SEMEN MICROBIOTA OF PATIENTS WITH ASTENOZOOSPERMIA AND HEALTHY CONTROLS: CLUSTER ANALYSIS OF REAL-TIME PCR DATA

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Male Contribution to Infertility Worldwide

- 15% of married couples have infertility;
- Total — about 50 mln couples;
- Male infertility — 50% of all cases:
  - 20-30 % “pure” male infertility;
  - 20-30% mixed male and female infertility.

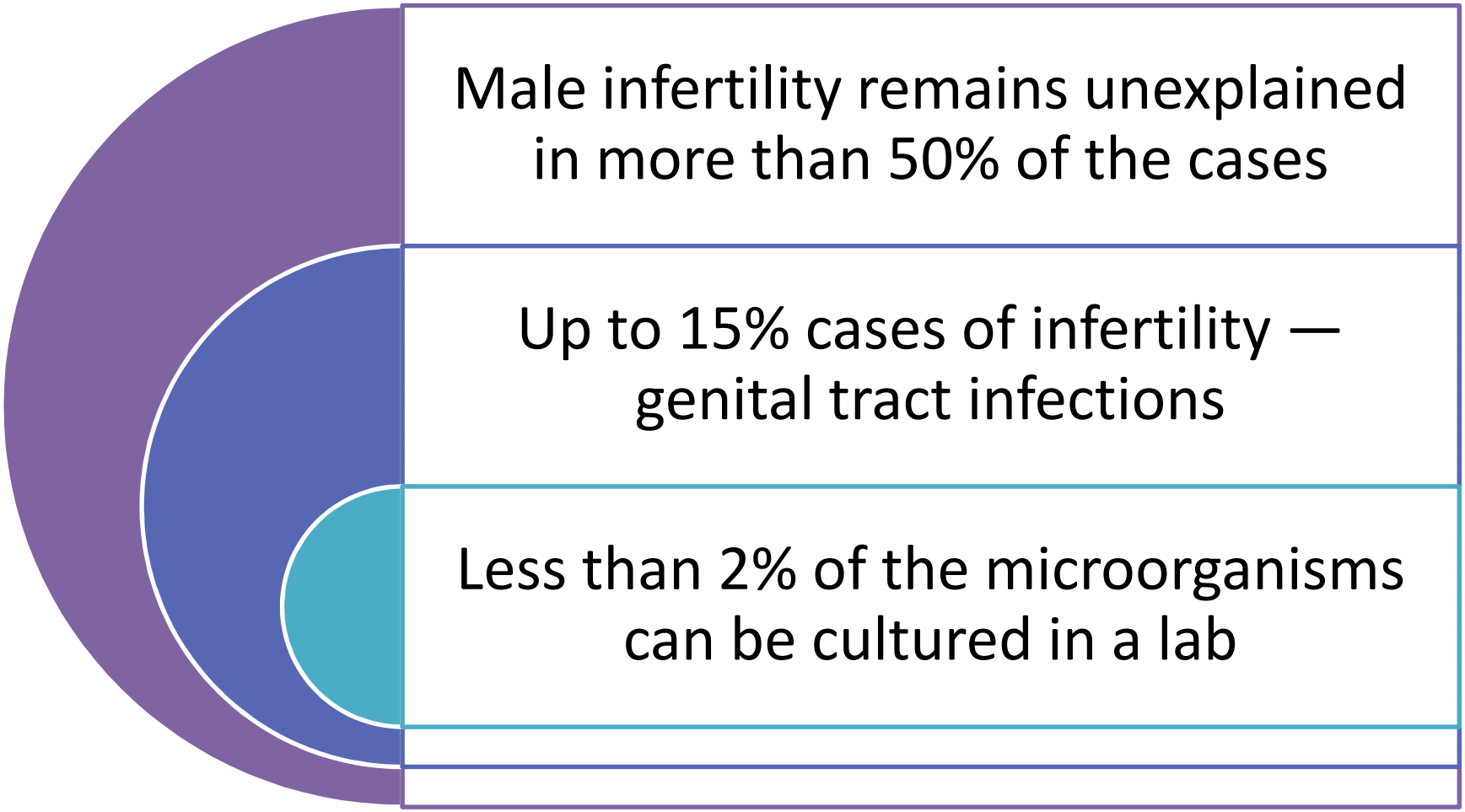
A. Agarwal, A. Mulgund, A. Hamada, M.R. Chyatte ***A unique view on male infertility around the globe*** *Reprod Biol Endocrinol, 13 (2015), p. 37*

