necrosis areas tend to form. The use of hydrogels to provide 3D configuration closer to that in the cartilage

would also improve nutrient diffusion. The aim of this study was to analyze the gene expression of chondrogenic markers to assess the differentiation of equine MSCs in 2 different 3D systems, classical pellet and alginate hydrogel, at 2 different time points, 14 and 21 d. Equine MSCs were pelleted (500,000 MSCs/pellet) or embedded in 1.5% sodium alginate (5×10^6 MSCs/construct). Cells in both conditions were cultured with chondrogenic induction media (10 ng/mL TGF-β3) for 14 or 21 d. Chondrogenic differentiation was confirmed in all conditions by Alcian Blue staining. Gene expression of the chondrogenic markers collagen type II α I (*COL2A1*) and Aggrecan (*ACAN*) was assessed by RT-qPCR. Results were normalized to the expression of 2 housekeeping genes, *GAPDH* and *B2M*, and undifferentiated cells were used as reference sample. Both *COL2A1* and *ACAN* were upregulated over undifferentiated cells under all conditions. Expression of both markers was higher in cells differentiated in alginate compared with the pellet at both time points. In addition, expression of *COL2A1* and *ACAN* in the pellets was similar between 14 and 21 d, whereas in the alginate constructs it was higher at 21 over 14 d. Our results suggest that alginate hydrogel may provide a more appropriate environment for chondrogenesis of MSCs, especially at 21 d. When working with cells in a matrix, studying their gene expression profile can provide highly useful information regarding the changes they are experiencing.

Key Words: horses and related species, cell biology, qPCR, gene expression, animal health

P271 Genetic variability of 2 native Sardinian horse populations analyzed by microsatellite markers. M. C. Cozzi*, P. Valiati, and M. Longeri, *Università degli Studi di Milano, Dipartimento di Medicina Veterinaria, Lodi, Italy.*

Giara (GRH) and Sarcidano (SRH) are 2 semi-feral local horse breeds living in Sardinia (Italy), a Mediterranean island rich in animal and plant biodiversity. This is a retrospective study, preliminary to further analyzes, on samples, collected in 1996 (GRH) and 2004 (SRH). The aim is to estimate the genetic variability of the 2 populations (sample numerosity: 60 GRH and 73 SRH) and to evaluate their inbreeding level using 17 microsatellite markers. Furthermore, genetic relationships among GRH, SRH, and 30 additional local/cosmopolitan breeds of the Mediterranean area, belonging to our long-term samples collection or online databases, were evaluated. Allelic frequencies, genetic equilibrium according to Hardy–Weinberg (P-val), and inbreeding coefficient (F_{IS}) were estimated using the GENEPOP software. The number of alleles (Na), the effective number of alleles (Ae), the observed (Ho) and expected (He) heterozygosity were calculated with POPGENE v.1.32. The genetic relationships among the 2 Sardinian populations and other Mediterranean horse breeds were analyzed using (1) GenAlex v. 6.503 for the dissimilarity matrix, the principal coordinates analysis (PCoA) and dispersion graph; (2) STRUCTURE software for the cluster assignment based on the Bayesian method; (3) POPULATION software for the individual Neighbor-joining dendrogram (NJD) using allele sharing distances. The Na were 6.000 and 6.813, whereas the Ae were 3.488 and 3.211 in GRH and SRH respectively. The Ho and the He mean values were 0.686 and 0.690 in GRH and 0.627 and 0.641 in SRH. Overall, well-defined population clusters and low levels of inbreeding within GRH (F_{IS} value 0. 003) and SRH (F_{IS} value 0.015) were recorded.

Key Words: horse, microsatellite, population structure, biodiversity, conservation

P272 Genetic diversity and phylogenetic relationships among the show Arabian horse populations of special interest to the breeder community. M. Machmoum*[1], D. Petit[2], B. Badaoui[3], M. A. El Alaoui[4], I. Boujenane[5], and M. Piro[1], [1]*Veterinary Genetic Laboratory, Department of Medicine, Surgery and Reproduction, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco,* [2]*Laboratoire Peirene, EA7500, University of Limoges, Limoges, France,* [3]*Biodiversity, Ecology and Genome Laboratory, Department of Biology, Mohammed V University, Faculty of Science, Rabat, Morocco,* [4]*Superior School of Technology, Ibn Tofail University, Kenitra, Morocco,* [5]*Department of Animal Production and Biotechnology, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco.*

This study aimed to understand the genetic relationship between the Desert breed, Straight Egyptian and Polish blood lines Arabian horse populations and to assess the accuracy of the traditional strains classification system as stated by the Bedouin culture. We collected 211 hair samples from stud farms, renown for the breeding of show activities Arabian horses, from KSA, Egypt, Bahrain, Qatar, Morocco, UAE and Poland. The sequencing of whole mitochondrial DNA D-loop (916p) followed by phylogenetic and network analyses highlighted a notable level of genetic diversity among the 3 Arabian populations. Discriminant analysis of principal components showed a relative separation between the horse populations, although we noted a considerable overlap among the populations. We found no correspondence between mitochondrial DNA sequence-based clustering and the traditional system of classification; more studies are warranted to clarify this finding.

P273 Quantitative trait loci associated with alternative gaits in Colombian Paso horses. M. Novoa-Bravo*[1,3], F. Serra-Bragança[2], R. Naboulsi[3], M. Sole[3], M. Rhodin[4], and G. Lindgren[3], [1]*Genética Animal de Colombia SAS, Bogotá, Colombia,* [2]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands,* [3]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [4]*Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

The lateral footfall pattern in gaited horses is mainly caused by the DMRT3 mutation. However, some horse gaits are not explained by this mutation suggesting that other loci could affect the footfall pattern and other gait parameters. The Colombian Paso horses show different gaits including the Paso Fino, Trocha, and Colombian Trot. Usually, these horses perform just one of the gaits per horse. The DMRT3 mutation is fixed in horses performing Paso Fino, but not in the other gaits. The Trocha is a 4-beat diagonal coupled gait with a lateral footfall pattern not explained by the DMRT3 mutation, whereas the Colombian Trot is a 2-beat diagonal coupled gait. We hypothesize that other loci may explain the footfall pattern and other differences in gait parameters between Colombian Trocha horses and Colombian Trot horses. To evaluate that, we selected 217 Colombian Paso horses, in several Colombian regions, performing Trocha (100 horses) or Colombian Trot (117 horses) according to the owner/trainer criterion. We measured these horses using objective gait analysis with inertial measurement units (Equimoves®) to estimate gait parameters and to classify the horse gaits by using a machine learning approach. In addition, we genotyped 95 horses (DMRT3 CC genotypes) from the 217 phenotyped horses using 670K Thermofisher Axiom Equine array and we did a preliminary GWAS by each parameter estimated and with the objective classification output. We calculated 40 gait parameters. Also, the classification algorithm objectively differentiated 53Colombian Trot horses (45% from the owner/trainer classification) and 158 Colombian Trocha horses from the 217 horses (6 horses were not classified). We used 85 horses and 360,755 markers per horse after quality controls. The GWAS employing different genomic models shows QTLs associated with the stride frequency ($P < 9.2 \times 10^{-9}$ after Bonferroni correction) and the Trot/Trocha classification ($P < 2.2 \times 10^{-9}$ after Bonferroni correction) in the chromosomes 10 (ECA10) and 16 (ECA16) respectively. In this preliminary study, we found other loci than DMRT3 associated with the lateral footfall pattern in horse gaits and with the stride frequency in Colombian Paso horses. This finding could be extrapolated to similar gaits to the Trocha in other horse breeds as Foxtrot in Missouri Foxtrotter, and Marcha batida in Mangalarga-Marchador breed, revealing genetic variants that potentially affect the locomotion of the horses. This needs further investigation.

Key Words: locomotion, horses, gait, QTL

P274 Transcriptomic markers of recombinant erythropoietin micro-dosing in Thoroughbred racehorses. A. Dahlgren*, H. Knych, and C. Finno, *School of Veterinary Medicine, University of California–Davis, Davis, CA, USA.*

Recombinant human erythropoietin (rHuEPO) is well known as a performance enhancing agent used in both human and, anecdotally, equine athletes. Like endogenous erythropoietin, rHuEPO stimulates red blood cell production in the bone marrow, increasing hemoglobin mass, arterial oxygen content, and thus aerobic power. To identify and prevent abuse of rHuEPO in horse racing, routine testing of samples from equine athletes has been performed with liquid chromatography tandem mass spectrometry (LC-MS/MS) based assays. However, these assays are cumbersome, the detection window is short, and micro-dosing is unlikely to be detected. To identify transcriptomic markers of rHuEPO dosing in Thoroughbred racehorses, we performed RNA sequencing of peripheral blood mononuclear cells (PBMCs) following repeated rHuEPO micro-dosing in 6 exercising Thoroughbreds as compared with 4 control Thoroughbreds over a 7-week time course. Three transcripts were significantly dysregulated ($\log_2$FC >1.5 and P_{FDR} < 0.05) between treatment groups when comparing wk 1 vs. d 0, d 3 and wk 4 time points. Following validation, these transcripts could serve as markers of rHuEPO micro-dosing in Thoroughbred racehorses.

Key Words: horse, RNA-seq, athletic performance, biomarker

P275 Withdrawn

P276 Assessment of genetic diversity using microsatellite markers to compare donkeys (*Equus asinus*) with horse (*Equus caballus*). S. Y. Lee[1] and G. J. Cho*[2], [1]*Racing Laboratory, Korea Racing Authority, Gwacheon, Korea,* [2]*College of Veterinary Medicine and Institute of Equine Medicine, Kyungpook National University, Daegu, Korea.*

The study aimed to evaluate the diversity of donkey populations by comparing with the diversity of Thoroughbred horse and Jeju Halla horse; identified breeding backgrounds can contribute to management and conservation of donkeys in South Korea Microsatellite DNA markers were used for individual identification and parentage verification in horse and donkeys. Total 1,000 horse samples (50 Thoroughbreds and 50 Jeju Halla horses) and 79 donkeys were genotyped with 15 microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG10, LEX3, and VHL20) The observed number of alleles per locus ranged from 1(ASB17, HMS1) to 14(AHT5), with a mean value of 4.87, 8.00, and 5.87 in Thoroughbreds, Jeju Halla horse, and donkeys, respectively. Of the 15 markers, AHT4, AHT5, ASB23, CA425, HMS2, HMS3, HTG4, HTG10, and LEX3 loci had relatively high PIC values (PIC >0.5) in these 3 populations. Mean levels of genetic variation were H_E = 0.6721 and H_O = 0.6600 in Thoroughbreds, H_E = 0.7898 and H_O = 0.7100 in Jeju Halla horse, and H_E = 0.5635 and H_O = 0.4861 in donkeys. Of the 15 loci in donkeys, 3 loci had negative inbreeding coefficients (FIS), with a moderate mean FIS (0.138). The FIS estimate for the HTG4 marker was highest (0.531) and HMS6 marker was lowest (−0.01). The total PE value of 15 microsatellite loci was 0.9996 in donkeys. Genetic cluster analysis showed that the genetic relationship among 79 donkeys was generally consistent with pedigree records. Among the 3 breeds, donkeys and Thoroughbred horse formed clearly different groups, but the group of Jeju Halla horse overlapped with that of Thoroughbred horse, suggesting that the loci would be suitable for donkey parentage testing. Therefore, the results of this study are a valid tool for genetic study and conservation of donkeys.

Key Words: donkey, horse, microsatellite marker, South Korea

P277 Genetic structure of maternal lines in Przewalski horses based on mtDNA variation. A. D. Musial*[1], K. Ropka-Molik[1], M. Stefaniuk-Szmukier[2], G. Mycka[2], A. Fornal[1], and N. Yasynetska[3], [1]*National Research Institute of Animal Production, Balice, Poland,* [2]*University of Agriculture, Krakow, Poland,* [3]*Biosphere Reserve, Askania-Nova, Ukraine.*

Przewalski's horses (*Equus ferus przewalskii*) are considered the last living population of wild horses, however, according to recent reports, modern Przewalski's horses may have descended from a feral line of horses domesticated about 5,000 years ago. Currently, the Przewalski horse population consists of no more than 2,000 individuals worldwide. Based on pedigree data, 14 founders of the species are distinguished, but the population cannot be completely genetically pure, because the mothers of 2 of the founders belonged to the *Equus caballus* species. To date studies on mitochondrial DNA (mtDNA) identified 3 unique haplotypes in Przewalski's horse, none of which are found in modern horses. The polymorphism of 2 hypervariable fragments of the mitochondrial DNA D-loop region, which contains about 10% of all nucleotide changes present in the mtDNA molecule, plays a unique role in verifying the origin of horses in terms of female genealogy. The aim of the study was the analysis of the mtDNA variability within the selected population of Przewalski's horses, using the sequence of the mitochondrial DNA hypervariable region. The hair samples were collected from 23 Przewalski horses living in the reserve Askania-Nova (Ukraine). All horses belonged to the 3 dam lines (Munich, Prague, Askanian) were tested on 46 SNP loci (single nucleotide polymorphism). The mitochondrial DNA hypervariable region with a total length of 1,165 bp were analyzed using Sanger method. Bioinformatic analysis allowed for assigning the examined Przewalski's horses to 3 distinctly different haplotypes. The first haplotype showed the greatest similarity to the *Equus caballus* reference, the second mtDNA profile was clearly separates from the genus *Equus*, while the third haplotype was similar to *Haringtonhippus*, an extinct species living in the Pleistocene in North America. This result may indicate an extensive history of the origins of the Przewalski horse species and requires further research. This research was funded by "Diamentowy Grant" no. 0211/DIA/2019/48, Ministry of Science and Higher Education, Poland.

Key Words: *Equus ferus przewalskii,* mitochondrial DNA, SNP, origin

P278 Will selection for elasticity maintain the allele causing fragile foals? M. Ablondi*[1,2], M. Johnsson[2], S. Eriksson[2], A. Sabbioni[1], Å. Viklund[2], and S. Mikko[2], [1]*Department of Veterinary, Università degli Studi di Parma Science, Parma, Italy,* [2]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Warmblood Fragile Foal Syndrome (WFFS) is an autosomal monogenic disease leading to a defective connective tissue, which in turn causes skin and mucosa lacerations, fragile skin, hyperextension of the articulations and hematoma. The WFFS is caused by a recessive lethal missense point mutation in the procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 gene (*PLOD1*, c.2032G>A). Foals homozygous for the WFFS recessive allele are not viable, and either aborted during late gestation, or have to be euthanized shortly after birth. Despite its harmful effect, a relatively high WFFS carrier frequency has been found among Warmblood breeds, suggesting a heterozygote advantage. Thus, the aims of this study were 1) estimate WFFS carrier frequency in the Swedish Warmblood breed (SWB), 2) evaluate the effect of WFFS genotype on estimated breeding values, and 3) simulate the potential effects of balancing selection and different selection strategies on future carrier frequency. The WFFS carrier frequency calculated from a cohort of 511 randomly selected SWB horses born in 2017 was equal to 7.4%, whereas it ranged from 0.0% to 14.0% among the whole set of tested SWB (1,811 horses) divided into 8 birth year classes starting from 1980 till 2019. Overall, we found a favorable effect of the WFFS allele for movements and dressage traits, highlighting potential balancing selection on the WFFS allele in SWB horses bred for dressage purposes. Via simulations, we proved that balancing selection could maintain a

recessive lethal over generations in populations similar to the SWB breed. The allele frequency of a recessive lethal allele is expected to slowly decline over generations but will decrease slower in the presence of balancing selection. Finally, we demonstrated that selection against carrier sires can over time give a more rapid decrease of the mutant allele frequency. Further research is needed to confirm the noticeable association between equine performance and the WFFS genotype. Identification of such associated genetic markers or novel causative mutations to horse performance traits might serve as new tools in horse breeding to select for healthy, sustainable, and better performing horses.

Key Words: *PLOD1*, mobility, movements, genotyping, candidate gene

P279 Towards a comprehensive horse Y-chromosomal tree: Signatures from local breeds and ancient DNA. E. Bozlak*[1,2], L. Radovic[1,2], D. Rigler[2], T. Kunieda[3], R. Juras[4], G. Cothran[4], and B. Wallner[2], [1]*Vienna Graduate School of Population Genetics, Vienna, Austria,* [2]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria,* [3]*Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Japan,* [4]*Department of Integrative Biosciences, College of Veterinary and Biomedical Sciences, Texas A&M University, College Station, TX, USA.*

The Y chromosome carries pivotal information about male-driven demography and is an indispensable tool for studying the historic development of domestic animals. Y haplotype patterns in modern-day domestic horses are characterized by the dominance of a recent 'Crown' clade, which is a consequence of extensive breeding with stallions of Oriental origin in the past 500 years. However, Northern European breeds, Asian horses, and presumably other populations in remote regions could have avoided recent Crown introgressions and retained autochthonous variation. Here we augmented the established modern horse Y-phylogeny with horses from remote regions around the globe. 82 samples were carefully selected from more than 2000 individuals by genotyping haplotype-defining mutations. We focused on horses that do not carry a 'Crown' haplotype. We performed target enriched resequencing (TES) of 5 Mb of the Y chromosome. NGS reads were mapped to the 6.4 Mb LipY764 reference, variants ascertained with GATK4, and filtered for single-copy regions in the reference and multiple quality parameters. Based on the remaining variants (>2,200), a parsimony tree was created. Most of the TES samples represented unique haplotypes that were reported for the first time. This proves, that genotyping prior sequencing was effective to catch low frequent HTs in remote populations. The extended Y phylogeny supports a pronounced radiation 'Dom-West', encompassing most domestic horse Y lineages, which substantiates previous findings. In order to investigate the origin and dissemination of the contemporary Y haplotypes, we combined the domestic horse Y phylogeny with data from a published set of whole-genome sequenced ancient males using the program PathPhynder. Data from ancient periods enable more accurate dating of branching points, disclose the lineages lost in recent times, and allow us to trace the origin of the present horses' lineages. The complemented Y-chromosomal phylogeny significantly reduces ascertainment bias when studying remote populations and it is the next step toward a better understanding of horse breeding via males.

Key Words: Y chromosome, horses and related species, phylogeny, resequencing, ancient DNA

P280 Transcriptomic and proteomic profiling of gluteal muscle of Standardbred horses between episodes of recurrent exertional rhabdomyolysis. D. Velez-Irizarry*[1], Z. Williams[1], M. Henry[1], H. Iglewski[1], K. Herrick[1], C. Fenger[2], and S. Valberg[1], [1]*Mary Anne MacPhail Equine Performance Center, Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA,* [2]*Equine Integrated Medicine PLC, Lexington, KY, USA.*

During an episode of acute rhabdomyolysis, studies have shown differential expression of calcium-regulatory, mitochondrial, and metabolic genes in the gluteal muscle. Whether this represents expression related to acute myodegeneration or a specific molecular signature of susceptibility to recurrent exertional rhabdomyolysis (RER) is currently unknown. We compared gluteal muscle histopathology, gene/protein expression, and antioxidant concentrations between Standardbreds with a history of, but not currently experiencing rhabdomyolysis, and race-trained controls to derive a molecular susceptibility profile. Transcriptomic (9 RER, 7 controls) and proteomic profiles were analyzed using RNA-seq and tandem mass tag LC/MS/MS. Glutathione and CoenzymeQ_{10} concentrations were determined by MS/MS. Muscle histochemistry revealed RER-susceptible horses had more type 1 fibers ($P = 0.007$) and more internalized myonuclei ($P = 0.002$) in mature fibers than controls without histopathologic evidence of degeneration or inflammation. There were 791 significantly differentially expressed genes (DEG), FDR ≤ 0.05. Upregulated DEG (n = 433) were associated with inflammation, cell proliferation, and oxidative stress. Downregulated DEG (n = 305) were associated with calcium ion regulation, electron transport, and metabolism. Differentially expressed proteins (DEP; ↑n = 50, ↓n = 12; FDR ≤ 0.10) were involved in the sarcomere (24% of DEP), electron transport (23%), metabolism (20%), inflammation (6%), and oxidative stress (7%). DE antioxidants included DEP ↑superoxide dismutase, ↑catalase, ↑peroxiredoxin 1, ↓peroxiredoxin 2, and DEG↑ glutathione peroxidase as well as cysteine-based redoxins ↓peroxiredoxin 2, ↑sulfiredoxin, ↑thioredoxin, and ↑thioredoxin reductase. Glutathione concentrations did not differ whereas CoenzymeQ_{10} concentrations were significantly higher in RER-susceptible versus controls. Numerous upregulated DEG encoded enzymes prerequisite for glutathione and CoenzymeQ_{10}synthesis. In conclusion, perturbation of pathways for calcium regulation, cellular/oxidative stress, inflammation, and cellular regeneration is present in muscle of RER-susceptible Standardbred horses weeks after an episode of rhabdomyolysis that could represent potential therapeutic targets.

Key Words: horse, recurrent exertional rhabdomyolysis, RNA-seq, proteomics

P282 Epigenetic characterization of horse centromeric domains in different tissues and individuals. E. Cappelletti*[1], F. M. Piras[1], R. Hijaz[1], L. Sola[1], J. L. Petersen[2], R. R. Bellone[3,4], C. J. Finno[3], T. S. Kalbfleisch[5], E. Bailey[5], S. G. Nergadze[1], and E. Giulotto[1], [1]*Department of Biology and Biotechnology, University of Pavia, Pavia, Italy,* [2]*Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE, USA,* [3]*University of California–Davis, School of Veterinary Medicine, Department of Population Health and Reproduction, Davis, CA, USA,* [4]*University of California–Davis, School of Veterinary Medicine, Veterinary Genetics Laboratory, Davis, CA, USA,* [5]*University of Kentucky, Gluck Equine Research Center, Lexington, KY, USA.*

The centromere is the locus required for chromosome segregation during cell division whose function is epigenetically specified by CENP-A, the centromere-specific histone H3 variant. The typical presence of satellite DNA at mammalian centromeres hampers a comprehensive molecular analysis of this locus. Our discovery that, in equid species, several centromeres are devoid of satellite DNA represented an important step forward and made equids a powerful model system for unraveling the epigenetic marks related to centromere function at the molecular level. In the horse, the centromere of chromosome 11 (ECA11) is the only one devoid of satellite DNA. We previously demonstrated that the position of its CENP-A binding domain is not fixed but slides within an about 500 kb region in different individuals, giving rise to positional alleles. These epialleles are inherited as Mendelian traits but their position can slide in one generation. This was the first demonstration that, in a native

mammalian centromere, the position of the functional CENP-A domain can slide within a relatively wide region suggesting that centromeric domains are characterized by positional instability which may be physically limited by epigenetic boundaries. As members of the equine community of the FAANG (Functional Annotation of ANimal Genomes) consortium, our first goal was to unravel whether centromere sliding can occur during development. To this purpose we characterized the position of the centromeric domains of ECA11 in tissues of different embryonic origin from 2 adult Thoroughbred mares. Our results demonstrated that the centromere is located in the same region in all tissues, suggesting that the position of the centromeric domains is maintained during development. We then evaluated the epigenetic and transcriptional profile of the centromeric locus, taking advantage of ChIP-seq, RNA-seq and microRNA-seq data sets produced by the consortium. This analysis demonstrated that the ECA11 centromere is transcriptionally silent across tissues and individuals, indicating that transcription is not a key feature of centromeric chromatin.

Key Words: horses and related species, Functional Annotation of Animal Genomes (FAANG), ChIP-seq, chromatin

P283 Satellite-less centromeres formation by centric fusion in equids. F. M. Piras*, E. Cappelletti, W. A. A. Ahmed, E. Raimondi, S. G. Nergadze, and E. Giulotto, *Department of Biology and Biotechnology, University of Pavia, Pavia, Italy.*

The centromere is a fundamental structure required for correct chromosome segregation during cell division whose function is epigenetically specified by the histone H3 variant, CENP-A. Centromeric DNA sequences are mainly represented by satellite DNA and vary among highly related species and even interchromosomally. We previously demonstrated that in the genus *Equus* an extraordinary number of centromeres are completely satellite-less and arose recently during evolution. We also showed that several of these neocentromeres originated from centromere repositioning events (shifting of centromere function without chromosome rearrangements). In equids, a mechanism which extensively contributed to karyotype reshaping is the Robertsonian fusion which leads to the formation of (sub)metacentric chromosomes from the fusion of 2 acrocentric chromosomes. These events involve inactivation of the original centromeres and formation of a new one. We show here that, in wild asses and zebras, several centromeres deriving from fusion are satellite-less. We then localized these centromeric loci by ChIP-seq with an antibody against CENP-A and found that the centromeric domain is very close (less than 500 kb) to the fusion point and that pericentromeric sequences are maintained as relics of the functional centromeres that were inactivated. Since we previously demonstrated that the position of the binding domains of CENP-A can slide, we hypothesize that these neocentromeres originated on the fusion point and then moved to a nearby region during evolution. In conclusion, we demonstrated that, besides centromere repositioning, Robertsonian fusion is an important mechanism for the generation of satellite-less centromeres in the genus *Equus.*

Key Words: horses and related species, evolutionary genomics, ChIP-seq, chromatin, chromosomal rearrangement

P284 Transcriptome analysis of 8 priority tissues in 2 Thoroughbred stallions for the Functional Annotation of Animal Genomes project. A. Barber*[1], S. Peng[2], A. Fuller[1], E. Giulotto[3], T. Kalbfleisch[4], C. Finno[2], R. Belone[2], and J. Petersen[1], [1]*University of Nebraska–Lincoln, Lincoln, NE, USA,* [2]*University of California–Davis, Davis, CA, USA,* [3]*University of Pavia, Pavia, Italy,* [4]*University of Kentucky, Lexington, KY, USA.*

The functional annotation of genomes will provide the tools necessary to perform applied research into complex traits such as disease, reproduction, and performance. The Functional Annotation of Animal Genomes (FAANG) project aims to identify all functional elements of the genome in domestic species, including the horse. The objective of this study was to utilize RNA sequencing data (RNA-seq) to determine tissue-specific gene expression from healthy Thoroughbred stallions across tissues. Transcriptome data are necessary to correlate gene activity with regulatory elements identified using other methodologies (e.g., ChIP-seq). To build a transcriptome database in the adult male horse, 102 tissues were collected from 2 Thoroughbred stallions and flash frozen. Eight tissues were prioritized for cross-species and between-sex comparisons: adipose, lamina, left ventricle, lung, liver, parietal cortex, skeletal muscle, and testis. RNA was isolated from tissues with RIN scores ranging from 7.6 to 9.7 (average = 8.8). Stranded, Poly-A$^+$ libraries were sequenced, and the resulting reads were trimmed and mapped to EquCab3.0. An average of 34.1M reads per sample were obtained [range 30.7M (testis) to 38.8M (adipose)]. On average, 92% of reads uniquely mapped to the genome [range 87.9% (left ventricle) to 93.6% (testis)]. Combined with data previously collected from 2 adult Thoroughbred mares, these transcriptome data will allow for analysis of gene expression across tissues, between sexes, and among species. Integrating these data with other functional annotation data will provide a basis for future studies. Furthermore, these data provide an understanding of the transcriptome in these prioritized tissues, which will aid in identification of aberrant gene function related to various traits of interest in the horse.

Key Words: horses and related species, RNA-seq, gene expression, genome annotation

P285 Debunking the genetic origins of the Mangalarga through Turbante J.O. L. Patterson Rosa*[1], F. Araujo[1], M. Vierra[1], S. Brooks[2], and C. Lafayette[1], [1]*Etalon Inc., Menlo Park, CA, USA.,* [2]*University of Florida, Gainesville, FL, USA.*

The written history of the origins of the Brazilian Mangalarga, or "Mangalarga Paulista" horse breed is controversial. Although breed historians and horse breeders are often able to recite pedigrees and ancestry of their horses better than their own family records, there are numerous inconsistencies and ancestor's divergence between farms, owners or historical records. In particular, the breed's popular history claims that one of its most notorious individuals, Turbante J.O., was sired by an unknown Hanoverian stallion. Turbante J.O. (1969–1998) sired over 1,678 offspring and is one of the Mangalarga's most influential sires, being present in about 71% of the male pedigrees and 17.4% of the breed. To compare the genomic ancestry to the pedigree records, we evaluated Turbante J.O. and genotyped data of 15 Mangalarga individuals using a commercially available ancestry service (Etalon Diagnostics, Menlo Park, CA) and pedigree-based estimates of owner-reported lineage. Turbante J.O.'s DNA was extracted from frozen semen samples collected in 1990, privately stored by the owner. Other breed-average genomic ancestries were provided by Etalon Diagnostics for the Arabian, Thoroughbred, Saddlebred and Hanoverian for comparison. Pedigree records for the individual were provided by the owner, through the Pedigree Online's All Breed Pedigree Database (www.allbreedpedigree.com) and analyzed with Pedigraph v2.4. While the stallion's pedigree inbreeding coefficient is estimated at 18.5%, genomic inbreeding coefficient is at 33%. Pedigree coancestry coefficients estimate that about 4% of his ancestry should be in common to the Thoroughbred and Arabian, while the genomic ancestry of Turbante J.O. resulted in 100% Iberian, and 99% in common with other Mangalarga individuals. These results demonstrate the increased accuracy and specificity of genomic-based coefficients of ancestry and inbreeding in detriment of pedigree-based. We also demonstrate higher pedigree inbreeding coefficient errors than previous reports (8.8–13.1%) for this individual horse, and due to the genomic ancestry relatedness to other breed individuals, these results may be applicable for other individuals of this breed.

Key Words: ancestry, horse, breeds, inbreeding, sire

P286 Withdrawn

P287 Withdrawn

P288 Historical biogeography of Philippine native pigs and the perplexing mitochondrial DNA variation in Philippine wild pigs. J. Layos*[1,2], C. Godinez[1,3], L. Liao[4], Y. Yamamoto[1], and M. Nishibori[1], [1]*Laboratory of Animal Genetics, Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan,* [2]*College of Agriculture and Forestry, Capiz State University, Capiz, Philippines,* [3]*Department of Animal Science, Visayas State University, Leyte, Philippines,* [4]*Laboratory of Aquatic Ecology, Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan.*

The Philippines is an archipelago of 7,641 islands situated in Island Southeast Asia at the crossroads of past human migrations in the Asia-Pacific region. It was believed to have never been connected to the Asian continent, even during the severe Quaternary sea-level drops. As a result, the history of pig dispersal in the Philippines remains controversial due to the limited molecular studies and the absence of archeological assemblages that exhibit signs of pig domestication. This study provides the first comprehensive screening of the mitochondrial DNA of Philippine native pigs (n = 175) and Philippine wild pigs (n = 9) to resolve their earlier dispersal history by conducting phylogenomic analysis altogether with domestic pigs and wild boars corresponding roughly to their geographic origin. Results revealed a demographic signal of pig ancestry exhibiting a close genetic affiliation from mainland Southeast Asia and Northeast Asia Regions which corroborates a gene flow that might have arisen through human migration and trade. Here, we proposed 2 possible dispersal routes. One is through Northeast Asia paralleled with the Neolithic expansion in Island Southeast Asia and Oceania, and the other is through mainland Southeast Asia, which may have traversed through the Sundaic Region to Palawan and the Sulu Archipelago. Despite geographical barriers to migration, numerous genetic lineages have persisted on various Philippine islands and even warrants the recognition of a Philippine Lanyu-type. The prehistoric population size dynamics predate a demographic expansion during the Late Pleistocene ages as the Southern regions to be the probable origin, eventually expanded toward the Central regions. The intriguing signal of disparity detected among the molecular result, morphology, and distribution range of the numerous Philippine endemic wild pigs opens a new challenging approach in shedding the complexities between these animals.

Key Words: genetic diversity, historical biogeography, mitochondrial DNA, Philippine native pigs, phylogenomics

P289 Reassessing phylogeny and Bayesian divergence dating provide new insights on the evolutionary history of chickens in Southeast Asia. C. J. P. Godinez*[1,2], J. K. N. Layos[1,3], Y. Yamamoto[1], L. M. Liao[4], M. Duangjinda[5], and M. Nishibori[1,2], [1]*Laboratory of Animal Genetics, Hiroshima University, Higashi-Hiroshima, Japan,* [2]*Visayas State University, Leyte, Philippines,* [3]*Capiz State University, Capiz, Philippines,* [4]*Laboratory of Aquatic Ecology, Hiroshima University, Higashi-Hiroshima, Japan,* [5]*Khon Kaen University, Khon Kaen, Thailand.*

The domestication of chickens has contributed various benefits to the sustenance and cultural development of mankind. The profound timings of their domestication have attracted wide interest in molecular phylogeny and phylogeography studies as it remains debatable up to today. Previous studies indicated that island Southeast Asia (SEA) sits as the region where specific haplogroup D mitochondrial lineage diversified, while most of the chicken populations in the mainland SEA observed to have diverse maternal lineages. However, population history and lineage-specific divergence time estimates of these populations in the aforesaid regions are not well studied. Here, we analyzed 332 complete mitochondrial DNA control-region sequences sampled in the mainland SEA and 144 sequences represented island SEA and Oceania. One hundred 27 haplotypes were newly identified and were distributed across major divergent haplogroups except haplogroup C. Phylogenetic analyses based on neighbor-joining method, maximum likelihood, and Bayesian inference revealed newfound divergent sub-haplogroup V2 sampled from the domestic chickens in Cambodia and Laos and red junglefowl from Thailand at the basal position. Significant posterior probability supported the Philippine-Pacific sub-clade, suggesting a Philippine origin of Pacific chickens. Bayesian divergence time estimates revealed split time of Pacific chickens congruent to the increase of effective population size of Philippine chickens and corroborated the prehistoric human expansion events in the region. The newly identified sub-haplogroup V2 diverged later from the previously identified haplogroup V classified by most red junglefowl species sampled from Thailand and Cambodia. Our results suggest a high level of genetic variability of these chicken populations in the region which demonstrates conservation significance.

Key Words: divergence time estimate, evolutionary genomics, mitochondrial DNA, phylogeny, poultry and related species

P290 Genetic diversity and runs of homozygosity in Rendena cattle. E. Somenzi*[1], N. Franceschi[1], M. Barbato[1], L. Colli[1], E. Partel[2], M. Komjanch[2], A. Achilli[3], H. C. Hauffe[2], and P. Ajmone Marsan[1], [1]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*Edmund Mach Foundation, San Michelle all'Adige, Trento, Italy,* [3]*Pavia University, Pavia, Italy.*

Rendena cattle are an autochthonous cattle breed from Val Rendena in the northern Italian region of Trentino Alto Adige. The breed is considered a dual-purpose breed (milk and meat) but recent selection has emphasized milk production and quality. The breed is characterized by a small-medium size, good fertility and high longevity, and is well adapted to the harsh Alpine environment. In this study 140 Rendena cows sampled in 31 different farms were genotyped with the GGP Bovine 100K SNP-chip (Neogen). In addition, their mitochondrial DNA was fully sequenced. Genotype data were used to estimate within-breed diversity and inbreeding, and were compared with SNP data from 56 Eurasian cattle breeds to evaluate population structure and relationships. Principal Component Analysis (PCA) and Neighbor-net analyses performed on the Eurasian data set indicated the Rendena cattle shared ancestry with cattle breeds of the Original Brown Swiss group. The analysis also revealed an original genetic makeup, thus confirming previous evidence from mitochondrial control-region data and lower density SNP profiles. Within-breed PCA highlighted the absence of substructure and excluded the presence of crossbred individuals or outlier animals. Some inbreeding was detected using a genome-wide analysis of runs of homozygosity (ROH) (F_{ROH} = 0.08 ± 0.03). The ROHs distribution across chromosomes appeared homogeneous and related to chromosome size. Most ROHs were private or common to very few animals. Exceptions were found in 3 genomic regions - on BTA6, BTA10 and BTA16 - where ROHs were shared by > 25% of the animals tested. Noticeably, the ROH on BTA6 consisted in 33 consecutive homozygous SNPs shared by >50% of the animals. This region was found to harbor genes relevant for meat (NCAPG, LCORL) and milk production (LAP3). The mitochondrial DNAs mostly belonged to the T3 haplogroup, widespread in Central Europe. Exceptions were 10 animals with T2 haplogroups and single occurrences of the T5 and Q haplogroups.

Key Words: diversity, alps, mtDNA, ROH

P291 Genetic relationships among Canarian, African, and European goats using SNPs. M. Macri*[1,2], A. Martínez[2], M. G. Luigi[3], J.

Capote[4], A. Canales[1,2], M. Amills[3], J. V. Delgado[2], and M. R. Fresno[4], [1]*1Animal Breeding Consulting, S.L, Cordoba, Cordoba, Spain,* [2]*Department of Genetics, University of Córdoba, Cordoba, Cordoba, Spain,* [3]*3Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, University of Barcelona, Bellaterra, Barcelona, Spain,* [4]*ICIA, Canary Islands Institute for Agricultural Research, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain.*

Majorera, Palmera and Tinerfeña are 3 local goat breeds reared in the Canary Islands which are specialized in the production of milk to elaborate cheese. Several studies have shown that the founder population of Canarian goats had an African origin, with posterior admixture, particularly in the 20th century, from European goats. The goal of this research is to determine the genetic contributions of African and European goats to the Canarian gene pool by using a high-throughput genotyping approach. Blood samples from 72 Canarian goats (24 Majorera, 24 Palmera and 24 Tinerefeña) were analyzed using the GoatSNP50 BeadChip (Illumina) and compared with 654 goats from 30 different breeds investigated in the ADAPTmap Project (http://www.goatadaptmap.org/). The SNPs were filtered using the PLINK v 1.9 program, excluding SNPs with a MAF less than 0.05, missing genotype data higher than 0.01 and significant departure from HWE (*P*-value <0.001), retaining a total of 23,527 SNPs. The PLINK v1.9 program was used to calculate H_O, H_E and inbreeding coefficient (F_{IS}) as well as to perform principal component analysis (PCA). The PCA was visualized with the ggplot2 R package. Reynolds genetic distance and F_{ST} between pair of breeds were obtained with Arlequin v3.1. and a Neighbor-Net was visualized using the Splits-tree4 v4.14.4 software. In both the Neighbor-net tree and PCA, the Canarian goats were located closer to the Central and North African breeds than to the Spanish and remaining European ones, being the North African closer to the Canarian goats than the central African breeds. These results agree with the African origin of Canarian breeds that, combined with insularity, has contributed to create a unique gene pool. This study was funded by the RTA2014-00047-00-00 Project (INIA).

