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Oogenesis Anomalies Induced by Heavy Metal Contamination in Two Tenebrionid Beetles (*Blaps polycresta* and *Trachyderma hispida*)

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Original article

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Histological and ultrastructure analysis of the ovaries of *Blaps polycresta* and *Trachyderma hispida* were performed to illuminate the effect of industrial pollution from a plastic factory located in Khorshed district, Alexandria, Egypt. Detection of heavy metals in the ovarian tissues was achieved by using X-ray microanalysis. The study revealed various anomalies in oocytes and trophocytes. These basic anomalies included exfoliation and vacuolation of follicular epithelium, vacuolated trophocyst, nuclear abnormalities and morphological changes in cytoplasmic organelles. Conclusively, the study was initiated to assess the extent of reproductive corruptions induced by industrial pollution in two insects as a biomarker of exposure which may impair reproduction in humans.

Keywords: Oogenesis anomalies; Heavy metals; Histological and ultrastructure analysis; Beetles.

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Toxic chemicals used by industries in processing and manufacturing are the main cause of industrial pollution. These toxic chemicals are released into the environment and are hazardous to human health (THOMPSON *et al.* 2009) and affect natural populations (OEHLMANN *et al*. 2009). Plastic has the potential to transfer toxic substances to the food chain if plastic debris are ingested (TEUTEN *et al.* 2009). Their additives can interfere with hormone function (HU *et al*. 2009). Some toxic chemicals in plastic such as phthalates and bisphenol-A (BPA) affect reproduction and impair development (in crustaceans, mollusks, and amphibians) (OEHLMANN *et al*. 2009). Hazardous emissions from plastic industries include bromide and color pigments that contain heavy metals (chromium, copper, cobalt, selenium, lead, and cadmium) (VERMA *et al.* 2016). Cytotoxic effects of heavy metals were proven to have an adverse effect on human health even at low concentrations (TCHOUNWOU *et al*. 2012). In addition, their accumulation causes reproductive and physiological oddities (BEDNARSKA & STACHOWICZ 2013; XIE *et al*. 2014; KHEIRALLAH *et al*. 2016). Insects have been used as ecological bioindicators that function as gauges of environmental alterations due to heavy metal pollution (NUMMELIN *et al*. 2007; AZAM *et al*. 2015; EL-SAMAD *et al*. 2015; KHEIRALLAH 2015; OSMAN *et al*. 2015; KHEIRALLAH *et al*. 2016; GHANNEM *et al*. 2017; OSMAN & SHONOUDA 2017; SHONOUDA & OSMAN 2018; EL-SAMAD *et al*. 2019). They give a quick and tactful response to the accumulation of heavy metals (CERVERA *et al.* 2004). The intake of heavy metals by insects could be through integument, respiration or ingestion (BALLAN-DUFRANÇAIS 2002).

Energy dispersive X-ray spectroscopy (EDX) is a very effective tool to specify the heavy metal load in tissues and for monitoring of heavy metal pollution (BISTRICKI & MUNAWAR 1982; KHEIRALLAH 2015; OSMAN & SHONOUDA 2017; SHONOUDA & OSMAN 2018). Intensification of heavy metals in insects causes cell injury and leads to pyknosis which could be investigated through histological and ultrastructure analyses (SUN *et al*. 2007; FONTANETTI *et al.* 2010; KHAN *et al.* 2012; KHEIRALLAH 2015;

KHEIRALLAH *et al.* 2016; OSMAN *et al.* 2015; OSMAN & SHONOUDA 2017; SHONOUDA & OSMAN 2018). Ovaries are an important organ in the accumulation of environmental toxicants (SUTER-EICHENBERGER *et al.* 1998; WAN *et al.* 2007; LIN *et al.* 2013; OSMAN & SHONOUDA 2017).

Few studies have reported the effect of environmental contaminants on gonads in insects (KHEIRALLAH *et al*. 2006, KHEIRALLAH 2015, KHEIRALLAH *et al*. 2016; OSMAN & SHONOUDA 2017; SHONOUDA & OSMAN 2018). Coleoptera-Polyphaga have telotrophic meroistic ovaries which contain germ cells and nurse cells (trophocytes) (BÜNING 2006). In our study, we used two species of beetles (*B. polycresta and T. hispida*) as biomarkers of exposure to elucidate the tremendous hazards resulting from plastic industry on the inhabitants of the Khorshed district. This objective was clarified through histological and ultrastructure analysis to disclose oogenesis anomalies. Detection of heavy metals in the ovaries was performed by X-ray microanalysis.

Materials and Methods

Study sites

Insects were sampled from two sites. The reference site (site A) was the backyard of the Faculty of Science Moharram Bek, Alexandria University, Alexandria, Egypt, with cultivated plants (EL-SAMAD *et al*. 2015). The polluted site (site B) was a juxtaposition of the plastic factory in Khorshed district (Latitude: 31.203945, Longitude: 30.038928) which is a residential area at the eastern edge of Alexandria, Egypt.

Specimen identification

Blaps polycresta and *Trachyderma hispida* (Forskal, 1775) were the main coleopteran insects inhabiting the selected sites. Specimen identification was performed at the Entomology Department, Faculty of Agriculture, Alexandria University. Insects belong to Coleoptera: Tenebrionidea.

Sampling procedure

100 insects from both species were collected randomly from each site. After sexual differentiation of the specimens, about 20 females from each site and species were kept alive in domestic soil and plants in glass containers. Absolute ethanol (95%) was used to anesthetize the beetles. Dissection was performed under a dissecting microscope in a drop of Ringer's physiological solution and the ovaries were taken out from the abdominal cavity. Procedures for protection and use of laboratory animals were done in compliance with ethical guidelines. The methodology was approved by the Ethics Committee of Alexandria University (protocol approval number is 0302440).

Bioaccumulation of metals in ovarian tissues

Detection of heavy metals in ovaries was performed in un-coated specimens using a Jeol scanning electron microscope-5300 equipped with a Link-Isis energy dispersive X-ray micro-