Change in Sperm Count

- From 1973 to 2011 sperm count declined by 50–60% in the Western countries (North America, Europe, Australia, New Zealand);
- This trend was not characteristic of other countries.

H. Levine, N. Jørgensen, A. Martino-Andrade, et al. ***Temporal trends in sperm count: a systematic review and meta-regression analysis*** *Hum Reprod Update, 23 (2017), pp. 646-659*

Relevance of Studying Semen Microbiota



Assessment of Semen Microbiota

- 16S rRNA gene specific Next generation sequencing
- Qualitative PCR
- Quantitative PCR (real-time PCR) – «ANDROFLOR®» Real-time PCR KIT (DNA-Technology, Russia)

«ANDROFLOR®» Real-time PCR KIT

Sexually transmitted pathogens (qualitative analysis)	**C.trachomatis, N.gonorrhoeae, T.vaginalis, M.genitalium**
Gram-positive facultative anaerobes (quantitative analysis)	•Staphylococcus spp. •Streptococcus spp. •Corynebacterium spp.
Obligate anaerobes (quantitative analysis)	•Gardnerella vaginalis •Atopobium cluster •Megasphaera /Veilonella/Dialister • Sneatia/Leptotrichia/Fusobacterium •Bacteroides/Porphyromonas/Prevotella •Anaerococcus •Peptostreptococcus/Parvimonas •Eubacterium

«ANDROFLOR®» Real-time PCR KIT

Genital mycoplasma (quantitative analysis)	**•Ureaplasma urealyticum •Ureaplasma parvum •Mycoplasma hominis**
Gram-negative facultative anaerobes (quantitative analysis)	•Haemophilus spp. •Pseudomonas aeruginosa/Ralstonia/Burkholderia
Enterobacteriaceae / Enterococcus spp. group	•Enterobacteriaceae spp./Enterococcus spp.
Yeast-like fungi (quantitative analysis)	•Candida spp.
Transient microbiota (quantitative analysis)	Lactobacillus spp.

Study Design

- 301 patients, who came to the “Garmonia” Medical Center (Yekaterinburg, Russia) either seeking preconception care or for infertility treatment.
- Depending on the spermiogram results, they were divided into two groups.
  - Group 1 (n=171) — asthenozoospermia,
  - Group 2 (n=130) — normospermia

Methods: Semen Microbiota Evaluation

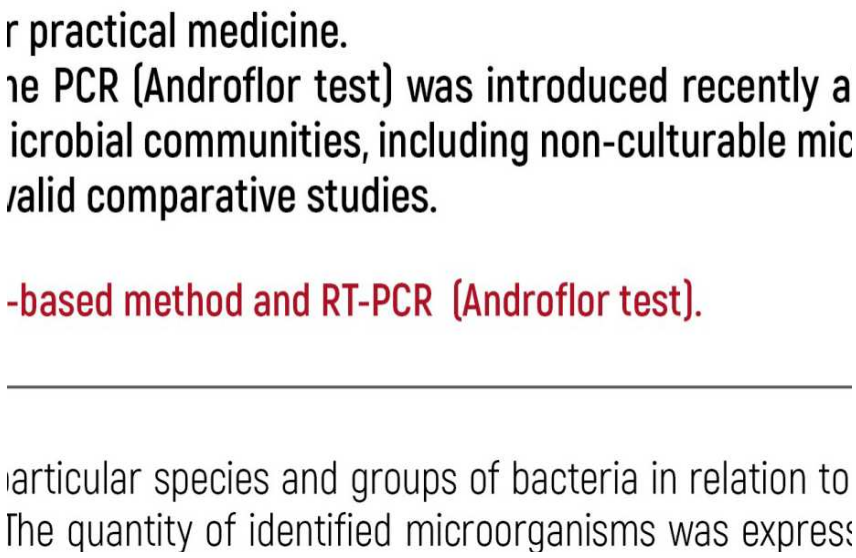
- **DNA extraction**
  - PREP-NA PLUS extraction kit (DNA-Technology, Russia)
- Real-time PCR
  - Androflor® REAL-TIME PCR Detection Kit (DNA-Technology, Russia)