Key Words: goat breeds, SNP, GoatSNP50 BeadChip, PCA

Livestock Genomics for Developing Countries Posters

P292 Genetic basis of thermotolerance in African indigenous chickens. A. A. Gheyas*[1], M. Rachman[2], A. Vallejo-Trujillo[2], O. Bamidele[3,4], A. Kebede[3,5], T. Dessie[3], J. Smith[1], and O. Hanotte[2,3], [1]*Centre for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Midlothian, Scotland, UK,* [2]*School of Life Sciences, University of Nottingham, Nottingham, UK,* [3]*LiveGene – CTLGH, International Livestock Research Institute, Addis Ababa, Ethiopia,* [4]*Kings University, Ode Omu, Nigeria,* [5]*Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia.*

Indigenous chickens in Africa are adapted to their harsh tropical environments. Heat stress is a major challenge to poultry survival and productivity in the tropical climate. The African continent, however, is not homogeneous in its temperature regimen but can show extreme conditions (High and Low) due to large variations in altitude (e.g., in Ethiopia). Elucidating the genetic basis of thermotolerance in African indigenous chickens has important implications for enhancing adaptive performance of dual-purpose improved breeds in tropical smallholder backyard poultry farming systems. Here we investigate the genomic signatures of positive selection in Nigerian and Ethiopian indigenous chickens to identify the genetic basis of thermotolerance. Genome sequence data from 12 Nigerian populations (87 samples), representing high temperature agro-ecologies (annual means: 24–29°C, max: 35°C) were analyzed in combination to identify genomic regions of low heterozygosity (using *Hp* method) as a signature of positive selection for heat tolerance. Contrarily from Ethiopia, we compared 4 populations (38 samples) from extreme low and high temperature regions (1–37°C; negatively correlated with altitude) to find selective sweeps showing genetic differentiations (using *XPEHH* and *Fst* approaches). Several highly plausible candidate genes for heat tolerance were detected from the Nigerian analysis but few from extreme population comparisons from Ethiopia. Instead, the Ethiopian analysis detected several strong candidates for high altitude/cold tolerance adaptation. Important heat stress candidate genes from the literature were also checked in the studied populations. Heat shock protein genes (*HSP70* and *HSP90)*, which have shown differential expression between heat tolerant and non-tolerant chicken breeds, failed to show any genetic differentiation between extreme populations from Ethiopia. This lack of genetic differentiation between extreme groups indicates another mechanism of regulation of heat tolerance genes (e.g., epigenetic). Future studies should, therefore, aim toward assessing the role of epigenetic regulation of heat tolerance traits.

Key Words: poultry and related species, genome biology, genome sequencing, adaptation, genetic improvement

P293 A within- and across-country assessment of the genomic diversity and autozygosity in South African and Eswatini Nguni cattle. S. Lashmar[1], C. Visser*[1], M. Okpeku[2], N. Mapholi[3], and E. Van Marle-Köster[1], [1]*University of Pretoria, Pretoria, South Africa,* [2]*University of KwaZulu-Natal, Durban, South Africa,* [3]*University of South Africa, Pretoria, South Africa.*

Within southern Africa, the Nguni cattle breed is classified as an indigenous and transboundary animal genetic resource that manifests unique adaptation abilities across distinct agroecological zones. In South Africa (SA) and Eswatini (ES), Nguni cattle are integral to local food security and breed-specific beef production is achieved through both natural grazing (NG) and feedlot (FL) systems. The genetic integrity of the breed, and its various ecotypes, is under threat because of both indiscriminate crossbreeding and uncontrolled inbreeding. The aim of this study was to assess both the genetic diversity and autozygosity that exists 1) across countries and 2) within-country (SA), between different production systems. Subsets of 96 ES, 96 SA-FL (representing purebred animals), and 96 SA-NG with a common set of 40 930 genotyped SNPs were used to study diversity parameters and runs of homozygosity (ROH). Across-country, principal component coordinates indicated clear distinction between SA and ES populations whereas within-country, SA-FL were tightly clustered with overlapping but interspersed SA-NG animals. The observed heterozygosity ranged from 0.318 (both SA populations) to 0.320 (ES). Inbreeding coefficients indicated low inbreeding (F_{ROH} range: 0.030 for SA-FL - 0.031 for ES). Short ROH (1–4Mb) were most prevalent (range: 0.454 for SA-FL - 0.480 for ES), and SA-NG had the lowest proportion of ROH ≥ 16Mb (0.144) but the highest number of ROH per animal (mean n = 10.72). Larger ROH are indicative of recent inbreeding; the higher incidence for SA-FL (0.198) is indicative of stronger directional selection compared with extensively farmed SA-NG. For ES, the higher incidence of ROH ≥ 16Mb (0.196) may result from reduced numbers of purebred animals. Overall, results illustrated that genetic distinctiveness in the Nguni resulted from both geographic isolation and exposure to different production strategies. Although no impending threat to genetic diversity was observed, further heterozygosity loss within respective countries and production system should be monitored.

Key Words: admixture, homozygosity, inbreeding, indigenous

P294 Signatures of selection in South African Nguni and Bonsmara cattle breeds. B. Bhika Kooverjee*[1,2], P. Soma[1], M. A. van der Nest[3], F. W. C. Neser[2], M. M. Scholtz[1], and M. D. MacNeil[4], [1]*Agricultural Research Council-Animal Production, Irene, South Africa,* [2]*Department*

of Animal, Wildlife, and Grassland Sciences, University of Free State - Bloemfontein, South Africa, [3]*Agricultural Research Council-Biotechnology Platform, Onderstepoort, South Africa,* [4]*Delta G, Miles City, MT, USA.*

Nguni, an indigenous cattle breed is highly adapted to the harsh South African weather conditions and requires less maintenance. In contrast, the Bonsmara cattle breed represents a popular beef breed. Given this, these 2 breeds show great potential to be included in crossbreeding programs to mitigate the effect of climate change while producing high-quality meat. The aim of this study was to identify selection signatures within and between Nguni and Bonsmara cattle in relation to production and adaptation. For this purpose, genomic 150K SNP data from Nguni (n = 231) and Bonsmara (n = 252) cattle was obtained. Extended haplotype homozygosity (EHH) based analysis was executed within each population, using integrated haplotype score (iHS). The Rehh package in R was used for detecting selection signatures across the 2 populations (iES Rsb) and cross-population EHH (XP-EHH) with $P < 0.0001$. A total of 121 regions of selection signatures were detected in the Bonsmara and Nguni populations. This included regions harboring genes related to DNA methylation (e.g., *METTL21C*, *DNMT3B*), heat stress (e.g., *Hsp40*, *Hsp70*, and *Hsp110*), as well as immunity (e.g., *IRGQ*, *IRGC*). Genes related to female fertility were detected in the Nguni population (e.g., *WEE2*, *SLBP2*), while male fertility-related genes were identified in both populations (e.g., *TEX101*, *TSSK3*, *TESK2*, *TEX52*). These regions also included QTLs associated with residual feed intake, residual gain, carcass weight, stature, and body weight in the Bonsmara, while QTLs associated with conception rate, shear force, tenderness score, juiciness, temperament, heat tolerance, and age at puberty were identified in Nguni. Results of this study coincide with Nguni and Bonsmara breed characteristics and performance, and furthermore, support informative crossbreeding programs to enhance livestock productivity in South Africa.

Key Words: indigenous cattle, beef breed, crossbreeding, genetic diversity, climate change

P295 Whole-genome sequence analysis to detect potential candidate genes for reproduction in South African beef cattle. K. Nxumalo*[1,2], M. B. Malima[1], J. Grobler[2], M. Makgahlela[1,3], J. Kantanen[4], C. Ginja[5], D. R. Kugonza[6], N. Mohamed[7], R. P. M. A. Crooijmans[8], and A. A. Zwane[1], [1]*Animal Breeding and Genetics, Agricultural Research Council-Animal Production, Pretoria, South Africa,* [2]*Department of Genetics, University of the Free State, Bloemfontein, Free State, Bloemfontein, South Africa,* [3]*Department of Animal, Wildlife and Grassland Sciences, University of Free State, Bloemfontein, Bloemfontein, South Africa,* [4]*Animal Production Research, Agricultural Research Centre (MTT), Jokioinen, Finland.,* [5]*CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal,* [6]*Department of Agricultural Production, School of Agricultural Sciences, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda,* [7]*Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt,* [8]*Animal Breeding and Genomics Group, Wageningen University and Research, Wageningen, The Netherlands.*

Indigenous cattle of South Africa are well known for their great production under seasonally harsh environmental conditions. The breeds consist of unique morphological features that differentiate them from other breeds, which includes the color patterns and horn shape. However, there is need to account for the changing climate, therefore, new and better adaptation improvements are required for various livestock species. This study investigated genes associated with reproduction traits in South African indigenous cattle breeds namely; Afrikaner, Bonsmara, Nguni and Tuli. The Illumina whole-genome sequences from 43 individuals (Afrikaner (n = 10), Tuli (n = 8), Bonsmara (n = 10), Nguni (n = 15)) were generated at 10X coverage. The iHS statistical method was used to detect the selective regions within the breeds. Selection of signatures analysis revealed 54, 72, 86, and 92 genomic regions under positive selection in the Nguni, Afrikaner, Tuli and Bonsmara cattle, respectively. Within these regions, there were genes involved in the regulation of reproductive performance. From 322 genes identified, the analysis revealed 4 common significant genes (*YWHAZ*, *KHDRBS2*, *KDM4B*, and *SEMA5B*) associated with cow fertility under the threshold of 4%. Gene ontology enrichment analysis revealed that several biological pathways could be involved in the variation of fertility in female cattle. The identification of genes responsible for fertility may facilitate the better understanding of reproduction-associated traits in this South African indigenous cattle breed.

Key Words: fertility traits, candidate genes, South African cattle, whole-genome sequencing

P296 Withdrawn

P297 Copy number variations and their association with coat color phenotypes in South African Nguni cattle. N. M. Dlamini*[1,2], E. F. Dzomba[2], and F. C. Muchadeyi[1], [1]*Biotechnology Platform, Agricultural Research Council – Onderstepoort Veterinary Institute, Onderstepoort, Pretoria, South Africa,* [2]*Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Scottsville, South Africa.*

Copy number variations (CNVs) are a major source of genomic structural variation and can be utilized as markers to investigate the genetics of phenotypes of economic importance. This study reports a high-resolution CNV scan screened using Illumina's 777k Bovine HD Beadchip for South African Nguni cattle, a hardy multi-purpose breed that has undergone little synthetic breeding and is uniquely adapted to different ecological regions of South Africa. Population cluster analysis revealed 3 genetic clusters and after stringent quality control and filtering, and CNV screening using PennCNV software, a total of 1,742, 8524 and 1,225 CNVs in 26 animals in cluster 1, 91 animals in cluster 2 and 16 animals in cluster 3, respectively, were reported. From these CNVs, 3,432 CNV regions (CNVRs) covered a total of 270.48 Mb of the cattle genomic sequence and corresponded to 10.87% of the ARS-UCD1.2/*bosTau*9 genome assembly. DAPC analysis of the identified CNVRs was conducted to associate CNVRs to 5 coat color phenotypes (base coat color, color sidedness, forehead stripe, % body white and presence or absence of forehead stripes) and showed that CNVRs are involved in the development of these phenotypes with the animals clustering on the basis of these phenotypes. The associated CNVRs overlapped with a total of 4,199 different genes and 12 of these genes were associated with coat color/pigmentation mechanisms. The study identified variants that are potentially associated with traits under selection in Nguni cattle, particularly in genomic regions harboring QTLs affecting coat color providing baseline information for further analysis.

Key Words: copy number variation, Nguni cattle, CNVRs, coat color genes

P298 Population structure, inbreeding and admixture for indigenous goats within a pilot community-based breeding program in Pella, North West, South Africa. T. Mtshali*1,3, F. Muchadeyi[2], O. Mapholi[3], E. Dzomba[4], and K. Hadebe[2], [1]*Agricultural Research Council, Vegetable and Ornamental Plants, Pretoria, South Africa,* [2]*Agricultural Research Council, Biotechnology Platform, Onderstepoort, Pretoria, South Africa,* [3]*University of South Africa, Florida, Johannesburg, South Africa,* [4]*University of KwaZulu-Natal, Scottsville, Pietermaritzburg, South Africa.*

The level of inbreeding, genetic relatedness and population structure of goats within a community-based breeding program (CBBP) are crucial to guide decision making and sustainability of any breeding program. The current study investigates population structure, admixture, levels of inbreeding and runs of homozygosity (ROH) for indigenous goats within a pilot CBBP in Pella village, North West province, South Africa. Sixty-three goat samples from 38 participating households were genotyped using Illumina Goat50K SNP array. Principal Component Analysis and admixture ($K = 3$) revealed at least 2 genetic backgrounds which are

linked to geographic distances between the different households. Inbreeding levels ranged from 0 to 0.36. A total of 637 ROH with a mean of 29.97 ± 10.99 was observed in the study. Chromosome 1 had the highest number of ROH (n = 49), followed by chromosome 8 (n = 36) and the lowest was observed for chromosome 6. The smallest length classes < 5 Mb were more abundant (48%) than the longest segments (>40) which occurred less frequently (3%). High ROH coverage within the short category may indicate an ancient family relatedness. The low number of breeding bucks in the area should be considered a threat to the population's diversity. The study findings provided insights into the demographic history and the diversity of the goats within the CBBP and will guide future buck selection and exchange.

Key Words: relatedness, goat improvement, community-based breeding program

P299 Correlation between resilience and tolerance in Angus females exposed to *Rhipicephalus* (*Boophilus*) *microplus*. C. D. S. Arce*[1], F. R. Araújo Neto[2], A. M. Maiorano[1], L. G. Albuquerque[1], and H. N. Oliveira[1], [1]*Universidade Estadual Paulista "Júlio de Mesquita Filho," Jaboticabal, Sao Paulo, Brazil,* [2]*Instituto Federal Goiano, Rio Verde, Goias, Brazil.*

Resilience (R) is a function of resistance and tolerance (T). This study aimed to estimate the correlation between R and T traits of female Angus exposed to *Rhipicephalus B. microplus* using preweaning weight gain (PWG) as phenotype. Records of 546 animals were collected in 2014. Tick counts (TC) were performed as described in Wharton and Utech (1970). Two TC were performed with an interval of 41 d to avoid medical treatment residual between the counts. Animals were genotyped with the GPP Bovine 150k Illumina panel. Samples with call rate below 0.90 and SNPs located in the same positions were excluded. We used only SNPs located in autosomal chromosomes with call rate values greater than 0.98, minor allele frequency greater than 0.03, and Hardy–Weinberg values greater than 10^{-7}, totalizing 71,237 SNPs. Phenotypic observations that deviated from the mean by ± 3 standard deviations were removed. Contemporary groups (CG), based on the CG average for PWG and postweaning weight gain, were formed considering management group, year, and farm. R was computed in a 2-step analysis: first, the environmental gradients (EGs) were computed by applying a mixed regression to the log(TC + 1) values, based on estimated CGs effects; second, EGs were normalized using Z-Score and measured as resilience with a Reaction Norm Model (RNM). T was measured using log(TC + 1) as slope in an RNM. Genomic breeding values (gEBVs) were estimated using GBLUP method. Pearson correlation test was applied to the obtained gEBVs for R and T. The correlation coefficient was significant, high, and positive (0.95; $P < 0.05$), indicating that resilient animals are also tolerant. However, this result should be interpreted with caution since the maternal effect was not included in the analysis of PWG. Therefore, it is not possible to state that the linear association between these traits is due entirely to the direct genetic effect. The inclusion of maternal effect is recommended to elucidate its influence on the studied traits.

Key Words: animal breeding, cattle and related species, environment, genetic improvement

P300 Candidate positive signature of selection and environmental adaptation in indigenous African cattle: A review. S. Kambal*[1,2], A. Tijjani[3], and O. Hanotte[3,4], [1]*National University Biomedical Research Institute, National University, Sudan,* [2]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [3]*LiveGene – CTLGH, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [4]*School of Life Sciences, University of Nottingham, Nottingham, UK.*

The environmental adaptation traits of indigenous African cattle breeds have been the subject of much research in the last decades. Several studies have highlighted genomic regions under positive selection that are likely associated with adaptation to environmental challenges (e. g. trypanotolerance, thermotolerance and resistance to tick-borne diseases). However, little attention has focused on pinpointing these regions with candidate causative variant(s) controlling the traits of interest. This review compiled the results of 24 signature of positive selection studies involving indigenous African cattle breeds. In addition, it highlights candidate regions and genes that have been identified based on within and between population genome-wide selection scan approaches. Notably, candidate genomic regions on BTA7 and BTA5 contain the highest number of candidate genes. Furthermore, the reported candidate genes are involved in biological pathways related to innate and adaptive immunity (e.g., *BoLA*s, *SPAG11B*, *IL-7*, and *GFI1B*), heat (e. g. *HSP*s, *SOD1* and *PRLH*) and oxidative stress responses (e. g. *BDNF*, *TFRC*, and *OLA1*). This comprehensive insight may further guide studies assessing the importance of mutation within regulatory and protein-coding genome regions to understand the biological mechanisms underlying African cattle unique adaptive traits.

Key Words: African cattle, adaptation, genome, selection, candidate gene

Microbiomes Posters

P301 The effect of a total fishmeal replacement by *Athrospira platensis* on the microbiome of African catfish (*Clarias gariepinus*). S. Rosenau*[1], E. Oertel[1], A. C. Mott[1], and J. Tetens[1,2], [1]*Department of Animal Science, Goettingen, Germany,* [2]*Center of Integrated Breeding Research, Goettingen, Germany.*

An increasing number of fishmeal supplements are becoming the focus of aquaculture research, with a special emphasis on microalgae such as Spirulina being considered as sustainable alternatives. New feed ingredients can have a far-reaching impact on the intestinal microbiome and therefore play an important role in the development and health of fish. However, the influence of these alternatives on the microbiome is largely unknown. We undertook a 10-wk feeding experiment on 120 African catfish with an initial body weight of 50.1 ± 2.95 g. To understand the effect of the Spirulina supplementation, 2 isoenergetic experimental diets were formulated, containing either fishmeal (FM100) or Spirulina (SP100) as a protein source. Bacterial DNA was extracted from 18 intestinal fecal probes with a QIAamp Fast DNA Stool Mini Kit. 16S rRNA sequencing was used to analyze the intestinal bacteria microbiota. Results show that the observed richness indicated no significant statistical difference ($P < 0.05$), but Chao1, ACE, Shannon and Simpson indicate a possible increase in bacterial richness for SP100. The most abundant bacteria in FM100 and SP100 probes was *Fusobacteriia* with the only taxa from the genus *Cetobacterium* which plays an important role in the intestinal microbiome, due to its physiological benefits of synthesizing vitamin B-12 and antimicrobial metabolites. The bacterium from genus *Romboutsia* was more likely to be found in the microbiome of FM100. Since this genus was found to be reduced in low protein diets, we discussed the bioavailability of the Spirulina protein. In SP100, the genus *Plesiomonas* and *Bacteroides* were the most dominant microbes observed. Even though some genera were more abundant in the SP100 group, the overall microbial community structure was not affected by diets, but remarkably, we observed a high animal-to animal variation, which could be due to host genetic effects. Our sample was too small to estimate genetic effects, but this will be subject to further studies.

Key Words: fish, microbiomics, DNA sequencing, biodiversity, aquaculture

P302 Response to selection on fecal microbiota composition in Large White piglets. C. Larzul*[1], M. Borey[2], Y. Billon[3], M.-N. Rossignol[2], G. Lemonnier[2], J. Estelle[2], and C. Rogel-Gaillard[2], [1]*Université de Toulouse, INRAE, ENVT, GenPhySE, Castanet-Tolosan, France,* [2]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [3]*INRAE, GenESI, Surgères, France.*

Pig gut microbiota displays high interindividual variability and it remains an open question to determine to what extent its taxonomic composition relies on host genetic determinism and not only on environmental conditions. We carried out a study to demonstrate coevolution of the host and its gut microbiota established 1 mo post-weaning, by directional selection over 2 generations. The gut microbiota was characterized by sequencing the V3-V4 variable region of the 16S rRNA gene from fecal samples collected on 60-d-old Large White piglets. Amplicon sequence variants were inferred from amplicon data and the microbial community was further studied at the genus level. Based on the stratification of the initial population (generation G0) according to the 2 major pig enterotypes, characterized by relative overabundance of either *Prevotella* and *Mitsuokella* or *Ruminococcus* and *Treponema*, we used the relative abundance of these 4 genera as selection criteria. From the G0 population of 317 piglets, we selected 6 males and 30 females per line and produced 2 successive generations (G1 and G2) of approximately 130 pigs per line. We consistently confirmed a moderate heritability for each of the selected genera (h^2 = 0.3 to 0.4). We also estimated the heritability values of the relative abundances for 64 additional bacterial genera, which ranged from 0.1 to 0.5. We showed significant differences between the 2 lines in the relative abundance of the 4 bacterial genera at G1 ($P < 0.001$, from 0.6 genetic standard deviation for *Treponema* to 1.3 for *Prevotella*). In the following generation G2, response to selection was maintained for *Prevotella* and was even increased for the 3 other genera. The observed contrasts were in the expected direction for the genera under direct selection, and we extended the analysis to the 64 other bacterial genera with estimated heritabilities higher than 0.1. All these results confirm a significant influence of host genetics on the composition of gut microbiota at 60 d of age in pigs, and a capacity of directional selection over generations that will be further explored together with early and late host traits.

Key Words: pig, microbiomics, heritability, genetic improvement

P303 The impact of host genetics, independently of environmental factors, on porcine gut microbiota composition. A. Heras-Molina*[1], J. Estellé[2], A. López-García[1], J. L. Pensantez-Pacheco[1,3], S. Astiz[1], C. Garcia-Contreras[1], M. Vazquez-Gomez[4,5], B. Isabel[4], A. Gonzalez-Bulnes[6], and C. Ovilo[1], [1]*INIA (CSIC), Madrid, Spain,* [2]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [3]*School of Veterinary Medicine and Zootechnics, Faculty of Agricultural Sciences, University of Cuenca, Cuenca, Ecuador,* [4]*Faculty of Veterinary Medicine, UCM, Madrid, Spain,* [5]*Nutrition and Obesities: Systemic Approaches Research Unit (NutriOmics), INSERM, Sorbonne Université, Paris, France,* [6]*Departamento de Producción y Sanidad Animal, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, Valencia, Spain.*

Pig microbiota is associated with the host's breed, with lean and fatty breeds showing relevant differences in their productive parameters. However, in previous studies aiming to investigate the microbiota differences between both breed groups, involved animals were gestated in their corresponding lean or fatty mother, leading to maternal influences. The present study aimed to elucidate the importance of host's genotype on the gut microbiome composition without maternal confounding factors. Sixteen Iberian (IB; fatty breed) sows were inseminated with heterospermic semen (from Iberian and Large White [LW; lean breed] boars). Offspring was sampled at 60 d old (n = 36; 22 IB×IB and 14 IB×LW) and at 210 d old (n = 31; 18 IB×IB and 13 IB×LW) to obtain fecal microbiota composition which was analyzed by sequencing the 16SrRNA gene (V3-V4 amplicon) in an Illumina MiSeq. Bioinformatic analyses were done with QIIME2, and biostatistics analysis were performed using phyloseq and metagenomeSeq R packages. Firmicutes and Bacteriodetes were majority at the phylum level, while *Prevotella* and *Treponema* were the most abundant genera. Observed α diversity was only affected by age ($P < 0.0001$; higher at 210 d). For β diversity there was a genotype × age interaction ($P < 0.01$) and at 210 d, IB×IB animals showed higher β diversity than IB×LW ($P < 0.05$). The differential abundance analysis showed 156 significant (q <0.05) over-abundant amplicon sequence variants (ASVs) in IB×IB animals and 71 in IB×LW. At the genus level, *Anaerovibrio* and *Lachnospiraceae* were more abundant in IB×LW. At 60 d, most over-abundant ASVs observed in IB×IB animals belong to *Ruminococcus* genus, and at 210 d, IB×LW had the most over-abundant ASVs belonging to *Prevotella*. At the genus level, *Agathobacter*, *Parasutterella* and *Lachnospiraceae* were more abundant at 60 d old and *Ruminococcus* at 210 d old, all in IB×IB. These different abundant bacteria are involved in adipogenesis, feed efficiency, digestibility and inflammation, and could be related to breeds' phenotypic differences. Thus, the host genotype affected pig's gut microbiota composition, in a model that exclude maternal confounding factors.

Key Words: pigs and related species, metagenomics, breed diversity, pregnancy, meat production

P304 Assessment of the fecal microbiota from sow to piglet and the impact of different ratios of dietary polyunsaturated fatty acids. M. Cau*[1,2], A. Agazzi[3], T. X. Nguyen[3], M. McLaughlin[2], and A. S. Bonastre[1], [1]*Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain,* [2]*College of Medical, Veterinary and Life Sciences, School of Veterinary Medicine, University of Glasgow, Glasgow, UK,* [3]*Department of Health, Animal Science and Food Safety "Carlo Cantoni" (VESPA), Universitá degli Studi di Milano, Lodi, Italy.*

The microbiome can influence animal production and changes in the intestinal microbial status can lead to a deterioration or improvement of the animal's health status and therefore its quality as a food product. These changes can occur due to various factors including stress, antibiotics and diet. In the current study, we hypothesized that: 1) alteration in the fatty acid composition of the diet influence the gut microbiota of the sows, modify their milk and/or gut microbiota composition; 2) the milk from the sows influence the microbiota profile of the piglets; 3) the fatty acid composition and seaweed derived supplements of the solid diet, post weaning, can influence the microbiota of piglets over a longitudinal period (0–21 d). Microbial 16S DNA from 270 fecal samples from sows and their piglets were extracted and sequenced using the MiSeq system (Illumina) and analyzed with QIIME2. Sows were fed with a diet containing poly unsaturated omega fatty acid (PUFAs) at a ratio of ω6:ω3 = 4 (low ratio diet, LR) and one group fed with a ratio ω6:ω3 = 13 (control ratio diet, CR). At post weaning, piglets were subdivided and one group received additional dietary supplement derived from seaweed (*A. nodosum*), which can promote animal health and development. Statistically significant differences (q-value <0.05) were found in the evenness of the microbiome profile in the LR-fed sows at time points 0 and 14, and 0 and 21; a longitudinal change was not detected in CR-fed sows. With regard to piglets, there was no statistical evidence comparing piglets from LR and CR sows at time point 0 (after one week of lactation); this may suggest that the milk feeding did not influence the offspring's microbiota. A statistical difference is observed in piglets that received seaweed supplementation (SW) from sows of both diets during time point 7. We are currently examining the fecal metabolome of the cohorts of sows and piglets that demonstrate altered microbiome to gain further insight into the impact of diet on the mother and offspring gut composition and physiology. The results from this study form part of the European Joint Doctorate in Molecular Animal Nutrition (MANNA).

Key Words: microbiota, microbiome, pig, 16S, bioinformatics

P305 Mapping the livestock microbiome. M. Watson*, L. Glendinning, A. Warr, and J. Mattock, *The Roslin Institute, University of Edinburgh, Edinburgh, UK.*

The microbiome is the entire complement of microorganisms that exist in a given environment, including bacteria, archaea, microbial eukaryotes such as fungi and protozoa, and viruses. Despite their abundance and importance, the majority of microbial species remain undiscovered, and we lack the tools to study them. Genome assembly and binning of metagenomic data has become the dominant way of characterizing unknown and uncultured components of the microbiome, and recent studies have computationally isolated hundreds of thousands of bacterial and archaeal genomes from chickens, pigs, and ruminants. I will present our work in unraveling the livestock microbiome, including an analysis of currently available data sets, their strengths and weaknesses, discussion of bioinformatics tools for the reconstruction of genomes from metagenomes, a discussion of scale and potential solutions for producing more accurate metagenomic bins. I will also present evidence linking elements of the microbiome with traits of interest in livestock

Key Words: livestock, microbiome, trait

P306 Cecal microbiota composition of experimental laying hens infected with infectious bronchitis virus differs according to genetics and vaccination. M. Borey*[1], B. Bed'Hom[1,2], N. Bruneau[1], J. Estellé[1], F. Larsen[3], F. Blanc[1], M.-H. Pinard-van der Laan[1], T. Dalgaard[3], and F. Calenge[1], [1]*Université Paris-Saclay, INRAE, AgroParisTech, UMR GABI, Jouy-en-Josas, France,* [2]*Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France,* [3]*Aarhus University, Department of Animal Science, Tjele, Denmark.*

Interactions between the gut microbiota and the immune system may be involved in the responses to vaccination and infection. We studied the correlations between cecal microbiota composition and parameters describing the immune response in 6 different experimental laying hen lines vaccinated against the infectious bronchitis virus (IBV) and further infected with the pathogen. A cohort of 96 animals was vaccinated at 2 and 5 weeks of age (w.o.a) before an IBV infectious challenge at 8 w.o.a. We quantified peripheral leukocyte subsets and expression of cell surface markers through flow cytometric assay. We characterized the cecal bacterial communities with a 16S rRNA gene amplicon sequencing approach performed on animals killed 1 wk after the IBV infectious challenge. Based on the tracheal IBV load measured the first 5 d after the infectious challenge, 2 lines were considered as high responders to IBV vaccination. The host genetic background was only slightly associated with microbiota composition. In contrast, the effect of vaccination on cecal microbiota composition was strong and similar in all the lines, with a reduced abundance of OTU from the *Ruminococcaceae* UCG-014 and *Fecalibacterium* genera, and an increased abundance of OTU from the *Eisenbergiella* genus. The main association between the cecal microbiota and the immune phenotypes involved $TCR_{\gamma\delta}$ expression on $TCR_{\gamma\delta}^{+}$ T cells, which especially shared negative associations with OTU from the *Escherichia-Shigella* genus. These results confirm the existence of a complex interaction between cecal microbiota and immunity after vaccination.

Key Words: microbiota, infectious bronchitis virus (IBV), infection, vaccine, chicken

P307 Withdrawn

P308 Characterization of cecum microbiome between Silky Fowl and White Leghorns in the late laying period. X. Yang*, Y. Tai, Y. Ma, and X. Deng, *Laboratory of Animal Genetic Resources and Molecular Breeding, China Agricultural University, Beijing, China.*

In the late stage of laying, the intestinal digestion and absorption function of laying hens continues to decline, which has a certain impact on the production performance. The cecum microbiome to chicken health and productivity is important, especially in food conversion and resistance to disease. Eight 48-week-old Silky Fowl (SF) and 8 White Leghorn (WL) were selected under the same feeding conditions. Cecum microbiome composition at slaughter was analyzed by resequencing the V4-V5 region of the 16S rRNA gene in an Illumina Miseq. Amplicon sequencing analysis revealed that the richness (Chao 1 index) of cecum microbiome was higher in the SF than in the WL. PCOA results showed that there were significant differences in cecum microbial community structure between the 2 chicken breeds. *Bacteroidetes* and *Firmicutes* were predominant bacterial phyla in the 2 chicken breeds. *Bacteroidaceae* and *Veillonellaceae* were significantly differed at family level ($P < 0.05$). At the genus level, the relative abundance of *Bacteroides* was 1.57-fold enriched in WL compared with SF and significantly differed ($P < 0.05$). Using linear discriminant analysis effect size (LEfSe), under nonparametric Kruskal–Wallis and Wilcoxon rank-sum test with LDA >3.5, *Bacteroides* was a significantly enriched species in WL, which positively correlated with glycan biosynthesis and metabolism, carbohydrate transport and metabolism, carbohydrate degradation and glycan degradation; while in SF were *Veillonellaceae* and *Parabacteroides*, which positively correlated with fat acid and lipid degradation, amino acid degradation, lipid transport and metabolism, TCA cycle and xenobiotics biodegradation and metabolism. Collectively, our results revealed that several bacterial taxa abundances in cecum significantly differed between SF and WL, which caused differences in metabolic pathways, suggesting the potential roles of cecum microbiome contributing to maximizing the advantages of beneficial bacteria for the prevention of disease and the feeding and management of laying hens.

Key Words: cecum microbiome, 16S rRNA sequencing, late laying period, chicken

P309 Rumen eukaryotes are the main risk factors for larger methane emissions in dairy cattle. A. Saborío-Montero*[1,2], M. Gutiérrez-Rivas[1], R. Atxaerandio[3], A. García-Rodríguez[3], I. Goiri[3], J. López-Paredes[4], J. A. Jiménez-Montero[4], and O. González-Recio[1,5], [1]*Departamento de Mejora Genética Animal, Instituto Nacional de Tecnología Agraria y Alimentaria, Madrid, Spain,* [2]*Centro de Investigación en Nutrición Animal y Escuela de Zootecnia, Universidad de Costa Rica, San Pedro, San José, Costa Rica,* [3]*Department of Animal Production NEIKER-Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Campus Agroalimentario de Arkaute s/n., País Vasco, Spain,* [4]*Departamento Técnico de Confederación de Asociaciones de Frisona Española (CONAFE), Valdemoro, Madrid, Spain,* [5]*Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain.*

Mitigation of methane emissions from dairy cattle is relevant to reduce environment impact and increase profitability through improvement of energy usage. The objective of this study was to estimate how microbiome composition determines large methane concentration (MET) and methane intensity (MI, ppm CH_4/kg of milk) in comparison to more traditional proxies (*i.e.* milk yield and conformation traits). A total of 1,359 Holstein cows from 17 herds in 4 northern regions of Spain were included in this study, the microbiome composition data were a subset containing 437 cows from 14 herds. Cows were classified in quartiles for MET and MI, according to individual records of methane measurements during the cow's visit to the automatic milking system unit. A probit approach under a Markov chain Monte Carlo (McMC) Bayesian framework was used to determine risk factors for high MET and high MI. Genetic merit for methane concentration and microbiome composition (86 phylum and 1,240 genus) were the main drivers for a cow to be classified as high MET and MI. Reducing MET and MI genetic merit by one SD decreased the probability of being classified in the upper quartile by 35.2% (33.9% to 36.4%) and 28.8% (27.6% to 29.6%), respectively. A reduction in probabilities was observed as the relative abundance of most bacteria increased (i.e., Firmicutes 9.9% (8.3 to 11.3) for MET and 7.1% (6.2 to 8.2) for

MI, per unit of SD). An opposite effect occurred with eukaryotes. Larger abundance of most eukaryote became a risk factor to be classified as a high emitter animal (*i.e.*, Oomycetes 14.2% (11.7% to 16.4%) for MET and 11.8% (9.4% to 14.0%) for MI, per unit of SD). An increment of one unit of SD in milk yield increased the probability of being classified in the upper quartile for MET by 3.7% (2.3% to 4.2%) and reduced the probability for MI by 12.6% (12.2% to 13.3%). Structure and capacity traits were not main drivers of being classified in the higher quartile of methane emission and intensity, with risk odds lower than 2% per unit of SD. After genetic merit, microbiome composition was the most relevant risk factor for larger methane emissions. This study suggests that mitigation of MET and MI could be addressed through animal breeding programs including genetic merits and strategies that modulate the microbiome.

Key Words: genetic merit, microbiome, methane, dairy cow, risk factor

P310 Could the gut microbiome modulate environmental variance and animal resilience? C. Casto-Rebollo*[1], M. Argente[2], M. García[2], A. Blasco[1], and N. Ibáñez-Escriche[1], [1]*Institute for Animal Science and Technology, Universitat Politècnica de València, València, Spain,* [2]*Departamento de Tecnología Agroalimentaria, Universidad Miguel Hernández de Elche, Orihuela, Spain.*

The gut microbiome and their derived metabolites could influence the immune response and affect the host health. Environmental variance of traits (VE) has been related to the immune system and the animal resilience. Animals with a low VE seem to cope better with environmental disturbances, being more resilient. The aim of this study was to identify the metabolites from gut microbiota with different concentration levels between 2 divergent rabbit lines selected for a high and a low VE of litter size (LS). Untargeted metabolites from cecum samples of 28 does (14 from each rabbit line) were obtained using the Discovery HD4 platform of Metabolon. A total of 725 metabolites were identified. After quality control, 631 from 26 animals were maintained in the data set. We combine a 2-sided Mann-Whitney test and the logarithm to base 2 of the fold change to identify the metabolites with differences in means concentration levels between lines. Two partial-least square-discriminant analyses (PLS-DA) were performed to identify the relevant metabolites showing the largest contribution to the classification of the rabbit lines. The classification performance of the PLS-DA was computed using a 4-fold cross-validation. A total of 16 metabolites allowed a classification performance for the PLS-DA higher than 95%. From them, we highlighted the metabolites behenoylcarnitine, equol, ethyl-glucopiranoside, glycerophosphoglycerol, and dimethylglycine because also showed relevant differences in mean between the rabbit lines. These metabolites are involved in the lipid metabolism, xenobiotic metabolism, and amino acid metabolism. These could be relevant to modulate the VE and the animal resilience. However, further studies are needed to understand the effect of this metabolites in these rabbits. Currently, a shotgun metagenomic analysis from the same cecum samples are being performance to identify the bacteria and genes with differences in abundances between the rabbit lines. The final aim is to understand how the selection for the VE of LS has affected the gut microbiome and what if the gut microbiome can be an important factor to modulate the animal resilience.

Key Words: gut microbiome, environmental variance, metabolites, resilience, rabbit

P311 The potential of using rumen microbial profiles for the prediction of enteric methane emissions traits for commercial livestock breeding. T. Bilton*[1], M. Bastiaanse[1], M. Hess[1], J. Budel[2], G. Noronha[2], H. Henry[1], S. Hickey[3], G. Pile[1], P. Janssen[4], J. McEwan[1], and S. Rowe[1], [1]*AgResearch, Mosgiel, New Zealand,* [2]*Universidade Federal do Pará (UFPa), Belém Do Pará, Brazil,* [3]*AgResearch, Ruakura, New Zealand,* [4]*AgResearch, Palmerston North, New Zealand.*

The rumen microbiome has been associated with important livestock production traits. Breeding that targets the rumen microbiome composition, therefore, has potential to make further genetic gains in these traits. For this to be implemented, a low cost and high-throughput measure is required that has prediction power across systems and time. Hess et al. (2020) developed a low-cost, high-throughput restriction enzyme reduced representation sequencing (RE-RRS) method for metagenomic sequencing of rumen microbiomes. The gold standard (GS) approach for preserving rumen samples, however, requires laborious and low-throughput processing steps before sequencing, limiting utility for large scale phenotyping. We investigate the performance of rumen microbial profiles from RE-RRS for trait prediction and the feasibility of using a TNES lysis buffer solution (solution A) for preserving rumen samples before DNA extraction. Rumen samples from 3,139 sheep of different ages and on different diets was generated using RE-RRS. Different training sets were formed based on different combinations of ages and diets. To estimate prediction accuracies, 2 independent data sets of 150 different animals from the same flock sampled at 2 time points (PD1) and 93 animals from an entirely different flock (PD2) were generated using RE-RRS. Rumen samples were preserved using both the GS method and solution A. Methane phenotypes in CH_4 g/day were measured immediately before rumen sampling and rumen microbiome profiles were generated using the reference-free approach by Hess et al. (2020). A linear mixed model with a microbial relationship matrix was used for prediction. Prediction accuracies ranged between 29 and 38% within the training sets, between 23 and 51% for PD1 and between 8 and 28% for PD2. The difference in prediction accuracies using solution A compared with GS was 0.2% ± 2.7% for PD1 and 0.5% ± 3.4% for PD2. These results indicate that the rumen microbial profile can be used for trait prediction across flocks and suggests solution A is a viable, low-cost preservative method for rapid high-quality microbial DNA extraction. This development could significantly advance the implementation of rumen microbiomes as a phenotype for breeding in ruminant livestock.