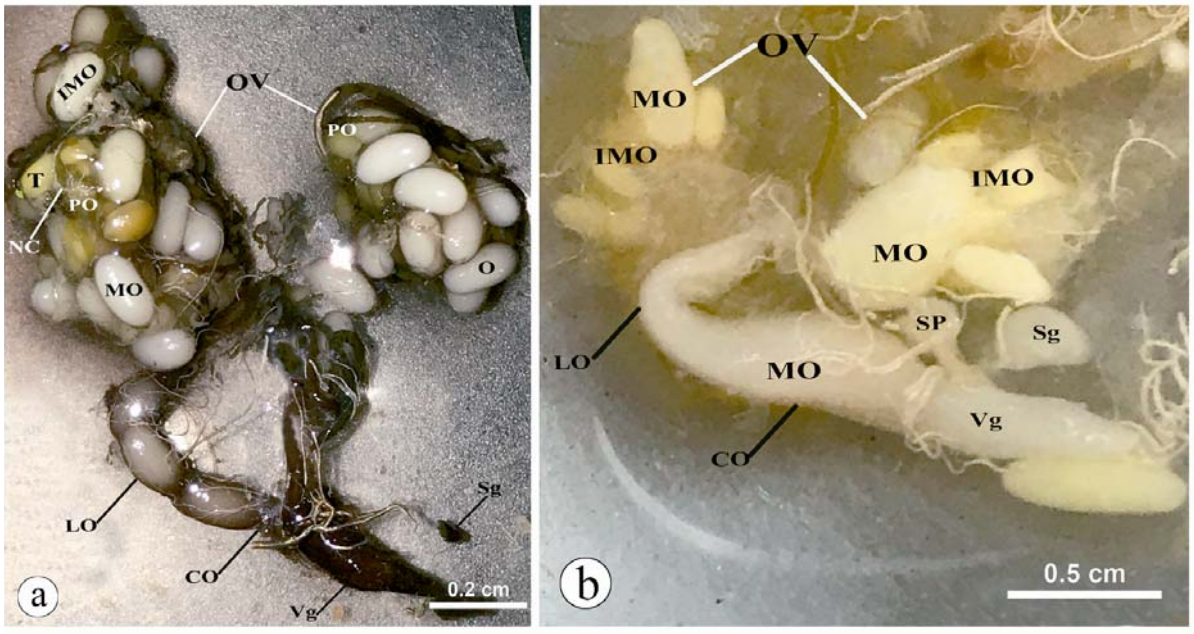

Fig. 1. Photograph of the reproductive system of the normal adult female of *B. polycresta* (a) and *T. hispida* (b) collected from the reference site (site A). Ovary (OV), trophocyte (T), nutritive cord (NC), pro-oocyte (PO), immature oocyte (IMO), mature oocyte (MO), lateral oviduct (LO), common oviduct (CO), spermatheca (Sp), vagina (Vg).

analyzer. A static spot (X500) was analyzed randomly for 110 sec. Due to the divergent distribution of trace metals, three samples of ovarian tissues (each from 3 females, with a total number of 9 insects) were analyzed from each site. The identity of each peak was assigned automatically by the SEM–EDX software. The line intensities were measured for each element in the sample and for the same elements in calibration standards of known composition.

Histological and ultrastructure analysis

Histological analysis followed ANDERSON and GORDON's (1996) method of dehydration, clearing and paraffin embedding. Xylene was used as a cleaning agent. Ovaries were fixed in paraffin wax (65-60°C) and sectioned 5 μm thick, then stained with hematoxylin and eosin. Ultrastructural assemblies for the ovaries started by the fixation in $_4F_1G$ (4% formaldehyde and 1% glutaraldehyde) in phosphate buffer solution (pH 7.2) at 4°C for 3 hours and post-fixed in 2% OsO_4 in the same buffer for two hours. The buffer was used to wash the samples, then they were dehydrated at 4°C through a series of ethanols. Specimens were submerged in Epon-Araldite mixture in labeled beam capsules. For semithin sections, an LKB ultramicrotome was used (5 μm thick). Sections were mounted on a glass slide and stained with toluidine blue. Examination with the light microscope was performed to determine the orientation and the structural characteristic. Ultra-thin sections (6-7 nm thick) were cut for TEM then picked upon 200 mesh naked copper grids. Grids were stained with uranyl for half an hour and lead citrate for 20-30 min. (REYNOLDS 1963).

Statistical analysis of the data

Data were analyzed using IBM SPSS software package version 20.0. **(**Armonk, NY: IBM Corp) (KIRKPATRICK & FEENEY 2013). The Shapiro-Wilk test was used to verify the normality of distribution of variables. The Student t-test (SOKAL & ROHLF 1981) was used to compare the two studied groups for normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level.

Results and Discussion

X-ray microanalysis

Accumulation of metals in the ovarian tissues of the two insects was determined by X-ray microprobe analysis collected from both sites. 12 elements were detected by the X-ray spectra inclusive of Na, Mg, Al, P, S, K, Cd, Ca, Ni, Cu, Zn, and Pb. In *B. polycresta*, a significant elevation in the percentages of Al, Cu, and Zn were noticed in the polluted site (site B) compared with those of the reference site (site A) (Table 1). High percentages of Cd and Pb in the polluted site (site B) were noticed (Table 1). In *T. hispida*, high and significant proportions of Al, Cu and Zn in the polluted site were observed. Moreover, Pb, Cd, and Ni were discerned in the polluted site (site B) (Table 1). Percentages of Mg, P, K and Ca were significantly lower when compared with controls of the two insects.

High percentages of heavy metals detected in the ovarian tissues of the two insects collected from the polluted site were evoked from industrial pollutant delivered to the medium. These metals cannot be broken down by an insect's metabolism (AZAM *et al.* 2015). Furthermore, concentrations of some heavy metals in the tissues were positively correlated with their concentrations in the soil (WAN *et al.* 2014; GHANNEM *et al*. 2018). Metals bind to metal-binding sites on the cell surface, and thus exert a toxifying mechanism that causes cell death (VENTER *et al.* 2017). NUMMELIN *et al.* (2007) and GHANNEM *et al.* (2016) studied the accumulation of heavy metals in different predatory insects located close to a steel factory and stated that all insect groups can be used as heavy metal indicators. Several researchers have shown that heavy metals are responsible for histological and ultrastructure anomalies (BEDNARSKA *et al.* 2009; KHEIRALLAH *et al.* 2006; TALARICO *et al.* 2014; KHEIRALLAH 2015; OSMAN *et al.* 2015; KHEIRALLAH *et al.* 2016; OSMAN & SHONOUDA 2017; SHONOUDA & OSMAN 2018).

Macroscopic findings

The reproductive system of female *B. polycresta* and *T. hispida* consist of a pair of telotrophic meroistic ovaries (BÜNING 1994; BÜNING 1998; BÜNING 2006; TRAUNER & BÜNING 2007; OSMAN & SHONOUDA 2017). The ovaries are composed of numerous ovarioles (Figs 1a, b). Each ovariole is composed of a germarium which contains the trophocyte (confined to the trophic chamber), and the vitellarium with immature and mature oocytes in a single row, asynchronously in development. Trophocytes which supply nutrients to oocytes are connected to pro-oocytes and oocytes by a nutritive cord (TELFER *et al*. 1982). Two lateral oviducts at the end of each ovary unite to form a common oviduct. The common oviduct leads to the vagina housing a spermatheca with a spermathecal gland opened in it (Figs 1a, b). A vast amount of mature oocytes in the ovaries of *B. polycresta* were observed. In old females, almost all ovarioles develop synchronously (NGERNSIRI *et al*. 2015). No morphological abnormalities were

Table 1

Element percentages in ovarian tissues of *B. polycresta* and *T. hispida* collected from reference and polluted sites using energy dispersive X-ray micro-analysis (EDX)

Element	*B. polycresta*			*T. hispida*		
	Site A	Site B	p	Site A	Site B	p
Na	1 ± 0.04	16.7 ± 0.06	<0.001*	5.6 ± 0.06	10.9 ± 0.06	<0.001*
Mg	3.4 ± 0.06	ND	–	ND	ND	–
Al	ND	6.9 ± 0.06	–	4.9 ± 0.06	8.2 ± 0.06	<0.001*
P	65.7 ± 0.06	55.7 ± 0.06	<0.001*	41.6 ± 0.06	31.3 ± 0.06	<0.001*
S	1.7 ± 0.06	10.20 ± 0.06	<0.001*	32.6 ± 0.06	15.3 ± 0.06	<0.001*
K	12.10 ± 0.06	ND	–	12.8 ± 0.06	ND	–
Pb	ND	1.2 ± 0.06	–	ND	2.9 ± 0.06	–
Cd	ND	9.3 ± 0.06	–	ND	8.3 ± 0.06	–
Ca	7.20 ± 0.06	0.10 ± 0.01	<0.001*	5.1 ± 0.06	0.10 ± 0.01	<0.001*
Ni	ND	1.5 ± 0.06	–	ND	1.10 ± 0.06	–
Cu	3.6 ± 1.1	6.7 ± 0.06	0.098	3.7 ± 0.06	9.2 ± 0.06	<0.001*
Zn	2.2 ± 0.06	5.8 ± 0.06	<0.001*	2.6 ± 0.06	7.9 ± 0.06	<0.001*

For each element, the percentage expressed by using minimum–maximum values and mean (N = 3) using Student t-test, p: p value for comparing between site A and site B, *: Statistically significant at $p \leq 0.05$, ND: Not detected.

observed in the ovarian structures of the two insect species collected from the polluted site.