Methods: Cluster Analysis

- Cluster analysis was performed for the samples with (total=301: asthenozoospermia=171, normospermia = 130) the total bacterial load (TBL) of at least 10^3 GE/ml.
- Cluster analysis was conducted using the k-means++ algorithm, scikit-learn.
- The Silhouette index and the Davies–Bouldin index (DBI) were used to confirm the stability of clusters.

Example of Lab Report after Testing the Semen Microbiota Using Real-time PCR Kit «ANDROFLOR®»

- Total bacterial load: 4.2 log GE/ml
- 10 groups of microorganisms detected in quantity > 3.0 log GE/ml



r practical medicine.
ıe PCR (Androflor test) was introduced recently a
icrobial communities, including non-culturable mic
/alid comparative studies.

-based method and RT-PCR (Androflor test).

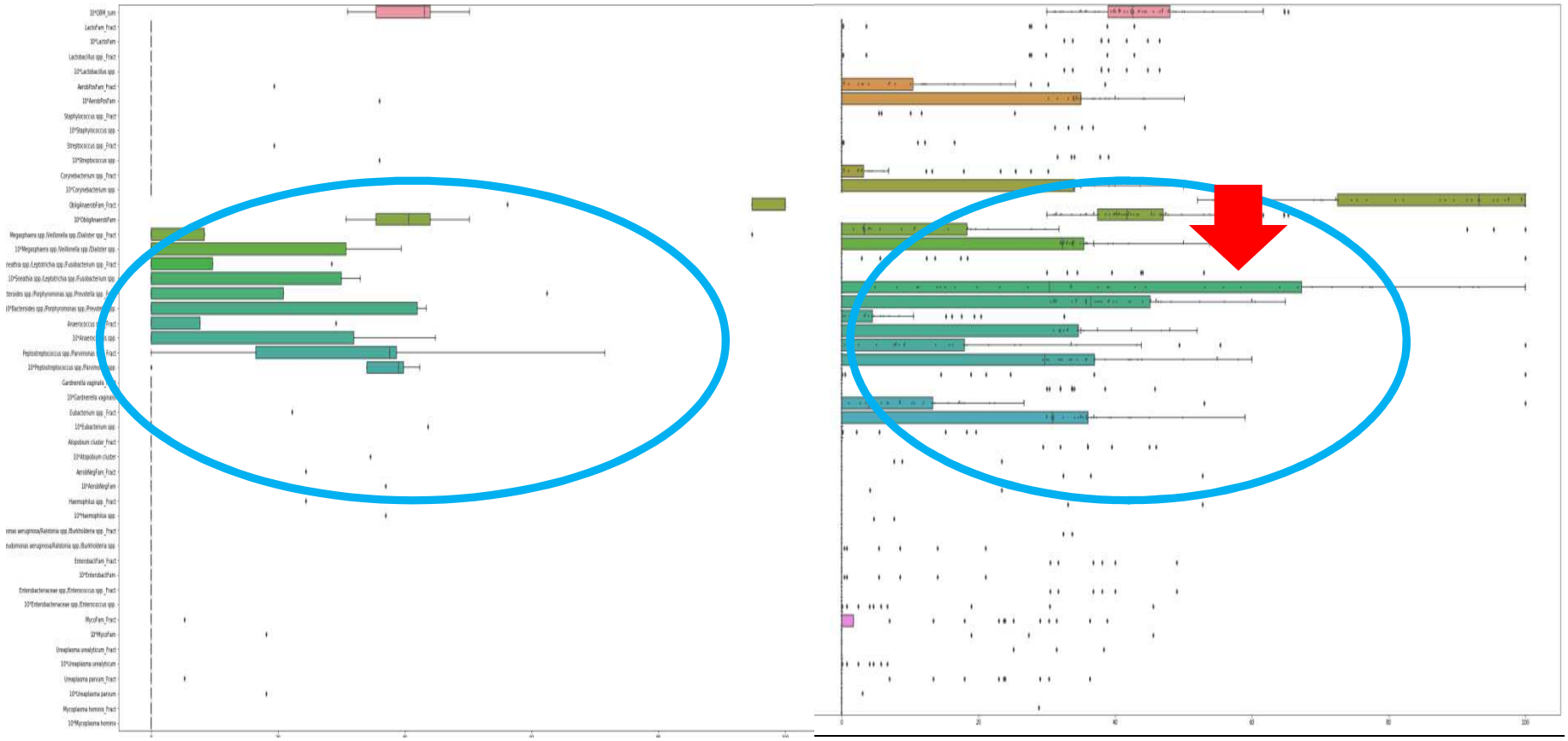
ıarticular species and groups of bacteria in relation to
The quantity of identified microorganisms was expres