Key Words: metagenomics, restriction enzyme reduced representation sequencing, sample preservation, genomic prediction

P312 Overcoming host contamination in bovine vaginal metagenomics studies with efficient host depletion, extraction and sequencing methods. C. Ong*[1], C. Turni[1], P. Blackall[1], G. Boe-Hansen[2], E. Ross[1], B. Hayes[1], and A. Tabor[1,3], [1]*The University of Queensland, Centre of Animal Science, Queensland Alliance for Agriculture and Food Innovation, Brisbane, Queensland, Australia,* [2]*The University of Queensland, School of Veterinary Science, Brisbane, Queensland, Australia,* [3]*The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Queensland, Australia.*

The reproductive tract metagenome plays a significant role in the various reproductive system functions, including reproductive cycles, health and fertility. One of the major challenges in bovine vaginal metagenome studies is host DNA contamination, which limits the sequencing capacity for metagenomics content and reduces the accuracy of metagenomic profiling. This is the first study comparing the effectiveness of different host depletion methods, DNA extraction methods and sequencing methods for bovine vaginal metagenomics samples. Soft-spin and QIAamp were most effective in reducing the amount of cattle genomic content in bovine vaginal samples. The DNA extracted from "Vaginal swab+Spike" with Soft-spin and QIAamp constituted 75.06% of 16S rDNA. The high microbe-to-host ratio in the extracted DNA increased the sequencing depth for microbial reads. Bovine vaginal samples extracted with Soft-spin and QIAamp presented taxonomical profiles which closely resembled the mocked microbial composition, especially for the recovery of gram-positive bacteria. Additionally, samples extracted with Soft-spin and QIAamp presented extensive functional profiles with deep coverage, for example "Vaginal swab+Spike" samples were reported with an average 2.65×10^7 functional annotations. Overall, a combination of Soft-spin and QIAamp provided the most robust representation of the vaginal microbial community in cattle while minimizing host DNA contamination. We also compared the efficiencies of various sequencing methods, includ-

ing Illumina 16S amplicon, Illumina shotgun, Nanopore and Nanopore adaptive sequencing. On the read-based level, Nanopore adaptive sequencing method consistently recovered higher percentage taxonomical hits from various taxonomic classification tools, including Kraken2 and taxMaps. Additionally, Nanopore adaptive sequencing method also recalled a higher percentage of functional annotations than Illumina shotgun and regular Nanopore sequencing methods. Our study evaluated the most efficient host depletion, extraction and sequencing methods to overcome the host contamination issue and for harvesting a more accurate representative metagenomics profile from bovine vaginal metagenome samples.

Key Words: cattle and related species, metagenomics, microbiomics, nucleic acid amplification, fertility

Pig Genetics and Genomics Posters

P313 Global analysis of the association between pig muscle fatty acid composition and gene expression using RNA-seq. J. Valdés-Hernández*[1,2], L. Criado-Mesas[1], Y. Ramayo-Caldas[3], A. Castelló[1], M. Passols[1], A. Sanchez[1,2], and J. M. Folch[1,2], [1]*Centre for research in agricultural Genomics (CRAG), Plant and Animal Genomics, CSIC-IRTA-UAB-UB Consortium, UAB Campus, Bellaterra, Spain,* [2]*Autonomous University of Barcelona (UAB), Department of Animal and Food Science, Faculty of Veterinary Medicine, Bellaterra, Spain,* [3]*Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Departament de Genètica i Millora Animal, Torre Marimon, Caldes de Montbui, Barcelona, Spain.*

Fatty acids (FA) are crucial for living organisms with a role in cell signaling, signal transduction, cellular differentiation, metabolic homeostasis, body energy homeostasis, protection of digestive tract mucosa. FA provided by the diet or derived from de novo lipogenesis are modified by the action of desaturases and elongases in lipogenic tissues (including muscle). We aimed to characterize the porcine *longissimus dorsi* (LD) muscle transcriptome and to study its association with FA composition in muscle. In a total of 129 backcross pigs (25% Iberian and 75% Duroc) the FA profile in LD muscle was determined by gas chromatography of methyl esters. RNA-Seq analysis was performed using an Illumina HiSeq 4000 instrument and sequence alignment with the Sscrofa11.1 porcine reference genome. Association analysis was performed with the limma (trend and voom approaches, Law et al., 2014) and ELMSeq (type I penalty function, Liu et al., 2018) programs. Here, we consider the FA composition and indices (n = 36) as the response variable, and the gene expression, sex and batch as the explanatory variables. A benchmarking reveals FA trait associated genes (FAAGs) identified with the 3 methods showing variable FAAGs number, even between limma-voom and limma-trend. Only 35.14% of the FAAGs were common among methods. We observed that he ELMSeq was able to detect more FAAGs (BH adjusted *P*-values <0.05). In summary, by ELMSeq we find an association of gene expression profiles in traits as: C18:2*n*-6/C18:3*n*-3 (*FADS2*, *SC5D*, *FOXO4*, *ILK*, *ACSL1*, *MDH1* and *GOT1*); ω6/ω3 (*FADS2*, *SC5D*, *LPIN1*, *NCOA2*, *ACSL1*, *MDH1* and *GOT1*); C16:1*n*-7 (*PLIN1* and *LEP*); C18:1*n*-9 (*ELOVL6*, *MDH1*, *PLIN1 and LEP*); C20:1*n*-9 (*PTGR1*, *ILK* and *MDH2*); MUFA (*ELOVL6*, *MDH1*, *PLIN1* and *LEP*); C20:2*n*-6 (*SC5D*, *PLIN1* and *ACAA1*); MUFA/PUFA (*ELOVL6*, *PLIN1* and *LEP*); C16:0 (*FBP1*); C18:0 (*FBP1*, *NMNAT2* and *ADSL*); SFA (*FBP1*, *NMNAT2*, *UGP2* and *IDH3A*) and unsaturation index (*FBP1*, *NMNAT2* and *IDH3A*). The current results contribute to elucidate the relationship between gene expression levels and variation of phenotypic fatty acid response traits in pig muscle.

Key Words: lipid metabolism, meat quality, swine, porcine, quantitative traits

P314 Genetic analysis of protein efficiency in Swiss Large White pigs. E. O. Ewaoluwagbemiga*[1,3], G. Bee[2], H. Pausch[3], and C. Kasper[2], [1]*Animal GenoPhenomics Group, Agroscope, Posieux, Switzerland,* [2]*Swine Research Unit, Posieux, Switzerland,* [3]*Animal Genomics, ETH Zurich, Lindau, Zurich, Switzerland.*

The improvement of protein efficiency (PE) is an essential factor in the development of a sustainable pig production, especially considering its contribution to environmental pollution through nitrogen excretion. Traits such as feed conversion ratio (FCR) and residual feed intake (RFI) are used to characterize feed efficiency in livestock. However, improving feed efficiency with the aim of reducing nutrient excretion may not be as efficient as selecting on the nutrient trait itself. The aim of this study was therefore to understand the genetic architecture of PE in Swiss Large White pigs by estimating its heritability and its genetic correlations with meat quality traits and production traits. A total of 541 pigs from an ongoing experiment were used to estimate the heritability of protein efficiency and 276 pigs were used to estimate its genetic correlations with meat quality and production traits. Pigs were slaughtered at 100 kg BW and carcass lean mass was measured with a dual-energy X-ray absorptiometry scanner. PE was calculated as the ratio of protein in the carcass to total dietary protein consumed. The production traits taken were average daily gain (ADG), feed conversion ratio (FCR), and average daily feed intake (ADFI). Meat quality traits such as meat color [lightness (L*), redness (a*) and yellowness (b*)], shear force, water holding capacity, as well as backfat thickness were recorded. The heritability of carcass PE was 0.40 [0.24, 0.59] and a common environmental (litter effect) variance of 0.21 [0.15, 0.34]. The heritability of PE clearly indicates the potential for selective breeding of PE in Swiss Large White pigs. Favorable relationships exist between PE and ADFI and FCR, which means protein efficient pigs are expected to consume less feed and efficiently convert the feed to lean mass. The quality of meat is not expected to be influenced by an improvement in the PE of Swiss Large White pigs, as no significant genetic correlations exist between PE and meat quality traits. Future work should aim at identifying SNPs associated with PE, as this will help to understand genes that underlie this trait, and thus accelerate genetic improvement of protein efficiency.

Key Words: protein efficiency, swine, heritability, genetic correlations

P315 An exon-intron split framework to prioritize miRNA-driven regulatory signals and its application to study energy homeostasis in pigs. E. Mármol-Sánchez*[1], S. Cirera[2], M. J. Jacobsen[2], Y. Ramayo-Caldas[3], C. B. Jørgensen[2], M. Fredholm[2], and M. Amills[1,4], [1]*Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain,* [2]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark,* [3]*Animal Breeding and Genetics Program, IRTA, Torre Marimón, Caldes de Montbui, Barcelona, Spain,* [4]*Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

MicroRNAs (miRNAs) are small noncoding RNAs that play a key role in posttranscriptional regulation of targeted mRNAs. Bulk sequencing of RNA transcripts has typically been used to quantify gene expression levels in different experimental systems. However, linking differentially expressed (DE) mRNA transcripts to gene regulators, such as miRNAs, remains challenging, as in silico or experimental interactions are commonly identified post hoc after selecting differentially expressed genes of interest, thus biasing the interpretation of underlying gene regulatory mechanisms. In this study, we performed an exon-intron split analysis to muscle and fat RNA-seq data from 2 Duroc pig populations subjected to fasting-feeding conditions and with divergent fatness profiles, respectively. We compared the number of reads from exonic and intronic regions

for all expressed protein-coding genes, and divided their expression profiles into transcriptional and posttranscriptional components, considering intronic and exonic fractions as estimates of the abundance of nascent and mature mRNA transcripts, respectively. In this way, we obtained a prioritized list of genes showing significant posttranscriptional regulatory signals. After removing unreliable in silico predicted miRNA-mRNA interactions, protein-coding mRNA genes with reduced exonic fractions and high posttranscriptional signals were significantly enriched for target sites of upregulated and DE miRNAs, as opposed to other downregulated mRNA genes. Moreover, these genes showed enriched expression covariation for the exonic but not for the intronic fractions. Among the set of loci displaying miRNA-driven post-transcriptional regulatory signals, we observed genes related to glucose homeostasis (*PDK4*, *NR4A3* and *CHRNA1*), cell differentiation (*MYO9A*, *KLF5* and *BACH2*) or adipocytes metabolism (*LEP*, *SERPINE2* and *RNF157*). Our results highlight an efficient framework to prioritize mRNA genes showing posttranscriptional signals linked to miRNA-driven downregulation using exonic and intronic fractions of commonly available RNA-seq data sets from domestic species.

Key Words: pig, genome regulation, microRNA, RNA-seq

P316 E2-ER system positive feedback induces *CYP19A1* expression to inhibit porcine granulosa cells apoptosis. Q. Li*, X. Du, Q. Zeng, L.-F. Wang, and Q.-F. Li, *Nanjing Agriculture University, Nanjing, China.*

Estrogen secreted from ovarian granulosa cells (GCs) has been proved to be a crucial regulator for the fate (maturation and ovulation, or atresia and degeneration) of follicular development in mammals. However, its action mechanism is largely unclear. In this study, we first showed that 17β-estradiol (E2), the principal representative form of estrogen, controls porcine GC apoptosis, the main inducement of follicular atresia. A total of 897 differentially expressed genes (DEGs) were identified in E2-treated porcine GCs by using RNA-seq, and DEGs were mainly enriched in the pathways associatedwith cell states and functions. Interestingly, we noticed that *CYP19A1*, encoded the key rate-limiting enzyme for estrogen synthesis, was upregulated in E2-treated porcine GCs. Furthermore, we demonstrated that E2-ERβ system and CYP19A1 forms a positive feedback regulatory loop which further inhibits porcine GC apoptosis. Mechanically, ERβ induces the transcription of *CYP19A1* by directly binding to the ER responsive elements (ERE) within the promoter of the porcine *CYP19A1* gene. The enrichment of ERβ on the promoter of *CYP19A1* gene is significantly increased after E2 stimulation. In addition, we also proved that the polymorphism of c.949G>A within the ligand binding domain (LBD) of ERβ is associate with sow reproductive traits (NBA, the total number of piglets born alive; NSB, number of stillborn) in a Large White pig population, through altering its sensitivity to E2 stimulation. Overall, our study uncovers a new mechanism underlying E2 regulation of sow reproduction, and provides a theoretical basis for improving sow fertility.

Key Words: sows, E2-ERβ system, *CYP19A1*, granulosa cell apoptosis, feedback regulation

P317 A SYBR Green qPCR for evaluation of factors affecting porcine semen sex ratio. N. R. Sahoo*[1,2], A. Santhosh[1], V. Yadav[1,3], M. V. Darji[1], N. Srivastava[1], P. Kumar[1], and G. K. Gaur[1], [1]*ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India,* [2]*ICAR-DFMD-ICFMD, Bhubaneswar, Odisha, India,* [3]*ICAR-National Dairy Research Institute, Karnal, Haryana, India.*

An alternative approach to semen sexing is often advocated in pigs owing to species-specific inherent limiting factors. This involves semen sex ratio enrichment in desired direction by modulating the factors influencing gametic sex ratio. We designed a qPCR-based protocol to determine the semen sex ratio (i.e., proportion of X- and Y-bearing spermatozoa). The target genomic regions from the sex chromosome were selected based on sex specific (Non-PAR) region and reports of single copy number as well as lack of involvement in structural aberrations. The primers were designed from 6 genes located in target region of either sex chromosome and screened for sex specific PCR amplification using genomic DNA of 30 (15 each from either sex) cyto-screened crossbred pigs (75% Landrace + 25% Ghurrah pigs) having numerically normal karyotype to find out a suitable X and Y specific primer set. A set of 7 designed reference gene primer pairs for relative copy number profiling of chromosomal genes were evaluated for copy number stability with SYBR green qRT-PCR using selected sex specific primer set. The relative copy number was characterized by *Ct* values in 3 technical replicates using 6 pigs from either sex. The stability was evaluated using various algorithms which revealed the most stable gene for normalization of copy number data (*IGF1R* > *FSHB* > *TCF24* > *IL4* > *ARMC1* > *SRSF4* > *BRMS1L*) across the samples. We also optimized a sperm DNA extraction method from those used for mammalian somatic cells to overcome the high degree of nuclear compaction in sperm. The effect of genetic group and processing on primary sex ratio was studied using the designed qPCR which revealed significant difference between 2 genetic groups (Landrace and Landlly) indicating breed influence. However, no significant variation was observed within group neither among the sires nor between ejaculates across spermatogenic cycles. Further, swim up technique produced a significant difference by X sperm enrichment with respect to control, whereas Percoll density gradient failed to show any.

Key Words: boar semen, gametic sex ratio, qRT-PCR, sire and processing factors

P318 Association of *LEP* and *CTSF* genotypes with levels of meat quality of Large White pig breed. V. Balatsky*, Y. Oliinychenko, K. Pochernyaev, A. Saienko, T. Buslyk, and I. Bankovska, *Institute of Pig Breeding and Agro-Industrial Production, National Academy of Agricultural Sciences of Ukraine, Poltava, Ukraine.*

Polymorphisms in leptin (*LEP*) and cathepsin F (*CTSF*) genes can influence meat quality traits in pigs. This study aimed to determine the distribution of genotypes by polymorphisms of the leptin (*LEP* SNP g.3469T>C, *LEP* SNP g.2845A>T) and cathepsin F (*CTSF* SNP g.22C>G) genes according to the quality levels: PSE (light, pale, soft, exudative), DFD (dark, firm, dry) and NOR (normal quality) meat in Large White pig breed and to find associations of genetic markers with the total indicator of meat quality ($TM = 5.4 + pH + 0.65 \cdot Z + 0.35 \cdot K$, where Z is staining intensity; *K* is consistency). 120 meat samples (*m. longissimus dorsi*) were studied from pigs of Large White breed raised to the weight at slaughter of 120 ± 5 kg. The ranking of muscle tissue was carried out according to the TM. PCR-RFLP analysis was used for DNA typing. Genetic population analysis of Large White breed pigs of Ukrainian selection by genetic markers *LEP* SNP g.3469T>C, *LEP* SNP g.2845A>T and *CTSF* SNP g.22C>G was carried out. The informative value of *LEP* SNP g.2845A>T and *CTSF* SNP g.22C>G was optimal for associative studies (PIC = 0.311 and 0.373, respectively). The distribution of meat samples by quality levels PSE, NOR and DFD was performed. Most of them had traits of moderately expressed (n = 22) and weakly expressed (n = 59) PSE defect. The calculated coefficients of Chuprov's mutual conjugation between the genotypes for the studied SNPs and meat quality levels showed a moderate relationship between the genotypes for *LEP* SNP g.2845A>T and *CTSF* SNP g.22C>G and meat quality levels, $K = 0.26$ and 0.24, respectively. According to the results of ANOVA, the differences were found between homozygous and heterozygous *CTSF* SNP g.22C>G genotypes in terms of the total indicator of meat quality. The meat of heterozygous pigs for *CTSF* SNP g.22C>G (g.22GC) is characterized by a higher total indicator of meat quality (4.6) compared with the meat of homozygous animals g.22GG (4.2, $P \leq 0.05$) and g.22CC (3.9, $P \leq 0.01$).

Key Words: pig, genetic marker, meat quality

P319 The common warthog (*Phacochoerus africans*) reference genome and sequence variation. L. Eory[1], P. Wiener[1], H. A. Finlayson[1], K. Gharbi[2], S. Girling[3], C. Palgrave[1], E. Okoth[4], T. Burdon[1], M. Watson[1], and A. L. Archibald*[1], [1]*The Roslin Institute and R(D)SVS, University of Edinburgh, Midlothian, UK,* [2]*Edinburgh Genomics, University of Edinburgh, Edinburgh, UK,* [3]*The Royal Zoological Society of Scotland, Edinburgh, UK,* [4]*International Livestock Research Institute, Nairobi, Kenya.*

The common warthog (*Phacochoerus africanus*) is an endemic, omnivorous species of savanna and woodlands of sub-Saharan Africa with conservation status of 'least-concerned'. The species harbor genotypes adapted to the environmental challenges posed by the hot and arid environment as well as by endemic pathogens. Common warthogs are known to be reservoirs of African Swine Fever Virus that causes a hemorrhagic disease with high mortality in domestic pigs while warthogs tolerate ASFV infections. High molecular weight DNA from a female individual was sequenced using Pacific Biosciences Sequel instruments yielding 150Gbp of raw data with a read N50 of 13.9kbp (ca. 60x coverage of the genome). A contig level assembly was generated using Falcon and Falcon-unzip. The contigs were scaffolded using Bionano optical mapping data and Bionano Solve software and proximity ligation data from a Hi-C library created using Dovetail Genomics kit. The scaffolds were refined with Dovetail Genomics' HiRise pipeline. Gaps in the assembly were filled with PBJelly using the PacBio long-read data. Error correction was done with Pilon using Illumina short-read data from the reference individual. The final assembly (GCA_016906955.1) comprises 2.4 Gbp in 718 contigs (contig N50 10.6 Mbp) with 1 scaffold for each of the 16 autosomes (PAF1–16) and PAFX plus 177 unplaced scaffolds (scaffold N50 141.88 Mbp). Comparisons with the pig (*Sus scrofa*) Sscrofa11.1 genome assembly confirmed the expected homologies including PAF1 representing a fusion of SSC13 and SSC16 and PAF3 representing a fusion of SSC15 and SSC17. RNA-seq data from 15 tissue samples from the reference individual have been used to identify the gene content of the genome. BUSCO analysis indicates that the assembly is highly complete with 94.9% BUSCOs (Benchmarking Universal Single-Copy Orthologs) complete. Whole-genome shotgun short-read sequence data were generated from 6 further warthogs at ca. 50x genome coverage. Analysis of these data plus ca. 100x coverage of the reference animal and 7x coverage of a further individual in the public databases with the GATK pipeline revealed 23,278,680 high-quality single nucleotide polymorphisms (SNPs).

Key Words: common warthog, *Phacochoerus africans,* genome sequence, single nucleotide polymorphisms (SNP)

P320 GBLUP-GWAS identifies candidate genes and polymorphisms for age at puberty in gilts. H. R. Wijesena*, D. J. Nonneman, W. M. Snelling, G. A. Rohrer, B. N. Keel, and C. A. Lents, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA.*

Age at puberty is the earliest known indicator for sow reproductive longevity. Gilts that reach puberty earlier have a greater probability of having more lifetime litters. Identifying genetic variants associated with age at puberty will facilitate genomic prediction for other related sow fertility traits expressed later in life. Gilts with puberty records and imputed genotypes for 71,634 SNP (n = 4,986) were used in a genomic BLUP (GBLUP) based genome-wide association (GWAS) for age at puberty. Twenty-one genome-wide significant SNP located in SSC1, SSC2, SSC9, and SSC14 were identified explaining 3.7% of the phenotypic variation of age at puberty. Some loci confirmed associations previously identified for age at puberty in gilts. Several genes within QTL regions (*GMFB*, *EWSR1*, *MEF2C*, *SOSTDC1*, *AHR*) were differentially expressed in ovaries of gilts with delayed puberty. The QTL on SSC9 (83.5 to 86.5 Mb) was characterized by long range LD (r^2 = 0.75 to 0.99) and estimated effects for significant SNP on SSC9 ranged from −1.14 to 1.35 d (P < 0.001). *AHR* located within this locus is a ligand activated transcription factor that regulates expression of *CYP1A* genes necessary for estrogen metabolism. *AHR* activation negatively affects ovarian function and association of *AHR* variants with age at puberty was previously reported in this population. The SNP with the largest effect on age at puberty on SSC2 (ALGA0116099; 82.7 Mb; P < 0.0001) had an estimated −1.8 d allelic substitution effect. *ANKRA2* located upstream of this SNP (~50Kb) acts as a corepressor for *AHR* by binding to C-terminus of *AHRR*, suggesting it is involved in mediating negative effects of *AHR* on puberty. Other genes with known effects on ovarian follicular development (*BMP4; SSC1*), gonadotropin secretion (*FOXD1; SSC2*), and pregnancy (*LIF; SSC14*) were identified within QTL regions. Transcriptomic data confirmed the presence of several polymorphisms in these genes, including 2 missense SNP in *ANKRA2*, that could act as potential functional variants affecting age at puberty. USDA is an equal opportunity provider and employer.

Key Words: gilts, age at puberty, GWAS, QTL

P321 A pangenome of commercial pig breeds. M. Derks*[1,3], B. Harlizius[2], M. van Son[2], M. Lopes[1], E. Grindflek[2], E. Knol[1], E. Sell-Kubiak[4], and A. Gjuvsland[2], [1]*Topigs Norsvin Research Center, Beuningen, the Netherlands,* [2]*Norsvin SA, Hamar, Norway,* [3]*Wageningen University and Research, Wageningen, the Netherlands,* [4]*Poznan University of Life Sciences, Poland.*

In recent decades, high-quality reference genomes have become available for most important livestock species. The availability of the reference genome (of Duroc origin) together with gene annotation have revolutionized pig genomics and genetics research over the past decade. However, sequences deviating considerably from the reference (i.e., from other breeds) will be interpreted as low-quality, so-called reference bias. One consequence of this is that a lot of (structural) variation not present in the reference genome is often missed. Structural variation includes various types of variation in which part of the DNA is altered (e.g., genomic deletions, duplication). Structural variants can have a large effect on phenotypes but they are often ignored or remain unidentified. Hence, the extensive degree of variation in pigs shows that a single reference genome does not represent all genomic variation within pigs, and a pig pangenome will be the future standard. The pig pangenome is particularly useful to identify presence/absence variations, structural variation, and other, miscellaneous variations. In this study we produced a pig pangenome by sequencing 4 pigs from different genetic backgrounds using the Nanopore long-read sequencing technology. The breeds comprise 2 dam lines, Large White and Landrace and 2 sire lines, Duroc and a synthetic line. We generated ~150X coverage Nanopore sequencing data (read N50: 40 kb) to produce the assemblies. We produced chromosome-level assemblies (using the Flye software) comparable to the current 11.1 in terms of completeness and continuity. We further identified between breed structural variation (using Syri and Sniffles tools), which gives a unique insight in the genomic structural variation that define and differentiate the breeds. In addition, we generated lower coverage long-read sequence data for 29 animals distributed over the 4 breeds to facilitate the discovery of within-breed structural variation. Together we have produced a pig pangenome covering 4 elite breeds from Topigs Norsvin. The pig pangenome will facilitate in the discovery of novel (structural) variation which provides a unique fundamental insight into breed genomic characteristics, which can subsequently be utilized to improve pig breeding.

Key Words: pangenome, Nanopore sequencing, structural variation

P322 Density gradient centrifugation to purify ejaculated sperm has a mild but noticeable impact on the pig semen transcriptome. Y. Lian*[1], M. Gòdia[1], A. Castello[1,2], J. E. Rodriguez-Gil[3], S. Balasch[4], A. Sanchez[1,2], and A. Clop[1,5], [1]*Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Barcelona, Catalonia, Spain,* [2]*Unit of Animal Science, Department of Animal and Food Science, Autonomous University of Barcelona, Barcelona, Catalonia, Spain,* [3]*Unit of Animal Reproduction, Department of Animal Medicine and Surgery, Autonomous*

University of Barcelona, Barcelona, Catalonia, Spain, [4]*Grup Gepork S.A., Barcelona, Catalonia, Spain,* [5]*Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Catalonia, Spain.*

The study of the sperm transcriptome is often preceded by the removal of non-sperm cells carrying a larger load of RNA. This purification is usually done by density gradient centrifugation to obtain viable spermatozoa from fresh ejaculates or artificial insemination doses, which limits the processing rate and the remoteness of the samples that can be analyzed in one experiment. To evaluate the impact of purification by density gradient centrifugation with BoviPure on the pig sperm, 4 boar ejaculates were purified with this commercial reagent. The purified and paired nonpurified samples were sequenced by RNA-seq and their transcriptomes were compared. 7,519 protein-coding genes were detected. Correlation, cluster and principal component analysis showed high resemblance between the purified and the nonpurified ejaculates. However, 368 and 4 genes displayed decreased and increased RNA levels after purification, respectively. There was an enrichment of translation, transcription and metabolic processes and specifically, of ribosomal protein genes. Moreover, the catalogs of differentially abundant genes and unaltered genes were contrasted against a list of genes with tissue-specific expression in pigs (ArrayExpress expression atlas from the European Bioinformatics Institute; E-MTAB-5895) and humans (GTEx portal). The list of differentially abundant genes was clearly enriched (1.3%) for genes from epididymal origin when compared with the list of unaltered genes (0.2%). On the contrary, most of the testis specific genes present in the boar sperm, showed unaltered RNA levels after purification. The list of differentially abundant genes also included several genes playing important roles during spermatogenesis. We detected no differences between both gene lists in the proportions of prostate, white blood, lymph node, tonsil, duodenum, skeletal muscle, liver and mammary gland specific genes. In summary, the BoviPure-based purification impacts on the RNA levels of a small number of genes, which is most likely caused by the removal of non-sperm cells or seminal exosomes with a distinct load of RNAs.

Key Words: swine, sperm purification, RNA-seq, differentially abundant genes

P323 Genomic variations of porcine cathelicidin PR-39 and determination of copy numbers using real-time PCR. B. Ahn, H. Jeon, M. T. Le, M. Kang, and C. Park*, *Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul, South Korea.*

Copy number variations (CNVs) of antimicrobial peptides (AMPs) in livestock could influence the innate immune response of individuals against infiltrating pathogens. We conducted sequence-based analyses of the genetic variations of *PR39* from cloned *PR39* amplicons from 4 pigs. The analysis identified diverse genomic sequences corresponding to 9 different CDS sequences (4 functional and 5 pseudogenes) encoding 7 different protein sequences (3 functional and 4 nonfunctional forms). In addition, we developed a genomic DNA-based typing method of *PR39* CNV using real-time PCR. Typing results of 44 pigs of 6 breeds showed a significant variation in PR39 copies in their genome, ranging 2 to 10 copies per individuals across all commercial breeds except for wild boar. The average copy number was largest in Korean native pigs (n = 8). The level of *PR39* expression increased in animals with higher *PR39* copy number, suggesting functional importance of the CNV. We also showed the structural conservation of the cathelicidin cluster containing region in mammals from analyzing 8 genomes. The current gap associated with the cathelicidin cluster of the porcine genome assembly Sscrofa11.1 may be attributed to the assembly failure due to the CNV of the *PR39* gene. The characterization of the AMP CNVs could benefit improving the genetic potential of animals against infiltrating pathogens especially for livestock animals in which genetics has been a critical tool for phenotypic improvements.

Key Words: *PR39*, copy number variation, antimicrobial peptide, swine, innate immunity

P324 Native pigs from Angola: Insights into their origins and unique genetic features. P. Sá[1], D. Santos[1], A. Leitão[1], J. M. M. Cordeiro[2], L. T. Gama[1], and A. J. Amaral*[1], [1]*CIISA - Centro de Investigação Interdisciplinar Em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisbon, Portugal,* [2]*Faculdade de Medicina Veterinária do Huambo, Huambo, Angola.*

The history of pigs in sub-Saharan Africa has been poorly studied. Previous studies with mitochondrial DNA suggest that Eastern and Western African pig breeds have different origins, most likely as a consequence of European colonization and trade routes. DAD-is (FAO) accounts for a large number of African indigenous breeds, most with an unknown status. Angola, in the western coast of Africa, has been through dramatic social events that have led to the disappearance of indigenous swine populations and the recent introduction of European exotic breeds has also contributed to the erosion of this indigenous swine repertoire. As an effort to investigate the genetic basis of remnant indigenous pig populations in Angola (ANG) we have generated whole genomes (10x depth) from animals (n = 4) of a remote local pig population in Huambo province which we have compared with 78 genomes of European and Asian pig breeds as well as European and Asian wild boar that are currently in public domain. The analyses of genetic diversity showed that ANG pigs still display high levels of genetic diversity (0.68) that are at the same level of European commercial breeds (~0.70) and higher than Iberian breeds (0.55). The principal component analysis showed that ANG pigs clustered together with the European cluster and clearly separated from Asian pig breeds. The F_{ST} for all breed pairs ranged from 0.13 to 0.25, and, as expected, ANG pigs display lower levels of genetic differentiation in comparison with European breeds. Finally, we have identified genomic regions that display outlier levels of genetic differentiation and of nucleotide diversity between ANG pigs and European breeds. While considering the comparisons ANG vs Iberian and ANG vs Commercial, we have identified a total 458 genes, of which 59 genes were common whereas the remaining correspond to selection signatures that are exclusive of each comparison. These exclusive sites harbor genes related to alternative splicing when ANG pigs were compared with Iberian and to genes related to immune system-derived pathways when ANG pigs were compared with commercial pigs. This study presents the first assessment of the genetic relationship between ANG pigs and other world breeds and uncovers unique selection signatures that may indicate adaptation features unique to this important genetic resource. These results are very important for the conservation of this population and for the establishment of a sustainable production chain.

Key Words: pigs, Africa, genome

P325 TGFBR2 is a novel substrate and indirect transcription target of deubiquitylase USP9X in granulosa cells. L. Yang*, X. Du, and Q. Li, *Nanjing Agricultural University, Nanjing, JiangSu, China.*

The X-linked deubiquitylating enzyme USP9X has emerged as an epigenetic regulator of diverse biological processes in development and disease. Recent reports demonstrated that several SMAD proteins, such as SMAD3 and SMAD4, of the TGF-β signaling pathway are substrate of USP9X. In this study, we report that TGFBR2, the type II receptor of the TGF-β signaling pathway, is a novel substrate of USP9X in porcine granulosa cells (GCs). Meanwhile, we also show that USP9X also indirectly increases TGFBR2 expression at posttranscriptional level through SMAD4/miR-143 axis in GCs. Western blotting showed that the protein levels of TGFBR2, were decreased significantly, while the protein levels of TGFBR1, were not changed significantly in USP9X-silencing GCs, indicating TGFBR2, not TGFBR1 is a potential substrate of deubiquitylase USP9X in GCs. IP assay with Ub-specific antibody showed that silencing of USP9X results in ubiquitination of TGFBR2 protein in GCs. Furthermore, MG132 could inhibit TGFBR2 degradation caused by USP9X-siRNA, indicating that TGFBR2 is a novel substrate of USP9X in porcine GCs. In addition, we demonstrated that USP9X controls TGFBR2-mediated TGF-β signaling pathway and GC apoptosis. Interestingly, we also

found that TGFBR2 mRNA levels were decreased in USP9X-silencing GCs. miR-143 has been shown to be a miRNA targeting porcine TGFBR2 gene, and a direct target of transcriptional factor SMAD4 in porcine GCs, we therefore speculate USP9X controls TGFBR2 transcription through SMAD4-miR-143 axes in porcine GCs. qRT-PCR and Western blotting showed that knockdown of USP9X suppresses SMAD4 protein levels and miR-143 expression, but reversed by SMAD4 overexpression. Furthermore, miR-143 inhibited TGFBR2 expression, and TGFBR2-mediated GC apoptosis, revealing that TGFBR2 is a functional target of miR-143 in GC apoptosis. miR-143 also inhibited TGF-β signaling pathway by suppressing TGFBR2 in GCs. In addition, we also demonstrated that miR-143 mediates the feedback regulation of TGFBR2 by SMAD4 in porcine GCs.

Key Words: porcine, TGFBR2, USP9X, TGF-β signaling pathway, granulosa cell apoptosis

P326 Differentiating pigs from wild boars from Poland based on *NR6A1* and *MC1R* gene polymorphisms. A. Koseniuk*, G. Smolucha, M. Natonek-Wisniewska, A. Radko, and D. Rubis, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

Conscious consumers search for food products consisting of defined reliable components. Wild boar meat products appear to be healthier than farmed pork products, because the animals grow in natural environment without any artificial nutritional additives. Although a porcine component can be simply identified, the differentiation between wild boar and domestic pig in meat products is still challenging. This preliminary study aimed to differentiate domestic pigs from wild boars based on *MC1R* and *NR6A1* polymorphisms and to identify admixture between these genomes. We studied samples obtained from wild boars from 2 regions of Poland and 5 pig breeds: Polish Landrace, Polish Large White, Zlotnicka White, Pulawska and Duroc. Along the MC1R gene sequence, we identified 4 polymorphic loci, comprising 3 codons. The "wild type" allele was found mostly in wild boar, but also in the Duroc and Zlotnicka White breeds. Non-wild-type alleles were identified in the vast majority of domestic pig samples, but also in 2 wild boar samples. Based on MC1R profiles, we conducted a population study, and revealed admixture between both genomes using STRUCTURE and NETWORK Software. Interestingly, an allelic discrimination assay with NR6A1 g.748C>T TaqMan probes revealed a clear separation of samples into 2 groups, the first including wild boar samples representing the C allele, and the second covering domestic breeds representing the T allele. This first study of NR6A1 g.748C>T in Polish pigs and wild boars did not demonstrate hybrid profiles. This might be an effect of strong selective pressure for body elongation to increase meat production and improve reproductive efficacy. As this is a preliminary study, such discrepancies should be clarified in a further study conducted on a larger number of samples. This could improve the power of both differentiating tests, which are conducted based on 4 polymorphic sites

Key Words: pig, wild boar, *MC1R*, *NR6A1*, genome admixture

P327 The genomic inbreeding trends in Italian heavy pig breeds over the last 25 years. G. Schiavo*[1], S. Bovo[1], A. Ribani[1], S. Tinarelli[1,2], V. Utzeri[1], M. Cappelloni[2], M. Gallo[2], and L. Fontanesi[1], [1]*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Bologna, Italy,* [2]*Associazione Nazionale Allevatori Suini (ANAS), Roma, Italy.*

The control of inbreeding is fundamental in managing livestock species. A high level of inbreeding leads to a decline in performance and fitness, a phenomenon known as inbreeding depression, and to the emergence of deleterious or lethal alleles that, in absence of inbreeding, would remain at a low frequency in the population. The Italian pig industry, mainly oriented to the production of the Protected Designation of Origin (PDO) dry-cured ham, is based on 3 heavy pig breeds, namely Italian Large White (ILW), Italian Landrace (IL) and Italian Duroc (ID). The breeding program of these breeds started about 25 years ago and information about pedigree has been recorded in the last decades. Inbreeding coefficient (F_{PED}) is traditionally calculated using pedigree records. With the advent of high-throughput genotyping platforms, new methods to calculate the genomic inbreeding have been developed, directly using genome information. One of the most effective methods is the detection of long stretches of the genome that is homozygous at each adjacent locus, called runs of homozygosity (ROH). The proportion of the autosomal genome covered by ROH can be used to estimate the level of genomic inbreeding (F_{ROH}) and the history of the population. In this work, we retrospectively analyzed F_{PED} and F_{ROH} over this period in these 3 breeds. A total of 3,400 ILW, 1940 ILA and 1,100 ID pigs born over the last 25 years have been genotyped with the 70K Illumina GGP Porcine HD and Illumina Porcine60K SNPchips. F_{PED} was computed with Inbupgf90 software from pedigree data. ROH were identified with PLINK version 1.9. Then F_{ROH} and F_{PED} were averaged over all animals born by year. Averaged F_{ROH} over all considered years was higher in ID; the trend of F_{PED} and F_{ROH} was increasing during years for all breeds. It is worth to notice that F_{PED} trend had a strongest slope with respect to F_{ROH}, indicating that, at a biological level, decades of selection did not worsen the inbreeding level of the breeds. The results indicated that both F_{ROH} and F_{PED} can be used to manage inbreeding levels in Italian heavy pig breeds and provided information that could be useful to manage these pig genetic resources.

Key Words: pigs and related species, population genomics, genotyping, inbreeding, single nucleotide polymorphism (SNP)

P328 Identifying muscle transcriptional regulatory elements in the pig genome. D. Crespo-Piazuelo*[1], O. González-Rodríguez[1], M. Mongellaz[2], H. Acloque[2], M.-J. Mercat[3], M. C. A. M. Bink[4], A. E. Huisman[5], Y. Ramayo-Caldas[1], J. P. Sánchez[1], and M. Ballester[1], [1]*Animal Breeding and Genetics Program, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Spain,* [2]*Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Génétique animale et biologie intégrative (GABI), Jouy-en-Josas, France,* [3]*IFIP-Institut du porc and Alliance R&D, Le Rheu, France,* [4]*Hendrix Genetics Research Technology and Services B.V., Boxmeer, the Netherlands,* [5]*Hypor B.V., Boxmeer, the Netherlands.*

The present work is part of H2020 GENE-SWitCH project which aims to deliver new functional genome information of two main monogastric farm species (pig and chicken) to better understand the genetic determinants of complex traits. Specifically, in this work, we aimed to identify regulatory elements of muscle transcriptome in the genome of 300 pigs (100 Duroc, 100 Landrace, and 100 Large White) using expression genome-wide association studies (eGWAS). For that purpose, muscle samples were obtained at slaughter and RNA was extracted using spin column-based kit and sequenced on the Illumina NovaSeq6000 platform. Counts were quantified by RSEM/1.3.0 and normalized by TMM (trimmed mean of M-values). Low expressed genes and those not present in at least 5% of the animals were removed, remaining 13,887 genes for further analysis. Through whole genome sequencing (NovaSeq6000 platform), 44,127,400 polymorphisms (SNPs and indels) were found among all the individuals. A total of 25,315,878 polymorphisms were kept after filtering out those with missing genotype data >0.1 and minor allele frequency <0.05. eGWAS were conducted between the filtered polymorphisms and the normalized expression data using the fastGWA tool from GCTA/1.93.2. After Bonferroni correction, a total of 8,099,604 significant associations were found between 4,814,732 polymorphisms and the expression of 7,496 genes. A total of 25,642 polymorphisms were associated with more than 10 genes and were considered hotspot regulatory elements. Regarding their genomic position, 3,283,835 polymorphisms (68%) were annotated as cis-regulatory elements, as they were located at 1Mb or less than their associated gene. The most significantly associated variant (adj.