Histological and ultrastructure archetypes observed in ovaries of *B. polycresta* and *T. hispida* collected from the reference and polluted sites (site A & B)

In both insects collected from the reference site (site A), the histological archetypes revealed that the tropharium consists of trophocytes immersed in a loose net of interstitial cells (Figs 2a, b, c & Figs 3a, b, c) (TRAUNER & BÜNING 2007; OSMAN & SHONOUDA 2017). Trophocytes are spherical cells with a rounded nucleus, whereas the interstitial cells are small asymmetrical cells among them (Figs 2b, c & Figs 3b, c) (MOHAMED *et al*. 2015). The oocytes are enfolded by the follicular wall. The follicular epithelial cells are cuboidal cells with a rounded nucleus (Figs 2d, e & Figs 3a, d). The ooplasm is stacked with yolk granules and a distinctive germinal vesicle (Figs 2d, e & Figs 3a, d), in addition to the appearance of lipid and fat droplets (Fig. 4d). Our results were compatible with MOHAMED *et al*. (2015) and OSMAN & SHONOUDA (2017), who worked on the ovarian structure of the coleopteran insect *Callosobruchus maculatus* and *Blaps polycresta,* respectively.

The ultrastructure archetypes of insects collected from the reference site accentuated that the trophocytes appeared with a heterochromatic rounded nucleus with uniform nuclear envelope (Fig. 4a, & Fig. 5a). The cytoplasm contains cytoplasmic organelles and dense vesicles (Figs 4a, b & Figs 5a, b) and appeared more electron lucent and granulated in *T. hispida* (Figs 5a, b). Lysosomes were also observed in the cytoplasm of the trophocytes in *T. hispida* (Fig. 5a). Follicular epithelial cells that ensheathed the vitellogenic oocyte in *B. polycresta* appeared with a euchromatic nucleus and regular nuclear envelope (Figs 4c, d). Rounded mitochondria, RER, SER and vesicular bodies occurred in the cytoplasm (Figs 4c, d).

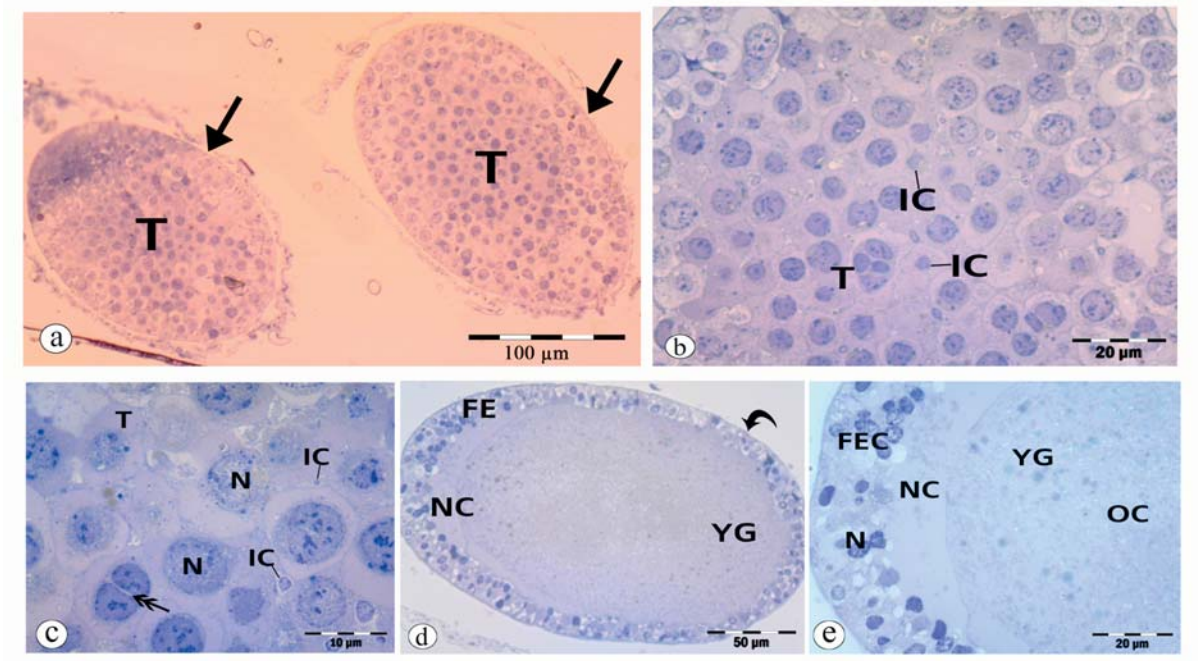

Fig. 2. Semithin sections of the tropharium (a, b, c) and vitellogenic oocyte (d, e) of the control adult female of *B. polycresta*. Trophic chamber (arrow), oocyte (curved arrow), trophocytes (T), trophocyte in mitotic division (double head arrow), interstitial cell (IC), nucleus (N), follicular epithelium (FE), follicular epithelial cells (FEC), nutritive cord (NC), oocyte (OC), yolk granules (YG).