4 Stable Microbiota Clusters were Distinguished

- Cluster 1 - with predominance of obligate anaerobes (OA).
- Cluster 2 - with predominance of gram-positive facultative anaerobes (GPFA)
- Cluster 3 – with predominance of *Lactobacillus spp.* (LB)
- Cluster 4 – with predominance of *Enterobacteriaceae/Enterococcus* (EE)

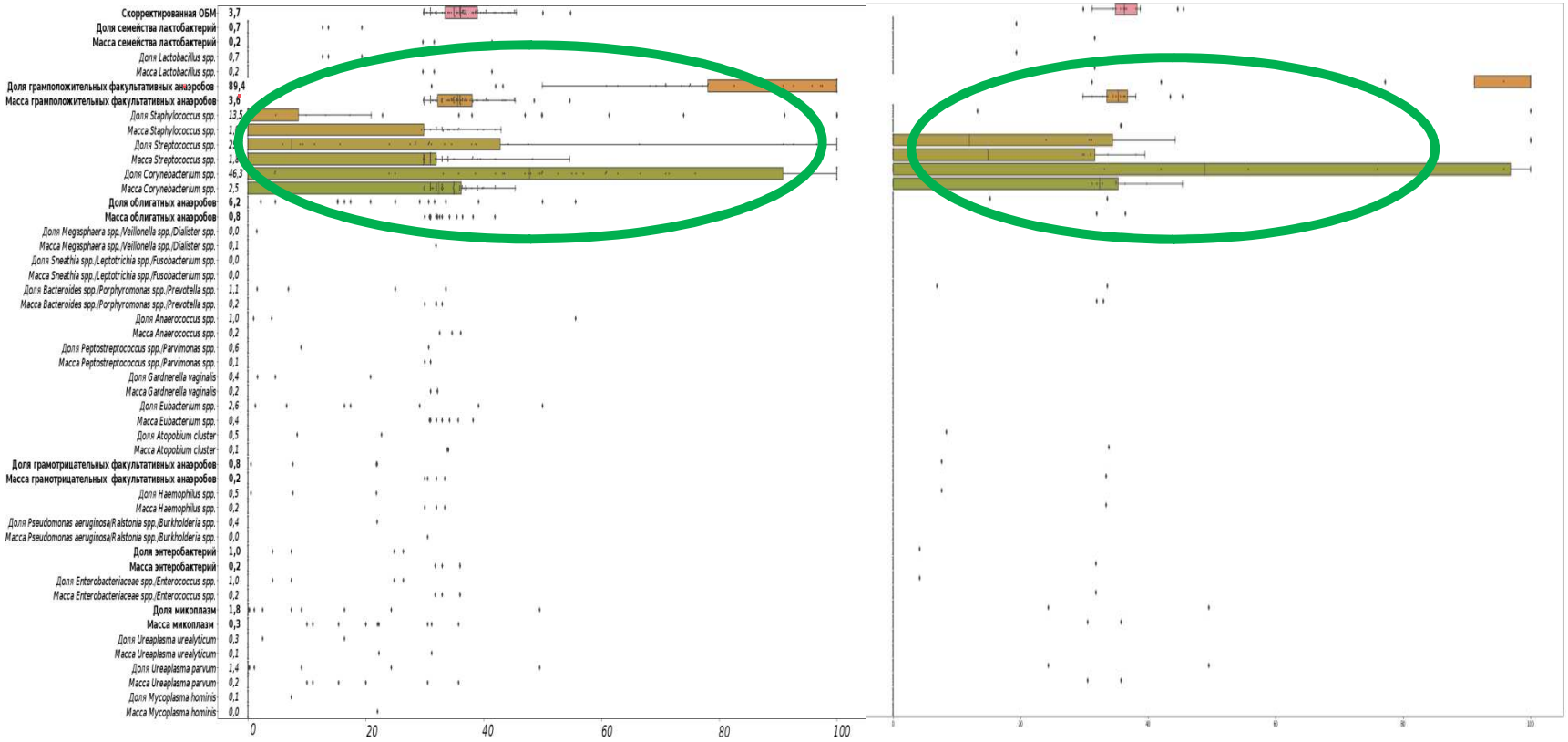
Cluster 1 – with Predominance of Obligate Anaerobes