P-value = 2.66× 10^{-178}) was located on an intronic region of the SRSF protein kinase 3 (*SRPK3*) gene, which encodes a protein associated to muscle development. Our results identified key regulatory elements associated with gene expression in muscle that may be included in new predictive models to increase the accuracy of genomic predictions, speeding up the rate of genetic improvement of economically important traits in pigs. This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the grant agreement no. 817998.

Key Words: pigs and related species, system genetics (eQTLs), RNA-seq, muscle, regulatory element

P329 Characterization of circulating microRNAs profile in Iberian pigs with and without heat stress. M. Muñoz*[1], A. Fernández-Rodríguez[2], F. García[1], A. García-Cabrero[1], C. Caraballo[1,3], G. Gómez[4], G. Matos[4], C. Óvilo[1], and J. García-Casco[1,3], [1]*Animal Breeding Department, INIA (CSIC), Madrid, Spain,* [2]*Unit of Viral Infection and Immunity, National Center for Microbiology, Institute of Health Carlos III, Majadahonda (Madrid), Spain,* [3]*Centro de Investigación en cerdo Ibérico INIA-Zafra (INIA, CSIC), Zafra (Badajoz), Spain,* [4]*Sánchez Romero Carvajal—Jabugo, SRC, Huelva, Spain.*

In the last century, the average surface temperature of the planet rose almost one degree, being 2016 the warmest year since records are available. One of the consequences of climate warming is heat stress, that alters the physiology of animals reducing female and male reproduction. Iberian pigs have been adapted to high temperatures during centuries, however, a seasonal effect on reproductive traits such as the number of piglets born alive or the number of weaned piglets has been observed. Circulating microRNAs (ECmiRNAs) are small regulatory RNAs (size <22 nt) proposed as biomarkers reporting physiological estates. We have analyzed the circulating microRNA profile in plasma samples of 9 Iberian sows and 6 sires at 2 different months: June (heat stress; HS) and November (without heat stress; NHS) with an average temperature of 23.84°C and 9.84°C, respectively. Small RNA libraries were built with NEBNext Small RNA Library kit using 1x50nts single ends and sequenced in an Illumina HiSeq2500. After quality control and trimming, the sequences were processed using miRDeep2 (v. 0.0.7) software which characterizes known and novel microRNAs. Afterward, differentially expressed ECmiRNAs between HS and NHS months were detected using edgeR software. DIANA-miRPath v3.0 was used to detect the in silico targets of the differentially expressed ECmiRNAs and a pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG). We obtained a total of 11.63 million of reads per sample on average and 8.64 million of reads after trimming and quality control. On average, a total of 5.32 million reads (60.67%) mapped against the reference porcine genome assembly (Sscrofa11.1), being a 14.14% identified as microRNAs. A total of 249 known and 4 novel ECmiRNAs were identified in the sample set. Differential expression analyses stratified by sex were carried out, being 4 ECmiRNAs differentially expressed in females (ssc-miR-30c-5p, ssc-let-7a, ssc-miR-361-5p and ssc-let-7e) and none in males. A total of 39 KEGG pathways such as oocyte meiosis or fatty acid biosynthesis and metabolism affected by these miRNAs were observed. These results suggest the 4 differentially expressed ECmiRNAs could be used as biomarker for heat stress in Iberian pigs.

Key Words: pigs and related species, microRNAs, environment, reproduction

P330 Rate of rejection of first-degree relationships for assigning parent-offspring relationships and estimation of genotyping errors with an HD array in pigs. L. Gomez-Raya*[1], E. Gomez Izquierdo[2], E. de Mercado[1], and W. M. Rauw[1], [1]*INIA-CSIC, Madrid, Spain,* [2]*ITACyL, Hontalbilla, Spain.*

A first-degree relationship shares about 50% of their genes. Individuals with a first-degree relationship cannot share the 2 alleles at a genetic marker; that is, they cannot be homozygotes for alternative alleles. Applying this concept to HD arrays typed in individuals with a mixture of first and other relationships may allow detection of first-degree relationship (parent-offspring or full-sibs). We define genome-wide rate of rejection of first-degree relationships as the rate at which 2 individuals typed for a large number of SNPs do not share at least one allele. Although first-degree relationships would impede markers to be homozygotes for alternative alleles, in practice, genotyping errors occur. A model of mixture of 2 binomials with parameters of the rate of genotyping errors and a general rate of rejection is proposed. A solution is found via Expectation-Maximization algorithm and tested with a crossbred experiment of Iberian x Duroc together with an Affymetrix 600K array. There were 9 candidate sires and 55 dams that produced 214 burrows. We were able to establish paternity and maternity of 75 and 101 piglets, respectively. A lower bound of the genotyping error rate among autosomal SNPs was estimated in 0.1243%. There were 8,652 SNPs that were rejected in 6 or more truly first-degree relationship and were eliminated for further analysis. This study shows that animal experiments allow to establish or to verify first-degree relationships as well as to estimate genotyping errors.

Key Words: swine, HD array, SNP, paternity testing, genotyping errors

P331 SNP discovery and association study for growth and fatness traits in crossbred Iberian Pigs. C. Ovilo*[1], N. Trakooljul[2], F. Hadlich[2], E. Murani[2], M. Ayuso[1], C. García-Contreras[1], M. Vázquez-Gomez[3], R. Benítez[1], Y. Núñez[1], A. Rey[3], A. González-Bulnes[1], B. Isabel[3], K. Wimmers[2], and M. Muñoz[1], [1]*INIA (CSIC), Madrid, Spain,* [2]*FBN, Dummerstorf, Germany,* [3]*UCM, Madrid, Spain.*

Duroc × Iberian crossbred pigs are the main genotype employed to produce high-quality Iberian meat products, although their meat quality is lower than in pure Iberians. Besides, Iberian pigs are characterized by highly heterogenic developmental traits. The objective of this work was to discover and evaluate a wide panel of DNA markers for traits involved in muscle growth, fatness and meat quality. We used 18 crossbred Iberian pigs with divergent postnatal growth patterns for whole-genome sequencing. An average of 83 million paired-reads were generated per sample by Illumina sequencing, with a mean 8.4X coverage. After quality control, 98% of the reads were mapped to the reference genome (Sscrofa11.1) using BWA, and over 13 million variants were detected with GATK tools. MAF and variant quality filters were applied. Annotation was performed with VEP software. We focused on missense SNPs located on annotated genes and showing divergent frequencies between growth pattern groups. Around 1,000 SNPs were prioritized. Besides, we complemented this panel with 192 candidate SNPs obtained from the bibliography and from pure and Duroc-crossbred Iberian muscle RNA-seq data. The selected markers were genotyped by genotyping-by-sequencing in 480 Iberian × Duroc pigs from a commercial population, in which phenotypes were obtained for several traits including sequential measurements of body weight (BW), average daily gain (ADG), fatness and tissue fatty acid (FA) composition. The association study was performed for the 1,072 successfully genotyped SNPs showing segregation within our population. The results confirmed the effects of several known SNPs in candidate genes (such as *LEPR*, *ACACA*, *FTO*, *ADIPOQ*, *IRX3*, *MYOD1*, *LIPE* or *SCD* on fatness, growth and FA composition) and also disclosed interesting effects of new SNPs in less known genes; such as *LRIG3*, *DENND1B*, *NMNAT1*, *SOWAHB*, *TFRC* or *NFE2L2* affecting BW and ADG at different ages, or *KRT10*, *NLE1*, *KCNH2* or *AHNAK* affecting fatness and FA composition. The results provide valuable tools for the future implementation of marker-assisted selection strategies and contribute to a better understanding of the genetic architecture of relevant traits.

Key Words: SNP, association, Iberian pig, sequencing

P332 Transcriptomics integrated with metabolomics reveal the complex molecular regulatory network involved in meat quality in Enshi Black pigs. Y. Ma*, H. Zhan, S. Xie, X. Li, and S. Zhao, *Key Lab-*

oratory of Agricultural Animal Genetics, Breeding, and Reproduction of the Ministry of Education and Key Laboratory of Swine Genetics and Breeding of the Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei, China.

Improving meat quality is a crucial purpose of commercial production and breeding system. Here, we employed Enshi Black pigs to perform the multi-omics analysis in meat quality. Enshi Black pig is an important Chinese indigenous pig breed, found in the southwest of Hubei Province – Enshi Tujia and Miao Autonomous Prefecture. Enshi Black pig is famous for its excellent production traits; for example, elasticity meat texture, great adaptability, and fat storage ability, making it one of the typical meat-lard breeds. Depending on its admirable flavor, Enshi Black pig is widely sold as upscale production and quality raw materials, but excessive diversity in meat quality will badly affect the sale and obstruct the extension of Enshi Black pig pork industry. Samples selected based on IMF and meat color were examined for gene expression through RNA sequencing and metabolite composition through a nontargeted approach using high-resolution mass spectrometry. To investigate the complex biological processes associated with phenotype, differentially expressed genes and significantly changed metabolites identified based on target traits were applied into correlation and gene-metabolite network analysis. The functional pathways enriched concentrated on the processes of fat accumulation and regulation of lipolysis, and nicotinate and nicotinamide metabolism process which may regulate meat color. The results demonstrate a list of candidate genes involved in trait-related pathways. Besides, it presents a series of metabolites as available biological indicators to measure meat quality. This research provides further insight into the detection of intramuscular fat accumulation and meat color variation, and provides a reference for molecular mechanisms in the regulation of IMF and meat color.

Key Words: pork, IMF, meat color, transcriptomics, metabolomics

P333 MSTN regulates fat distribution in different pathways through GR. Y. Niu*, X. Wen, W. Lin, L. Zhang, and J. Chen, *College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China.*

Intramuscular fat content is one of the key factors affecting pork quality. However, because the deposition of intramuscular fat and subcutaneous fat presents a moderately positive correlation, increasing the intramuscular fat content often leads to excessive accumulation of subcutaneous fat. Our previous studies found that the muscle secreted factor MSTN regulated intramuscular fat deposition through the smad4/miR-124-3p/GR pathway. Research showed that body fat distribution was a better way to predict insulin resistance and related complications than total fat mass. To further study the mechanism of fat distribution, smad4 binding element (SBE) knockout mice in miR-124-3p promoter and miR-124-3p binding element (miRBE) mutation mice in GR 3′UTR were made using the CRISPR/Cas9 system. To SBE knockout mice, GR and PPARγ expression were significantly higher in abdominal fat tissue and not different in subcutaneous fat tissue after treated with MSTN compared with nontreatment, while GR and PPARγ expression were not different in abdominal fat tissue but significantly lower in subcutaneous fat tissues of wild mice. And the weight of abdominal fat tissue was significantly higher in SBE knockout mice after treated with MSTN compared with nontreatment, but the weight of subcutaneous fat tissues was not significantly different. To miRBE mutation mice, there were no differences in GR and PPARG expressions in cultured abdominal and subcutaneous fat tissues after treated with MSTN compared with nontreatment, while GR and PPARG expressions were significantly lower in cultured abdominal and subcutaneous fat tissues of wild mice. And the diameters of mature adipocytes were no significantly different in cultured abdominal and subcutaneous fat tissues of miRBE mutation mice after treated with MSTN compared with nontreatment, which were significantly lower in cultured abdominal and subcutaneous fat tissues of wild mice. Our results indicated that MSTN regulated abdominal and subcutaneous adipose deposition both through miR-124-3p/GR pathway, but the pathway between smad4 and miR-124-3p regulating abdominal and subcutaneous adipose deposition was complex, also we will further investigate the difference in abdominal and subcutaneous adipose deposition.

Key Words: MSTN, GR, pathways, fat distribution.

P334 Alteration of expression of miRNA and mRNA transcripts in fetal muscle tissue in the context of sex, mother, and variable fetal weight. S. Ponsuksili*[1], A. Ali[1], F. Hadlich[1], E. Murani[1], and K. Wimmers[1,2], [1]*Leibniz Institute for Farm Animal Biology (FBN), Institute for Genome Biology, Dummerstorf, Germany,* [2]*Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany.*

Prenatal embryonic and fetal development are important processes that are closely linked to birth weight and piglet survival, as well as subsequent growth performance and final carcass quality. In particular, skeletal muscle growth and mass are largely determined during the prenatal period, when the number of muscle fibers is almost fixed. Prenatal muscle growth is regulated by complex molecular pathways that are not well understood. MicroRNAs (miRNAs) have emerged as key regulators of vital pathways and biological processes, including developmental processes such as myogenesis. The study aimed at elucidating molecular routes of developmental processes mediated by miRNAs and the expression of mRNA targets in fetal muscle tissue at the background of variable fetal weights. Analyses were performed in an experimental population based on reciprocal crossing of German Landrace (DL) and Pietrain (Pi) to obtain a 3-generation pig F_2 population. The sows were slaughtered and the F_2 fetuses (n = 118) were extracted from the uteri and their weight was recorded. Expression profiling for mRNAs and miRNAs of M. longissimus dorsi was done using microarrays. The effect of sex, dam and fetal weight on the expression levels of transcripts was analyzed using a linear model (GLM procedure, SAS 9.4 Software, SAS Inc., Cary, USA containing the fixed effects (sex and mother) and fetal weight as a covariate. Transcripts with FDR <0.05 were considered as a significant threshold. The abundance of 13 mRNA transcripts was found to be significantly affected by sex, most of them were located on the sex chromosome, except ANKS1B and LOC100155138. Moreover 853 and 275 probe-sets were influenced by the dam and fetal weight at 63 dpc, respectively. Only miR-153 was differentially expressed due to sex, the expression of 13 miRNAs was influenced by dam and 6 miRNAs by fetal weight. In addition, we identified 343 pairs of 12 miRNAs and 152 in silico predicted and negatively correlated mRNA target transcripts that showed correlation with fetal weight. The correlated miRNAs and their target genes were enriched in key KEGG pathways and biological processes. These results demonstrate triangular relationships between miRNA expression in fetal skeletal muscle tissues, their mRNA targets and fetal weight at 63 dpc of prenatal development in pigs.

Key Words: fetal weights, pig, miRNA, mRNA

P335 Integrated transcriptomes and functional analyses in porcine oviductal tissue through porcine estrous cycle. M.-J. Jang*, C. Lim, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

Reproductive traits of pigs including litter size, litter per sow per year are closely related to breeding goals. The estrous cycle is regulated by hormones and understanding the complex changes in the reproductive system is important in solving the problem of reproduction. To understand the regulatory mechanisms through the dynamic changes in gene expression of the oviduct according to the estrous cycle, which is the place where the fertilization occurs, and the fertilized egg moves to the uterus for implantation, whole transcriptome was examined by RNA-seq using oviduct tissue samples of day 0, 3, 6, 9, 12, 15 and 18 of estrous cycle. Pig genome was annotated using Sus scrofa 11.1.98 and gene expression profiling was performed using R package edgeR. Differentially expressed

genes (DEGs) were extracted by FDR <0.05, |$\log_2$ fold change (FC)| ≥ 1. As a result, 7,623 significant DEGs were detected (upregulated genes 2,692, downregulated genes 4,931) and the genes were partitioned into 3 clusters according to the expression pattern. Cluster 1 and 2 (1,222 and 1,146 genes) showed overall physiological changes through the estrous cycle. Cluster 1 majorly involved in PI3K-Akt signaling and steroid hormone biosynthesis pathways and Cluster 2 were involved ECM-receptor interaction and protein digestion. The expression pattern of Cluster 3 was uniquely downregulated at luteal phase and network was constructed with 1,000 genes. KEGG pathway enrichment analyses revealed that DEGs in Cluster 3 were associated with cell cycle, calcium signaling, oocyte meiosis etc. As a result of gene set enrichment analysis (GSEA) networking, calcium signaling pathway and oocyte meiosis were also significantly observed in the luteal phase. Calcium signaling pathway which calcium causes sperm hyperactivity by progesterone and helps oocyte transport through smooth muscle contraction and oocyte meiosis affect oocyte migration and fertilization. As genes directly related to fertilization, the most important role in the oviduct, were relatively downregulated in the luteal phase, it can be inferred that genes are relatively upregulated in the follicular phase and play a prominent role in reproductive tissues during estrous cycle.

Key Words: pigs and related species, functional genomics, RNA-seq, gene expression, reproduction

P336 Time serial ovarian transcriptome analysis for entire porcine estrous cycle reveals changes of steroid metabolism and corpus luteum development. Y. Park*, Y.-B. Park, S.-W. Lim, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

The estrous cycle (estrus, metestrus, diestrus, and proestrus) is a physiological process that occurs in most mammalian females under the effect of reproductive hormones. This cycle affects reproduction and causes many changes, especially in the reproductive organs. Among them, an ovary is an important place where ovulation, luteinization, CL development, and luteolysis take place. For a more in-depth study of the dynamic changes in gene expression, the transcriptome of the porcine ovary was observed in the estrous cycle at intervals of 3 d from d 0 to d 18. A total of 4,414 DEGs were identified at 7 time points of the estrous cycle, and these were classified into 3 clusters according to the transcriptome expression pattern. During diestrus, the expression of the transcriptome increased rapidly, and cluster 1 was upregulated at that period, whereas clusters 2 and 3 tended to be downregulated. We performed functional analysis of the genes included in each cluster, selected KEGG pathways related to the gene ontology (GO) terms, and identified significant genes among them. In cluster 1, GO results were found that included terms such as intestinal absorption and sterol biosynthesis, and based on this, steroid biosynthesis was selected for the significant KEGG pathway. In cluster 2, cytokine-cytokine receptor interaction was chosen as important KEGG pathways based on GO outcomes such as neutrophil chemotaxis and regulation of timing of cell differentiation. Finally, morphogenesis and embryo development-related terms were shown in cluster 3, and the hedgehog signaling pathway was selected. Our study exhibited the dynamic changes and a comprehensive understanding of the porcine ovary during the estrous cycle through DEG profiling and transcriptome analysis. Especially, we found several genes that were affected to hedgehog signaling pathway and consequently, this suggests that genes that influence embryonic development during the diestrus are expressed in the ovary. Further studies should be conducted with every estrous cycle to understand the mechanism of the porcine ovary.

Key Words: pigs and related species, functional genomics, gene expression, RNA-seq, reproduction

P337 Overview of long noncoding RNA and mRNA annotation throughout swine estrous cycle in reproductive tissues. Y.-B. Park*, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

Long noncoding (lnc) RNAs were reported to regulate target genes at transcriptional, posttranscriptional, and posttranslational regulation levels in a wide variety of species. The reproductive traits of porcine are very closely related to economics among economic traits, and it has been understood through various preliminary studies that several reproductive tissues are involved in a complex manner. However, unlike mRNA, lncRNA annotation has not been sufficiently performed, and understanding at the level of protein-coding gene has limitations in determining the mechanism of reproductive traits of porcine. Therefore, we investigated lncRNAs of porcine ovary, oviduct and endometrium at d 0, 3, 6, 9, 12, 15, or 18 of the estrous cycle. We focused on analyzing the expression patterns of mRNA and lncRNA and finding the key lncRNA through network analysis. The patterns of differentially expressed mRNA and differentially expressed lncRNA were similar, and we identified 10 key lncRNAs. Our analysis suggests lncRNAs and mRNAs that are differentially expressed according to the porcine reproductive tissue and estrus cycle and are involved in the reproductive trait mechanism by regulating pathways such as steroid hormone synthesis.

Key Words: pigs and related species, bioinformatics, noncoding RNA, RNA-seq, gene expression

P338 Signature of stress-related characteristics according to changes in pig breeding condition through transcriptome analysis. S.-W. Lim*, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

Pig breeding condition, such as density-stress, is one of the important factors in terms of pig productivity. However, the molecular mechanisms for the level of whole-genome expression depending on the pig breeding condition has not been well studied. The purpose of this study is to identify functional mechanisms according to changes in pig breeding condition through transcriptome analysis. In this study, we accepted samples from 3 conditions (control, welfare and density) and observed their transcriptomic changes using the RNA-seq method. Gene alignment and gene transfer format of the entire pig genome was annotated using Sus scrofa 11.1.102. Whole gene expression profiling was performed using a general linear model using edgeR of R package. The differentially expressed genes (DEGs) were extracted by the *P*-value <0.01 with absolutely expressed for double changes to each comparison group. As a result, for each group genes were identified into the DEGs (welfare vs. control; a total of 109 genes, downregulated genes 62 and upregulated genes 47, density vs. control; a total of 199 genes, downregulated genes 126 and upregulated genes 73, welfare vs. density; a total of 135 genes, downregulated genes 55 and upregulated genes 80). Gene Ontology (GO) functional enrichment analyses distinguished the DEGs primarily associated with metabolism, signaling molecules and circulatory systems. KEGG pathway enrichment analyses revealed the DEGs primarily associated with oxidative stress signaling and immune signaling. Therefore, the biological process in breeding condition suggests that the expression of stress-related genes through metabolic and immune signals were high. We believe that further research would be required to understand more precise mechanisms.

Key Words: pigs and related species, functional genomics, RNA-seq, animal welfare, behavior

P339 Muscle proteomics of preweaning piglets from sows fed diets with extreme ω-6/ω-3 fatty acid ratios. Y. Manaig*[1,3], A. Agazzi[3], S. Panseri[3], G. Tedeschi[3], J. Folch[1,2], A. Sanchez[1,2], and G. Savoini[3], [1]*Universitat Autònoma de Barcelona, Barcelona, Spain,* [2]*Centre for Research*

in Agricultural Genomics, Barcelona, Spain, [3]Università degli Studi di Milano, Milan, Italy.

An optimal ratio of omega-6 and omega-3 polyunsaturated fatty acids (PUFA) plays an essential role to maintain metabolic modulations and homeostasis, mainly due to their contrasting inflammatory functions. At present, there are a few studies on how sow nutrition directly affects piglet muscle deposition, especially before weaning. The study was conducted to determine on how the sow's milk, fed with extreme ω-6/ω-3 FA ratio diets, directly affects the expression profiles of genes, abundance of proteins, and their biological pathways. A total of 8 multiparous sows were used and divided between 2 dietary treatments with ω-6/ω-3 FA ratios of 13 (SOY) and 4 (LIN), mainly derived from soybean and linseed oil, respectively. Piglets were nourished only with sow's milk during lactation. At the end of lactation period, a total of 24 longissimus dorsi muscle from piglets (12 males and 12 females) were collected and subjected to proteomics analysis based on nano liquid chromatography coupled to high-resolution tandem mass spectrometry (nLC-HRMS) and FA profiles were determined using GC-MS. Of the 412 proteins identified by Proteome Discoverer 2.5 software, 4 proteins (haptoglobin, phosohoglycerate kinase-2, interferon-induced GTP-binding protein Mx2, and prophenin and tritrpticin precursor) were over-abundant ($P < 0.05$) in SOY compared with LIN. Enrichment analysis of identified proteins for gene ontologies (GO) and pathways ($P < 0.05$) showed annotation on GO terms related to fatty acid β-oxidation, high-density and very low density lipoprotein particle assembly, citrate cycle, and glycolysis/gluconeogenesis. These pathways are involved in glucose and lipid metabolism and are mostly regulated by FAs. Moreover, the observed over-abundancy of haptoglobin, an acute phase protein, in sows fed the SOY diet could be related to the proinflammatory role of ω-6 FA. Fat deposition on muscle showed a great resemblance to the diets – 15.3 vs 8.6, SOY vs LIN. A separate in-depth transcriptomics analysis are ongoing and thus will further elucidate the effect of extreme ω-6/ω-3 FA ratio on the expression profiles of genes and microRNAs on piglet muscle.

Key Words: piglet, sow milk, fatty acids, proteomics, muscle

P340 Structural genetic basis of differential gene expression in loin muscle of Iberian pigs. A. López-García*[1], R. Peiro[1], M. Muñoz[1], C. García-Contreras[1], M. Vázquez-Gómez[2], B. Isabel[3], A. Rey[3], A. González-Bulnes[1], and C. Óvilo[1], [1]*INIA (CSIC), Madrid, Spain,* [2]*INSERM (UPS), Paris, France,* [3]*UCM, Madrid, Spain.*

Iberian pig production has acquired growing importance in recent years, due to the increasing demand for high-quality dry-cured products. However, farm productivity is lower compared with other commercial breeds, mainly due to the low prolificacy and uterine capacity of Iberian pigs. Variation in prenatal growth and birth weight can increase this problem, as prenatal muscle development and intramuscular adipogenesis are determinant in postnatal growth. Recent studies of prenatal muscle transcriptome in Iberian pigs have shed light on the molecular basis of the differences in prenatal growth between Iberian and crossbred pigs. Our next step focuses on the genetic basis of these gene expression differences by genetic variant discovery. Muscle RNA-seq data obtained from purebred and crossbred Iberian fetuses at d 77th of pregnancy were used to identify allelic variants between breeds and between high and low weight fetuses. Variant calling was performed using GATK pipeline. Variant filtering was performed, by multiple quality attributes, including minor allele frequency and missingness per variant. 197,732 polymorphisms were detected, and Variant Effect Predictor (VEP) was then used to annotate variants. We kept variants associated with genes reported as differentially expressed (DE) between Iberian and crossbred fetuses (645 genes) or between high and low weight fetuses (35 DE genes for Iberian and 60 for crossbred). PLINK toolset was used to recalculate allele frequencies. Final data set included 8,278 SNPs (14.57 average SNPs per gene, 24% missense variants and 13.5% deleterious), and 866 indels (3.10 average indels per gene, 3.5% coding alteration indels) associated with DE genes. Genes with the highest expression differences between purebred and crossbred fetuses were studied in depth. For instance, we found interesting variants associated with FOXO3 (24 SNPs, 1 indel, 1 missense), LEPR (27 SNPs, 2 indels, 5 missense) or APOD (1 SNP, deleterious). The work done provides useful SNP data and functional information for future association studies in strong candidate genes for early development-related traits in pigs.

Key Words: Iberian, RNA-seq, variant, SNP

P341 *AGPAT5* gene influences fat content and composition in pigs. E. Molinero*, R. N. Pena, J. Estany, and R. Ros-Freixedes, *Departamento de Ciencia Animal, Universidad de Lleida – AGROTECNIO-CERCA Center, Lleida, Spain.*

The 1-acylglycerol-3-phosphate O-acyltransferases (AGPATs) are enzymes that catalyze the conversion of lysophosphatidic acid to phosphatidic acid. Phosphatidic acid is a precursor of triacylglycerol (the main fat reservoir in mammals) and various glycerophospholipids, as well as a signaling molecule involved in multiple regulatory processes. Therefore, we investigated the role of *AGPAT5* gene variants on fat content and fatty acid composition in pigs. We used sequence data to search for variants in the *AGPAT5* gene. A single nucleotide polymorphism in exon 6 (rs196952262, A>G), which produces a missense mutation, was selected as a tag variant for the 11 identified variants in the *AGPAT5* gene that segregate at a frequency higher than 0.05. The effect of this variant was validated in muscle, subcutaneous fat and liver samples of 1,073 pigs from a Duroc line genotyped using a high-resolution melt protocol. The A allele showed a positive additive effect for intramuscular fat (+0.29% ± 0.09 in the *gluteus medius* muscle and +0.24% ± 0.09 in the *longissimus* muscle, $P < 0.01$) and backfat thickness (+0.48 mm ± 0.20 at 180 d of age and +0.60 mm ± 0.24 at slaughter, $P < 0.05$). We also observed a significant effect on fat composition, resulting in more monounsaturated fatty acids and less polyunsaturated fatty acids as a consequence of the increased intramuscular fat content. The increase of monounsaturated fatty acids is desirable, because it is positively related with sensorial, technological and nutritional attributes of pork. However, only the arachidonic acid, which is particularly abundant in membrane phospholipids, was associated with the A allele after accounting for intramuscular fat content (−0.04% ± 0.02 in *gluteus medius* muscle, $P < 0.05$). We showed that the *AGPAT5* gene is involved in fatty acid deposition in pigs and that variations in the gene sequence affect fat content (backfat thickness and intramuscular fat), with specific effects on the arachidonic acid content.

Key Words: pigs and related species, animal breeding, DNA sequencing, fat/lipid, genomic selection

P342 Functional variant identification of cis-eQTL associated with pig *NUDT7* gene and its association analysis with meat color traits. X. Xu*[1,2], L. Liu[1,2], L. Liu[1,2], T. Ma[1,2], Z. Zheng[1,2], and X. Xu[1,2], [1]*Huazhong Agricultural University, Huazhong Agricultural University, Wuhan, Hubei Province, China,* [2]*Key Lab of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei Province, China.*

Pork color is an important indicator affecting consumption choice, but the candidate genes and molecular markers that regulate pork color are rarely reported. M. Taniguchi et al. localized the QTL affecting pork color (measured by heme content) on chromosome 6, and experimentally proved that the overexpression of *NUDT7* gene may lead to downregulation of heme biosynthesis in skeletal muscle. Our previous cis-eQTL study in skeletal muscle identified a significant signal associated with the expression level of *NUDT7*. Based on the relative difference of FPKM values, individuals with extreme high values and extreme low values were found to have significant differences in the expression levels of exons 3 and 4 and transcripts. Amplification and sequencing results show that the high group of rs326029010 is GA heterozygous, and the low group is GG homozygous. Furthermore, we carried out PCR-RFLP analysis on 416 individuals, and association analysis showed that the polymorphism of

rs326029010 was significantly associated with drip loss (P = 0.026); and meat color L value (P = 1.65E-04), A value (P = 1.65E-08) and C value (P = 1.01E-05). In this study, we identified a functional mutation site rs324204832 located in the promoter region, which may the causal variant of the cis-eQTL. We found that there is a certain linkage disequilibrium between rs324204832 and rs326029010. The present study lays the foundation for further analysis of the genetic mechanism of meat color.

Key Words: pig, cis-eQTL, *NUDT7* gene, meat color, functional mutation

P343 Integration analysis of molecular phenotype QTLs speeds up the identification of functional mutations affecting pork quality. Y. Liu*[1,2], T. Ma[1,2], Z. Zheng[1,2], and X. Xu[1,3], [1]*Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education and College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China,* [2]*The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, China,* [3]*Key Lab of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Wuhan, China.*

In the post-GWAS era, genetic analysis of molecular phenotypes (MolQTL) was a promising approach for characterizing of functional genes or variants of complex traits like meat quality. In the present study, we conducted genotyping using the DNA microarray, resequencing and RNA sequencing using 170 porcine longissimus muscle of Duroc × Luchuan F_1 generation. With these data, we characterized gene expression QTL (eQTL) and other MolQTLs, such as Allele-specific expression (ASE), transcript expression QTL (TreQTL), splicing QTL (sQTL), alternative polyadenylation QTL (APAQTL) and CNV-eQTL. Totally, we identified 2,696 cis-genes, 2,246 cis-transcripts, 266 cis-AS events, 296 cis-APA events, 434 CNV target cis-genes and 1,520 ASE-genes. Interestingly, those MolQTL target genes could significantly enriched in muscle system process, muscle structure development, metabolism of lipids, oxidation-reduction process, carbohydrate metabolic process. Of those genes, we identified a splicing mutation (rs325100969) which could directly change the "GT-AT" motif and displayed a great impact on the expression level of *ZFAND2B* (P = 4.26E-17). Additionally, we highlighted a TreQTL (rs330629900) that could provide a new "AG" motif affecting the expression level of *MYLPF* (P = 1.20E-12). Furthermore, a coupled INDEL mutations including a 17-bp exonic insertion and an 11 bp deletion (rs790284184) were identified to be associated with the expression level of *TNNC2–201*. In conclusion, the present study provided new clues of candidate genes and functional mutations for genetic analysis of meat quality traits.

Key Words: pig, meat quality, MolQTL, eQTL, ASE

P344 Withdrawn

Ruminant Genetics and Genomics Posters

P345 Genetic and genomic factors influencing gestational length in beef cattle. H. Bolen* and M. Asai-Coakwell, *Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

The ability for beef cattle producers to maximize herd profitability is impacted through reproductive efficacy on farm. Gestation length is a trait which directly influences other production traits such as postcalving interval, calving difficulty, and has genetic associations with birth weight. It has moderate to high heritability estimates, making it an ideal candidate to be included in selection systems. Much of the research surrounding gestation length has primarily been conducted in dairy cattle, and to the best of our knowledge, no study has been conducted in vivo to examine the efficacy of selection based solely on expected gestation length. We selected 5 Hereford bulls who were identified from producer records as having short to long predicted offspring gestation lengths (~281–288 d). Angus and Hereford heifers (n = 153) were bred at random using artificial insemination to compare the observed gestation lengths of the offspring to the predicted. Data were collected at calving from the offspring (n = 107) including calving difficulty score and calf birth weight. Five candidate genes (*SIGLEC5*, *CTU1*, *ZNF613*, *FOXD2*, *EXOC4*) identified from previously published association studies in beef and dairy cattle were investigated for their influence on gestation length variation, based on their predicted function. Single nucleotide polymorphisms (SNPs) that differed between the sires' coding sequences were identified and association of the SNPs of interest examined. Predictive mapping of the variant effects on protein structure which vary between short and long gestation length animals will yield an understanding of the candidate genes involvement in the traits variation. Our work will investigate the effect of shorter or longer gestational periods on calf traits to determine the economic impact of gestation length variation. Identification of a genotype associated with the short or long gestation length phenotype would allow for selection of the trait to be made on farm.

Key Words: cattle, animal breeding, reproduction, gestation length

P346 Genomic regions of polygenic selection in Nelore cattle revealed by whole-genome sequencing. A. M. Maiorano*, C. D. S. Arce, W. B. Santos, F. C. Silvério, L. G. Albuquerque, and H. N. Oliveira, *Universidade Estadual Paulista "Júlio de Mesquita Filho," Jaboticabal, Sao Paulo, Brazil.*

This study aimed to use whole-genome sequencing and an allelic differentiation approach to detect large polygenic genomic regions in Nelore animals raised in Brazil. We compared purebred bulls selected for racial characterization (PO) and bulls selected for economically important traits who received the Brazilian Special Certificate of Identification and Production (CEIP). From a total of 150 sequenced animals, a hierarchical clustering analysis was performed to choose the more unrelated animals from each category (16 PO and 40 CEIP). We considered genotypes at 37,617,713 biallelic SNPs in the 29 bovine autosomes. The Weir and Cockerham's F_{ST} estimator (wcFst) was computed for SNPs with MAF greater than 0.01. The segmentFst function from the GPAT++ software was used to identify polygenic selection. The cutoff parameter (-s) was defined based on the 99.50% percentile of wcFst values and was equal to 0.3. A total of 1,713 highly differentiated genomic regions were identified and overlapped with 271 genes. The polygenic selection signals were spread across the genome. Considering SNPs with the highest significant values, we found a variant located within the 3′ region of the *BOLA-DRB3* gene as a promising candidate polymorphism. Within relevant genomic regions, we found the genes *GRIP1*, *HELB*, *IRAK3*, *LLPH*, *HMGA2*, *TMBIM4*, *ENSBTAG00000053419*, *ENSBTAG00000052954* (Chr5: 47,225,234 - 47,860,420 bp), *BOLA-DQA2*, *BOLA-DQB*, *BOLA-DQA5*, *LOC100848815*, *BOLA-DRB3*, *ENSBTAG00000038397* (Chr23: 26,126,226 - 26,235,160 bp), and GABRG3 (Chr21: 4,991,976 - 5,159,260 bp). The genes on chromosome 23 are among the Bovine major histocompatibility complex (MHC) class II gene family, representing good candidates for immune response and adaptation to tropical conditions. Some genes were associated in other studies with adaptation to tropical environments (*HELB*), growth and navel score (*HMGA2*), fat deposition and domestication (*IRAK3*), and feed efficiency and postmortem carcass traits (*GABRG3*). The regions here identified should contribute to our knowledge regarding genes and variants that have an important role in complex traits selected in this breed.

Key Words: adaptation, animal breeding, candidate gene, genome sequencing, selection scan

P347 The genetic complexity of scurs development in cattle. I. A. S. Randhawa*[1], R. E. Lyons[2,1], B. J. Hayes[3], and M. R. McGowan[1], [1]*The University of Queensland, Gatton, QLD, Australia,* [2]*Agri-Genetics Consulting, QLD, Australia,* [3]*Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, St Lucia, QLD, Australia.*

Scurs are miniature horns present in small proportions of several farmed bovid species and usually grow unattached to the skull. In cattle, generally scurs develop in genetically heterozygous (Pp) animals, which carry an allele of either Celtic (Pc) or Friesian (Pf) mutations at the POLL locus on autosome one. However, understanding of scurs genetics remains limited because it is not obvious yet that why some Pp animals remain polled. This study has investigated a population of 17,725 animals of different cattle breeds to find effect of sex (male vs female), POLL mutation (Pc vs Pf) and the "scur genes" on scurs development. Overall, the sample population was consisted of 15.4% were heterozygotes (Pp) but only 3% with scurs and the remaining majority were polled. Scurs development in males and females differed significantly by 29% and 7%, respectively. Similarly, higher occurrence of scurs was found in Pc carrying heterozygotes (24%) as compared with Pf (15%) animals. Genome-wide scans for association mapping and selection signatures by using 770K SNP genotypes of heterozygous polled vs scurred animals found 4 regions coincide with previously detected regions as well as found several novel genomic regions. However, none of the significant regions harbor genes of profound effect on scur development. Overall, these results and lack of concordance among the previous studies suggest that scur genetics has complex inheritance likely underlying multiple genes, whereas the sex influence and POLL locus mutations interact variably in different populations to develop scurs.