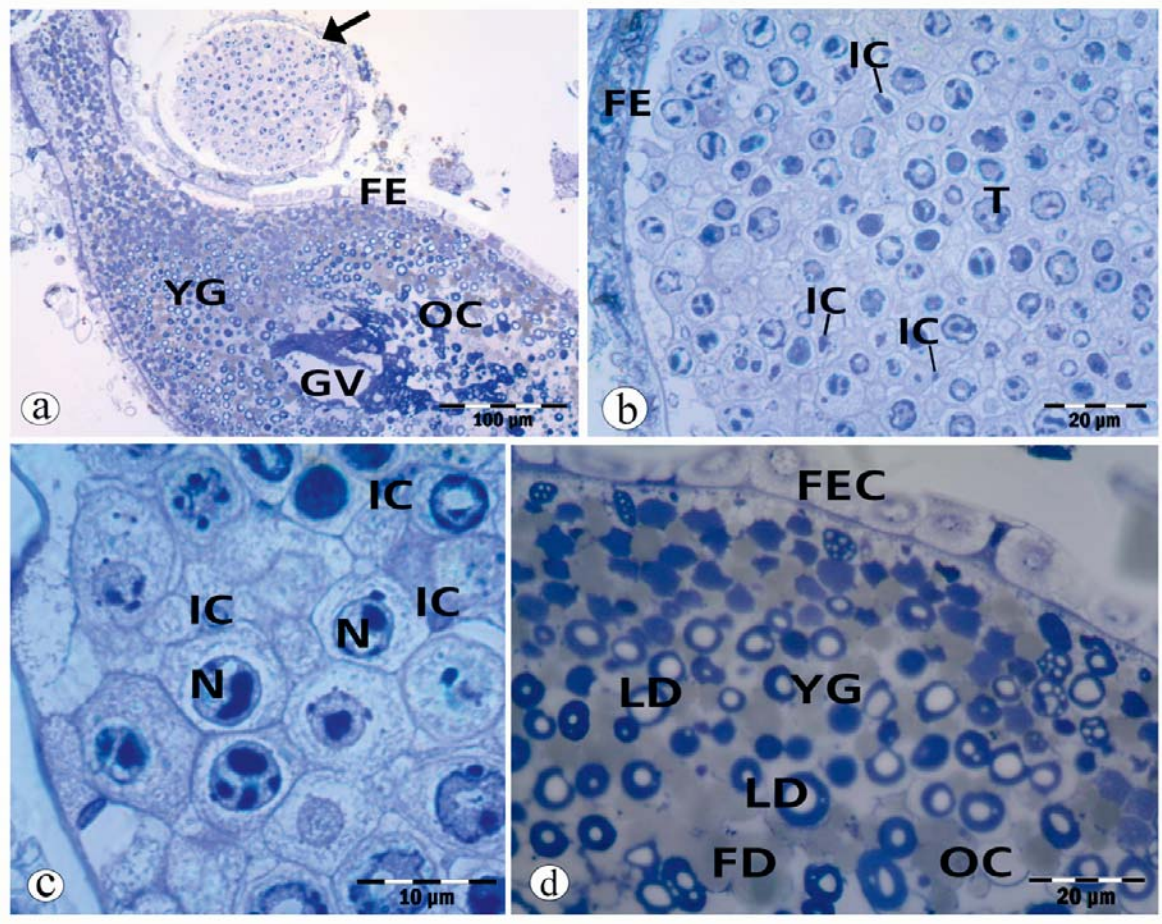

Fig. 3. Semithin sections of the tropharium (a, b, c) and vitellogenic oocyte (a, d) of the control adult female of *T. hispida*. Trophic chamber (arrow), trophocytes (T), interstitial cell (IC), nucleus (N), follicular epithelium (FE), follicular epithelial cells (FEC), nutritive cord (NC), oocyte (OC), yolk granules (YG), germinal vesicle (GV), lipid droplets (LD), fat droplets (FD).

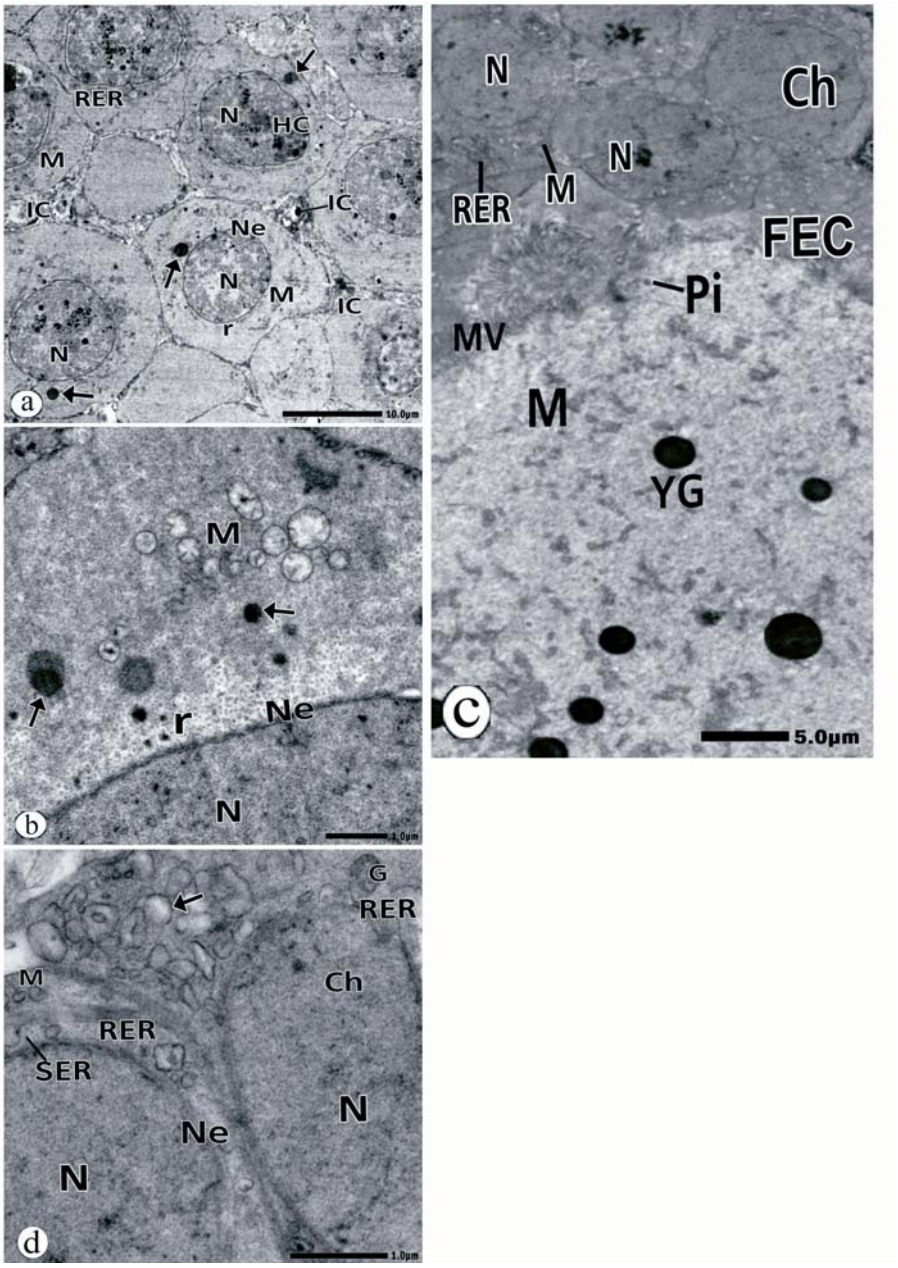

Fig. 4. Electron micrographs in ovarian cells of the control adult female of *B. polycresta*. a – trophocytes with normal nucleus (N), heterogeneous heterochromatin (HC), regular nuclear envelope (Ne), mitochondria (M), dense vesicle (arrow), rough endoplasmic reticulum (RER), free ribosomes (r), interstitial cell (IC); b – magnified part of Fig. 4a; c – vitellogenic oocyte with normal follicular epithelial cells (FEC), rounded nucleus (N), homogenous chromatin (CH), regular nuclear envelope (Ne).mitochondria (M), rough endoplasmic reticulum (RER), microvilli (Mv) with brush border, ooplasm with yolk granules (YG), mitochondria (M), pinosomes (Pi); d – rounded nucleus of the follicular epithelial cell (N), homogenous chromatin (CH), regular nuclear envelope (Ne), mitochondria (M), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), semicircular vesicles (arrow).

The plasma membrane of the follicular cells enfolded into finger-like microvilli which interlocked with the microvilli of the oocyte plasma membrane, thus forming channels. This region is called the brush border. Sometimes the ends of the long channels are filled with condensed materials and cut off forming dense granules in the ooplasm, the pinosomes (Fig. 4c). The pinosomes grow in size towards the interior of the oocyte forming a voluminous dark globule (Fig. 4c). The ooplasm was also filled with mitochondria and yolk granules. The vitellogenic oocyte in *T. hispida* contained a heterochromatic nucleus of the follicular cells and festoon brush border (Figs 5c, d). Cytoplasmic organelles are well recognized in the cytoplasm of the follicular epithelial cells (Figs 5c, d). Pinosomes, mitochondria, dark globules and yolk granules appeared in the ooplasm as well as fat and lipid droplets (Fig. 5c).