Normospermia **Asthenozoospermia**



Cluster 2 - with Predominance of Gram-positive Facultative Anaerobes

Normospermia **Asthenozoospermia**



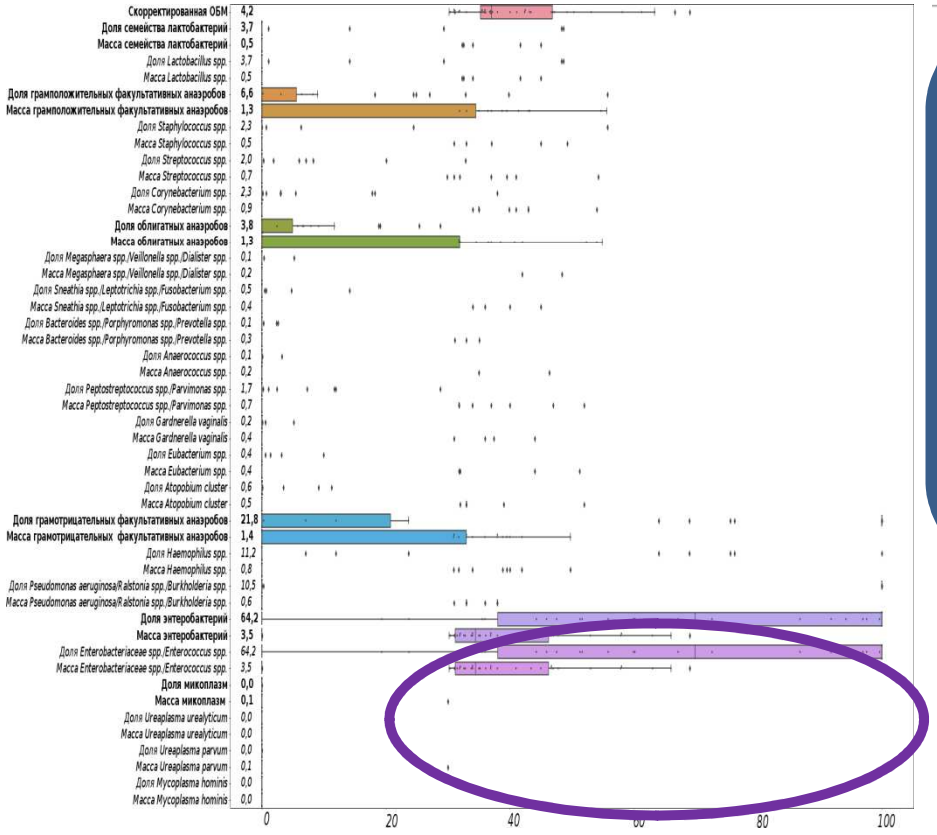
Cluster with Predominance of *Lactobacillus spp.*