Key Words: scur inheritance, horn-like structure, polygenic trait, sex influenced trait

P348 Genome-wide investigations reveal the population structure and selection signatures of Nigerian cattle adaptation in the sub-Saharan tropics. D. H. Mauki[1,3], A. Tijjani[4,5], C. Ma[1,3], S. I. Ng'ang'a[1,3], A. I. Mark[6], O. J. Sanke[7], A. M. Abdussamad[13], S. C. Olaogun[8], P. M. Dawuda[9], R. R. Kazwala[10], P. S. Gwakisa[11], Y. Li[12], M.-S. Peng[1,2], A. C. Adeola*[2,13], Y.-P. Zhang[1,14], [1]*State Key Laboratory of Genetic Resources and Evolution and Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China,* [2]*Sino-Africa Joint Research Center, Chinese Academy of Sciences, Kunming, China, Kunming, China,* [3]*Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, China,* [4]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [5]*Centre for Genomics Research and Innovation, National Biotechnology Development Agency, Kunming,* [6]*Ministry of Agriculture and Rural Development, Secretariat, Ibadan, Nigeria,* [7]*Taraba State Ministry of Agriculture and Natural Resources, Jalingo, Nigeria,* [8]*Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria,* [9]*Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine, University of Agriculture Makurdi, Makurdi, Nigeria,* [10]*Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania,* [11]*Sokoine University of Agriculture, Department of Microbiology, Parasitology and Biotechnology/ Genome Science Center, Morogoro, Tanzania,* [12]*State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, School of Life Sciences, Yunnan University, Kunming, China,* [13]*Centre for Biotechnology Research, Bayero University, Kano, Nigeria,* [14]*Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming, China.*

Cattle are considered to be the most desirable livestock by small scale farmers. In Africa, although comprehensive genomic studies have been carried out on cattle, the genetic variations in indigenous cattle from Nigeria have not been fully explored. In this study, genome-wide analysis based on genotyping-by-sequencing (GBS) of 193 Nigerian cattle was used to reveal new insights on the history of West African cattle and their adaptation to the tropical African environment, particularly in sub-Saharan region. The GBS data were evaluated against whole-genome sequencing (WGS) data and high rate of concordances between the 2 platforms was evident. The genetic structure of Nigerian cattle was observed to be homogeneous and unique from other African cattle populations. Selection analysis for the genomic regions harboring imprints of adaptive traits revealed genes associated with immune responses, growth and reproduction, efficiency of feeds utilization, and heat tolerance. Our findings depict potential convergent evolution between African cattle, dogs and humans with adaptive genes *SPRY2* and *ITGB1BP1* possibly involved in common physiological activities. The study presents unique genetic patterns of Nigerian cattle which provides new insights on the history of cattle in West Africa based on their population structure and the evidence of parallel adaptation between African cattle, dogs and humans in Africa which require further investigations

Key Words: cattle, genotyping-by-sequencing, genome, convergent evolution, Africa

P349 A comprehensive catalog of regulatory variants in the cattle transcriptome: A case study for the FarmGTEx Project. G. E. Liu*, *Animal Genomics and Improvement Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, MD, USA.*

The systematic characterization of genetic regulatory variants on the transcriptome of livestock is essential for interpreting the molecular mechanisms underlying traits of economic value, and to increase the rate of genetic gain through artificial selection. The Farm Animal Genotype-Tissue Expression (FarmGTEx) Consortium is a collaborative endeavor to provide a comprehensive atlas of tissue-specific gene expression and genetic regulation in livestock species. In its pilot phase, by uniformly analyzing publicly available sequence data using our newly-developed transcriptome pipeline as described in the cattle GTEx preprint (https://www.biorxiv.org/content/10.1101/2020.12.01.406280v1), we build a cattle Genotype-Tissue Expression atlas (cGTEx, http://cgtex.roslin.ed.ac.uk/) for the research community, based on 11,642 RNA-seq data sets from over 100 cattle tissues. We describe the landscape of bovine transcriptome across tissues and report thousands of *cis* and *trans* genetic variants (like expression QTL, or eQTL), associated with gene expression and alternative splicing for 24 tissues. We evaluate the specificity/similarity of these genetic regulatory effects across tissues, and functionally annotate them using a combination of multi-omics data. Finally, we link gene expression in different tissues to 43 economically important traits using a large transcriptome-wide association (TWAS) study to provide novel biological insights into the molecular regulatory mechanisms underpinning agronomic traits in cattle. This study provides a showcase for the FarmGTEx Project in other major farm animal species including pigs, chickens, sheep, and goats. The research plan for the next phases of FarmGTEx in ruminants will be discussed and highlighted. *This is a presentation about Cattle Genotype-Tissue Expression Atlas on behalf of the FarmGTEx consortium. The contributors include Shuli Liu, Yahui Gao, Oriol Canela-Xandri, Sheng Wang, Ying Yu, Wentao Cai, Bingjie Li, Erola Pairo-Castineira, Kenton D'Mellow, Konrad Rawlik, Charley Xia, Yuelin Yao, Xiujin Li, Ze Yan, Congjun Li, Benjamin D. Rosen, Curtis P. Van Tassell, Paul M. Vanraden, Shengli Zhang, Li Ma, John B. Cole, George E. Liu, Albert Tenesa, Lingzhao Fang, etc from multiple international institutions.

Key Words: cattle, expression QTL, GWAS, RNA-seq, transcriptome-wide association (TWAS)

P350 Confirmation of quantitative trait locus location on BTA19 for the percentage of oleic acid in beef based on effects of 5 polymorphisms and linkage disequilibrium analysis in 2 Japanese Black cattle populations. F. Kawaguchi*[1], F. Kakiuchi[1], K. Oyama[2], H. Mannen[1], and S. Sasazaki[1], [1]*Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan,*

[2]*Food Resources Education and Research Center, Kobe University, Kasai, Hyogo, Japan.*

The ratio of fatty acids, i.e. fatty acid composition, in beef is essential for beef quality. For marker-assisted selection, responsible polymorphisms for fatty acid composition were surveyed in previous studies, resulting in the identifications of a significant association between a polymorphism and fatty acid composition. In fact, 5 polymorphisms on bovine chromosome 19 (BTA19) were reported to be significantly associated with fatty acid composition in Japanese Black cattle. Two of the 5 polymorphisms were within *FASN* gene (g.841G>C and g.16024A>G), which was known to be likely responsible for fatty acid composition. The other polymorphisms were within *SREBP1* [c.1065+83 (84bp indel)], *STARD3* (c.1187C>T), and *GH* (c.379C>G) genes and might be however significantly associated with fatty acid composition due to a linkage disequilibrium (LD) with the *FASN* polymorphisms. Conducting LD analysis between these 5 polymorphisms could provide some evidence to clarify this possibility. However, doing this has not yet been possible because the previous studies about these 5 polymorphisms were conducted in different populations. Hence, this study aimed to verify the effects of these 5 polymorphisms on fatty acid composition, especially the percentage of oleic acid (C18:1) using the same Japanese Black cattle population and conduct LD analysis to determine the locations of the quantitative trait loci (QTLs). We used 2 populations which were bred in Hyogo and Gifu Prefecture, Japan (n = 441 and 443, respectively). The 5 polymorphisms were genotyped in the 2 populations to analyze the effects on C18:1 by ANOVA and calculate the LD coefficients (r^2). As the results, *SREBP1* and *STARD3* polymorphisms were significantly associated with C18:1 ($P < 0.001$) in Hyogo population. Meanwhile, both of *FASN* polymorphisms and *GH* polymorphism were significantly associated with C18:1 ($P < 0.01$) in the Gifu population. LD analysis subsequently detected the positions of the QTLs, which did not include *FASN* polymorphism and ranged from 32.2 to 46.4 Mbp and 47.8 to 52.1 Mbp in the Hyogo and Gifu populations, respectively. These results provide valuable information to identify novel responsible polymorphisms and genes for fatty acid composition on BTA19.

Key Words: Japanese Black cattle, fatty acid composition, QTL

P351 Whole-genome sequencing reveals selection signals among Chinese, Pakistani and Nepalese goats. Y. Li*[1,2], S. Song[1,3], X. Liu[1,2], Y. Zhang[1,2], D. Wang[1,2], X. He[1,2], Q. Zhao[1,2], Y. Pu[1,2], W. Guan[1,2], E. Guangxin[4], C. Kumer[5], Y. Ma[1,2], and L. Jiang[1,2], [1]*Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, P. R. China,* [2]*CAAS-ILRI Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, P. R. China,* [3]*State Key Laboratory of Cardiovascular Disease Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, P. R. China,* [4]*College of Animal Science and Technology, Chongqing Key Laboratory of Forage and Herbivore, Chongqing Engineering Research Centre for Herbivores Resource Protection and Utilization, Southwest University, Chongqing, P. R. China,* [5]*Department of Animal Breeding and Genetics, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam, Pakistan.*

Domestic goats have an excellent adaptability to harsh environments and various traits of economic importance. Whole-genome sequencing is a powerful platform to rapidly gain novel insights into the identification of genetic basis underlying specific traits in goats. Here we collected whole-genome sequences of 115 domestic goats representing 15 breeds from China, Nepal and Pakistan. Comparative genomic analyses among goats varying in phenotypic traits, detected a set of candidate genes that might be associated with heat adaptation, milk production and cashmere growth. We also identified that a 505-bp indel variant at the *fibroblast growth factor 5 (FGF5)* gene locus appeared to be strongly associated with cashmere growth. Functional validation showed that the insertion variant may serve as an enhancer for transcription factor binding, resulting in increased transcription of the upstream *FGF5* gene in non-cashmere goats. Our study provides useful information of the genetic diversity and traits exploration for Chinese, Pakistani and Nepalese goats, and reveals that an indel mutation in the *FGF5* gene could potentially serve as a molecular marker for cashmere growth in cashmere goat breeding.

Key Words: whole-genome sequencing, heat adaptation, milk production, cashmere growth, *FGF5*

P352 Long-terminal repeat insertion as potential origin of allele-biased expression of the *APOB* gene in cholesterol deficiency carriers. D. Becker[1], A. Heimes[1], R. Weikard[1], M. Meyerholz[2,3], W. Petzl[2], H. Zerbe[2], H.-J. Schuberth[3], M. Hoedemaker[4], M. Schmicke[5], S. Engelmann[6,7], and C. Kühn*[1,8], [1]*Research Institute for Farm Animal Biology, Dummerstorf, MV, Germany,* [2]*Clinic for Ruminants with Ambulatory and Herd Health Services, Ludwig-Maximilians-University Munich, Oberschleißheim, Germany,* [3]*Institute of Immunology, University of Veterinary Medicine Foundation, Hannover, Germany,* [4]*Clinic for Cattle, University of Veterinary Medicine Foundation, Hannover, Germany,* [5]*Faculty of Natural Sciences III, Martin-Luther University Halle-Wittenberg, Halle, Germany,* [6]*Institute for Microbiology, Technical University Braunschweig, Braunschweig, Germany,* [7]*Microbial Proteomics, Helmholtz Centre for Infection Research, Braunschweig, Germany,* [8]*Agricultural and Environmental Faculty, University of Rostock, Rostock, Germany.*

Cholesterol deficiency (CD) is a genetic disorder affecting lipid metabolism in cattle caused by a long-terminal repeat (LTR) insertion into exon 5 of the *APOB* gene. Homozygous carrier calves usually die due to insufficient lipid uptake, emaciation and incurable diarrhea. Recent data indicate that the defect might not fully follow a recessive expression pattern, because cows carrying the mutant CD allele (CDC) have a significantly lower blood cholesterol level compared with homozygous wild-type cows (CDF). However, knowledge is limited about the specific *APOB* expression pattern in CDC cows and potential functional effects of the LTR insertion. In our study, we analyzed the hepatic transcriptome of cows after intramammary challenge with a mastitis pathogen (*S. aureus* or *E. coli*). Four CDC were compared with 20 CDF animals in the *S. aureus* group, and 3 CDC to 8 CDF in the *E. coli* group, respectively. In both pathogen challenge cohorts, the CDC animals displayed a significantly decreased hepatic *APOB* expression compared with CDF cows, which was also observed when analyzing exons 5 to 29 only. In contrast, across all exons upstream of the LTR insertion (exon 1–4) CDC animals showed a 10-fold higher expression than CDF cows ($P < 10^{-15}$). We further followed up, if the elevated expression of exon 1–4 might result from an activated compensatory transcriptional activity at the *APOB* locus, which should affect both haplotypes to the same extent. Illumina Bovine HD chip data across the *APOB* locus for CDF and CDC heifers from a common CDC sire enabled us to reconstruct the sire's haplotype phases and subsequently to conclude on the inherited haplotypes of the offspring. We used this information to perform an allele biased differential expression analysis in CDC animals testing the ratio of reads from the haplotype carrying the CD allele and versus reads from the haplotype with the wild-type allele. Our data clearly showed that *APOB* exons 1–4 on the haplotype carrying the LTR insertion were significantly higher expressed than the same exons from the haplotype carrying the wild-type allele, which indicates a potential cis-acting effect of the LTR insertion.

Key Words: cattle, genetic defect, long-terminal repeat, gene expression, RNA-seq

P353 Comparison of sequencing and assembly strategies for the cattle pangenome effort. A. Leonard*[1], Z.-H. Fang[1], B. Rosen[2], D. Bickhart[2], T. Smith[2], and H. Pausch[1], [1]*ETH Zürich, Zürich, Switzerland,* [2]*ARS, USDA, Beltsville, MD, USA.*

Selective breeding and natural selection have resulted in over a thousand diverse cattle breeds, grouped into 2 subspecies: *Bos taurus*

taurus and *Bos taurus indicus*. Currently, the cattle reference genome is based on a single Hereford-breed animal, limiting comparisons of diversity from more distantly derived lineages. As efforts continue to increase the number of breeds with reference-quality assemblies, we examined various sequencing technologies and assembly methods for cattle trios to identify efficient approaches. We assembled 5 distinct breed-specific cattle genomes, including a low-heterozygosity purebred Original Braunvieh, a medium-heterozygosity F_1 cross of Nellore and Brown Swiss, and a high-heterozygosity interspecies cross of gaur and Piedmontese. Both PacBio's High-Fidelity (HiFi) and Oxford Nanopore Technology's (ONT) long reads produce high-quality assemblies across multiple tested assemblers. However, we highlight several differences between the sequencing technologies, like the quantity of telomeric or centromeric sequence and expected gene content found in the assemblies. We also demonstrate the effectiveness of graph-based over direct trio-binning assembly approaches across different scales of heterozygosity, with average N50 values of 70 Mb and 30 Mb respectively. Effect of sequence coverage depth on assembly completeness, contiguity, and accuracy was assessed to optimize the balance of quality and cost. These assemblies also represent the first reference-quality long-read assemblies for their respective breeds. Using the minigraph tool and the current cattle reference genome as the backbone, we selected the best HiFi and ONT assembly for each of the 5 breeds for iterative incorporation into the assembly graph. Through the graph, we identified approximately 70k breed-specific structural variants that were used to construct a relationship dendogram among the breeds that showed high agreement with previous SNP-based phylogenies. The results provide insight into predicted effects of sequencing platform and experimental design selection for pangenome projects in cattle and other diploid species.

Key Words: cattle, genome assembly, sequence variation, bioinformatics, phylogeny

P354 Genome-wide association analyses for maternal weaning weight in South African Bonsmara cattle. J. Reding[1], E. van Marle-Köster*[1], and D. Berry[1,2], [1]*University of Pretoria, Hatfield, South Africa,* [2]*Teagasc, $Dublin, Ireland.*

Beef cow milk production is a major contributor to calf weaning weight. Genetic gain in beef cow milk production is hampered by the trait being both sex-limited and recorded relatively late in life. Genomic insights into direct and maternal weaning weight (MMW) could help resolve the known antagonism between both traits. A genome-wide association for direct (2,777 animals) and maternal (1,454 animals) weaning weight was undertaken using 134,421 imputed single nucleotide polymorphism (SNP) genotypes. Both measures were represented by weighted deregressed estimated breeding values in the association analysis with each SNP separately included in a linear mixed model; relationships among animals was accounted for via a genomic relationship matrix. A total of 23 significant SNPs at a genome-wide threshold ($P = 1 \times 10^{-7}$) were associated with MWW collapsing into 13 QTL across 8 autosomes and a QTL on the X chromosome. Significant SNPs resided in or near 7 genes, with the frequency of favorable alleles varying from 0.57 and 0.96. A QTL on BTA 16 encompassed *SUSD4* and *TLR5*. Eight SNPs were located in a single QTL on BTA 8 ($P = 7.9 \times 10^{-9}$), all upstream in the protein-coding regions of *PCSK5.* The *HERC3* gene has been reported in several cattle breeds associated with antral follicle count, body weight (heifers) and α2-casein in Holstein. *HERC3* is involved in ubiquitin-protein transferase activity and signals 26S proteasome dependent degradation. *STK32A* and *TLR5* were associated with host resilience to *Eimeria* spp. and inflammatory responses to ticks. *SUSD4* is linked with epistatic interactions with IGF-1 in Brahman cattle related to growth. No associations with *HERC2*, *SUMF1* or *PCSK5* have been previously reported in cattle. *HERC2* is associated with spermatogenesis, while *SUMF1* is involved in protein oxidation and posttranslational modification. *PCSK5* has serine-type endopeptidase activity involved in bone mineralization and embryonic implantation; it has been associated with milk, protein and fat yield in buffalo. Genes previously associated with milk, growth and resilience traits in Bovid types illustrate genetic similarities; with novel genes being specific to the SA Bonsmara breed.

Key Words: cattle, fertility QTL

P355 Investigating gene and allele-specific expression in early development in sheep to identify functional variants associated with growth traits. S. Woolley*, M. Salavati, and E. Clark, *The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, UK.*

The sheep transcriptome offers numerous opportunities for understanding the biological function and the roles of genes in maintaining health and productivity. Growth traits in sheep are important for production and are often polygenic, making the identification of trait-specific genomic markers for genomic selection challenging. This study primarily aims to use RNA sequencing to identify transcriptionally active genes involved in healthy growth and development in Texel x Scottish Blackface sheep. Tissues were collected from fetal sheep at d 23, 35 and 100 of gestation, postnatally from lambs at birth, one week and 8 weeks of age, and from adult sheep. An initial RNA-seq data set of 48 samples was generated from whole d 23 embryos, skeletal bicep muscle, liver, ovary, placentome and caruncle tissue. Samples were sequenced at an expected depth of 60 million reads per sample on the Illumina NovaSeq platform. Transcript per million expression estimates were generated using Kallisto based on the gene models from the Oar_rambouillet_v1.0 genome (Ensembl v103), and network cluster analysis was performed in Graphia. Gene-to-gene network analysis revealed large clusters of genes expressed in ovary, liver and skeletal bicep tissues. One of the largest clusters showed high expression levels in lambs from birth to 8 weeks of age in skeletal bicep muscle tissue. Genes within this cluster were associated with GO terms for muscle fiber organization and contraction. The next stage of this study will involve analyzing allele-specific expression (ASE) to identify variants exhibiting an imbalance in expression from either parent. The RNA-seq and ASE information will then be integrated with GWAS data to identify functional expressed variants located in genomic regions associated with growth traits in sheep. The results from this study will not only provide a foundation for the investigation of transcriptional differences across developmental stages, but will also identify functional variants that could be integrated into future genomic breeding programs for sheep.

Key Words: allele-specific expression, RNA-seq, transcriptome, functional variants, sheep

P356 Whole-genome gene expression profiling of muscle tissue of Nellore cattle with divergent meat cooking loss. M. Serna-García*[1], L. F. S. Fonseca[1], D. B. S. Silva[1], P. I. Schmidt[1], A. F. B. Magalhães[2], N. A. Marín-Garzón[1], B. M. Salatta[1], G. B. Frezarim[1], and L. G. Albuquerque1,3, [1]*Faculty of Agricultural and Veterinary Sciences, São Paulo State University, FCAV/UNESP, Jaboticabal, São Paulo, Brazil,* [2]*APTA Beef Cattle Center, Animal Science Institute, Sertaozinho, São Paulo, Brazil,* [3]*National Council for Science and Technological Development (CNPq), Brasília, Brazil.*

The aim of this study was to use RNA-seq approach to identify differentially expressed genes (DEG) related to water loss in Nellore cattle muscle tissue after cooking. Cooking loss is due to the meat heating treatments, which causes the shrinkage of the muscle fibers, resulting in loss of content. This process results in a great impact on the meat quality attributes, such as juiciness and tenderness of the final product. Moreover, evaluation of this trait is difficult and expensive, requiring the slaughter of the animals and laboratory tests. Twenty samples from *longissimus thoracis* muscle were used to perform the RNA-seq. The samples were separated in 2 significantly (t-test < 0.05) divergent to Cooking Loss (CL): 10 samples with Low CL (23.07 ± 1.39) and 10 with High CL (33.51 ± 1.75). The CL analysis were performed as described by Wheeler et al. (1995). Total RNA was extracted using RNeasy Lipid Tissue Kit (Qiagen)

according to manufacturer's instructions. The libraries were constructed and sequenced with the Illumina HiSeq 2500 system. The raw data were filtered for quality control and assembled. The HiSat was used to map the fragments and aligning with the reference genome (UMD3.1) assembly. The fragments mapping and normalization (FPKM) were performed using the Cufflinks2 program and for the analysis of DEG, the Cuffdiff2 program was used (FDR less than 5%). Pathway Studio (Elsevier) was used for the analysis of enrichment. Thirty-two DEG were found in Low CL vs High CL group. The *OXT*, *SOCS-3*, and *SERPINE1* genes are related to biological processes, as regulation of the immune system, regeneration of cell damage, adipogenesis and proteolysis. The *OXT* is directly involved in increasing water retention and development of myogenesis, stimulating the recruitment of glucose in the skeletal muscle cells. Differences in the immune system between the groups seems to be an essential topic, since dehydration changes the fluidity of blood, what directly affects the immunity action. The acquired knowledge about the biological mechanisms related to CL in Nellore cattle muscle tissue could, in the future, open new insights to improve meet quality in this breed.

Key Words: bovine, longissimus thoracis, meat quality, production, RNA-seq

P357 The eastward dispersal of domestic goats and their introgression, population stratification, and genetic adaptation in East Asia. Y. Cai*, W. Fu, Z. Zheng, X. Liu, Y. Jiang, and X. Wang, *Northwest A&F University, Yangling, Shaanxi, China.*

Goat domestication began ~11,000 years ago from a mosaic of wild bezoar populations. Following domestication, goats dispersed throughout the world, along with human migration. In Asia, domestic goats were present in China more than 4,000 years ago. However, their migration route to East Asia and their local evolutionary history remain poorly understood. Here, we sequenced 101 modern *Capra* genomes and 31 ancient Chinese goat genomes. Together with the publicly available genomic resources for modern and ancient goats, we revealed that the ancient Chinese goats descended from the eastern Fertile Crescent and began to migrate into China about 7,000 to 6,000 years ago. Compare with European and African goats, we found Asian goats have a specific higher level of introgression from markhor. Modern Chinese goats were divided into a northern and a southern group, coinciding with the most prominent climatic division in China. Northern Chinese goats have a lower level of introgression than other Asian goats. And, compared with northern Chinese goats, southern Chinese goats maintained a gene pool more similar to that of ancient Chinese goats. The gene flow from European goats into northern Chinese goats may contribute to this divergence. The 2 most divergent genes between northern and southern Chinese goats are both related to hair follicle development, which may play an important role in their environmental adaptation. And, the evolutionary trajectories of these genes were uncovered through ancient DNA. Our findings add to our understanding of the eastward dispersal of domestic goats and the specific evolutionary process of East Asian goats.

Key Words: introgression, ancient genomes, population genomics, environmental adaptation, genomic selection

P358 Single-cell transcriptomic analyses of cattle ruminal epithelial cells before and after weaning. Y. Gao[1], L. Fang[3], R. L. Baldwin[1], E. E. Connor[4], J. B. Cole[1], C. P. Van Tassell[1], L. Ma[2], C. J. Li*[1], and G. E. Liu[1], [1]*ARS, USDA, Beltsville, MD, USA,* [2]*University of Maryland, College Park, MD, USA,* [3]*University of Edinburgh, Edinburgh, UK,* [4]*University of Delaware, Newark, DE, USA.*

Integration of genomic, transcriptomic, and other regulatory and functional information offers crucial justification for a comprehensive platform that allows convenient investigation of complex relationships across multiple genes and traits. Comprehensive analyses of tissues at the single-cell level will benefit our understanding of genetic bases for complex traits. Although single-cell RNA sequencing (scRNA-seq) has been widely explored in humans and other model species, its applications in livestock like cattle are still largely unreported. Here we present an initial effort of single-cell transcriptomic analyses of cattle ruminal epithelial cells before and after weaning. Using the 10X Genomics Chromium Controller, we obtained 5064 and 1,372 cells from Holstein ruminal epithelial cells before and after weaning, respectively. We detected 6 distinct cell clusters and designated their cell types using Human Cell Atlas/Blueprint reference cell data sets. We also found thousands of differential expressed genes (DEG) among cell clusters and between the feed schemes. We then performed cell cycle, pseudotime trajectory, regulatory network, weighted gene co-expression network, and gene ontology analyses. We proposed assigning Clusters C1 as active dividing epithelial stem cells, C0 as resting poised epithelial cells, C4 as keratinized epithelial cells during their terminal differentiation, and C5 as muscle-like vascular cells, C2 and C3 their intermediate populations for these differentiated extremes. We also reported distinct cell markers for these cell types, such as BCRA1, HMMR, MKI67, and EZH2 for C1 and the TGF-β pathway, and the keratin gene family C4. By integrating these DEG with Holstein GWAS signals, we found that all clusters, especially C5 and C0, were enriched for animal production and body type traits. Additionally, we confirmed their cell identifies by comparing them with the human and mouse stomach epithelial cells. This study provides a comprehensive resource for bovine rumen research, genomic activities in bovine rumen development, functional annotation of the bovine genome. It enables discoveries about tissue/cell type roles in complex traits at single-cell resolution.

Key Words: cattle, ruminal epithelial cell, single-cell RNA-seq, transcriptome

P359 The detection of transmission ratio distortion signals in the goat genome is strongly affected by SNP calling quality. M. Luigi-Sierra[1], J. Casellas[2], A. Martínez[3], J. Delgado[3], J. Alvarez[3], F. Such[2], J. Jordana[2], and M. Amills*[1,2], [1]*Centre for Research in Agricultural Genomics (CRAG), Bellaterra, Spain,* [2]*Universitat Autònoma de Barcelona, Bellaterra, Spain,* [3]*Universidad de Córdoba, Córdoba, Spain.*

Transmission ratio distortion (TRD) refers to instances in which the 2 alleles harbored by a parent are not transmitted in equal proportions to the offspring. Transmission ratio distortion can arise as a consequence of multiple factors related with germline selection during mitosis of germ cells, meiotic drive, gametic competition of sperm cells to fertilize the oocyte, embryonic death and faulty imprinting, among others. Obviously, TRD is mostly explained by the existence of deleterious alleles with adverse effects on embryo and fetal viability of domestic animals, thus influencing reproductive success, one of the main parameters that determines the income of farmers. In 2012, a novel methodology to detect TRD at a genome-wide scale was developed, being further refined in the following years. One appealing feature of this statistical approach is that TRD detection can be performed even in pedigrees with incomplete trios. This can be achieved by calculating the probability that the missing parent has a given genotype for a SNP based on its frequencies in the general population. Here, we have investigated the effect of SNP calling quality on the identification of genomic regions associated with TRD in the Murciano-Granadina goat breed. Seventeen bucks and their offspring (n = 305) were genotyped with the Goat SNP50 BeadChip by following standard procedures, while dams were not genotyped. By carrying out a genome-wide scan in these 305 individuals, we found 36 SNPs displaying significant evidence of TRD. Interestingly, 25 of these SNPs showed GenTrain scores below 0.8. Even more, we also observed, for these 25 markers, that allele frequencies inferred for ungenotyped dams did not correlate with allele frequencies estimated in the genotyped offspring. These findings suggest that TRD signals associated with these 25 markers are most likely spurious. Our main conclusion is that SNP calling quality is a fundamental parameter to be taken into account to obtain reliable results in TRD scans.

Key Words: GenTrain score, transmission ratio distortion, goat SNP50 BeadChip, SNP

P360 miR-2382-5p regulates lipid metabolism by targeting *NDRG2* and *KLF6* genes in bovine mammary epithelial cells. L. Xia*[1], Z. Zhao[2], X. Fang[1], and R. Yang[1], [1]*College of Animal Science, Jilin University, Changchun, Jilin, China,* [2]*College of Coastal Agricultural Sciences, Guangdong Ocean University, Zhanjiang, Guangdong, China.*

The dairy industry is one of the most dynamic and fastest developing industries in animal husbandry. The regulation of milk fat metabolism is of important significance for the improvement of dairy milk quality, and is a hot spot in the research field of dairy cow genetics and breeding. The levels of gene and miRNAs expression in bovine mammary epithelial cells (bMECs) can affect the deposition of fat in milk. miR-2382-5p mimics, inhibitor and Nc were transfected with bMECs, respectively, showing a significantly lower triglyceride content in the miR-2382-5p mimics groups than that in the Nc group ($P < 0.05$). Furthermore, miR-2382-5p regulated the expression of *LPL*, *PPARGC1B*, *HSL*, and *PPARγ*, which are known to effect triglyceride decomposition in lipid metabolism. Meanwhile, in the bMECs transfected with miR-2382-5p mimics, the expression of miR-2382-5p increased significantly ($P < 0.05$), and the mRNA expression levels of *NDRG2* and *KLF6* decreased ($P < 0.05$). However, in the bMECs transfected with miR-2382-5p inhibitor, the expression of miR-2382-5p decreased significantly ($P < 0.05$), and the mRNA expression levels of *NDRG2* and *KLF6* increased ($P < 0.05$). Luciferase detection results showed that the luciferase activity was significantly higher in control group than in the WT group ($P < 0.05$), indicating that miR-2382-5p regulated *NDRG2* and *KLF6* genes through a target sequence located at 3′-UTR. Furthermore, the intracellular triglyceride and cholesterol content upregulated in pBI-CMV3-NDRG2 and pBI-CMV3-KLF6 groups, and the intracellular triglyceride and cholesterol content downregulated in pGPU6/GFP/Neo-NDRG2 group, indicating the expression levels of the *NDRG2* and *KLF6* genes are positively correlated with the intracellular triglyceride and cholesterol content. This experiment indicated that miR-2382-5p could regulate the expression of lipid metabolism-related genes, such as *LPL*, *HSL*, *PPARγ*. In addition, *NDRG2* and *KLF6* were positively correlated with the intracellular triglyceride and cholesterol content, which were targeting regulated by miR-2382-5p. Above all, we speculated that miR-2382-5p affects the lipid metabolism by targeting the *NDRG2* and *KLF6* genes and regulating the expression of lipid metabolism-related genes in bMECs. Acknowledgments: National Natural Science Foundation of China (31972993).

Key Words: triglyceride, miR-2382-5p, *NDRG2*, *KLF6*, bovine mammary epithelial cells

P361 Genome-wide association analysis for milk production traits in German Black Pied cattle (DSN). P. Korkuc*[1], D. Arends[1], K. May[2], S. König[2], and G. Brockmann[1], [1]*Humboldt University Berlin, Berlin, Germany,* [2]*Justus-Liebig-University of Giessen, Giessen, Germany.*

The dual-purpose German Black Pied cattle (DSN, "Deutsches Schwarzbuntes Niederungsrind") is an ancestor breed of the predominant Holstein dairy cattle breed. Because of its ~2,500 kg lower milk yield compared with Holstein, DSN cattle were almost entirely replaced by Holstein. With about 2,550 herdbook cattle, DSN is an endangered population. Thus, DSN breeders are financially compensated for the lower milk yield by the German government to preserve the breed as a gene reserve. The identification of genomic loci affecting milk production is a first step toward genetic improvement of the small population. We conducted a genome-wide association analysis of 30 milk production traits on 1,490 DSN cows genotyped with the Illumina BovineSNP50 BeadChip which was recently published in Frontiers in Genetics (doi: 10.3389/fgene.2021.640039). Association analysis was performed using multiple linear regression models in R. We identified 61 (41 significant and 20 suggestive) SNPs associated with milk production traits and additional 15 SNPs for protein content which are less reliable due to high inflation. The most significant effects on milk yield were detected on chromosomes 1, 6, and 20. The haplotype corresponding to the association on chromosome 6 overlapped with the *CSN3* gene (kappa casein). No associations were detected for SNPs in the regions of *DGAT1* or other known milk genes although DSN and Holstein are closely related. Among the identified SNPs, 15 have previously been associated with milk production and 16 SNPs with exterior, health, meat carcass, and reproduction traits in diverse other breeds. The results of this study show that many SNPs that are associated with milk traits in other breeds also affect milk traits in DSN cattle. This confirms the reliability of the results obtained in a small population. The favorable alleles can be used as a basis for selection decisions to improve milk yield and thus to increase the market chances of DSN.

Key Words: GWAS, 50k SNP chip, milk genes, Holstein, *DGAT1*

P362 Assessing the impact of genome assemblies on livestock genomic analyses. A. Lloret-Villas*, M. Bhati, N. K. Kadri, A. S. Leonard, and H. Pausch, *Animal Genomics, Institute of Agricultural Sciences, ETH, Zürich, Switzerland.*

Reference-quality assemblies are generated for a wide range of species and applications at an unprecedented pace. As sequencing technologies improve and more powerful tools are developed, breed- and population-specific reference-quality assemblies are available. To understand the benefits of targeted reference genomes in livestock species, we assessed how different reference genome assemblies affect downstream analyses in the Brown Swiss cattle (BSW) breed. First, we generated 3 haplotype-resolved BSW assemblies through the trio-binning approach. The assemblies were generated using either ONT or Pacbio HiFi long reads and different assemblers (Shasta, Hifiasm and Canu). We then assessed how suitable these breed-specific assemblies are as reference genome in contrast to assemblies from other breeds (Hereford, Angus). Short-read sequencing data of 161 BSW individuals were aligned against the current Bos taurus reference genome (ARS-UCD1.2), a highly continuous Angus-based assembly (UOA_Angus_1) and the 3 BSW assemblies. Mapping statistics indicate higher alignment rates against BSW assemblies than other haplotype-resolved and primary bovine reference genome, especially when Pacbio HiFi reads were used for the assembly. Sequence variant genotypes were obtained for the different assemblies. We then carried out genome-wide association studies (GWAS) between imputed genotypes and 6 traits (stature and 5 dairy traits). Neither genotyping accuracy nor significantly trait-associated variants substantially differed across assemblies. However, the choice of reference may indeed have a large impact on detecting signatures of selection that already reached fixation. Our evaluation is the first to compare sequence variant discovery from primary and haplotype-resolved assemblies. Our findings show that haplotype-resolved reference-quality assemblies may readily serve as reference genomes for linear read mapping and variant genotyping.

Key Words: bovine reference genome, genome assembly, haplotype-resolved assemblies, read mapping, variant discovery

P363 Long noncoding RNA-420 competitively binds to miR-129-5p and targets *DLK1* inhibiting lipid metabolism in bovine preadipocytes. J. Mi*, W. He, X. Lu, X. Fang, and R. Yang, *College of Animal Science, Jilin University, Changchun, Jilin, China.*

The study of lipid metabolism is a very important item in breeding improvement of bovine meat quality traits. Noncoding RNA, especially long noncoding RNA, has also been confirmed to play different regulatory roles at different stages of development. Our study indicated that lncRNA-420 has an inhibitory effect on the lipid metabolism by ceRNA model. Preadipocytes were isolated from the pelvic fat of 1-wk-old bulls, after passaging, the cells were transfected with pBI-CMV3-lncRNA-420 and miR-129–5p to explore the effect on lipid metabolism. The triglyceride detection kit is used to determine the change of triglyceride content in cells, and the expression of target gene *DLK1* and lipid metabolism-related genes in cells are verified by quantitative PCR. The dual-luciferase report experiment is to verify the complementary binding between lncRNA-420 and miR-129-5p. LncRNA-420 has high tissue and cell ex-

pression specificity. It is significantly higher in liver, muscle and pelvic fat, and its expression level in preadipocytes is significantly higher than that in adipocytes. When lncRNA-420 is overexpressed in bovine preadipocytes, the mRNA and protein expression levels of target gene *DLK1* increased. The content of triglyceride in preadipocytes decreased, the accumulation of lipid droplets decreased, and the expression level of LPL, ATGL, HSL related to triglyceride hydrolysis increased ($P < 0.05$). At the same time, fatty acid composition analysis showed that the FFA content in the cells of the lncRNA-420 overexpression group increased, and the content of PUFA were significantly increased ($P < 0.05$). LncRNA-420 and miR-129-5p competitively regulate *DLK1* through the same targeting site CAAAAA. LncRNA-420 rescued miR-129-5p effect on *DLK1* gene suppression, and suppressed the increase in triglycerides caused by miR-129-5p. This study proved that lncRNA-420 reduced the inhibitory effect of miR-129-5p and further upregulated *DLK1* as a competing endogenous RNA (ceRNA) to regulate fat metabolism in preadipocytes. We have confirmed the interaction between lncRNA and target gene and proved the inhibitory effect of lncRNA on lipid metabolism. Therefore, lncRNA as a starting point for studying the effect on preadipocytes is also a new direction of our genetic research. Acknowledgment: National Natural Science Foundation of China (31672389)

Key Words: long noncoding RNA, miR-129-5p, *DLK1*, preadipocyte, lipid metabolism

P364 Isolation of bovine milk-derived exosomes and identification of miRNAs associated with mastitis. X. Lu*, X. Zhang, J. Mi, W. He, X. Fang, and R. Yang, *College of Animal Science, Jilin University, Changchun, Jilin, China.*

Exosomes are nanoscale vesicles secreted by cells which can be transferred from donor cells to recipient cells and play significant role in communication between cells. In this experiment, exosomes from milk samples of the mastitis and healthy groups were separated by differential ultracentrifugation so as to detect the expression levels of immune-related miRNAs. Then exosomes were cocultured with bMECs to detect the influence of miRNAs on immune-related genes, to predict the signature immune-related miRNAs of bovine mastitis through milk-derived exosome screening. The results indicated that the optimized ultracentrifugation method could successfully separate 2 groups of dairy cow milk-derived exosomes. According to the characterization and analysis of exosomes, the morphology of milk-derived exosomes isolated was round and the size of exosomes was between 50 and 150nm, mainly distributed in the range of 100nm. Both groups of milk-derived exosomes expressed a large amount of exosomal surface marker proteins TSG101 and CD81, but there was no difference indicating the milk-derived exosomes were successfully isolated. This test detected the expression levels of immune-related miRNAs in the milk-derived exosomes of 2 groups. The results showed that the expression levels of immune-related miRNAs in the mastitis group had significant changes compared with the healthy group. The expression of miR-103, miR-146a and miR-29a was strongly increased ($P < 0.01$), and the expression of miR-200b was significantly lower than that of the healthy group ($P < 0.01$). After milk-derived exosomes and bMECs coculture, it was found that the expression levels of miR-146a and miR-29a in the milk-derived exosomes group of both mastitis and healthy dairy cows were significantly increased compared with the blank control ($P < 0.01$). Meanwhile, the mRNA expression levels of immune-related genes TLR2 and TLR4 genes in the cells of mastitis milk-derived exosomes treatment group, healthy milk exosomes treatment group, LPS treatment group and blank control group showed significant decrease trend ($P < 0.01$). In summary, it is shown that miR-146a and miR-29a in milk-derived exosomes are candidate markers of bovine mastitis. This study will provide theoretical and technical basis for more timely and accurate diagnosis of cow mastitis. Acknowledgments: National Natural Science Foundation of China (31972993).

Key Words: bovine mastitis, exosomes, microRNA, bovine mammary epithelial cells

P365 Identification and characterization of miRNAs in spleens of animals subjected to repetitive vaccination. E. Varela-Martínez[1], M. Bilbao-Arribas[1], N. Abendaño[1], A. Guisasola-Serrano[1], J. Asín[2,3], M. M. Pérez[2], L. Luján[2], and B. M. Jugo*[1], [1]*University of the Basque Country (UPV/EHU), Leioa (Bizkaia) Spain,* [2]*University of Zaragoza, Zaragoza, Spain,* [3]*University of California, Davis, CA, USA.*

Aluminum adjuvants, and aluminum hydroxide specifically, are the most widespread compounds used to enhance the immune response in human and veterinary vaccines. Despite their extended use, the underlying mechanism of action remains partially unclear. To characterize the mechanism of action of Al hydroxide as adjuvant, the molecular signature activated in the spleen of sheep samples after a long-term exposure to multiple vaccines is analyzed by miRNA sequencing (miRNA-seq). Animals were treated with either commercial vaccines (vaccine group), Al hydroxide only (adjuvant group) or PBS (control group), entailing a total of 19 inoculations through 475 d. Except control animals, each animal received the same amount of Al hydroxide as adjuvant in vaccines or alone. Spleen samples from 4 animals per group were used for sequencing in the NGS platform HiSeq2500 (Illumina). All the sequences were aligned to the new ovine reference genome (Oar_rambouillet_v1.0) and miRNA characterization and expression estimates were achieved with sRNAtoolbox. Four different tools were used for miRNA target predictions: miRanda, PITA, TargetScan and TarpMir. 46 and 16 miRNAs were found dysregulated in the vaccine vs. control and adjuvant vs. control comparisons, respectively. Among the enriched GO terms within the predicted targets of the most highly expressed miRNAs, terms related mainly with immune signaling functions such as antigen receptor-mediated signaling pathway and immune response-regulating cell surface receptor signaling pathway, and terms related with chemotaxis such as positive regulation of macrophage migration and positive regulation of cellular component movement were found. The functions of the predicted targets highlight the importance of miRNAs in regulating the immune response in this tissue.