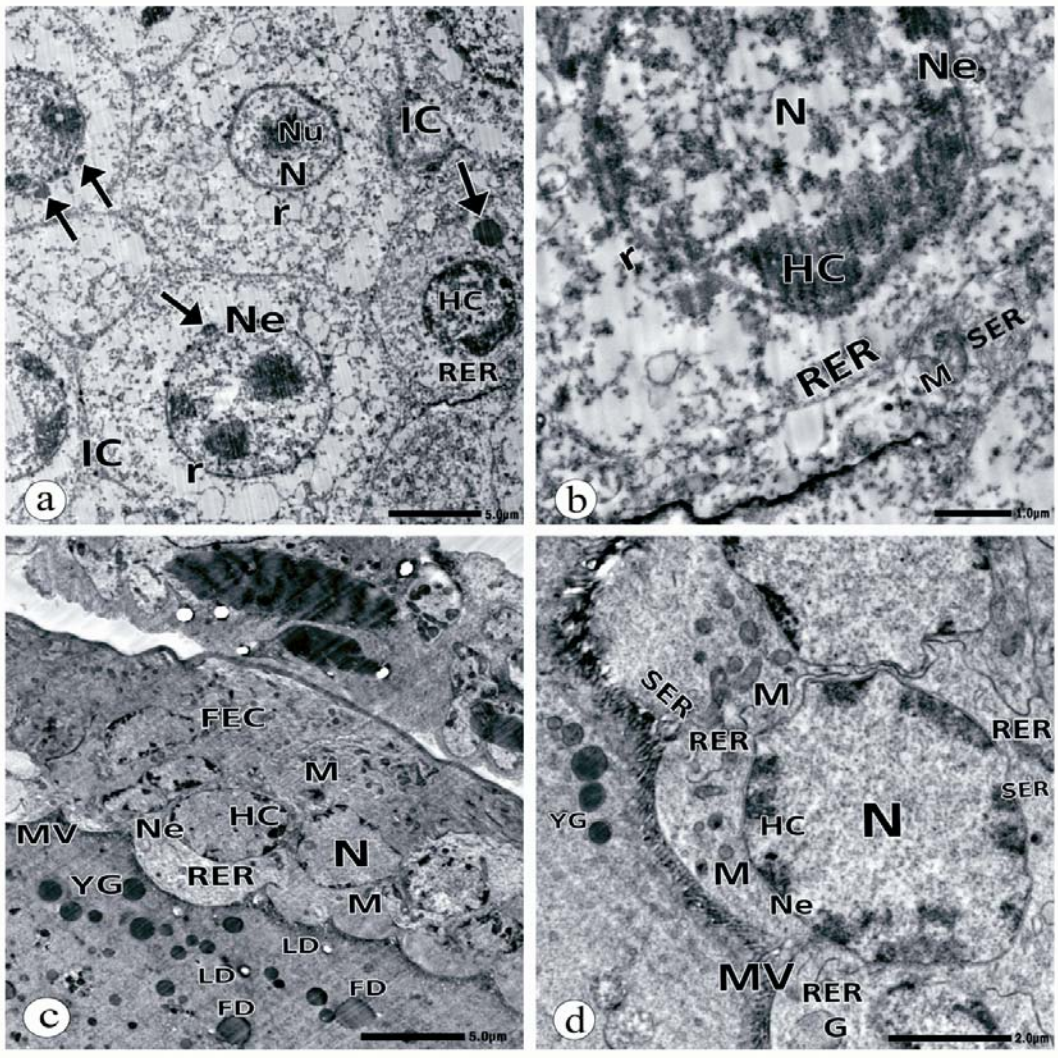

Fig. 5. Electron micrographs in ovarian cells of the control adult female of *T. hispida*. a – trophocytes with rounded nucleus (N) and electron dense heterochromatin (HC), nucleolus (Nu), regular nuclear envelope (Ne), electron-lucent cytoplasm with dense vesicles (arrow), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), ribosomes (r), lysosomes (L). Note interstitial cells (IC); b – magnified part of Fig. 5a showing mitochondria (M); c – vitellogenic oocyte with normal follicular epithelial cells (FEC), nucleus (N), homogenous chromatin (CH), nuclear envelope (Ne).mitochondria (M), rough endoplasmic reticulum (RER), microvilli (Mv), ooplasm with yolk granules (YG), lipid droplets (LD), fat droplets (FD), pinosomes (Pi); d – nucleus of the follicular epithelial cell (N), heterochromatin (HC), nuclear envelope (Ne), mitochondria (M), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), Golgi complex (G), microvilli (Mv), ooplasm with yolk granules (YG), mitochondria (M) and pinosomes (Pi).

These observations were compatible with the findings of MOHAMED *et al.* (2015) and OSMAN & SHONOUDA (2017), who worked on the ovarian archetypes of the beetles *Callosobruchus maculatus* and *Blaps polycresta*, respectively. Trophocytes supply RNA to the oocytes via trophic cords. At a late stage of vitellogenesis, they are broken down by the action of lysosomes (DE WILDE 1964), and their products are assimilated into oocytes to provide protein, RNA, and other materials to the oocytes (ENGELMANN 1970). In the vitellogenic oocytes, microvilli unite with those of the follicle cells indicating the active involvement of follicle cells during vitellogenesis (MOHAMED *et al.* 2015). The existence of pinosomes at the oocyte surface suggests the transfer of material from hemolymph through the follicle cells. Materials such as the yolk proteins, synthesized in the fat bodies and released toward the follicle cells, are then secreted into the oocyte (BRENNAN *et al.* 1982; ISAAC & BOWNES 1982; BUTTERWORTH *et al.* 1992).

Some authors have reported pinocytosis in insects (KAMEL *et al*. 2005; MOHAMED *et al*. 2015). The presence of extensive mitochondria in the oocyte surface reflect high energy production during the active transport of materials. Yolk granules resulted from the fusion of pinosomes (TELFER & SMITH 1970; KAMEL *et al*. 2005). Peripheral ooplasm of the oocyte is rich with microvilli, which