Normospermia

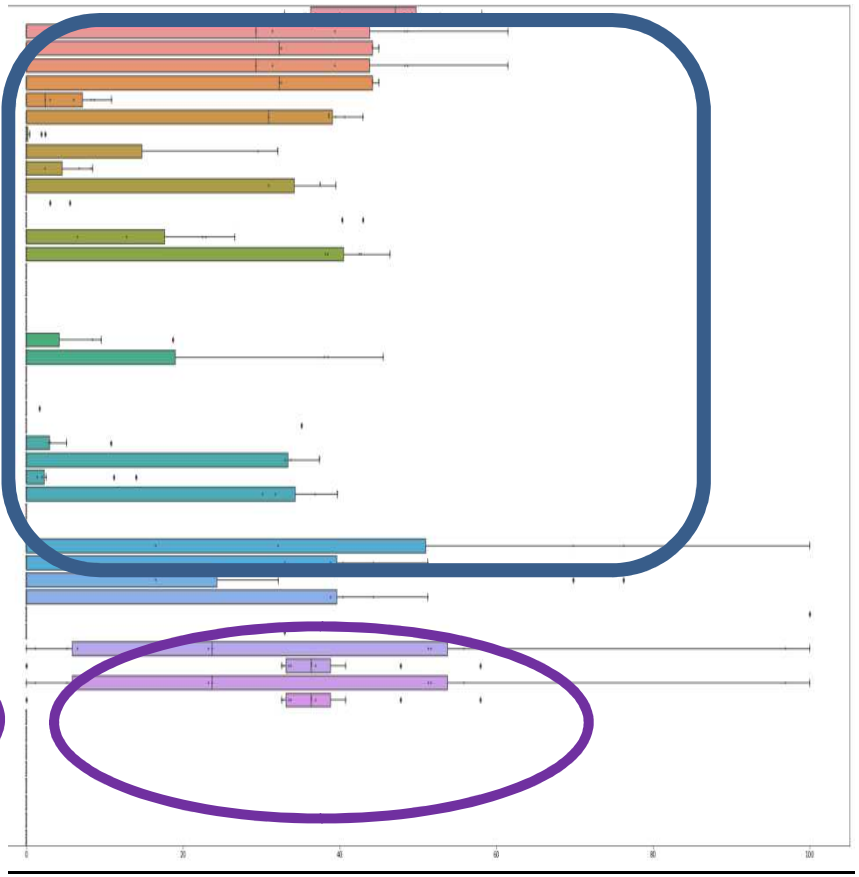
Asthenozoospermia

Cluster with Predominance of *Enterobacteriaceae/Enterococcus* (EE)