Key Words: miRNA-seq, sheep, aluminum, vaccine, computational biology

P366 Genetic history and convergent evolution of the northernmost cattle from Siberia. L. Buggiotti[1], A. A. Yurchenko[2,9], N. S. Yudin[2,9], C. J. Vander Jagt[3], N. V. Vorobieva[4], M. Kusliy[4], S. K. Vasiliev[5], A. N. Rodionov[6], O. I. Boronetskaya[7], N. A. Zinovieva[6], A. S. Graphodatsky[4], H. D. Daetwyler[3,8], and D. M. Larkin*[1,9], [1]*Royal Veterinary College, University of London, London, UK,* [2]*The Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia,* [3]*Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia,* [4]*Department of the Diversity and Evolution of Genomes, Institute of Molecular and Cellular Biology SB RAS, Novosibirsk, Russia,* [5]*Paleometal Archeology Department, Institute of Archaeology and Ethnography SB RAS, Novosibirsk, Russia,* [6]*L. K. Ernst Federal Research Centre for Animal Husbandry, Podolsk, Russia,* [7]*Timiryazev Russian State Agrarian University, Moscow Agrarian Academy, Moscow, Russia, Moscow, Russia,* [8]*School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia, Bundoora, Victoria, Australia,* [9]*Kurchatov Genomic Center, Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Science, Novosibirsk, Russia.*

Native cattle breeds represent an important cultural heritage. Evolutionary processes that occur in response to extreme environmental conditions could also be better understood using adapted local populations. Herein, different evolutionary histories of the world northernmost native cattle breeds from Russia were investigated. We found that Yakut cattle

separated from European taurine cattle ~5,000 years ago and contain numerous ancestral and some novel genetic variants allowing their adaptation to harsh conditions of living above the Polar Circle. Scans for selection signatures pointed to common gene pathways related to adaptation to harsh climates in several breeds. But genes affected by selection from these pathways were mostly different. A Yakut cattle breed-specific missense mutation in a highly conserved *NRAP* gene involved in climate adaptations in a variety of animals, represents a unique example of a young amino acid residue convergent change shared with at least 16 species of hibernating/cold-adapted mammals from 6 distinct phylogenetic orders. To our best knowledge this is the first revealed case of a convergent evolution event along the mammalian phylogenetic tree showing fast fixation in a single isolated cattle population exposed to a harsh climate.

Key Words: cold-adapted cattle, convergent evolution, hibernation, *NRAP*, Siberia

P367 Genome-wide association studies in the Reggiana cattle breed identify several candidate genes affecting pigmentation-related traits, stature and udder defects. S. Bovo*[1], G. Schiavo[1], H. Kazemi[1], G. Moscatelli[1], A. Ribani[1], M. Ballan[1], M. Bonacini[2], M. Prandi[2], S. Dall'Olio[1], and L. Fontanesi[1], [1]*Department of Agricultural and Food Science, Division of Animal Sciences, University of Bologna, Bologna, Italy,* [2]*Associazione Nazionale Allevatori Bovini di Razza Reggiana (ANABORARE), Reggio Emilia, Italy.*

Reggiana is an Italian autochthonous cattle breed reared mainly in the Emilia Romagna region (North of Italy) and utilized for the production of a mono-breed Parmigiano-Reggiano cheese. Local production systems and breeding strategies have shaped the genome of Reggiana animals and resulted in the selection of exterior traits characterizing the breed (e.g., the typical solid red coat color). However, Reggiana animals still present a degree of phenotypic variability, opening the possibility to better characterize the genetic architecture of this breed. As such, we investigated at the genome-wise level the within-breed variability affecting different exterior traits and defects, including 3 pigmentation-related traits (intensity of red coat color, piebaldism and muzzle color), stature, presence/absence and the number of supernumerary teats and teat length. A total of 1,776 animals was genotyped with the GeneSeek GGP Bovine 150k SNP array and single-marker and haplotype-based genome scans were carried out. The genome scans for coat color included animals characterized by 3 intensity of red (light-red 10.7% of the animals; normal-red, 83.7%; dark-red, 5.6%) and highlighted associations with DNA markers located on the bovine chromosome (BTA) 5, BTA7, BTA13 and BTA14. Piebaldism was observed in 4.3% cattle and associated with *KIT* gene markers (BTA6). The genome scans for muzzle color pointed out the involvement of the *MC1R* gene markers (BTA18) and other DNA markers on BTA3 and BTA20. Stature was associated with DNA markers located upstream to the *NCAPG-LCORL* genes (BTA6). Another suggestive peak of association was closed to the *PLAG1* gene (BTA14). Teats related traits associated with DNA markers on BTA8, BTA10, BTA17. Overall, this study returned a series of genetic elements determining the main phenotypic differences characterizing Reggiana and that contribute to clarify the genetic mechanisms regulating relevant exterior traits in cattle and that can be applied in the conservation and selection of this breed.

Key Words: cattle and related species, genome-wide association, single nucleotide polymorphism (SNP), breed diversity, conservation

P368 An integrated long noncoding RNA transcriptome during the sheep immune system activation. M. Bilbao-Arribas*, E. Varela-Martínez, and B. M. Jugo, *Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Basque Country, Spain.*

Long noncoding RNAs (lncRNAs) are involved in several biological processes, including the immune system response to pathogens and vaccines. The annotation and functional characterization of lncRNAs is much more advanced in human than in livestock species such as sheep. Here, we take advantage of the increasing number of high-throughput functional experiments deposited in public databases to uniformly analyze, profile unannotated lncRNAs and integrate 451 available ovine RNA-seq samples of blood cells, lymphoid organs and other immune cells. We identified 14,674 expressed novel lncRNA genes and 781 annotated lncRNAs from the assembled transcriptome, with many of them being lowly expressed. Lymphoid tissues had the highest number of expressed lncRNAs. We performed a differential expression (DE) analysis between unstimulated samples and samples with any stimulation such as infection or vaccination. In blood cells, there were 479 differentially expressed genes, including 176 lncRNAs, and in lymph nodes, there were 1,322 differentially expressed genes, including 448 lncRNAs. Differential transcript usage (DTU) analysis revealed a lower number of significant genes, with 30 and 20 genes showing DTU, respectively. Interestingly the most statistically significant isoform switch in the lymph node samples has been previously characterized in human myeloid cells. We performed a gene co-expression analysis to analyze the relation between activated gene clusters and lncRNAs. Overall, using a diverse set of immune system samples we identify several unannotated ovine lncRNAs dysregulated during the response to an external stimulus. Taken together, these findings expand the lncRNA catalog in sheep and provide evidence that lncRNAs are implicated in the general immune response, as it has been shown in other organisms.

Key Words: sheep, functional genomics, noncoding RNA, RNA-seq, immune system

P369 Online Mendelian Inheritance in Animals (OMIA): Updated variant tables for key livestock species. I. Tammen*[1], K. L. M. Eager[1,2], S. A. Woolley[1,2], S. M. Y. Shields[1], S. Hermesch[3], B. A. O'Rourke[2], and F. W. Nicholas[1], [1]*The University of Sydney, Sydney School of Veterinary Science, Sydney, NSW, Australia,* [2]*The Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia,* [3]*University of New England, Animal Genetics Breeding Unit, Armidale, NSW, Australia.*

Online Mendelian Inheritance in Animals (OMIA, https://omia.org/home/) is a freely available, curated, online database. OMIA provides up-to-date summary information on all known harmful and beneficial variants in animals, together with background information on all known inherited disorders and non-disease traits, which are called 'phenes'. OMIA curation focuses on phenes with confirmed and suspected Mendelian modes of inheritance. Several phenes caused by somatic mutations, chromosomal abnormalities, genetic modifications, or phenes with unknown or complex modes of inheritance are also included. OMIA is reciprocally hyperlinked to Online Mendelian Inheritance in Man, and is hyperlinked to resources at the National Center for Biotechnology Information (NCBI), including PubMed, NCBI Taxonomy and NCBI Gene. In 2017, downloadable tables of all known likely causal variants were added to OMIA, using Human Genome Variation Society (HGVS) nomenclature (https://varnomen.hgvs.org/). Information on many variants was incomplete due to limited published information. Here we report updated variant tables for cattle, sheep, goats and pigs. The updates include DNA, RNA and protein variant locations for recent reference genome releases, to facilitate the development of diagnostic tools. If published information is insufficient, variant locations and predicted effects on proteins were determined through various methods, including coordinate remapping and confirmation of the variant effect using in silico variant effect prediction. When authors publish variant information, we recommend they use HGVS nomenclature, including DNA, RNA and (predicted) protein locations and changes, and submit variant information to the European Variation Archive (EVA), because EVA automatically updates variant information, which OMIA can then include in its variant tables. If a variant requires updating in OMIA, please contact the first and last authors of this abstract.

Key Words: databases, monogenic traits, ruminants, pigs

P370 Single-cell RNA sequencing reference datasets of isolated bovine milk cells and cultured primary mammary epithelial cells. D. Becker*[1], R. Weikard[1], F. Hadlich[1], and C. Kühn[1,2], [1]*Leibniz Institute of Farm Animal Biology (FBN), Institute for Genome Biology, Dummerstorf, Germany,* [2]*University of Rostock, Faculty of Agricultural and Environmental Sciences, Rostock, Germany.*

For decades, the transcriptional regulatory processes of bovine mammary function, development and immune response have been extensively studied using mammary gland tissue and primary bovine mammary epithelial cells (pbMECs). Unfortunately, collection of mammary tissue and pbMECs often involves invasive procedures (i.e., biopsy) or even requires killing the animal. Additionally, the bulk transcriptome is impacted by cell type composition of the tissue and the animal's genetic background. An alternative approach to study mammary gland (patho)physiology is the analysis of the milk cell transcriptome. Milk cells comprise a mixture of somatic and epithelial cells, and milk cell number, reported as number of somatic cells, varies between individuals. However, similarly to mammary tissue, it is difficult to determine if changes in the transcriptome are based on varying cell composition or on a change in the expression of a particular gene in a specific cell type. In consequence, knowledge about the variation in gene expression profiles of cell populations and their effect on function is limited. Here, we used single-cell RNA sequencing of isolated milk cells and pbMECs to identify and characterize subpopulations of cells in a complex cell community. We used the 10 x Chromium Single-cell 3′ workflow to process the cells and sequenced single-cell libraries on an Illumina HiSeq 2500 platform. Subsequent data analysis was performed using the scRNA-seq analysis tool Seurat. In total, we generated high-quality data for 7,119 milk cells and 10,549 pbMECs. The median number of expressed genes per cell was 829 and 1,614 for milk cells and pbMECs, respectively. Clustering of the pbMEC data set resulted in 14 indefinite clusters displaying intrapopulation heterogeneity. In contrast, milk cells formed 14 distinct clusters that expressed classic immune cell and epithelial cell marker genes. Our data sets provide the first single-cell profiles of pbMECs and first insights into milk cell composition and gene expression. They can serve as reference data sets for further studies of milk cell analysis and contribute to a molecular cell atlas.

Key Words: cattle and related species, Functional Annotation of Animal Genomes (FAANG), single-cell RNA-seq, transcriptome, milk cells

P371 RNA sequencing transcriptomic analysis of Maedi-Visna and scrapie coinfected sheep. A. Hernaiz*[1], D. Martínez[1], B. Marín[2], B. Ranera[3], P. Zaragoza[1], J. J. Badiola[2], R. Bolea[2], B. Moreno[2], and I. Martín-Burriel[1,2], [1]*Laboratorio de Genética Bioquímica (LAGENBIO), Facultad de Veterinaria, Universidad de Zaragoza-IA2, IIS, Zaragoza, Spain,* [2]*Centro de Encefalopatías y Enfermedades Transmisibles Emergentes (CEETE), Facultad de Veterinaria, Universidad de Zaragoza-IA2, IIS, Zaragoza, Spain,* [3]*Facultad de Ciencias de la Salud, Universidad San Jorge, Zaragoza, Spain.*

Scrapie and Maedi-Visna (MV) are small ruminant diseases characterized by long incubation periods. Scrapie is a neurodegenerative disorder, belonging to the group of prion diseases, caused by the misfolded isoform (PrP^{Sc}) of the cellular prion protein (PrP^{C}). The etiology of MV infection is a lentivirus targeting mainly the lungs, mammary glands, joints and the central nervous system (CNS). PrP^{Sc} deposits accumulate mainly in the CNS but have also been found in lesser amount in other tissues such as lungs and mammary glands. Although the pathogenesis of both diseases has been well studied, little is known about Scrapie and Maedi-Visna coinfections. We present here an RNA sequencing analysis of lung samples from 12 female sheep: 5 controls, 4 MV infected sheep and 3 Scrapie and MV coinfected sheep. A total of 12,729 genes were coexpressed between controls, MV infected and MV-scrapie coinfected animals. Regarding differentially expressed genes (DEGs), 18, 29 and 69 genes were identified to be differentially expressed between MV and control sheep, MV-scrapie and control sheep and MV-scrapie and MV sheep, respectively. In comparison to healthy sheep, the majority of the DEGs were upregulated in MV and MV-scrapie sheep. Conversely, in MV-scrapie coinfected animals most of the identified DEGs were downregulated comparing to MV infected animals. Furthermore, enrichment analysis revealed that Maedi-Visna DEGs were significantly associated with homeostatic processes, cellular protein modification process, ion binding and catabolic pathways, whereas Maedi-Visna/Scrapie DEGs were significantly related to transmembrane transport, immune and nervous system processes, ion binding, protein-containing complex and oxidoreductase activity. Among the Maedi-Visna/Scrapie DEGs, some of them had functions that could be related to prion pathology. A set of these genes has been selected for a qPCR validation study that is currently being carried out. Although further studies are still needed, these data indicate that there is an interaction between Maedi-Visna and scrapie diseases with some genes that could favor PrP^{Sc} accumulation in non-target scrapie tissues such as lungs.

Key Words: sheep, RNA-seq, Maedi-Visna, scrapie

P372 Differential allele-specific expression in SNPs related to meat quality traits in *Bos indicus* muscle. J. Bruscadin*[1], T. Cardoso[2], M. De Souza[3], J. Afonso[2], J. Malheiros[4], T. Porto[1], J. Petrini[5], and L. Regitano[2], [1]*Federal University of São Carlos, São Carlos, São Paulo, Brazil,* [2]*Embrapa Southeast Livestock, São Carlos, São Paulo, Brazil,* [3]*Iowa State University, Ames, IA, USA,* [4]*Federal University of Latin American Integration, Foz do Iguaçu, Paraná, Brazil,* [5]*University of São Paulo/ESALQ, Piracicaba, São Paulo, Brazil.*

Allele-specific expression (ASE) studies contribute to the knowledge of *cis*-regulatory variants, but little is known about the relationship between ASE and livestock traits. We identified SNPs showing differential ASE (DASE SNPs) between contrasting groups for mineral content, carcass, and meat quality traits to better understand this scenario. The RNA-seq from Nelore (*Bos indicus*) muscle tissue, genotype and phenotype data used here were obtained previously. Skeletal muscle transcriptional profiles from 20 Nelore steers from both extremes of the distribution of estimated genomic breeding values (GEBVs) for different traits were classified as belonging to the HIGH (n = 10) or LOW (n = 10) groups. The DASE analysis was executed using DESeq2 software, comparing the reference and alternative allele counts ratio of 2,832 heterozygous SNPs between the contrasting groups. A total of 27 DASE SNPs (FDR <0.05) was identified considering the contrasting groups for ribeye area (REA), intramuscular fat (IMF), backfat thickness (BFT), tenderness measured at 24h (WBSF0), at 7 d (WBSF7), and 14 d (WBSF14) after slaughter, calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), manganese (Mn), sodium (Na), and zinc (Zn) content. The g26:34088221 and the g19:29461624 DASE SNPs were identified with differential ASE between contrasting groups for IMF and Ca traits, where the allelic imbalance was observed only in the LOW IMF and WBSF14 groups and the HIGH Ca group. The g26:34088221 DASE SNP showed the most differential ASE for WBSF14, expressing only the reference allele in the LOW group. The g3:22857282 DASE SNP also showed a monoallelic pattern in the LOW group for Ca and IMF, expressing only the reference allele. As LOW WBSF14 consists of more meat tenderness, and due to the known relationship of meat tenderization biochemical events and Ca, the similar allelic imbalance pattern of these SNPs should be better investigated. Thus, based on the differential allelic imbalance analysis, a new layer of information was obtained about the regulation of meat quality traits in bovine muscle, which can be explored to identify the most promising variants for beef improvement.

Key Words: cattle and related species, genome regulation, RNA-seq, allele-specific expression

P373 The intronic branch point sequence is depleted for mutations in the bovine and human genome. N. K. Kadri* and H. Pausch, *ETH Zurich, Zurich, Switzerland.*

Pre-mRNA splicing by the spliceosomal complex requires several cis-acting intronic features including the splice acceptor and donor sites, polypyrimidine tract, and the branch point sequence. Variations in these noncoding features have been implicated in complex trait variation and in human diseases. While the splicing sites at the exon-intron boundaries are readily accessible from standard gene annotation, the other 2 features are barely annotated and hence ignored generally in functional genomic studies. We herein, for the first time, computationally predict branch point sequences in 180,892 bovine introns and investigate their variability at nucleotide resolution. The bovine branch point is predominantly adenosine and is contained within a degenerate heptamer "nnyTr**A**y" (branch point in bold) located between 18 and 37 bp upstream of the 3′ splice acceptor site. In addition to the branch point at position 6, a thymine residue at the fourth position of the heptamer is also highly conserved. More than 90% of the bovine introns contain canonical branch point sequences with an adenosine residue acting as branch point and a thymine residue at position 4. Using a catalog of 29.4 million variants detected in 266 cattle, we show that the conserved thymine and adenine residues are targets of extreme purifying selection, harboring 39% and 41% less variants than coding sequences suggesting extreme purifying selection. We replicate these observations in human branch point sequences with a catalog of more than 115 million SNPs detected in 3,942 genomes from the gnomAD database. The strong evolutionary constraint on intronic branch point sequences unraveled in our study indicates that systematic assessment of sequence variation affecting this hitherto neglected intronic feature is warranted in functional genomic studies.

Key Words: cattle and related species, genome annotation

P374 Genome-wide local ancestry and direct evidence for cytonuclear disequilibria in hybrid African cattle populations (*Bos taurus*/*indicus*). J. A. Ward*[1], G. P. McHugo[1], M. J. Dover[1], T. J. Hall[1], S. I. Ng'ang'a[2,3], T. S. Sonstegard[4], D. G. Bradley[5], L. A. F. Frantz[2,3], M. Salter-Townshend[6], and D. E. MacHugh[1,7], [1]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [2]*Palaeogenomics Group, Department of Veterinary Sciences, Ludwig Maximilian University, Munich, Germany,* [3]*School of Biological and Chemical Sciences, Queen Mary University of London, London, UK,* [4]*Acceligen, Eagan, MN, USA,* [5]*Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland,* [6]*UCD School of Mathematics and Statistics, University College Dublin, Dublin, Ireland,* [7]*UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland.*

Cattle play an important role in African economies, culture, and society. Today, most African cattle are hybrids of the humpless *Bos primigenius taurus* (taurine) and the humped *Bos primigenius indicus* (indicine) types. These subspecies originated from independent domestications of *Bos primigenius primigenius* and *Bos primigenius namadicus*, respectively. The most recent common ancestor (MRCA) for the taurine and indicine lineages is estimated to have lived between 200,000 to 500,000 years ago, and as such there are significant differences between the *B. p. taurus* and *B. p. indicus* genomes. Despite substantial indicine nuclear genomic admixture in African cattle, they only carry taurine mitochondrial DNA (mtDNA). The efficient function of the vertebrate mitochondrion relies on fine-tuned interactions that exist between the products of over 1,000 nuclear genes and 37 mitochondrial genes. This bi-genomic system presents a challenge when there may be a mismatch between the nuclear and mitochondrial genomes, as may be the case in hybrid populations. Using high-density SNP data from over 500 cattle, representing 10 hybrid African cattle breeds, 4 pure taurine breeds and 4 pure indicine breeds we tested the hypothesis that there has been adaptive introgression of taurine ancestry at regions of the nuclear genome containing genes that encode proteins functionally associated with the mitochondrion. Our results support this hypothesis, demonstrating that mitonuclear disequilibria exists in hybrid African cattle populations. Using local ancestry analysis to infer ancestry at individual SNPs and then employing a bootstrap approach, we generated distributions of mean ancestry deviation at groups of nuclear-encoded mitochondrial genes. This analysis indicated that there is a significant retention of taurine alleles at nuclear-encoded mitochondrial genes in hybrid African cattle populations.

Key Words: cattle and related species, population genomics, admixture, mitochondrial DNA

P375 Genome-wide scan reveals pleiotropic effects on carcass and meat quality traits in crossbred beef cattle. F. Rezende*[1], E. Rodriguez[1], J. Leal-Gutiérrez[2], M. Elzo[1], D. Johnson[1], C. Carr[1], and R. Mateescu[1], [1]*University of Florida, Gainesville, FL, USA,* [2]*University of California, San Diego, CA, USA.*

While carcass quality is the primary factor determining the value of a carcass in the beef industry supply chain, consumers evaluate beef products at purchase time based on visual quality and at consumption time based on sensory quality. These traits are of even greater importance in crossbred cattle used in subtropical and tropical regions for their superior adaptability because they tend to underperform compared with their purebred counterparts. Genetic improvement of such traits is not viable through traditional phenotypic selection, but before genomic selection can be implemented in crossbred populations, it is important to explore if pleiotropic effects exist between carcass and meat quality traits. Therefore, the objective of this study was to identify genomic regions with pleiotropic effects on carcass and meat quality traits in a multibreed Angus-Brahman population. Data included phenotypes for 10 carcass and meat quality traits from 2,384 steers, of which 1,038 were genotyped with the GGP Bovine F-250. Single-trait genome-wide association studies were first used to investigate the relevance of additive genetic effects on each carcass, sensory and visual meat quality traits. A second analysis for each trait included all other phenotypes as covariates to correct for indirect effects, capturing genomic regions with pure direct effects on the trait under analysis. Five genomic windows on chromosomes BTA5, BTA7, BTA18 and BTA29 explained more than 1% of additive genetic variance of 2 or more traits. Moreover, 3 other suggestive pleiotropic regions were identified on BTA10 and BTA19. A total of 317 genes were identified across all pleiotropic regions. Many of the candidate pleiotropic genes encode anchoring and cytoskeletal proteins, important factors in muscle proteolysis, and key players in cell growth, muscle development, lipid metabolism and fat deposition. A functional analysis of these genes revealed GO terms directly related to carcass quality, meat quality, and tenderness in beef cattle, including calcium-related processes, cell signaling, and modulation of cell-cell adhesion. Our findings contribute with novel information about the complex genetic architecture and pleiotropic effects of carcass and meat quality traits in crossbred beef cattle.

Key Words: carcass attributes, sensory traits, visual traits, WssGBLUP, Angus × Brahman

P376 Imputation to whole-genome sequence by using a small reference population. J. Petrini*[1], B. G. N. Andrade[2], T. F. Cardoso[3], A. S. M. Cesar[4], B. Silva-Vignato[1], G. Morota[5], M. L. Spangler[6], L. C. A. Regitano[3], L. L. Coutinho[1], and G. B. Mourão[1], [1]*Department of Animal Science, College of Agriculture "Luiz de Queiroz," University of São Paulo/ESALQ, Piracicaba, SP, Brazil,* [2]*Department of Computer Science, Munster Technological University, Cork, Munster, Ireland,* [3]*Embrapa Southeast Livestock, São Carlos, SP, Brazil,* [4]*Department of Agroindustry, Food and Nutrition, College of Agriculture "Luiz de Queiroz," University of São Paulo/ESALQ, Piracicaba, SP, Brazil,* [5]*Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University,*

Blacksburg, VA, USA, [6]Animal Science Department, University of Nebraska–Lincoln, Lincoln, NE, USA.

Due to the high cost of sequencing, genotype imputation has been an important tool to increase the number of animals with genomic DNA sequence (DNA-Seq), enabling the use of these data in genetic studies. However, imputation can be challenging when the number of sequenced individuals is low, given the influence of the size of the reference set and SNP frequency on imputation accuracy. Thus, the aim of this study was to assess the quality of imputation to the whole-genome sequence from a high-density (HD) panel by using a small reference population. DNA-Seq data (at 8–21x of coverage) from 20 unrelated Nellore sires (mean relationship degree of −0.04) as well as the genotypes of 778 progenies obtained from a HD panel were used. Three strategies were investigated for imputation: EAGLE for haplotype phasing and Minimac3 for imputation, EAGLE for phasing and BEAGLE (v. 5.1) for imputation, and BEAGLE for both phasing and imputation. Imputation accuracy was assessed using a leave-one-out cross-validation procedure. Each sequenced sire was removed one at a time from the reference set and included as a target individual, considering only its HD genotypes to be imputed jointly with the progenies. Only markers mapped to bovine autosomes 1 and 29 were investigated. Higher imputation accuracy was obtained by using EAGLE and Minimac3. The means (standard deviations) of the correlation between true and imputed genotypes were 0.75 (0.323) and 0.72 (0.345) using EAGLE and Minimac3, 0.58 (0.428) and 0.53 (0.441) using EAGLE and BEAGLE, and −0.06 (0.335) and −0.05 (0.351) using BEAGLE, respectively. Independently of the strategy used, lower correlations were obtained for SNP with a frequency lower than 0.025. Differently from BEAGLE, Minimac3 considers recombination in the imputation process, not depending only on the haplotypes observed in the reference population. This is an interesting feature when imputation is based on a small reference population and/or the relationship between reference and target populations is low. The next step in this study is to include pedigree information in imputation and assess the accuracy of imputation in the progenies.

Key Words: animal breeding, cattle, DNA sequencing, Nellore, SNP

P377 C2C12 myotubes promote the migration of 3T3-L1 preadipocytes via the CCL5/CCR5 axis under coculture condition. W. Yu*, Y. Zhao, Y. Tian, M. Yan, W. Wei, L. Zhang, and J. Chen, *College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu Province, China.*

Increasing intramuscular fat content is important for improving meat quality, but the source of intramuscular adipocytes is still not completely clear. It has been shown that adipose stromal cells from subcutaneous fat could migrate to skeletal muscle tissue to form intramuscular adipocytes, but it is not clear whether the myotube promotes the migration of preadipocytes. The crosstalk between myotubes and adipocytes was evaluated using transwell assay in the present study. The result indicated that differentiated C2C12 myotubes showed more effective promotion effect for migration of 3T3-L1 preadipocytes compared with C2C12 myoblasts. As a myokine, the secretion and mRNA expression of chemokine (C-C motif) ligand 5 (CCL5) was significantly increased among the C2C12 myogenic differentiation. And CCL5-small interfering RNA significantly decreased the migration of 3T3-L1 preadipocytes. It indicated the CCL5-CCR5 axis played an important role in the regulation of migration from 3T3-L1 to C2C12 myotubes. These data suggest that intramuscular fat deposition may be regulated by the chemokines such as CCL5 in skeletal muscle.

Key Words: CCL5, intramuscular fat, preadipocytes, C2C12 myotube

P378 Mutations in transcription factors and cofactors associated with gene expression and feed efficiency-related traits in Nelore cattle. T. F. Cardoso*[1], J. J. Bruscadin[2], J. Afonso[1], J. Petrini[3], B. G. N. Andrade[4], P. S. N. de Oliveira[2], J. M. Malheiros[5], T. Porto[2], A. Zerlotini[6], G. B. Mourão[3], L. L. Coutinho[3], and L. C. A. Regitano[1], [1]*Embrapa Southeast Livestock, São Carlos, São Paulo, Brazil,* [2]*Postgraduate Program on Evolutionary Genetics and Molecular Biology, Federal University of São Carlos, São Carlos, São Paulo, Brazil,* [3]*Department of Animal Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo/ESALQ, Piracicaba, São Paulo, Brazil,* [4]*Munster Technological University, Ireland,* [5]*Federal University of Latin American Integration, Foz do Iguaçu, Paraná, Brazil,* [6]*Embrapa Agricultural Informatics, Campinas, São Paulo, Brazil.*

Cis-acting effects of noncoding variants on gene expression constitute significant factors influencing phenotypic variation in complex traits. To provide new insights into the impacts of single nucleotide polymorphisms (SNPs) on transcription factors (TFs) and transcription cofactors (TcoF) and identify *cis*-regulatory effects, we carried out a multi-omics analysis on Nelore cattle muscle gene expression and its association with feed efficiency-related traits. For that, we used RNA-seq data from 190 muscle samples, as well as imputed genotypes and phenotype data from 374 Nelore cattle. We adopted a prospecting window corresponding to 2 Kb upstream to the transcriptional start site (TSS) up to 3′ UTR region of TFs and TcoFs. Association analysis were performed using a univariate mixed model. We identified 6 tagSNPs predicted to impact gene expression and the analyzed traits. Among these, the rs134134361 was associated with *ZBTB32* expression and with average daily gain, and was predicted to disrupt the TTF1 binding site in the *ZBTB32* upstream sequence. Another tagSNP is the rs208255476, which showed association with *DDIT3* expression and relative growth rate. Additionally, rs135996356 tagSNP was associated with the *E2F4* gene expression and feed conversion ratio (FCR). Co-regulatory network analysis revealed that *E2F4* is correlated with many putative regulators of FCR, *e.g.*, *PRUNE2* and *ARKT1*, previously identified as candidate genes related to this trait. Three tagSNPs associated with the residual feed intake (RFI), *i.e.*, rs524832799, rs385089005, and rs137256008, were associated with *CBFA2T3*, *KAT6A*, and *EEF1A1* gene expressions respectively. In addition, genes of the collagen family (*e.g.*, *COL1A1*, *COL1A2*, and *COL16A1*) were positively co-expressed with the *EEF1A1* and were predicted as potential regulators of RFI. All associated SNPs overlapped with epigenetic marks and/or QTL regions related to these traits. Therefore, our analyses reinforce and contribute to a better understanding of the biological mechanisms underlying gene expression control of feed efficiency traits in bovine. These *cis*-regulatory SNPs can be used as biomarkers for feed efficiency in Nelore cattle.

Key Words: polymorphism, association analysis, regulatory genes, *Bos indicus*

P379 Bovine horn bud structure and gene expression at 58 days of fetal development. J. Aldersey*[1], Y. Ren[1], W. Low[1], R. Tearle[1], K. Petrovski[1], T. Chen[1], J. Williams[1,2], T. Sonstegard[3], and C. Bottema[1], [1]*Davies Livestock Research Centre, School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia,* [2]*Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Università Cattolica del Sacro Cuore, Piacenza, Italy,* [3]*Acceligen, Eagan, MN, USA.*

Horns are permanent cranial appendages in Bovids and are made of bone and keratin. Hornless or polled cattle are naturally occurring and polledness is caused by at least 4 different genetic variants located in a ~400 kb region on chromosome 1. In cattle, horn ontogenesis begins during embryo development but the genetic pathways involved are unknown. The horn bud region (HB) and frontal skin (FS) were biopsied from the head of 6 homozygous horned and 5 homozygous polled fetuses at 58 d of development for hematoxylin and eosin staining. All horned HB sections had good structural integrity, while only 25% of polled HB and FS tissues maintained structural integrity post-processing. HB and FS tissues from 4 horned and 3 polled fetuses were used for RNA sequencing (RNA-seq) which identified 97 genes that were differentially expressed (DE) between tissue types from horned fetuses ($P < 0.05$, log2 fold change (logFC) >1). Of these, 54 have increased expression in the HB relative to the FS. Only 2 genes were DE between the polled HB and FS ($P < 0.05$,

logFC >1). In the horned fetuses, 35% of the DE genes expressed more highly in the FS were involved in nerve development and the nervous system. However, there were also genes involved in nerve development that had higher expression in the HB. These DE genes may play a role in the development of the zygomaticotemporal and cornual branches that innervate the forehead and horns, respectively. About 30% of the DE genes expressed more highly in the horn bud of the horned fetuses coded for structural proteins. This coincides with the histological results as the horn bud had greater structural integrity and thicker epidermis than the frontal skin. The morphology of the horn buds was consistent with the morphology described in older fetuses in previous studies, except for a population of condensed cells within the horn bud mesenchyme below the thickened epithelium. This study provides a snapshot of the horn bud structure and gene expression at 58 d of fetal development and suggests substantial differences in nearby tissues even at this very early stage of development.

Key Words: cattle, horns, polled, RNA-seq, development

P380 Genomic breeding values from low-coverage Nanopore sequencing. H. J. Lamb, B. J. Hayes, L. T. Nguyen, and E. M. Ross*, *The Queensland Alliance for Agriculture and Food Innovation, St Lucia, Queensland, Australia.*

Turnaround time has been a major limitation to the use of genomic prediction in Australia's northern beef industry where cattle are often only handled once a year. Therefore, Australia's northern producers require an alternative to traditional SNP array genotyping, which takes 6–8 weeks. We have previously proposed using portable DNA sequencing technology to sequence animals on-farm to produce rapid genomic estimated breeding values (GEBVs). To investigate the feasibility of this approach we sequenced 19 Droughtmaster cattle to an average coverage of 5.5x on the minION (Oxford Nanopore Technologies; Guppy v4.2.2). The sequence was aligned to ARS v1.2 and SNP were called using a custom algorithm that accounts for variable read depth across loci. Genotypes at loci that overlapped with 641k high-quality markers from the Illumina Bovine 777k HD SNP array were extracted. Missing genotypes were imputed based on the weighted likelihood of each genotype given the allele frequency of the population. Marker effects at the same loci were taken from a population of 30k beef cattle (Brahman and crossbreeds) in Northern Australia for body weight. The marker effects were then used in a SNP-BLUP to calculate 2 EBVs for each animal, one using the genotypes obtained from the sequencing data, and one using genotypes from the 50K SNP array (imputed up to 641K). The SNP array and the sequencing-based EBVs were then compared with determine the level of concordance between the 2 methods. We observed a correlation between the Nanopore sequencing derived GEBVs and SNP array GEBVs of 0.96 for body weight and a regression slope of 1.02 when missing genotype calls in the sequencing data were not imputed. When missing genotypes were imputed based on the population allele frequencies the correlation increased to 0.97 with a regression slope of 1.06. This indicates that accurate GEBVs can be calculated at low sequencing depths using portable sequencing technologies. Methods to combat sequencing errors and regions of ultra-low coverage (1x-2x) could be implemented to further increase the accuracy of GEBVs derived from on-farm sequencing. Furthermore, investigations to reduce the cost of sequencing are underway.

Key Words: sequencing, Nanopore, cattle, GEBV

P381 Expression (e)QTL study to determine the association between omics and phenotypes for Warner-Bratzler shear force of longissimus dorsi in Hanwoo cattle. Y. Chung*[1], D. Seo[1], K.-Y. Chung[2], and S. H. Lee[1], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, South Korea,* [2]*Department of Beef Science, Korea National College of Agriculture and Fisheries, Jeonju, 54874, South Korea.*

Finding a causality of genetic variants controlling breeding traits would improve the genetic evaluation system in cattle breeding programs. Many studies have been performed about investigating genetic factors using genome-wide association study and RNA-seq analysis. However, there are limits to understanding the biological complexity and network in integrating genome, transcriptome, and phenotype to find out the causality of gene and genetic variants. In this study, an association study between Warner-Bratzler shear force (WBSF) of *longissimus dorsi* muscle and gene expression value and eQTL study was conducted using 20 Hanwoo cattle. To determine how much significant SNPs from an eQTL study explain genetic variance in 1,181 cattle, including 20 animals of eQTL study, heritability was estimated using a linear mixed model. As the results, we detected 166 WBSF-associated genes (*P*-value <0.01) from the association study. In eQTL analysis using the WBSF-associated genes, 819 and 854,675 SNPs with cis- and trans-effects respectively in 1 Mb has statistically significance (*P*-value <0.01). Also, heritability from all_777k was 0.45 ± 0.08, and heritabilities were 0.44 ± 0.08 and 0.01 ± 0.02 for exp_777k and cis_777k, respectively. Based on the results above, we selected 6 candidate genes with functional effects for WBSF and 11 variants in 5 kb for these genes; ASAP1 (1 intron variant), CAPN5 (1 intron variant), ELN (1 upstream gene variant), MGAT4A (7 intron variants), SUMF2 (1 upstream variant) and TTC8 (2 intron variants). For CAPN5, the expression value tends to be increased in the animals that have minor allele of rs41772707 (A/C), and with this change, WBSF tends to increase. Furthermore, the results showed that the intron variants in the noncoding region might affect the modulation of phenotype. Thus, finding the integrated relation could be useful in explaining phenotypic changes by genetic variation. In addition, further studies are needed to detect the genetic factors in the noncoding region.

Key Words: Hanwoo cattle, Warner-Bratzler shear force (WBSF), omics, system genetics (eQTLs), noncoding variants

P382 Whole-genome resequencing points to candidate variants and genes for body temperature maintenance under the cold stress in Siberian cattle populations. A. V. Igoshin*[1], N. S. Yudin[1,2], R. B. Aitnazarov[1], A. A. Yurchenko[1,2], and D. M. Larkin[1,3], [1]*The Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (ICG SB RAS), Novosibirsk, Russia,* [2]*Kurchatov Genomic Center, Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Science, Novosibirsk, Russia,* [3]*Royal Veterinary College, University of London, London, UK.*

Despite the economic importance of creating cold tolerant cattle breeds, our knowledge of the genetic basis of adaptation to cold environments in cattle is still scarce compared with information on other economically important traits. To gain insight into the genetic mechanisms underlying cattle tolerance to cold, we performed whole-genome resequencing of 12 animals from Siberian populations of Kazakh Whiteheaded and Hereford breeds showing contrasting temperature maintenance phenotypes under acute cold stress. Variant calling produced more than 16.8 million autosomal variants. To highlight genome regions contributing to cold tolerance phenotype, we conducted window-based F_{st} calculations between cold tolerant and sensitive groups (6 individuals each). To reveal candidate causative variants, we combined the F_{st} analysis results with functional variant annotations (FAETH score, Xiang et al., 2019) and allele frequency data. Each SNP was ranked according to combination of the F_{st} value of its region, FAETH score and differences in allelic frequencies between contrasting groups. As a result, we revealed multiple candidate variants and genes controlling body temperature in Siberian cattle populations, e.g., in *GAP43* (SNP BTA1:60917653, synonymous variant), *COX17* (SNP BTA1:65031883, missense variant), *KMT2D* (SNP BTA5:30945568, splice region variant), *KCNH3* (SNP BTA5:30453758, missense variant), *GSK3B* (SNP BTA1:65292044, missense variant). Our results could be useful for developing cattle breeding strategies in countries with harsh climates, including the Russian Federation.