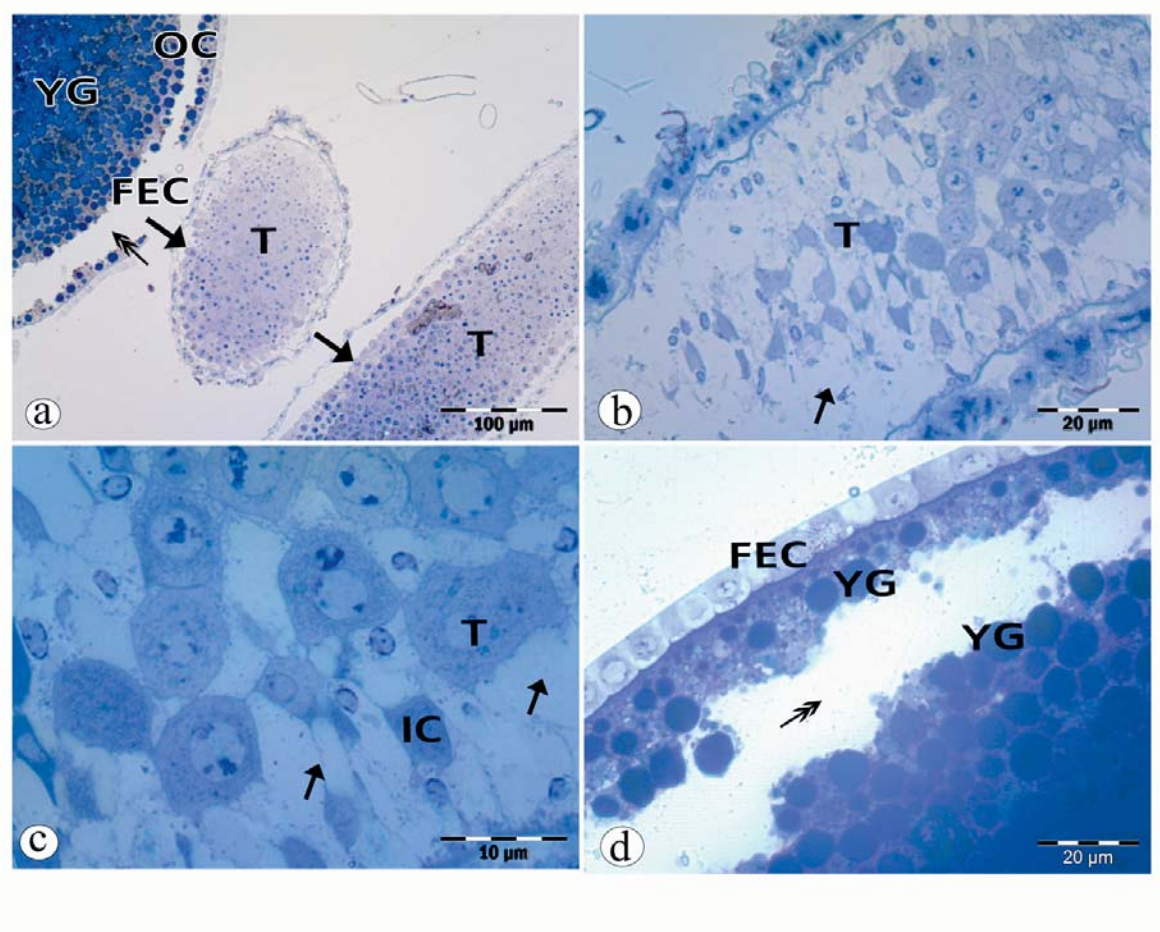

Fig. 6. Semithin sections of the tropharium and vitellogenic oocyte of an adult female of *B. polycresta* from the polluted site. a – reduction of trophic chamber (arrow) and vitellogenic oocyte (OC) with empty ooplasm (double head arrow); b & c – necrotic trophocytes (arrow); d – empty ooplasm (double head arrow). FEC – follicular epithelial cells, T – trophocyte, IC – interstitial cell, YG – yolk granules.

serve in the deposition of vitelline envelope, idem ELDA & ATTILIA (1995).

Several histological and ultrastructure corruptions were noticed in both insects collected from the polluted site. Histopathological observations of the ovarian structure of *B. polycresta* showed a reduction of the trophic chamber and the vitellogenic oocyte (compare Fig.6a, with Fig. 2a). The tropharium appeared with large vacuoles and morphologically altered trophocytes (Figs 6b, c). Thickening of the follicular epithelium, vacuolation in the epithelial cells and empty ooplasm were noticed in the vitellogenic oocyte (Fig. 6d). In *T. hispida*, signs of degeneration were detected in the trophocytes and in the follicular epithelial cells of the vitellogenic oocyte (Figs 7a, b, c). Also, exfoliation of the follicular wall (Fig. 7a) and an oocyte with empty ooplasm was observed (Fig. 7d).

These alterations may retard oocyte growth because of the impairment of the structure and function of trophocytes and follicular cells (OSMAN & SHONOUDA 2017). MC GEE *et al.* (1992) reported that vulnerable membranes are susceptible to toxic effects of xenobiotics by interacting with protein or lipid components of cell membranes. LAGISZ & LASKOWSKI (2008) stated that metal-pollution affects egg quality and unexposed offspring.

Disruptions at the subcellular level were observed in the trophocytes and the oocytes of the two insects. Abnormalities were observed in the nucleus and cytoplasmic organelles of both trophocytes and oocytes of the two insects. Trophocytes of both insects displayed irregularities of nuclear envelopes (Figs 8a, 9a) and karyolysed nucleus. Sometimes the nuclei appeared with patches of heterochromatin (Figs 9a, b) and globular inclusion bodies (Figs 9b, c). In addition, mitochondria with disintegrated cristae (Figs 9a, b), lysosomes and dense vesicles (Figs 8a, 9a, b, c) were observed. The vitellogenic oocyte in the two insects evinced with a lytic area in the follicular cells (Figs 8c, d, e, 9d, e, f, g). Some cells contained a a pyknotic nucleus (Fig. 8d) and other cells appeared with karolysed ones (Figs 9e, f, g). Globular inclusion bodies were observed at the nuclear envelope (Fig. 8e). In the cytoplasm, mitochondria with lysed ma-