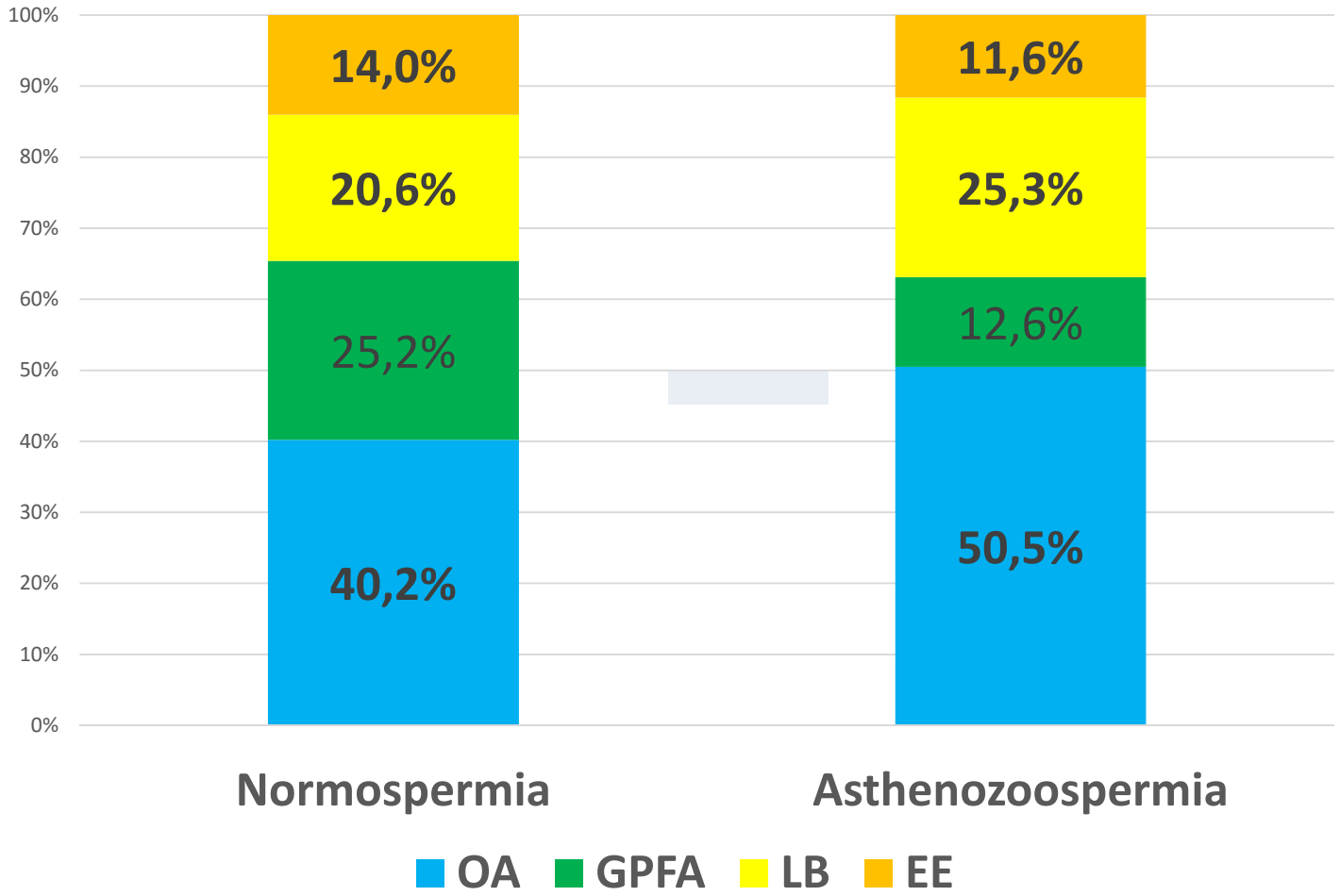
Normospermia



Asthenozoospermia



Cluster Detection Rate: Normospermia and Asthenozoospermia



Conclusion: Semen Microbiota in Patients with Normospermia

- Cluster formed by obligate anaerobes was not dominated by any bacteria group.
- Corynaebacterium spp., Streptococcus spp. are prevalent in the cluster formed by gram-positive facultative anaerobes.
- Lactobacilli prevalence is associated with the detection of obligate anaerobes and facultative anaerobes.
- Enterobacteriaceae spp./Enterococcus spp. group is associated with gram-positive anaerobes and obligate anaerobes, as well as low TBL.

Conclusion: Semen Microbiota in Patients with Asthenozoospermia

- One of the bacteria groups was prevalent in the obligate anaerobes cluster.
- Lactobacilli were present in the cluster where *Enterobacteriaceae spp./Enterococcus spp.* group was predominant.
- *Corynaebacterium spp., Streptococcus spp.* are prevalent in the cluster formed by gram-positive facultative anaerobes.
- Lactobacilli cluster was formed without other bacteria groups.