Key Words: body temperature, cattle, selection, cold adaptation

P383 Rare casein variants in goats identified by capture sequencing. S. Rahmatalla*[1,2], D. Arends[1], A. S. Ahmed[1], L. Hassan[3], S. Krebs[4], M. Reissmann[1], and G. Brockmann[1], [1]*Humboldt University, Berlin, Germany,* [2]*University of Khartoum, Khartoum, Sudan,* [3]*Wildlife Research Center, Khartoum, Sudan,* [4]*Ludwig Maximilian University, Munich, Germany.*

Casein protein variants have obtained substantial attention, since they are known to affect milk protein yield, milk composition, cheese processing properties, digestibility, and tolerance in human nutrition. Furthermore, milk protein variants are used for breed characterization, biodiversity, and phylogenetic studies. This study aimed to identify casein protein variants in 5 domestic goat breeds from Sudan (Nubian, Desert, Nilotic, Taggar, and Saanen) and 3 wild goat species (Bezoar ibex, Nubian ibex, and Alpine ibex). Thirty-three unrelated female goats were sequenced using high-density capture sequencing for casein genes. Casein genes were enriched by hybridization to a custom tiling and sequenced on a Hiseq1500 instrument in paired-end mode with a read length of 100 nt. The gene specific tiling array was created using the goat reference sequences (version LWLT01) available at the NCBI. Protein variants were predicted from DNA polymorphisms by using bioinformatics tools. Nine new casein protein variants were detected. In the α S1 casein, 3 novel casein variants were found in Saanen goats (*CSN1S1**C), Bezoar ibex (*CSN1S1**J), and Alpine ibex (*CSN1S1**K). A new β casein variant (*CSN2**F) was detected which was unique to Alpine ibex. In the α S2 casein, 4 novel protein variants were identified, which were segregating in all domesticated and wild goats (*CSN1S2**H), in Nubian and Desert goats (*CSN1S2**I), and in Nubian ibex only (*CSN1S2**J, *CSN1S2**K). A single novel casein variant of *CSN3* was detected in Alpine ibex (*CSN3**X). The identified novel protein variants are of interest not only for their effect on protein and milk composition but also for evolutionary studies on milk protein genes. Therefore, further investigation is necessary to examine the expression of the 9 new variants on protein level to validate and confirm this study's outcome.

Key Words: casein genetic variation, Sudanese goats, Saanen, Bezoar ibex, Nubian and Alpine ibex

P384 Simulating the likely impact of genome editing using a real multigeneration pedigree of genotyped animals. A. Reverter*, L. Porto-Neto, and J. Kijas, *CSIRO Agriculture and Food, Brisbane, Queensland, Australia.*

To date, the use of genome editing (GE) in livestock has focused on simple traits that are controlled by a few QTL with large effects. When used to improve quantitative traits that are controlled by many QTL, GE is referred to as PAGE for Promotion of Alleles by Genome Editing. Unlike other studies that use data simulated using assumptions that may not approximate commercial scenarios in terms of population structure features (e.g., generation intervals, mating and replacement ratios), the aim of this study was to use a real multigeneration data set of pedigreed and genotyped individuals to explore the potential of PAGE to increase the frequency of rare favorable alleles. We employ data from a population of ~80,000 Australian Angus cattle with genotypes for 50,000 SNP. Without loss of generality, we assume that the first allele "A" is the favorable yet minor allele. We then selected 10 SNP with a very low frequency of A (<5%) and another set of 10 SNP with a high frequency of A (>45% but <50%). Starting with the first and at each generation, we "gene-edit" the selected SNP in the top 10% sires in terms of the number of progeny and modify the genotype of the progeny accordingly. At the end of the process, we re-compute the allele frequencies of edited SNP and compared with their original frequencies. For SNP initially at low A frequency, the frequency of heterozygous AB individuals increased exponentially to 100% while that of homozygous AA individuals was mostly unchanged to ~1%. The opposite was true for high A frequency loci: the frequency of AB individuals remained unchanged to ~50% while the frequency of AA individuals experienced a rapid increased to 50%. Importantly, the frequency of the A allele never achieved fixation and found a maximum of 50% and 75% when its initial frequency was <5% and >45%, respectively. While further research is warranted, these preliminary results forecast the opportunity and limitation to achieve population wide allele frequency shifts using PAGE. Our results clearly indicate the need to preferentially use the progeny of edited sires as future sires to fully exploit the power of PAGE.

Key Words: genome editing, cattle and related species, QTL

P385 Withdrawn

P386 Genome-wide association and genetic parameter estimation studies for coat color of Chikso. J. M. Kang*[1], Y. K. Kim[1], D. H. Lee[1], S. H. Lee[2], T. J. Choi[2], M. N. Park[2], and S. H. Lee[1], [1]*Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea,* [2]*Animal Genetic Improvement Division, National Institute of Animal Science, Korea.*

Korean Brindle cattle (Chikso) is a Korean indigenous cattle breed, with a small population that is endangered. As genomic information is available, Chikso population is getting to be enlarged population size from controlling inbreeding, pedigree reconstruction and assortative mating using genomics. One of the breeding objective traits for this breed is their coat color (brindle color). This Chikso population has a quite big variation of brindle pattern from black to clear brindle. Therefore, we categorized a measuring of brindle pattern from 1 to 6 scale. In this study, a total of 1,118 Chikso with a brindle color phenotype were genotypes using Illumina Bovine Beadchip ver 2. Genome-wide association study attempted to identify genomic loci associated with brindle color and heritability estimation for brindle pattern were performed using linear mixed model. We proceed quality control based on minor allele frequency (<0.01) and missing rate (>0.1) and finally use 47,896SNPs for analysis. As a result, significant associations to brindle pattern were found on BTA 6, 13, and 18. Several SNPs, such as ARS-BFGL-NGS-72592 (6:67949582), ARS-BFGL-NGS-103077 (13:65006713) and ARS-BFGL-NGS-86599 (18:14359452) were detected as significant SNPs, of which ARS-BFGL-NGS-86599 showed the highest significance. Of these, adjacent loci of ASIP gene on BTA 13 confirmed black and brown coat color relationship and MC1R gene on BTA 18 detected brindle coat color relationships. Heritability was evaluated by estimating 118 Chikso test population in a reference population which is divided into 1,118 Chikso population. For the cross-validation, heritability estimation performed 5 repetitions by random sampling of the test group. The heritability of brindle pattern was quite high, ranging from 0.509 to 0.610, and genetic variance and residual variance represent 1.246 to 1.505 and 0.962 to 1.203 respectively. As a result, significant results for coat colors could be seen even though Chikso is a natural population, and further research could lead to better results.

Key Words: genome-wide association study (GWAS), coat color, SNP, Chikso, genetic parameter

P387 Multi breed genome-wide association for male fertility traits in tropical cattle. L. Porto-Neto*[1], J. Bertram[2], M. Fortes[3,4], P. Alexandre[1], M. McGowan[5], B. Hayes[4], and A. Reverter[1], [1]*Commonwealth Scientific and Industrial Research Organisation, Agriculture and Food, Brisbane, QLD, Australia,* [2]*Agriculture Consultant, Livestock management and breeding, Toowoomba, QLD, Australia,* [3]*The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, QLD, Australia,* [4]*The University of Queensland, Queensland Alliance of Agriculture and Food Innovation, Brisbane, QLD, Australia,* [5]*The University of Queensland, School of Veterinary Sciences, Gatton, QLD, Australia.*

The reproductive efficiency of cattle breeding directly affects the productivity of the entire beef supply chain. Worldwide, most beef breeding herds are bull mated, relying heavily on bulls' reproductive performance. Here, common fertility indicators, including scrotal circumference and semen traits, were explored in combination with 700K imputed SNP genotypes using a multi-breed GBLUP approach. The data set included

6,063 bulls from 6 Australian populations: Brahman, Tropical Composite, Santa Gertrudis, Droughtmaster, Ultra-Black, and Belmont Tropical Composite. Phenotypes were pre-adjusted using a model that included fixed effects (population, year of birth, and cohort) and the first 2 principal components of the genomic relationship matrix and age as covariates. We estimated the heritability of fertility indicators as moderate to high, in agreement with the literature. That is 0.46 for scrotal circumference (cm), 0.24 for the percentage of normal sperm (%), and 0.22 for the percentage of proximal cytoplasmic droplet (%), 0.21 for the mass movement of sperm in the ejaculate (1–5), 0.20 for density of ejaculate (1–5), and 0.19 for sperm progressive motility (%). Additionally, we calculated the effect of each SNP, estimated its significance, and plotted along the genome to identify genomic regions explaining a relatively high proportion of the genetic variance. A broad region on chromosome 5 and some regions of chromosome X were consistently significant across several traits, suggesting that these regions harbor genetic variations that affect the observed fertility indicators. The identification of the functional genomic variants and the definition of how to better integrate this new knowledge into breeding programs warrant future investigation.

Key Words: cattle, fertility, multibreed, GWAS, tropical

P388 Withdrawn

P389 Genotyping of *PRNP* gene in Spanish local goat breeds. A. Canales*[1,2], M. Macri[1,2], J. V. Delgado[2], and A. M. Martínez[2], [1]*Animal Breeding Consulting, S.L, Cordoba, Cordoba, Spain,* [2]*Department of Genetics, University of Córdoba, Cordoba, Cordoba, Spain.*

Scrapie is a fatal, neurodegenerative disease of sheep and goats. It is also the earliest known member in the family of diseases classified as transmissible spongiform encephalopathies (TSE) or prion diseases, which includes Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy and chronic wasting disease in cervids. Natural caprine scrapie has been discovered throughout Europe, with reported cases generally being greatest in countries with the highest goat populations, although PRNP variability in goats differs from that observed in sheep, the 2 species share several identical alleles. Moreover, while the ARR allele associated with enhancing resistance in sheep is not present in the goat PRNP, there is evidence for the existence of other PRNP variants related to resistance. In this work, analysis of the prion protein gene was performed, with the aim to obtain information about the genetic variability in Spanish local goat populations/breeds. This research could help to promote the adoption of selective breeding programs as a possible future strategy in scrapie control and outbreak prevention. Three codons recommended by the EFSA (European Food Safety Authority), i.e., D146, S146 and K222, where analyzed. Additionally, 4 codons were genotyped, i.e., I142, H143, R154 and R211. Primers to detect polymorphism in the 7 codons were designed: 142: (38T)GTGCCATGAGTAGGCCTCTTAT, 143 (24T) AAGTGCCATGAGTAGGCCTCTTATAC, 146 (22T)GGCCTCTTATACATTTTGGCA, 146.1 (16T)AGTAACGGTCCTCATAGTCAT (reverse complement), 154 (7T)TGACTATGAGGACCGTTACTATC, 211 (43T) GAAACTGACATCAAGATAATGGAGC and 222 GAGCAAATGTGCATCACCCAGTAC. Genomic DNA was extracted from whole blood, amplification was performed in 10 µL reaction volume containing 1.5 mM $MgCL_2$, 200 µM dNTPs, 0.5 µM of forward and reverse primers of the gen PRNP and 1U of MyTaq DNA Polymerase (BIOLINE). The samples were amplified with the following thermal profile: 95°C × 3 min followed by 32 cycles at 95°C × 30 s, 56°C × 30 s, 72°C × 30 s, and final extension 15 min at 72°C. PCR products were purified by the enzyme Fastp and exonuclease, following by SNaPshot technique using Applied Biosystems SNaPshot Multiplex Kit. The SNPs were detected in capillary electrophoresis ABI PRISM 3130 XL Genetic Analyzer and observed the difference fluorescence and polymorphism in the different populations.

Key Words: *PRNP*, goat breed, codon, polymorphism

P390 Single nucleotide polymorphisms in the calpain 1 gene (*CAPN1*) are associated with production traits in Irish beef cattle. K. Quigley*[1], D. F. G. Flores[1], L. O'Kane[1], R. D. Evans[2], T. J. Hall[3], D. E. MacHugh[3,4], and M. P. Mullen[1], [1]*Bioscience Research Institute, Athlone Institute of Technology, Athlone, Co. Westmeath, Ireland,* [2]*Irish Cattle Breeding Federation, Bandon, Co. Cork, Ireland,* [3]*Animal Genomics Laboratory, UCD School of Agriculture and Food Science, Belfield, Dublin, Ireland,* [4]*UCD Conway Institute of Biomolecular and Biomedical Research, Belfield, Dublin, Ireland.*

Genetic improvement of meat-eating quality attributes is both desirable and possible with routine genetic evaluations recently published in Ireland. The calpain 1 gene (*CAPN1*) has been associated with meat tenderness and postmortem tenderization and estimation of the associated effects of polymorphisms in *CAPN1* on other performance traits in beef cattle may inform breeding decisions. The objective of the current study was to estimate breed allele frequencies and the phenotypic effects of 3 single nucleotide polymorphisms (SNPs) at the *CAPN1* locus (*rs17872000*, *rs17871051* and *rs17872050*) on carcass and fertility traits in Irish beef cattle. The analysis encompassed 120,000 cows, 20,000 from 6 admixed cattle breeds: Aberdeen Angus, Belgian Blue, Charolais, Hereford, Limousin, and Simmental. *CAPN1* genotypes were obtained from the Irish Cattle Breeding Federation who also provided data for the 8 phenotypes, expressed as predicted transmitting abilities (PTAs). Associations between each SNP and PTA were analyzed in ASReml 4.1 using a weighted mixed animal model. The 3 *CAPN1* SNPs were polymorphic in each of the 6 breeds analyzed. The C, G and C alleles at *rs17872000*, *rs17871051* and *rs17872050*, associated with tenderness in the literature, exhibited frequencies between 0.09 to 0.21, 0.14 to 0.39 and 0.19 to 0.61, respectively. While no individual estimated SNP effects were greater than 0.4% of phenotypic variance, significant associations (*P*adj. < 0.05) were observed in several cattle breeds. Both *rs17871051* and *rs17872050* were significantly associated (*P*adj. < 0.05) with improved carcass weight, carcass conformation, cull cow carcass weight and increased gestation, while *rs17872000* was significantly associated (*P*adj. < 0.05) with decreased carcass weight, carcass conformation, cull cow carcass weight and decreased gestation. This candidate gene analysis, suggests that *CAPN1* polymorphisms in 6 popular Irish beef cattle breeds are associated with variation in economically important traits, thereby providing additional information to support future breeding goals and strategies.

Key Words: cattle and related species, animal breeding, quantitative genetics, candidate gene, meat production

P391 MicroRNA profiles in colostrum and newborn calves after colostrum ingestion. D. T. Hue*[1,2], Y. Ren[1], W. Y. Low[1], T. Chen[1], K. Petrovski[1], J. L. Williams[1,3], R. Tearle[1], and C. D. K. Bottema[1], [1]*Davies Livestock Research Centre, School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia,* [2]*Faculty of Animal Science, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi, Vietnam,* [3]*Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

MicroRNAs (miRNAs) are short noncoding RNAs that play a critical role in regulating gene expression and thereby affecting the physiology of animals. In this study, miRNA profiles were analyzed in colostrum and the blood of calves after colostrum ingestion. Dam colostrum (n = 4) was fed to their own calves (Group A, n = 4) and foster calves (Group B, n = 4) every 12 h for 3 d after birth. Pooled colostrum (n = 4) collected from multiple cows at d 0 – 4 postpartum was fed once to another group of calves (Group C, n = 4) after birth, and thereafter, these calves were fed bulk tank milk. Total RNA from the colostrum and calf blood before the calves received colostrum (d 0), and 24 h after feeding colostrum (d 1) was extracted, sequenced using Illumina short reads. The data were analyzed using a bioinformatics pipeline that incorporates miRDeep2. The number of miRNAs in the calf blood was 4-fold higher than in the colostrum. There were 303 miRNAs detected in colostrum (296 known and 7 novel). A similar

miRNA profile was found in dam and pooled colostrum with 76% in common. Only 4 miRNAs had significantly higher levels in the dam colostrum compared with the pooled colostrum. The top 100 most highly expressed miRNAs in colostrum are involved in several biological processes, but mainly they contribute to cellular and mammary gland functions rather than functions that might benefit the newborn calf. The total number of miRNAs in calf blood was 1,198 (1,004 known and 194 novel). Of these miRNAs, 94% were detected in the calf blood samples from both d 0 and 1, and only 22 miRNAs had significantly different levels between these 2 time points. Pathway analysis of these 22 miRNAs indicated that they are involved primarily in cellular membranes and growth. Of these 22 miRNAs, 3 had higher levels in the calf blood at d 1 after 2 colostrum feeds. However, the levels in the calf blood were not correlated with the levels in the corresponding colostrum. These findings suggest that miRNAs in the colostrum are not likely to be absorbed by the calves. Instead, the newborn calves appear to be synthesizing their own miRNAs.

Key Words: miRNA, bovine, calf blood, neonate

P392 Prediction of Hanwoo cattle phenotypes from genotypes using machine learning methods. B. I. Lopez*, S. Srivastava, M. Jang, H. Kumar, W. Park, H.-H. Chai, J.-E. Park, and D. Lim, *Division of Animal Genomics and Bioinformatics, National Institute of Animal Science, Wanju, Republic of Korea.*

Hanwoo is originally raised for draft purposes, but the increased in local demand for red meat turned that purpose into full-scale meat-type cattle and is now considered one of the most economically important species and a vital food source for Koreans. With the application of genomic selection in Hanwoo breeding program in recent years, it was expected to lead to higher genetic progress. However, better statistical methods that can improve the genomic prediction accuracy are required. Hence, this study aimed to compare the predictive performance of 3 machine learning methods, namely, random forest (RF), gradient boosting method (GBM) and extreme gradient boosting methods (XGB) when predicting the back fat thickness (BFT), carcass weight (CWT), eye muscle area (EMA) and marbling score (MS). Phenotypic and genotypic data from 7,324 commercial Hanwoo cattle that were slaughtered at the age of around 30 mo were used. Results revealed that the boosting method GBM showed the best predictive performance among the methods with average correlations of 0.43, 0.37, 0.46 and 0.49 for CWT, BFT, EMA and MS. Correspondingly, the mean correlations using XGB were 0.43, 0.23, 0.31 and 0.44, and 0.36, 0.24, 0.32 and 0.39 using RF. Gene functional annotation of the significant marker identified by GBM revealed that there were many pathways which were involved in biological metabolic process. ADCY2 and ADCY1 (Adenylyl cyclase) were identified, which plays an important role in flesh tenderization of carcass. Moreover, kinases such as PRKD1, PRKG1, PLCB1, MAPK8 were also identified which play essential role in body weight development.

Key Words: genomic prediction. Hanwoo, machine learning

P393 Pregnancy in goats induces a strong transcriptomic change in the olfactory bulb. M. G. Luigi-Sierra*[1], D. Guan[1], M. López-Béjar[2], E. Casas[2], S. Olvera[2], J. Gardela[2], M. J. Palomo[3], U. I. Osuagwuh[3], U. L. Ohaneje[3], E. Mármol-Sanchez[1], and M. Amills[1,4], [1]*Centre de Recerca Agrigenòmica (CRAG), Bellaterra, Barcelona,*

[2]Department of Health and Animal Anatomy, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [3]Department of Animal Medicine and Surgery, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [4]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.

The brain is an organ involved in essential processes such as the maintenance of body homeostasis, memory, learning, and behavior. Pregnancy induces physiological changes to allow the development of the embryo as well as the preparation of the female for motherhood, and the majority of these processes are tightly regulated in a brain-dependent manner. For instance, the establishment of offspring bonding and nurturing after parturition are regulated by behavioral changes at the central nervous system, which experiences substantial anatomical and transcriptomic changes. Studies performed in humans and mice have demonstrated that the brain of pregnant females experiences substantial anatomical and transcriptomic changes. In this work, we aimed to understand the transcriptomic changes induced by pregnancy in goats by bulk sequencing of total RNA from 12 brain regions in 3 pregnant (second month of gestation) and 4 nonpregnant Murciano-Granadina females. Subsequently, we extracted total mRNA from each tissue and performed sequencing. Quality control and removal of the adapters were carried out with FastQC and TrimGalore. Reads were aligned to the goat reference genome ARS1 with HISAT2 software, and mRNA expression profiles were estimated with StringTie tool. Differential expression analyses were performed with DESeq2. We observed that pregnancy does not significantly affect the transcriptomic profiles of the medulla oblongata, cerebellar trunk, cerebellar hemisphere, rostral collicle, hypothalamus, and neurohypophysis. In contrast, the olfactory bulb, adenohypophysis, pons, frontal neocortex, hippocampus, and pineal gland displayed significant changes in mRNA expression, with a total of 1,234, 203, 197, 85, 71, and 66 differentially expressed genes (DEGs), respectively. Among the 12 brain regions under analysis, the olfactory bulb transcriptome was the most heavily affected by pregnancy. These findings agree well with studies performed in mice during the 70s that demonstrated that bulbectomized mice during pregnancy neglected their litters and were unable to build well-constructed nests.

Key Words: goats and related species, RNA-seq, pregnancy

P394 Integrative QTL mapping and selection signature analysis identify positional candidate genes influencing headed white spotting in Groningen White Headed cattle breed. R. Gonzalez-Prendes*[1], C. Ginja[2], J. Kantanen[3], N. Ghanem[4], D. R. Kugonza[5], M. I. Makgahlela[6], M. A. M. Groenen[1], and R. P. M. A. Crooijmans[1], [1]*Animal Breeding and Genomics, Wageningen University and Research, Wageningen, the Netherlands,* [2]*CIBIO/InBIO-Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal,* [3]*Natural Resources Institute Finland, Jokioinen, Finland,* [4]*Animal Production Department, Cairo University, Cairo, Egypt,* [5]*Makekere University, Kampala, Uganda,* [6]*Agricultural Research Council, Pretoria, South Africa.*

Within the Optibov project, we investigated the genetic architecture of the Dutch traditional cattle breed Groningen White Headed (GWH) to identify genomic regions and candidate genes associated with Ambilateral Circumocular Pigmentation (ACOP). We implemented an integrative analysis approach, including: a) a genome-wide association study (GWAS) using GWH with ACOP, other white-headed breeds (Simmental and Hereford), and breeds without the white head phenotype (Dutch Belted, Deep Red, Meuse-Rhine-Yssel, Dutch Friesian and Holstein Friesian); b) scans for specific signatures of selection in GWH cattle by comparison with the other 4 Dutch traditional breeds (Dutch Belted, Deep Red Dutch Friesian and Meuse-Rhine-Yssel); and c) detection of candidate genes identified in these approaches or reported in literature for other species. Whole-genome sequences of 120 animals were generated using Illumina Hiseq6000, and the number of reads by animals varied between 203,644,434 (Fries Hollands) and 377,428,034 (Meuse-Rhine-Yssel). After mapping the reads to the reference bosTau9 genome, the depth of coverage per sample varied between 7X and 13X with an overall average across breeds of 9X. The number of breed-specific variants varied from 559,091 (Deep Red) to 148,224 (Holstein Friesian). Specific genetic effects in the GWH breed with the ACOP phenotype may be due to coincidences between the QTLs and the specific selective sweep of genomic regions on BTA5 (10.0–13.6Mb), BTA15 (55.6–59.7Mb) and BTA20 (10.9–20 Mb). Those regions also contained candidate genes associated with pigmentation, UV protection and retinal degeneration. This finding

will assist in characterizing the genetic background of the GWH breed and in understanding the genetic of coat color in cattle.

Key Words: whole genome, cattle, pigmentation, signature selection, candidate gene

P395 The distinct morphological phenotypes of domestic sheep are shaped by introgressions from their sibling wild species. H. Cheng*, J. Wen, Z. Zhang, and Y. Jiang, *Northwest A&F University, Yangling, Shaanxi, China.*

Domestic sheep, including thousands of breeds worldwide, exhibit distinct phenotypes. However, how are these phenotypic variations affected by introgression has not been systematically investigated. Here, we analyzed 1,403 whole-genome sequences of sheep containing 69 samples from all 7 wild species (snow sheep, bighorn, thinhorn, argali, urial, Asiatic and European mouflon) within *Ovis* genus, and 1,334 domestic sheep representing more than 170 worldwide breeds to assess the extent of introgression in domestic sheep. We identified on average 7.1%, 1% and 0.19% introgressive sequence for each domestic sheep from Asiatic mouflon, urial and argali, respectively. Introgression signals were detected in *RXFP2* from Asiatic mouflon and *MSRB3* from argali. *RXFP2* was well-known associated with horn status in sheep. *MSRB3* was proved to be strongly associated with the width of ear by using hybrid populations in our study. Furthermore, we found that introgressions in *RXFP2* and *MSRB3* resulted in 3 distinct haplotypes, which showed strong signature of selection among diverse domestic populations. Our results suggest that distinct haplotypes originating in wild species and introgressed into domestic sheep have played an important role in phenotype diversity and local population adaptation, and contribute to a depth understanding of occurrence timing, area and process of introgression events among *Ovis* species.

Key Words: domestic sheep, introgression, horn status, ear morphology

P396 Resequencing the Yaroslavl cattle genomes reveals signatures of selection and young genetic variants likely to be related to the breed phenotypes. D. Ruvisnkiy*[1,2], N. S. Yudin[1,2], and D. M. Larkin[1,3], [1]*The Federal Research Center Institute of Cytology and Genetics, Novosibirsk, Russia,* [2]*Kurchatov Genomic Center, Institute of Cytology and Genetics, Novosibirsk, Russia,* [3]*Royal Veterinary College, London, UK.*

Studying genomes of local livestock breeds could shed light on their genetic history, mechanisms of adaptations to the environments, and unique genetics. Herein we looked into genetics and adaptations of the Yaroslavl cattle, a native Russian breed formed in the 16th century, known for the highest milk yield among the Russian native cattle. Using the data of 10 resequenced individuals of the Yaroslavl cattle (~10x clean Illumina coverage) and comparing them to resequenced individuals from 3 additional breeds (Yakut, Kholmogory, and Holstein), and to the whole 1000 Bull Genome Data set (Run 8) we located candidate gene variants that contribute to Yaroslavl cattle phenotypes. F_{ST} calculations against the other 3 breeds identified over 1,000 highly differentiated ($F_{ST} > 0.40$) single nucleotide polymorphisms (SNPs) in 940 genes in the Yaroslavl cattle genome. Genes with highly differentiated alleles were enriched in *vascular smooth muscle contraction*, *GMP-PKG signaling pathway* and *salivary secretion* functional categories. High on the list was a missense mutation ($F_{ST} = 0.52$) in the *ADORA3* gene on BTA3. *ADORA3* is involved in lactation traits in cattle. F_{ST} comparison with the 1000 Bull Genome Data set (Run 8) revealed 2 high-frequency unique to Yaroslavl cattle missense mutations ($F_{ST} > 0.99$) in genes such as MSS51 (involved in skeletal muscle growth) and KAT6B (a skeletal gene). The later mutation is highly deleterious and affects a linker histone H1/H5, domain H15 suggesting potential effect on the Yaroslavl cattle carcass. A HapFLK analysis revealed 42 genome regions under selection in the 4 breeds (q-value <0.01). Of those regions, 11 were the Yaroslavl cattle specific. One of the strongest signals was found in the KIT gene likely explaining the Yaroslavl cattle coat color pattern. Other regions included candidate genes for constitution (ANXA13, ADAMTS14), feed efficiency (LRRIQ3), reproduction (PRAME), and immune function (AP3M1). Our work provides the cattle industry with candidate genetic variants to be tested for functional significance and breed improvement. The work was was supported by the Kurchatov Genomic Center of IC&G (075-15-2019-1662).

Key Words: native cattle, Yaroslavl, signatures of selection, Russia

P397 Preliminary genome-wide association study with sialyl oligosaccharides content in 4 cattle breeds. M. Milanesi*[1], C. Lovallo[2], C. Marchitelli[3], S. Claps[2], G. Chillemi[1], and A. Crisà[3], [1]*Dipartimento per la Innovazione nei sistemi Biologici, Agroalimentari e Forestali (DIBAF), University of Tuscia, Viterbo, VT, Italy,* [2]*Centro Ricerca Zootecnia e Acquacoltura (CREA), Consiglio per la Ricerca in agricoltura e l'analisi dell'economia agraria, Bella Muro, PZ, Italy,* [3]*Centro Ricerca Zootecnia e Acquacoltura (CREA), Consiglio per la Ricerca in agricoltura e l'analisi dell'economia agraria, Monterotondo, RM, Italy.*

Milk oligosaccharides represent a class of bioactive molecules with potential beneficial effects on human health. Sialyoligosaccharides (SOS) play an important role not only in brain development and increasing immunity in infants, but also in adults for the prebiotic action on the bacterial flora. One of the MIQUALAT project aims was to analyze the concentration of 3′-sialyllactose (3′-SL), 6′-sialyllactose (6'-SL), disialyllactose (DSL) and 6′sialyl-N-acetyllattosamine (6′-SLN) in milk of 4 breeds (Holstein, HO; Simmental × Holstein, SM×HO; Simmental, SM; and Podolica, POD), sampled at 60 and 120 d of lactation. Phenotypic results showed significant effects of the breed and sampling date. 3′-SL, 6′-SL, DSL, and 6′-SLN were higher at 60 than at 120 d, and in POD, compared with the other breeds ($P < 0.001$). Significant differences were observed for 3′-SL content in HO (63.86 mg/L), SM×HO (68.12 mg/L) and SM (73.14mg/L) ($P < 0.001$); 6′-SL content between SM×HO (19.82mg/L) vs SM (21.11mg/L) ($P < 0.05$); 6′-SLN was higher in SM (7.07mg/L) and statistically significant different from SM×HO (5.19mg/L) and HO (5.11mg/L) ($P < 0.001$). The animals were also genotyped. After quality control the data set comprise 113 animals (26 HO, 30 SM, 27 SM×HO and 30 POD) and almost 37,000 SNPs. GCTA software was used to perform association analyses. A SNP in BTA3 (FDR < 0.07), nearby *PGM1* gene, was associated with DSL, in HO, SM×HO and SM. Six SNPs in BTA9, nearby *SLC17A5* gene, were associated with 3′-SL in all the breeds at 60 d (FDR < 0.06). When only candidate genes were considered, a SNP nearby *ST3GAL5* gene (BTA11) was associated with 3′-SL at 60 d in HO and SM×HO (FDR < 0.23), and a SNP nearby *SLC35C1* gene (BAT15) was associated with 6′-SLN in SM and SM×HO (FDR < 0.12). All the identified signals are part of the SOS metabolism, both considering lactose synthesis or sialic acids transfer on sugar or sugar transport. These preliminary results could be used in a breeding program focused on milk healthiness. Acknowledgment: this research was funded by the MIPAAF in the national research project MIQUALAT (D.M. 16844/7100/2019).

Key Words: cattle, genome-wide association, milk composition, human health, multibreed

P398 Can SNPs associated with variation in the level of stress biomarkers be used for the selection of stress-resilient dairy cows? M. M. Passamonti*[1], M. Milanesi[4], J. Ramirez Diaz[1], A. Stella[2], M. Barbato[1], M. Premi[1], R. Negrini[1], A. Cecchinato[3], E. Trevisi[1], J. L. Williams[1], and P. Ajmone Marsan[1], [1]*Universitá Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*Consiglio Nazionale della Ricerca, Milan, Italy,* [3]*Universitá di Padova, Padua, Italy,* [4]*Università della Tuscia, Viterbo, Italy.*

Despite progressive improvements in management practices, animals are still exposed to physiological and environmental stressors, which are exacerbated by ongoing climate change. Selecting stress-resilient animals could increase animal welfare and production efficiency. At the physiological level, stress causes a change in homeostasis. Biomarker levels for metabolism, liver functionality and immune system which are modulated during stress response can be measured in the plasma [J1]. In a previous study we identified significant association of SNP mark-

ers with the plasma level of 3 proteins (ceruloplasmin, CP; paraoxonase, PON; and gamma-glutamyl transferase, GGT) in Italian Holstein and Italian Red Pied breeds sampled around mid-lactation. In all cases variation in the levels of these biomarkers was mainly driven by genetic variants mapping within or nearby genes coding for the proteins themselves. The aims of the present study were to confirm these associations in a different set of animals, and to understand if the SNPs associated with the level of biomarkers are also predictive of the animal response to stress. A total of 1,000 Italian Holstein dairy cattle were sampled at one farm in Italy and genotyped with the GGP Bovine 100K SNP panel (Neogen). Single-SNP, gene and haplotype-based GWAS were conducted using plasma-biomarker levels as intermediate phenotypes for stress response. Results confirmed genetic association between SNPs and plasma levels of paraoxonase (BTA4) and gamma-glutamyl transferase (BTA17). A novel association was discovered between SNPs and alkaline phosphatase (BTA2), while the association with CP was not confirmed. 100 cows having opposite homozygote genotypes at SNPs previously associated with CP, GGT and PON have been identified and are being sampled during the stressful peripartum period. Postpartum animals will be assessed for their metabolic response to parturition and early lactation stresses. The results of this investigation will shed light on the utility of intermediate phenotypes as proxies of complex traits and on the value of including them in genomic assisted breeding programs as novel traits.

Key Words: cattle, stress, genetics, GWAS

P399 Comparison of variant calling programs with short-read sequences from U.S. sheep. M. Stegemiller*[1], R. Redden[2], D. Notter[3], J. Taylor[4], N. Cockett[5], M. Heaton[6], T. Kalbfleisch[7], and B. Murdoch[1], [1]*Department of Animal, Veterinary and Food Sciences, University of Idaho, Moscow, Idaho, USA,* [2]*Department of Animal and Veterinary Science, University of Idaho, San Angelo, TX, USA,* [3]*3Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA, USA,* [4]*4United States Sheep Experiment Station, United States Department of Agriculture, Agricultural Research Service, Dubois, ID, USA,* [5]*Utah State University, Logan, UT, USA,* [6]*USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA,* [7]*7Maxwell Gluck Equine Research Center, College of Agriculture and Veterinary Sciences, Lexington, KY, USA.*

Accurately identifying genomic DNA sequence variation is a critical step in discovering causal variants for biological traits and genetic diseases. Several programs can be used to detect variants, but these programs use different quality metrics that may affect call accuracy. Our aim was to compare the performance of 2 commonly used variant detection programs (GATK HaplotypeCaller and Freebayes) and call variants from 13 sheep breeds: East Friesian x Lacaune, Hampshire, Polypay, St. Croix, Targhee, Dorper, Dorset, Finn, Katahdin, Rambouillet, Romanov, Texel and USMARC III Composite. Paired-end, short-read, whole-genome sequence (WGS) data from a cohort of animals from each breed was generated. The sequences were mapped to the OARv3.1 reference genome assembly with the Burrows-Wheeler Alignment tool. Variants were called with GATK HaplotypeCaller and Freebayes. Only the biallelic single nucleotide polymorphisms (SNPs) that had a phred quality score of 20 or greater were retained from the output. Quality filtering was accomplished with bcftools based on phred scores. The genotypes called from both programs and those obtained from bead arrays were compared with determine concordance. Preliminary results showed an average of 4.1% more SNPs called and 3.6 greater mean sequence read depth per SNP was used by Freebayes in comparison to GATK HaplotypeCaller which filtered out more reads. The Freebayes variant caller used more of the sequence data and identified more SNPs in this ovine WGS data sets; however, the false positive and negative rates remain unclear.

Key Words: GATK HaplotypeCaller, Freebayes, whole-genome sequencing, sheep

P400 Validation of selection signatures for coat color in an Italian grey beef cattle breed. G. Rovelli[1], V. Landi[2], F. Sbarra[3], A. Quaglia[3], F. Pilla[4], E. Lasagna*[1], and E. Ciani[5], [1]*Department of Agricultural, Food and Environmental Sciences (DSA3), University of Perugia, Perugia, Perugia, Italy,* [2]*Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy,* [3]*National Association of Italian Beef-Cattle Breeders (ANABIC), San Martino in Colle, Perugia, Italy,* [4]*Department of Agricultural, Environment and Food, University of Molise, Campobasso, Campobasso, Italy,* [5]*Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Bari, Italy.*

Taurine and indicine gray cattle represent a relevant livestock resource in many countries of the world. A gray coat color, combined with a pigmented skin, common in most of the gray cattle breeds, has been demonstrated to confer better adaptation to solar radiation and thermal stresses. In a previously published study, adopting the FST-outlier approach with BayeScan v2.0, we identified genomic regions differentially selected in a set of gray cattle breeds, including the Podolica Italiana, contrasted with 4 non-gray cattle breeds. The more supported signals were detected on BTA 2, 4, 14, and 26, encompassing more than 50 genes known to be directly or indirectly related to one or more steps in pigment biology. Here, we aimed at validating the previously observed signals by using the same methodological approach, applied to 3 Podolica Italiana new sample sets (30 animals each, selected at the ANABIC genetic station during the performance test, as representative of the Podolica Italiana population at 3 different time-frames, roughly separated by 10 years interval each), typed at 23,027 quality-controlled SNP loci. To this aim, we also analyzed the above data set using the haplotype-based approach implemented in the hapFLK v1.4 software. Both the FST-outlier and the hapFLK approaches consistently detected the signals on chromosomes 2, 4, 14, and 26 in all of the pairwise contrasts with the non-gray breeds. The only exception was observed for the signal on BTA4, that was not detected, by the FST-outlier approach, in the contrasts involving Angus. In addition, both methods detected additional signals on BTA 7 and 18, though the latter was not observed, by using the FST-outlier approach, in the contrasts involving Angus. We provide a further experimental support to the knowledge that the hapFLK approach, by using haplotype information and the hierarchical structure of populations, provides significantly improved detection power. A detailed analysis of the significant signals detected by hapFLK for the 3 Podolica Italiana data sets highlighted the presence of extra signals in the second (BTA29) and third set (BTA 3, 5, and 13) i.e., in the more recent ones, possibly suggesting an evolution in the current genetic makeup of the Podolica Italiana breed.

Key Words: *Bos taurus*, Podolica Italiana, single nucleotide polymorphism, FST-outlier, hapFLK.

P401 Gene network for thermotolerance in Brangus cattle. K. M. Sarlo Davila*, E. E. Rodriguez, F. M. Rezende, A. N. Nunez Andrade, and R. G. Mateescu, *University of Florida, Gainesville, FL, USA.*

Thermal stress in hot and humid conditions limits beef cattle production. Over 65% of the world's cattle (beef and dairy) reside in tropical or subtropical climates known for their hot and humid conditions. These economic losses are expected to increase as thermal stress increases due to climate change. Thermotolerance, the ability to maintain production under heat stress conditions, is a complex trait determined by many component traits making interpretation of genome-wide association studies (GWAS) on any one component challenging to interpret. The objective of this study was to combine traditional GWAS with gene network interactions theory to dissect the genetic architecture of thermotolerance utilizing association weight matrix/partial correlation information theory (AWM/PCIT) methodology to include SNP with relatively small effects which do not reach genome-wide statistical significance but are potentially linked to elements controlling the trait of interest. 2,114 Brangus heifers were genotyped with the Bovine GGP F250 array. Genotypes were imputed to high density and after quality control 751,149 SNP were available

for genome-wide association analyses for 8 thermotolerance traits using single-step methodology and the BLUPF90 family of programs. AWM/PCIT methodology was used to create a gene network from the results of GWAS. Top genes from the gene network include *RUNDC3A*, *TIGD7*, *YIPF1*, *PTPN21*, and *SESN2.* While only *PTPN21* has previously been associated with thermotolerance, all 5 of these genes have been associated with cellular stress response. These results indicate that there may be crucial genetic architecture responsible for thermotolerance and cellular stress response.