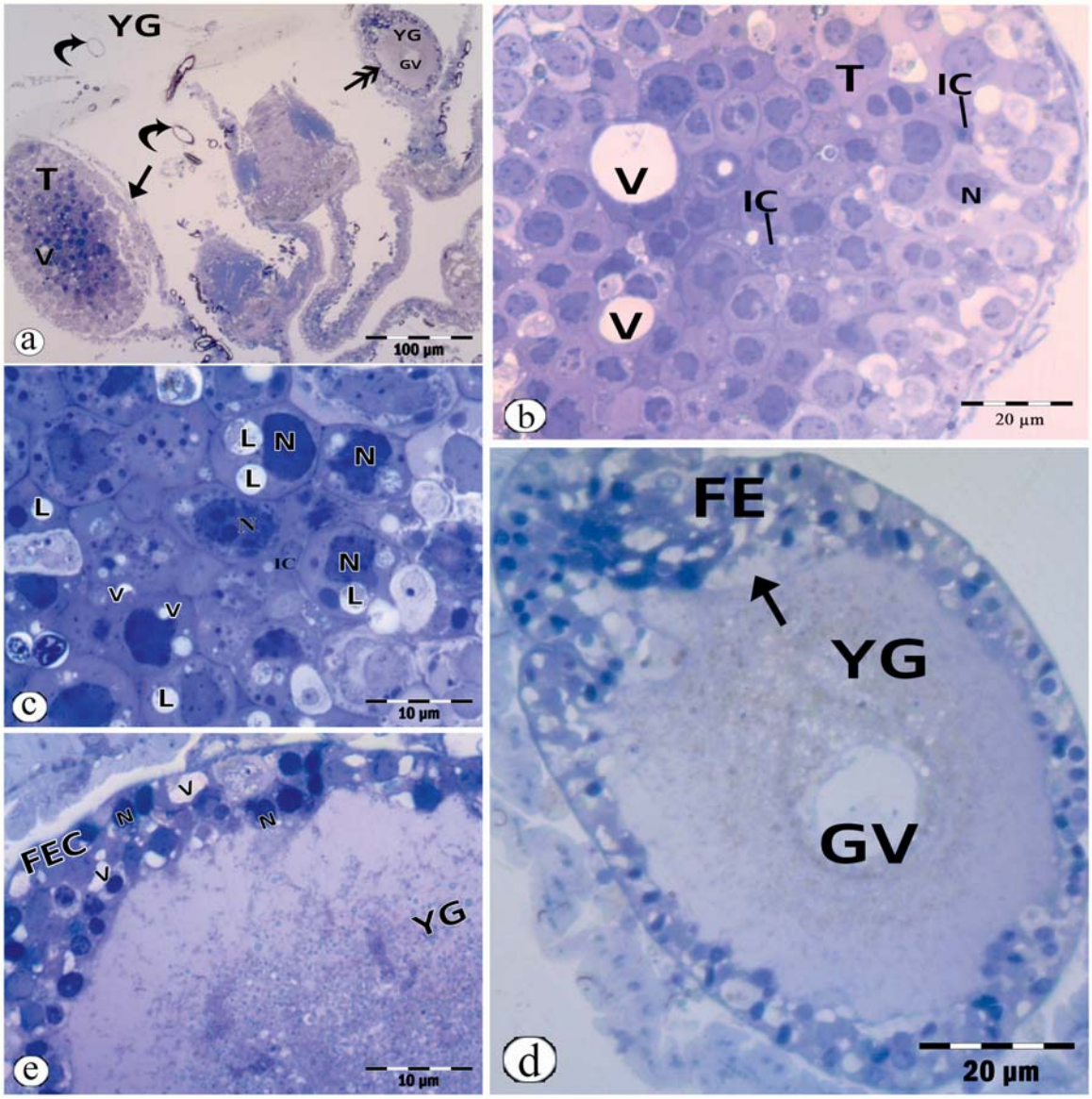

Fig. 7. Semithin sections of the tropharium and vitellogenic oocyte of an adult female of *T. hispida* from the polluted site. a – exfoliation of the follicular wall (arrow) and degenerated follicular epithelial cells (FEC); b – degenerated trophocytes and empty cytoplasm (arrow); c – magnified part of Fig. 7b showing degenerated trophocytes and empty cytoplasm (arrow); d &e – vitellogenic oocyte with damaged follicular epithelial cells (FEC) and empty ooplasm (arrow). T – trophocyte, OC – oocyte, IC – interstitial cell, YG – yolk granules.

trices and dilated smooth endoplasmic reticulum were also noted (Fig. 8e). Sometimes, the cytoplasm lacked cytoplasmic organelles (Fig. 9d). The brush borders of the microvilli were distorted and/or attenuated (Figs 8c, d, e, 9d, e, f, g). Degenerated yolk granules and lytic areas were observed in the ooplasm (Figs 8c, 9d, e, f, g). Fusion of yolk granules into patches was observed (Fig. 9g).

The results of the present study showed that tenebrionid beetles, *B. polycresta*, and *T. hispida*, living in the metal-contaminated environment have many ultrastructural alterations. The toxic effect of heavy metals leads to the accumulation of proteins and lipids which may disrupt vitellogenesis (Świątek 2005; Ben Ahmed *et al.* 2010; Ben Ahmed *et al.* 2013; Khaled *et al.* 2017). The majority of trophocytes and vitellogenic oocytes were affected by heavy metal pollution and underwent degenerative changes, such as cytoplasmic vacuolization and nuclear divergence. Nuclear abnormalities could be the first sign in the course of cell death. The irregularity of nuclear envelopes and the appearance of globular inclusion bodies which is a novelty in our study revealed cellular degeneration (Pazir *et al*. 2011). Synthesis of membranes within the nucleoplasm leads to the formation of globular tubules (Engedal *et al*. 1977). The appearance of lytic areas in the cytoplasm could be due to the activity of lysosomal hy-

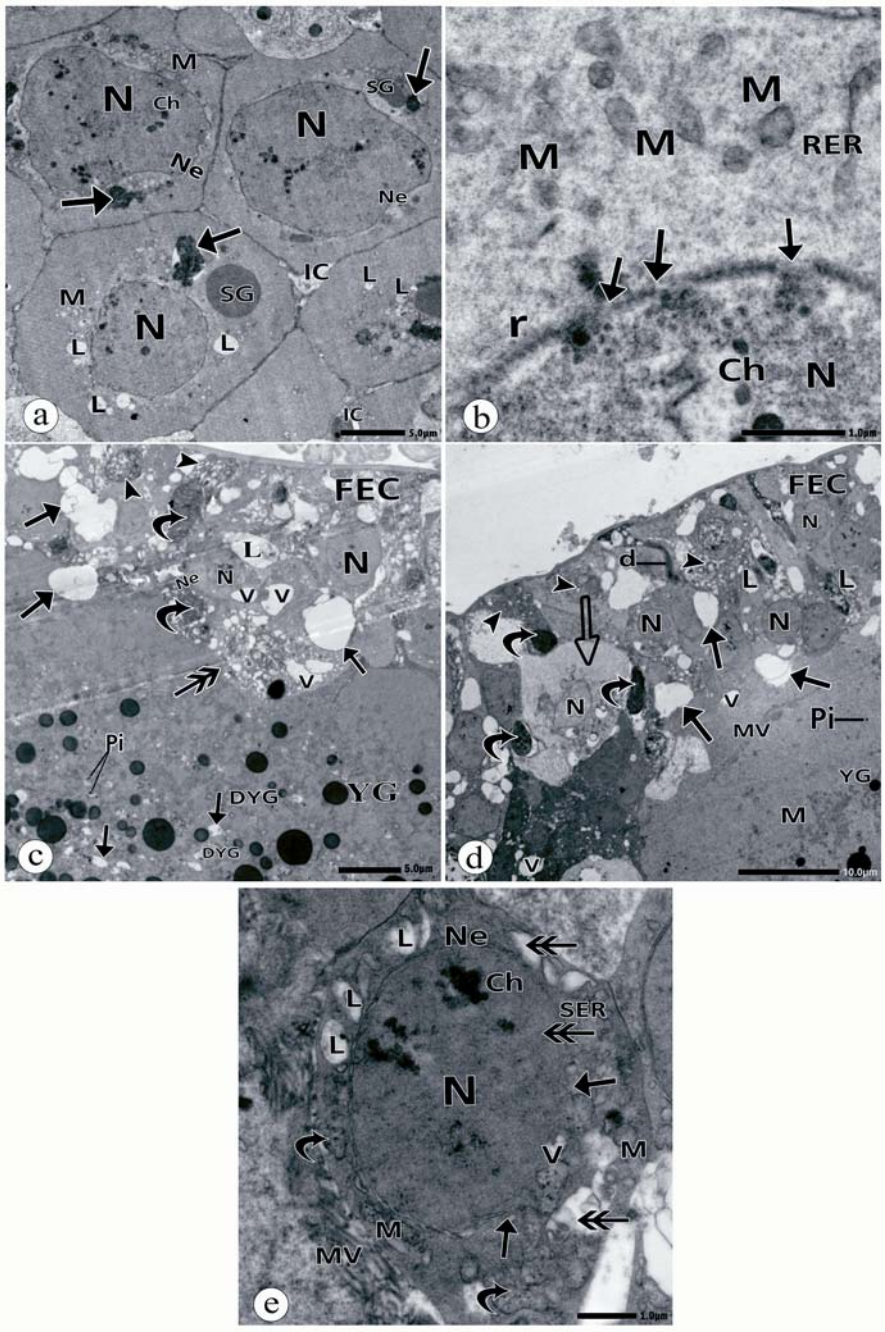

Fig. 8. Electron micrographs in ovarian cells of *B. polycresta* from the polluted site. a – abnormal trophocytes with indented nuclear envelope (Ne) and disintegrated mitochondrial cristae (M). N – nucleus, L – lysosomes, SG – large secretory granule, curved arrow: dense vesicle, IC – interstitial cell; b – magnified part of Fig. 8a showing nuclear pore (arrow) and mitochondria with disintegrated cristae (M). RER – rough endoplasmic reticulum, r – free ribosomes; c – abnormal vitellogenic oocyte showing lytic areas in the follicular wall and ooplasm (arrow), indentation of nuclear envelope (Ne), distorted microvilli (double head arrow) and degenerated yolk granules (DYG); d – Follicular epithelial cells (FEC) with apoptotic cells (hollow arrow), degenerated mitochondria (M) and lytic areas in the cytoplasm (arrow). Note: attenuated microvilli (Mv) and degenerated yolk granules (DYG). Desmosomes (d), secretory vesicles (head arrow), dense vesicle (curved arrow), vacuole (V), pinosomes (Pi), yolk granules (YG); e – Follicular epithelial cell with globular inclusions in the nucleus (arrow), vacuolated nucleoplasm (V), lytic areas in the cytoplasm, mitochondria with lysed matrices and dilated smooth endoplasmic reticulum (SER). Note distorted microvilli (Mv). Nucleus (N), nuclear envelope (Ne), a secretory vesicle (curved arrow), lysosomes (L).