P402 A comparative framework for comparing Hi-C datasets across ruminant livestock species. C. MacPhillamy*[1], H. Alinejad-Rokny[2,3], W. Pitchford[1], and W. Low[1], [1]*Davies Livestock Research Centre, School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia,* [2]*Biological and Medical Machine Learning Lab, The Graduate School of Biomedical Engineering, University of New South Wales, Sydney, NSW, Australia,* [3]*School of Computer Science and Engineering, The University of New South Wales, Sydney, NSW, Australia.*

The advent of high-throughput chromosome conformation capture and sequencing (Hi-C) has enabled researchers to probe the 3D architecture of mammalian genomes in a genome-wide manner. Hi-C and related techniques have enabled identification of active and inactive chromatin regions and the discovery of topologically associating domains (TADs), which are believed to have an important role in gene regulation. Moreover, the integration of 3D genome data with 1D genome data such as single nucleotide polymorphism (SNP), H3K27ac, H3K4me3 peaks and gene expression data are enabling researchers to gain a more complete understanding of how gene regulation and traits are controlled. Much of this 3D and 1D data has been generated in humans and mice with multiple replicates available for a variety of tissue types, cell lines and time points with many factors controlled, such as sequencing depth and restriction enzyme. This enables statistically meaningful comparisons to be made with relative ease. This is starkly contrasted by the availability of similar data for our most economically important ruminant livestock species. Only a small number of Hi-C data sets exist for cattle, goat and sheep, and the purpose for generating such data sets was mainly targeted at genome assembly scaffolding but not for probing chromatin structure. Additionally, these data sets were generated from different tissues and at different time points. It becomes apparent that there is a need for tools that enable meaningful, robust comparisons between different species and tissue types. Using information gleaned from high-resolution human Hi-C studies, we propose a framework for comparing 3D genomes across ruminant livestock species. By identifying syntenic regions between 2 species, normalizing sequencing depth of the matrix and performing downstream analysis on syntenic regions one can make meaningful comparisons between species. This framework helps researchers glean insights from ruminant livestock 3D genome data.

Key Words: cattle and related species, 3D genome architecture, Hi-C, computational workflow

P403 Dissection of the scurs phenotype to refine the mapping of scurs. G. Wang* and C. Gill, *Texas A&M University, College Station, TX, USA.*

Scurs and horns are 2 types of headgear in cattle. Scurs are corneous growths in the same location of the skull as horns, which range in size from buttons to horn-like structures, but generally do not fuse with the frontal skull. Expression of scurs is epistatic to horns and only heterozygous polled cattle can grow scurs. The objective of this study was to dissect the scurs phenotype and refine the mapping of scurs. *Bos taurus-Bos indicus* F_2 and reciprocal backcross mapping populations were used for this study. The status of headgear (horns, scurs, polled) was recorded on live animals or at 18 mo of age when cattle were harvested. Photographs were taken of the headgear and skulls. Blood samples were extracted for DNA and 50K genotypes were obtained for the F_2 and backcross cattle and HD genotypes were obtained for parents and grandparents that contributed to more than 10 offspring. Genotypes were imputed to HD scale using eagle and minimac. The Celtic *polled* locus was either directly genotyped or inferred from haplotypes. Only heterozygous polled cattle (n = 560) were used in this study. Scurs were categorized based on anatomy. We identified at least 3 distinct types of scurs, which were classified as "buttons," "sheaths," and "bony scurs," which reflect 3 milestones of headgear development. The frequency of the different types of scurs differs between sexes and different families tended to segregate for a single type of scurs. Genome-wide association studies were conducted within each sex for presence or absence of scurs and for the different types of scurs. We identified a major locus for presence of scurs (FDR-corrected *P*-value $<1 \times 10^{-15}$) on BTA 12 that is close to RXFP2, a well-known regulator of headgear in other ruminants. Other significant loci were found on BTA 17 and 22 for the different classifications of scurs. Based on differences in the GWAS results between sexes, the role of RXFP2 may differ in males and females, which could explain the sexual dimorphism of the scurs phenotype.

Key Words: cattle, scurs, GWAS, sexual dimorphism

P404 Withdrawn

P405 Genome-wide association study in bull semen production traits: A review. M. Modiba*[1,2], J. Wang[1], K. A. Nephawe[2], L. Wenfa[1], and B. J. Mtileni[2], [1]*Jilin Agricultural University, Jilin, China,* [2]*Tshwane University of Technology, Pretoria, South Africa.*

The fact that results of artificial insemination (AI) are declining in highly selected bull populations has added a renewed interest to the evaluation of male fertility. Identifying genomic regions, particularly individual genes that associates with semen quality traits, is regarded as very important in improving sire fertility through selective breeding. The aim of association studies, is to identify genes and their association to a certain trait. However, selecting animals directly based on their semen phenotypes can be difficult, because of low (0.04) to moderate (0.30) heritability of these traits. A lot of association studies conducted on complex traits have used Illumina BeadChip to help identify semen production traits. Analysis in these studies requires genotypic information, phenotypic information, a model that describes the factors that influence phenotype, and specification of prior distributions. Multiple studies have been conducted on the candidate genes associated with semen parameters, like sperm motility, sperm abnormal morphology, percentage of live sperm etc., however, little is known on the number of genes need to be discovered, the genetic variability in bull semen quality traits and their interrelationships. The use of genome-wide association study strategy can assist in identifying genes of significance and their association to semen production traits.

Key Words: bull, semen traits, GWAS, marker, model

P406 Identification of novel SNPs in litter size–associated genes in indigenous goat of Bangladesh. A. Das*, M. Shaha, and G. Miah, *Department of Genetics and Animal Breeding, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram, Bangladesh.*

Goat farming in Bangladesh is primarily based on indigenous Black Bengal, Jamnapari and their crosses. Black Bengal is highly prolific and frequently gives birth to twins, triplets, or more per kidding, while Jamnapari and crossbred goats are less prolific; they produce mostly single or twins, rarely triplets per kidding. Studies revealed many candidate genes associated with fecundity in goats. This study screened 150 indigenous goats of Bangladesh intending to identify single nucleotide polymorphism (SNP) in 3 reported litter size–associated genes: growth differentiation factor 9 (*GDF9*), bone morphogenetic protein 15 (*BMP15*) and cadherin 26 (*CDH26*). Genomic DNA was extracted, and PCR amplification of coding DNA sequence for the target genes was performed using primers designed or available in the literature. Direct sequencing was done to detect the genetic polymorphisms. Association between identified novel SNPs and litter size were analyzed employing a generalized linear model. We identified 9 novel SNPs in tested animals. Of the identified SNPs, 3 SNPs were in *GDF9* gene, 5 were in *BMP15* gene, and one was in *CDH26* gene. Association results (Table 1) show genotypes for one SNP in the GDF9 gene, 2 SNPs in *BMP15* gene, and one SNP in *CDH26* gene had a significant effect on litter size in different Bangladeshi goat. To our knowledge, this study, for the first time, discovered novel SNP loci in litter size–associated genes in indigenous Bangladeshi goats. These identified SNP loci will be a resource for future studies on the litter size trait in goats.

Table 1. Effects of genotypes of SNP loci on the litter size in different indigenous goats in Bangladesh

		Effect of genotypes on litter size		
Gene	SNP loci	Black Bengal	Jamnapari	Crossbred
GDF9	G1330T	NS	*	NS
BMP15	G754T	*	NS	NS
	C781A	***	NS	NS
CDH26	C975T	NS	*	*

*$P < 0.05$; ***$P < 0.001$; NS, not significant.

Key Words: goat, fecundity, candidate genes, SNP

P407 Study on fiber characteristics of different Inner Mongolia Cashmere goats. Z. Chongyan, X. Yuchun, G. Juntao, S. Xin, Z. Cun, Q. Qing, D. Dongliang, W. Zhixin, L. Jinquan, and L. Zhihong*, *Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China.*

Cashmere goat hair structure belongs to heterogeneous fleece, composed of long and thick medullary wool produced by primary follicle and short and thin medullaless cashmere grown by secondary follicle. Inner Mongolia Cashmere Goat is a big export province of cashmere. Its cashmere cellulose is called “soft gold” and “fiber gem”. Therefore, this study compared the fiber of Inner Mongolia cashmere goats of Alpas and Alashan type. The strength, fineness, elongation at break and coefficient of variation of fiber diameter of 2 varieties of cashmere goats were compared. The results show that the strength, elongation at break and coefficient of variation of fiber diameter of Alashan cashmere are higher than those of Alpas cashmere. The fineness of Alpas cashmere is higher than that of Alashan cashmere. In strength, fineness and coefficient of variation of fiber diameter, Alpas wool is higher than Alashan. The breaking elongation of Alashan wool is higher than that of Alpas. This shows that different types and kinds of fibers have their own characteristics and advantages and disadvantages, and it is most sensible to choose according to different production needs.

Key Words: fiber, strength, cashmere, wool

P408 Machine learning algorithm to predict coagulating milk factor through milk traits in 2 sheep breeds. H. Marina*, B. Gutiérrez-Gil, R. Pelayo, A. Suárez-Vega, C. Esteban-Blanco, and J. Arranz, *Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana, León, Spain.*

Sheep milk is mainly used to manufacture high-quality cheeses. In the last years, breeding programs aimed at increasing total milk solids due to the economic importance of cheese yield for the sheep dairy industry. Consequently, several studies focused on the milk coagulation properties (MPC) and cheese production parameters have been performed in dairy sheep. At the practical level, noncoagulation of sheep milk samples has been an important problem for the dairy industry. Previous studies in Spanish Assaf and Churra sheep breeds identified about 13% and 4% of noncoagulating samples within 60 min from the addition of the clotting enzyme, respectively. The routine measurement of MCP in commercial populations for inclusion as selection criteria in sheep breeding programs is expensive and not practical. Therefore, this study aimed to employ machine learning (ML) approaches to integrate the phenotypes registered by the official milk recording system and the pH of the milk to predict the coagulation behavior of individual milk samples as a binary trait (coagulating vs. noncoagulating samples) in the 2 dairy sheep breeds. A total of 1,039 and 973 milk samples were analyzed for Assaf and Churra breeds, respectively. The prediction ability of the selected traits was tested with ML applied to random forests through a 10-fold cross-validation procedure per breed using the R software. Our results revealed a global statistical sensitivity of 90% and 98% for Assaf and Churra breeds, respectively, on the validation set. In both breeds, the pH of the milk was the trait with the highest prediction ability for the coagulation behavior. Moreover, the second most relevant trait for coagulation prediction was the somatic cell count for Assaf and milk yield for Churra breeds. These results support the implementation of the ML methodologies to predict the coagulation behavior of milk samples without the need to measure MCP traits specifically. Future studies should increase the number of samples to achieve higher prediction efficiencies and assess whether this approach can be implemented in different dairy sheep breeds.

Key Words: sheep and related species, animal breeding, complex trait, machine learning, product quality

P409 Design of a low-density panel of SNPs to detect fraud in cured goat cheese. A. M. Martínez*[1], A. Canales[1,2], M. Macri[1,2], and J. V. Delgado[1], [1]*University of Cordoba, Cordoba, Spain,* [2]*Animal Breeding Consulting S.L., Cordoba, Spain,* [3]*Instituto Canario de Investigaciones Agrarias, Tenerife, Spain.*

Palmera goat is a local breed from La Palma (Canary Islands, Spain) whose main and most valuated product is the Palmero cheese, that is protected by a quality mark that guarantees that only milk from the Palmera goat is included in the elaboration process. The genotyping of 24 DNA samples of each Canarian breed (Majorera, Tinerfeña and Palmera) and other 6 Spanish and international breeds was carried out with the Goat SNP50 BeadChip in accordance with the instructions of the manufacturer. TRES software was used to stablish the minimum number of SNPs necessary to discriminate the Palmera, Majorera and Tinerfeña breeds, resulting a 3,385 SNPs set. Using this number of SNPs was possible to differentiate Palmera from the rest of the breeds included in the study as well. The reduced panel was tested in 110 samples of experimental cheeses containing different proportions of Palmera, Majorera and Tinerfeña milk, ranging from 10% to 100%. Furthermore, 22 samples of commer-

cial Palmero cheeses were analyzed. PLINK v. 1.90 software was used to calculate MAF values and the package Adegenet for the R software and fastStructure were used for inferring population structure. The results show that this panel is useful to distinguish the cheeses prepared with a 100% of milk of Palmera, 100% of Tinerfeña and 100% Majorera with assignment coefficients of 0.9962, 0.9999 and 0.9999 respectively. However other proportions of milks are not so clearly differentiated because it is difficult to discriminate mixtures of Tinerfeña and Majorera breeds. Anyway, this panel is highly efficient for identifying variable proportions of Palmera milk in all the experimental cheeses. In conclusion, the panel of 3,385 SNPs designed is a powerful and objective tool to detect milk from other genetically related goat breeds such us Majorera and Tinerfeña. The systematic analysis of milk or cheese with this set of markers can be used by the Palmero Cheese Denomination of Origin Regulating Council to ensure the quality and the authenticity of this product. This study was funded by the RTA2014–00047–00–00 Project (INIA).

Key Words: Palmera goat, quality, Majorera

P410 Extended haplotype homozygosity analysis reveals positive selection patters in 6 Spanish goat breeds. T. E. Ziegler[1,2], A. Molina[3], G. Anaya[3], and S. Demyda-Peyrás*[2,4], [1]*IGEVET, Instituto de Genética Veterinaria, La Plata, Buenos Aires, Argentina,* [2]*FCV-UNLP, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina,* [3]*Departamento de Genética, Universidad de Córdoba, Córdoba, Spain,* [4]*CONICET, Consejo Superior de Investigaciones Científicas y Tecnológicas, La Plata, Buenos Aires, Argentina.*

Goats are a major livestock resource in Spain. Their adaptability and resilience in adverse environments and their increased productivity even in intensive schemes make them valuable livestock resources. For this reason, several local breeds were developed, focused on meat or dairy production during the last decades. In this study, we determined the presence of selection fingerprints in dairy (n = 2) and meat (n = 3) Spanish goat breeds, by estimating the integrated haplotype score (iHS) of the extended haplotype homozygosity (EHH) analysis using SNP array information. Samples from178 Spanish individuals including 46 Malagueña (MLG), 43 Florida (FLO) and 25 Murciano-Granadina (MUR) dairy goats, and 24 Bermeya (BER), 20 Mallorquina (MLL) y 20 Blanca de la Rasquera (RAS) were genotyped using the Illumina GoatSNP50 BeadChip (55,000 markers). Data were pruned by LD and MAF using PLINK and analyzed (per breed) using the *REHH* package of the statistical environment R. Finally, candidate regions (selection sweeps, SS) were selected based on the iHS P-value with a minimum length of 1Mb. Results showed a clear and distinctive iHS peak in the CHI12, despite their productive ability. This selective signature, located in a chromosome previously associated with adaptability, could be related to a genetic ability to cope with the Spanish environment, in which all these breeds were bred during the last 50 years, characterized as harsh and with low levels of forage lands. In addition, meat breeds showed an increased number of selective sweeps but more diffuses than dairy breeds (19 vs 11 on average). In particular, the less selected breeds (RAS and MLL) showed more than 20 small selective sweeps located in 15 different chromosomes, whereas the most selected breed (FLO) showed only 8 candidate regions located in 5 different chromosomes, including a clear peak in CHI6. Overall, we demonstrated that iHS could be an interesting tool to analyze differences in adaptability and selection process in Spanish goats. Further research, including functional analysis of the regions detected, is necessary to obtain more precise conclusions.

Key Words: goat and related species, population genomics, breed diversity, homozygosity

P411 Unveiling genomic regions that underlie footrot resistance in Portuguese sheep Merino. D. Gaspar*[1,2], A. Usié[1,3], C. Leão[3,4], C. Matos[5], L. Padre[3], C. Dias[3], C. Ginja[2], and A. M. Ramos[1,3], [1]*CEBAL – Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo, Beja, Portugal,* [2]*CIBIO/InBIO – Research Centre in Biodiversity and Genetic Resources, University of Porto, Vairão, Porto, Portugal,* [3]*MED-Mediterranean Institute for Agriculture, Environment and Development, University of Évora, Évora, Portugal,* [4]*INIAV – Instituto Nacional de Investigação Agrária e Veterinária, Santarém, Portugal,* [5]*ACOS – Agricultores do Sul, Beja, Portugal.*

Footrot is an acute necrotic and highly contagious disease, caused by a co-infection of 2 g-negative anaerobic bacteria, *Dichelobacter nodosus* and *Fusobacterium necrophorum*. It affects the interdigital skin and hooves of sheep, being the main cause of lameness and a major animal welfare and economical concern for the wool, milk and meat sheep industries worldwide. Current effective strategies to control footrot are costly and rely on the use of antibiotics, which could result in the development of parasite resistance mechanisms in the long term. The development of genomic markers associated with footrot resistance can provide a more reliable strategy for classifying and selecting sheep with increased resistance, besides enhancing our understanding of the biology of this disease. We aimed to identify genomic regions and molecular mechanisms associated with resistance to footrot in Portuguese native Merino breeds. For this, a set of 50k single nucleotide polymorphisms (SNPs) was specifically designed based on whole-genome data obtained for 39 sheep (depth of coverage >22X). A total of 1,466 Portuguese Merino sheep were genotyped using this SNP array. Genome-wide association analysis was performed using a quantitative trait approach based on the modified Egerton system (scores from 0 to 5) for foot integrity and footrot lesions. Genome-wide significance was determined using corrected P-values for multiple testing and SNPs significantly associated with footrot resistance were filtered at a genome-wise false discovery rate of 5%. Our results revealed a set of promising SNPs associated with resistance to footrot that overlaps candidate genes related to immune response and wound healing. These findings contribute to better understanding the architecture of footrot resistance in Merino sheep and to enhance the development of genomic tools to control infections. Also, the whole-genome data were used to investigate the underlying population structure of these native Iberian Merino breeds in the context of worldwide sheep, which is useful to define conservation and management programs.

Key Words: sheep, Merino, footrot, GWAS

P412 Comparative transcriptome analysis between suckling lambs with different levels of perirenal adipose tissue in the carcass. M. Alonso-García[1], A. Suárez-Vega[1], J. Mateo[2], H. Marina[1], R. Pelayo[1], C. Esteban-Blanco[1], J. J. Arranz[1], and B. Gutiérrez-Gil*[1], [1]*Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, León, León, Spain,* [2]*Departamento de Higiene y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de León, León, León, Spain.*

Suckling lamb meat is very appreciated in the European-Mediterranean region. This meat is tender, juicy and shows a smooth texture. Suckling lamb carcass quality is positively related to the amount of perirenal adipose tissue, which is the predominant carcass internal fat depot. Quality traits of interest in this production show a strong influence of maternal effects as the lambs are fed exclusively of milk and slaughtered between 21 and 30 d of age. RNA sequencing (RNA-seq) has proven to help expand our understanding of the relationships between the transcriptome and the phenotype across different physiological, treatment, or disease conditions. The objective of the present study was to compare the perirenal fat transcriptome between lambs with high and low percentages of perirenal fat in the carcass. For that, 18 male Spanish Assaf lambs born in the same flock and lambing season from primiparous ewes were initially considered. After birth the lambs had colostrum access for 4 to 8 h, and they were then fed ad libitum with reconstituted milk replacer powder. The animals were slaughtered when they reached the market live-weight (9–12 kg). At slaughter, perirenal adipose tissue samples were collected from each lamb for RNA extraction. After a phenotypic characterization of the carcass composition, RNA samples from the 4 lambs with the high-

est percentage of perirrenal fat (high-PFP group) and the 4 lambs with the lowest PFP (low-PFP group) were analyzed through RNA-seq. The differential expression analysis performed with *DESeq2* identified a total of 101 differentially expressed genes (DEGs) ($p_{adj} < 0.05$). Enrichment analyses for the 58 genes with higher expression levels in high-PFP lambs than in low-PFP lambs highlighted terms related the fatty acid metabolism such as *fatty acid derivative biosynthetic process* and *response to fatty acid (GO Biological Process)*, *Sterol Regulatory Element-Binding Proteins* (SREBP) *signaling* (*Pathway*) and *Obesity (Disease)*. This study provides a preliminary evaluation of genes and physiological pathways that may underlie phenotypic variation of the levels of perirenal adipose tissue in Assaf suckling lambs.

Key Words: sheep and related species, transcriptome, fat/lipid, meat production

P413 Exploring differentially expressed genes in hypothalamic transcriptome in different sexual behavior phenotypes in rams using RNA-seq. K. Lakhssassi*[1,2], I. Ureña[3], B. Marín[4], M. P. Sarto[1], B. Lahoz[1], J. L. Alabart[1], J. Folch[1], M. Serrano[3], and J. H. Calvo[1,5], [1]*Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)-IA2, Zaragoza, Spain,* [2]*Institut National de la Recherche Agronomique, Rabat, Morocco,* [3]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain,* [4]*Universidad de Zaragoza, Zaragoza, Spain,* [5]*Fundación Agencia Aragonesa para la Investigación y el Desarrollo (ARAID), Zaragoza, Spain.*

The ram effect is commonly used to improve the out of season reproduction, showing that males with greater sexual behavior (mounts and services) produce a greater stimulus during seasonal anestrus, which lead into a higher percentage of mated ewes and higher fertility. However, there is a considerable variation in sexual behavior among rams. The main objective of this study was to investigate transcriptional changes in the hypothalamus (HT), key tissue in sexual activity regulated by photoperiod, in 2 groups of rams with different sexual behavior using RNA-seq. First, 59 rams were submitted to individual sexual behavioral pen tests, twice for each ram. Each ram was exposed to 3 estrous females for 20 min to observe their behavior, and count the frequency of mounts and services. Therefore, 2 homogeneous groups of rams were identified: active (A) (7.93 ± 3.56, average mounts ± sd) and not active (NA; without any mount). Six rams of each group were killed and total RNA was extracted from HT. Sequencing was carried out generating Illumina paired-end reads of 151 bp. Gene level quantification was estimated using HTSeq, while differential expression and pathway analysis to find regulated functional groups were performed with EdgeR and Gene Set Enrichment Analysis (GSEA), respectively, using the OmicsBox package from BioBam. In the comparison A *vs* NA, 52 differentially expressed genes (DEGs) were found being 41 and 11 genes up- and downregulated, respectively. One of the most outstanding upregulated genes was the *PDYN* that has been related to sexual motivation in male European starlings. Therefore, it has been proposed as one of the neuropeptides that are involved in the control of sexual behavior at the central level. Finally, enrichment analysis including 17,003 DEGs expressed in the HT yielded 130 overrepresented pathways at FDR q-value 5%. The most interesting GO was related to behavior (GO:0007610) with 133 enriched genes including *Neuropeptide Y* (*NPY*) and *Pro-Melanin Concentrating Hormone* (*PMCH*) genes, also involved in the control of sexual behavior whereas others genes such as *CIART* and *NR1D1*, belong to circadian rhythm pathways.

Key Words: sheep, RNA-seq, ram, sexual behavior

P414 Identification of a novel loss-of-function variant in the ovine *TMCO6* gene associated with motor neuron disease of North Country Cheviot sheep. A. Letko*[1], I. M. Häfliger[1], E. Corr[2], F. Brulisauer[2], S. Scholes[2], and C. Drögemüller[1], [1]*Institute of Genetics, Bern, Switzerland,* [2]*SRUC Consulting Veterinary Services, Penicuik, Midlothian, UK.*

Motor neuron diseases (MND) occur sporadically in farm animals including sheep. The aim of our study was to characterize the phenotype and the genetic etiology of an early-onset neurodegenerative disorder observed in several lambs of purebred North Country Cheviot sheep, a native Scottish breed. Affected lambs showed progressive ataxia and subsequent histopathological analysis revealed motor neuronal degeneration including cytoplasmic vacuolation. By whole-genome sequencing of 4 affected lambs, we identified a shared homozygous loss-of-function frameshift variant in exon 6 of the ovine *TMCO6* gene on chromosome 5. Herein we present evidence for the occurrence of a familial novel form of a recessively inherited MND in sheep due to a likely pathogenic 4bp deletion that is assumed to lead to a dysfunction of a transmembrane and coiled-coil domain-containing protein 6 (TMCO6: p.Leu215PhefsTer34). The uncharacterized TMCO6 protein is proposed to interact with the ubiquilin-1 (UBQLN1) protein, which plays an important role in the regulation of different protein degradation mechanisms and pathways and is reported to be associated with sporadic forms of amyotrophic lateral sclerosis (ALS). Therefore, these findings implicate an important role of TMCO6 for proper function and survival of motor neurons and provide a novel candidate gene for human ALS or similar motor neuron disease. Furthermore, these results enable selection against the fatal disorder in sheep population.

Key Words: neurogenetic disorder, rare disease, precision medicine, whole-genome sequencing, membrane trafficking

P415 Withdrawn

P416 The difference of lipid metabolism based on intestinal microbiome and transcriptome between Dorper and Tan sheep. Y. Ma*, X. Yang, G. Hua, and X. Deng, *National Key Laboratory of Animal Genetics, Breeding and Reproduction, China Agricultural University, Beijing, China.*

There are differences in metabolism between different breeds of sheep, which can be reflected in changes in fat content. Our research found that the IMF content of Tan sheep is significantly higher than Dorper. We infer that lipid metabolism plays a key role in the different fat content. Dorper and Tan sheep are kept in the same environment. We collected 4 Dorper and 4 Tan sheep cecum, colon contents samples from 8-mo-old ewes. First, we sequenced the 16sDNA of the bacteria in the intestinal contents to obtain the specific composition of the microorganisms in each sample at each classification level. Second, the metabolome uses LC-MS to analyze the intestinal contents, and differentially expressed metabolites are represented by Variable importance for the projection (VIP). Third, we performed mRNA sequencing on the liver, muscle, and brain tissues, and used FC >2 and $P < 0.05$ as screening criteria to obtain differentially expressed genes (DEG). In our study, we found that there are 700 OTUs shared by the cecum and colon tissues. The ratio of *Firmicutes* to *Bacteroides* in Tan sheep is higher than Dorper. LEfse analysis found that the proportion of *Rikenellaceae* in Tan sheep is lower. Spearman correlation analysis found that bile acid metabolites were significantly associated with different genus. In metabolomics analysis, the pathway of bile secretion was significantly enriched. The bile secretion in the intestine of Tan sheep is significantly higher than Dorper. Bile acids are closely related to liver lipid metabolism and circadian rhythm. The differential expression of *ABCB11*, *SREBF1* and *CYP1A1* genes in the liver plays a key role in lipid metabolism and bile acid secretion. *PPARGC1A* gene in muscle tissue and *NR1D1*, *PER1*, *PER3* gene in brain tissue are also thought to be related to metabolic rhythm. Our research shows that the difference in fat content between Dorper and Tan sheep may be caused by lipid metabo-

lism, and key bacteria, bile acid metabolism and liver tissue DEG jointly cause this phenomenon.

Key Words: fat content, microbial diversity, metabolome, transcriptome, bile secretion

P417 Identification of novel SNPs associated to litter size in Rasa Aragonesa sheep breed. K. Lakhssassi[1,2], J. Grimplet[1], M. P. Sarto[1], B. Lahoz[1], J. L. Alabart[1], J. Folch[1], M. Serrano[3], and J. H. Calvo*[1,4], [1]*Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) - IA2, Zaragoza, Spain,* [2]*Institut National de la Recherche Agronomique (INRA), Rabat, Morocco,* [3]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain,* [4]*Fundación Agencia Aragonesa para la Investigación y el Desarrollo (ARAID), Zaragoza, Spain.*

Rasa Aragonesa is an autochthonous Mediterranean sheep breed, mainly reared in extensive or semi-extensive farming systems and oriented to meat production. Three *FecX*-mutated alleles called *FecX*^{R}, *FecX*^{Gr} and *FecX*^{Ra} in *BMP15* gene have been described in this breed up to now. In a previous research, a genome-wide association study (GWAS) using 158 ewes (73 high prolific *vs* 85 low prolific ewes) genotyped with the Illumina AgResearch Sheep HD (680K) SNP array was performed, identifying the *FecX*^{Gr} allele at genome-wide level, and also 11 significant SNPs at chromosome-wide level. Therefore, the main objective of this study was to investigate the GWAS significant regions at chromosome level and identify new SNPs/genes associated with litter size in Rasa Aragonesa sheep breed. First, we annotated the genes identified in the 500 kb region on both sides of the significant SNPs at chromosome-wide level. Genes and SNPs were mapped on the Oar v3.1 (Texel) sheep genome. We identified 8 (*MTFMT*, *PTGER2*, *SLC51B*, *RASL12*, *KBTBD13*, *TXNDC16*, *GNPNAT1* and *ERO1A*), 1 (*ARID1B*), 4 (*CCT6B*, *NLE1*, *RAD51D* and *LIG3*) and 6 (*HK1*, *TACR2*, *AIFM2*, *TYSND1*, *ZMIZ1* and *POLR3A*) genes in chromosomes 7, 8, 11 and 25 respectively, related to reproduction. To identify putative SNPs related to litter size, we chose 8 prolific ewes without the *FecX* alleles to be sequenced using low coverage whole-genome sequencing (lcWGS; 10x). Analysis was run through Galaxy project: Trimmomatic was used to trim reads and remove adapter sequences, Bowtie2 to align reads to the reference genome Oar v3.1 and finally Freebayes was used for variant calling. In total, we selected 14 SNPs within the genes described above based on the impact of amino acid substitution on the structure and function of the protein using the PolyPhen-2 and Variant Effect Predictor (VEP) tools. The genotype of these SNPs obtained by lcWGS were validated using Sanger sequencing. The lcWGS results were confirmed for 7 SNPs, located within *TXNDC16*, *ZMIZ1* and *POLR3A* genes. Finally, 4 SNPs located in the *ZMIZ1* and *POLR3A* genes were genotyped to study their effect in the GWAS population.

Key Words: litter size, WGS, SNP, sheep

P418 Linkage disequilibrium (LD) pattern, effective population size and persistence in LD phase in multi-breed South African sheep. E. F. Dzomba*[1], M. A. Snyman[2], M. Chimonyo[3], and F. C. Muchadeyi[4], [1]*University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, Republic of South Africa,* [2]*Grootfontein Agricultural Development Institute, Middelburg, Eastern Cape, Republic of South Africa,* [3]*University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, Republic of South Africa,* [4]*Agricultural Research Council, Biotechnology Platform, Pretoria, Gauteng, Republic of South Africa.*

The accuracy of genome-wide association studies, genomic selection is dependent on the level of linkage disequilibrium (LD). This study investigated (i) LD between adjacent SNPs, (ii) LD decay with increased marker distance, (iii) trends in effective population size and (iv) consistency of gametic phase in 13 South African sheep breeds of mutton, wool, pelt, dual-purpose and unimproved types. LD (r^2) averaged 0.16 ± 0.021 and ranged from 0.09 ± 0.14 (SA Merino and Dohne Merino) to 0.28 ± 0.29 (Blackhead Persian). Chromosome 10, associated with genomic regions under selection for horn size and shape, had the highest LD ranging from 0.10 ± 0.15 (SA Merino) and 0.12 ± 0.18 (Dohne Merino) to over 0.28 in Blackhead Persian and SA Mutton Merino. Across breeds LD decayed from 0.27 ± 0.30 at 0–10Kb window to 0.02 ± 0.03 at 1000–2000Kb window. A progressive decrease in N_e across generations across all populations was observed, with N_e of <500 for all populations 66 generations ago decreasing to well below 100 13 generations ago. Highest correlations in gametic phase were observed within 0–10 kb window between pairs of Merino and Merino derived breeds. Highest correlation observed with Nguni sheep was with Dorper sheep (0.33) within 0 −10 kb, which was similar to that observed with Blackhead Persian and Dorper (0.32) within the same window. Overall, the study reported considerable LD persistent over short distances. The implications of the observed LD, LD decay and consistency in gamete phase on applications such as GWAS, QTL mapping and GS are discussed.

Key Words: multi-breed sheep, LD, gamete phase, Ovine SNP50K, genomic applications

P419 Identification of selection signatures on the X chromosome in East Adriatic sheep. M. Shihabi[1], B. Lukic[2], I. Drzaic[1], M. Ferencakovic[1], V. Brajkovic[1], L. Vostry[3], V. Cubric-Curik[1], and I. Curik*[1], [1]*University of Zagreb, Faculty of Agriculture, Zagreb, Croatia,* [2]*J. J. Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia,* [3]*Czech University of Life Sciences, Prague, Czech Republic.*

Sheep farming is one of the most important livestock sectors in the region of East Adriatic. Therefore, the identification of genomic regions showing signals of a positive selection signature is of economic importance. The aim of this study was to analyse regions showing selection signals in 59 sheep individuals representing the East Adriatic sheep populations (Dalmatian Pramenka; Dubrovnik Ruda Sheep; and Pag Island sheep) using the Ovine Infinium HD SNP BeadChip. Our analysis was limited to the X chromosome only (21,748 SNPs), as such analyses are rarely performed, with the exception of a few studies. Among sheep populations, the Balkan sheep group was never represented in such an analysis. After quality control, we performed 2 different methods, identification of extremely frequent SNPs in ROHs (eROHi) and Integrated Haplotype Score (iHS), based on the analysis of genomic variation within a large meta-population. Using iHS, we detected a weak selection signal in a narrow region (6 SNPs at position 25.6 Mb) indicative of the interleukin-1 receptor accessory protein-like 1 (*IL1RAPL1*) gene. In humans, ILRAPL1 has been linked to the hippocampal memory system and learning ability. Using eROHi (1Mb), we identify strong selection signals in a large region extending from position 69.9 to 74.6 Mb with a number of potential gene candidates (*POU3F4*, *CYLC1*, *RPS6KA6*, *HDX*, *APOOL*, *ZNF711*, *POF1B*, *TRNAC-GCA*, *CHM* and *DACH2*). *CHM*, DACH2, *RPS6KA6*, *ZNF711*, and *IL1RAPL1* were also identified as selection signals in some Chinese and European breeds. Nevertheless, the pattern of selection signals in the X chromosome when comparing iHS and eROHi was somewhat different from analyses performed on autosomes and needs to be further analyzed using other methods and on large samples.

Key Words: adaptation, sheep and related species, genetic improvement, genotyping, evolutionary genomics

P420 Polymorphism of the *MC1R* gene in sheep breeds from Poland. A. Piestrzynska-Kajtoch*[1], E. Klocek[2], and A. Kawecka[3], [1]*National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Malopolska, Poland,* [2]*University of Agriculture in Kraków, Faculty of Animal Science, Kraków, Malopolska, Poland,* [3]*National Research Institute of Animal Production, Department of Sheep and Goat Breeding, Balice, Malopolska, Poland.*

The animal coat color is the feature that often distinguishes a particular breed. On the basis of the different features, e.g., coat color, the individual can be even eliminated from the breeding stock, especially for the native breeds included in the Genetic Resources Conservation Programme. One of the genes connected with coat color and melanin pro-

duction is *MC1R* - Melanocortin 1 Receptor. We analyzed the variability of the *MC1R* gene in 90 sheep of 6 breeds from Poland: Old-type Polish Merino (MST), Colored Polish Merino (MPB), Colored Mountain Sheep (POGB), Polish Mountain Sheep (POG), Wrzosówka (WRZOS) and Romanov sheep (ROM). Four breeds were included in the Genetic Resources Conservation Programme (MST, MPB, POGB, WRZOS). We sequenced the fragment of the *MC1R* coding region (codons 1–289) and the gene 5′ flanking sequence with promoter. To obtain the sequence (about 1,250 bp), we used self-designed primers in 2 separate PCR reactions, BigDye Terminator v3.1 Cycle Sequencing Kit and Genetic Analyzer 3500xl (*Thermo Fisher Scientific*, USA). We analyzed sequences in Variant Reporter Software and BioEdit Sequence Alignment Editor. Eight different polymorphic sites (position according to Oar_rambouillet_v1.0, Ensembl) were present in the whole studied group: 15486875A>G (−279A>G), 15487124G>A (−31G>A), 15487372T>A (M72K), 15487438C>T (T95M), 15487515G>A (D121N), 15487583C>T (Y143Y; rs160910030), 15487754T>G (L200L; rs398814350) and 15487889C>T (I245I; rs412064209). The first 2 were located in the 5′ *MC1R* flanking region and 3 SNPs were missense mutations. The −279A>G and T95M seemed to be novel, as we did not find them in the literature. All WRZOS and ROM sheep were homozygotes ($G_{-279}A_{-31}T_{72}C_{95}G_{121}T_{143}G_{200}T_{245}$). M73K and D121N mutations, which according to Våge et al. [1999] determine the dominant black (E^D) *MC1R* allele, were present only in all POGB sheep. This might confirm the association of these mutations in POGB breed with its brown coat color. Three T95M heterozygotes were of POG and POGB breed. None of the *MC1R* SNPs seemed to be connected with the brown/black coat color of MPB or with white coat color of MST. Further studies on other genes connected with coat color will be perform.

Key Words: sheep and related species, DNA sequencing, polymorphism, coat color, MC1R

P421 A homozygous frameshift variant in *MFSD2A* associated with congenital brain hypoplasia in a Kerry Hill sheep family. G. Lühken*[1], A. Letko[2], M. Häfliger[2], M. J. Schmidt[3], C. Herden[4], L. Herkommer[4], J. Müller[4], and C. Drögemüller[2], [1]*Institute of Animal Breeding and Genetics, Justus Liebig University, Giessen, Germany,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [3]*Clinic for Small Animals, Neurosurgery, Neuroradiology and Clinical Neurology, Justus Liebig University, Giessen, Germany,* [4]*Institue of Veterinary Pathology, Justus Liebig University, Giessen, Germany.*

In several consecutive years, a German breeder observed male and female purebred Kerry Hill sheep lambs with severe ataxia and in some cases with convulsions. The lambs died after some days to weeks. All affected lambs descended from the same sire or one of his sons. We hypothesized an autosomal recessive inherited brain disorder underlying the observed deaths and aimed to identify the potential causal allele. Imaging with CT and MRT of 2 affected lambs revealed a reduced skull circumference compared with age matched controls. The forebrain appeared unusually small in relation to the cerebellum and midbrain. The cerebral cortical surface pattern was simplified (pachygyria). There was moderate ventriculomegaly. The cortical and subcortical white matters were thin and the contrast between white and gray matter was diminished. Pathological examination confirmed these findings. Additionally, a multifocal mild cortical dysplasia was found. CNS infection was ruled out. Genotyping 5 affected lambs on the 50 k ovine SNP array allowed us to localize the critical genome regions harboring the causative variant to 3 shared IBD segments of totally 8 Mb on different chromosomes by homozygosity mapping. By whole-genome sequencing of an affected lamb, homozygous private variants called in this single case were identified by comparison with 86 publically available control sheep genomes. This yielded 101 private homozygous protein-changing variants affecting 73 different genes. Within the critical intervals there was only a single variant predicted to be nonsynonymous. Genotyping using Sanger sequencing showed perfect co-segregating of this variant with the observed disorder in the studied Kerry Hill family, consistent with autosomal recessive inheritance. This private homozygous loss-of-function frameshift variant in exon 3 of *MFSD2A* (c.285dupA; p.Asp96fs) is predicted to truncate the encoded protein. MFSD2A is part of the blood-brain barrier. In humans, missense mutations in *MFSD2A* are associated with progressive microcephaly, spasticity, and brain imaging abnormalities.

Key Words: sheep and related species, genome sequencing, genetic disorder animal health

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