drolase (VANDENBULCKE *et al.* 1998). The dense vesicles which were detected in the ovarian cells of the polluted site denote the accumulation of metals in the lysosomes (LAUVERJAT *et al.* 1989; SUN *et al.* 2007).

Several authors reported that heavy metals interfere with cytoplasmic membranes and cause pathological consequences (HAWKINS *et al.* 1980; SEIDMAN *et al.* 1986; KAWAHARA *et al*. 1990; PAWERT *et al.* 1996; AU *et al.* 2003; KHEIRALLAH

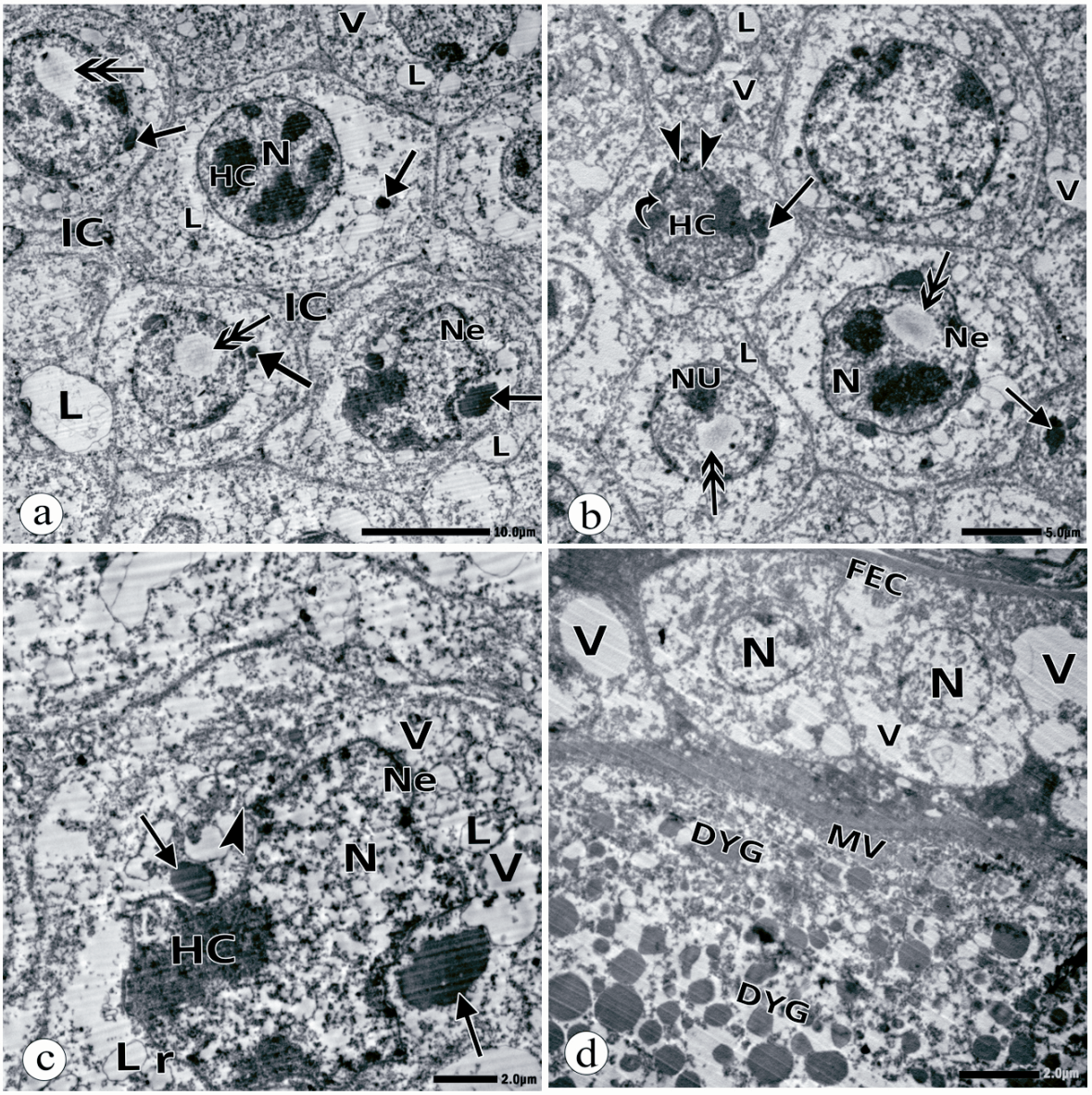

Fig. 9 (a-d) – Electron micrographs in ovarian cells of *T. hispida* from the polluted site. a – trophocytes with pyknotic nuclei (double head arrow), indented nuclear envelope (Ne), heterochromatin (HC), dense vesicle (arrow), lysosomes (L); b – trophocytes with pyknotic nuclei (double head arrow), globular inclusion bodies (head arrow), nuclear pore (curved arrow), indented nuclear envelope (Ne), heterochromatin (HC), dense vesicle (arrow), lysosomes (L), vacuoles (V); c – magnified part of Fig. 10b showing globular inclusion bodies (double head arrow) and indented nuclear envelope (Ne). Heterochromatin (HC), a dense vesicle (arrow), lysosomes (L), vacuoles (V), free ribosomes (r); d – vitellogenic oocyte showing follicular epithelial cells (FEC) with a karyolysed nucleus (N) and vacuolated cytoplasm (V). Note: cytoplasm devoid of cytoplasmic organelles. Note also: microvilli with distorted brush borders (Mv) and abnormal ooplasm with degenerated yolk granules (DYG).

et al. 2006; KHEIRALLAH 2015; OSMAN *et al.* 2015; KHEIRALLAH *et al.* 2016; OSMAN & SHONOUDA 2017; SHONOUDA & OSMAN 2018). These pathological ramifications encompass nuclear and cytoplasmic corruptions. The follicular epithelial cells showed signs of deterioration which is an eminent aspect found in the ovarian cells in the polluted site. This may retard oocyte maturation and leads to imperfect yolk deposition (ANDERSON 1971; SREELATHA & GEETHA 2010; OSMAN & SHONOUDA 2017). The distorted brush borders of the microvilli may block the passage of the materials toward the oocyte. Also, the deformity of yolk granules could obstruct vitellogenesis and result in a lower fecundity and egg viability (SREELATHA & GEETHA 2010).

A few studies have shown the influence of metal pollution on gamete quality and ovarian structure (LAGISZ & LASKOWSKI 2008; KHALED *et al.* 2017). Our results are in line with those of OSMAN

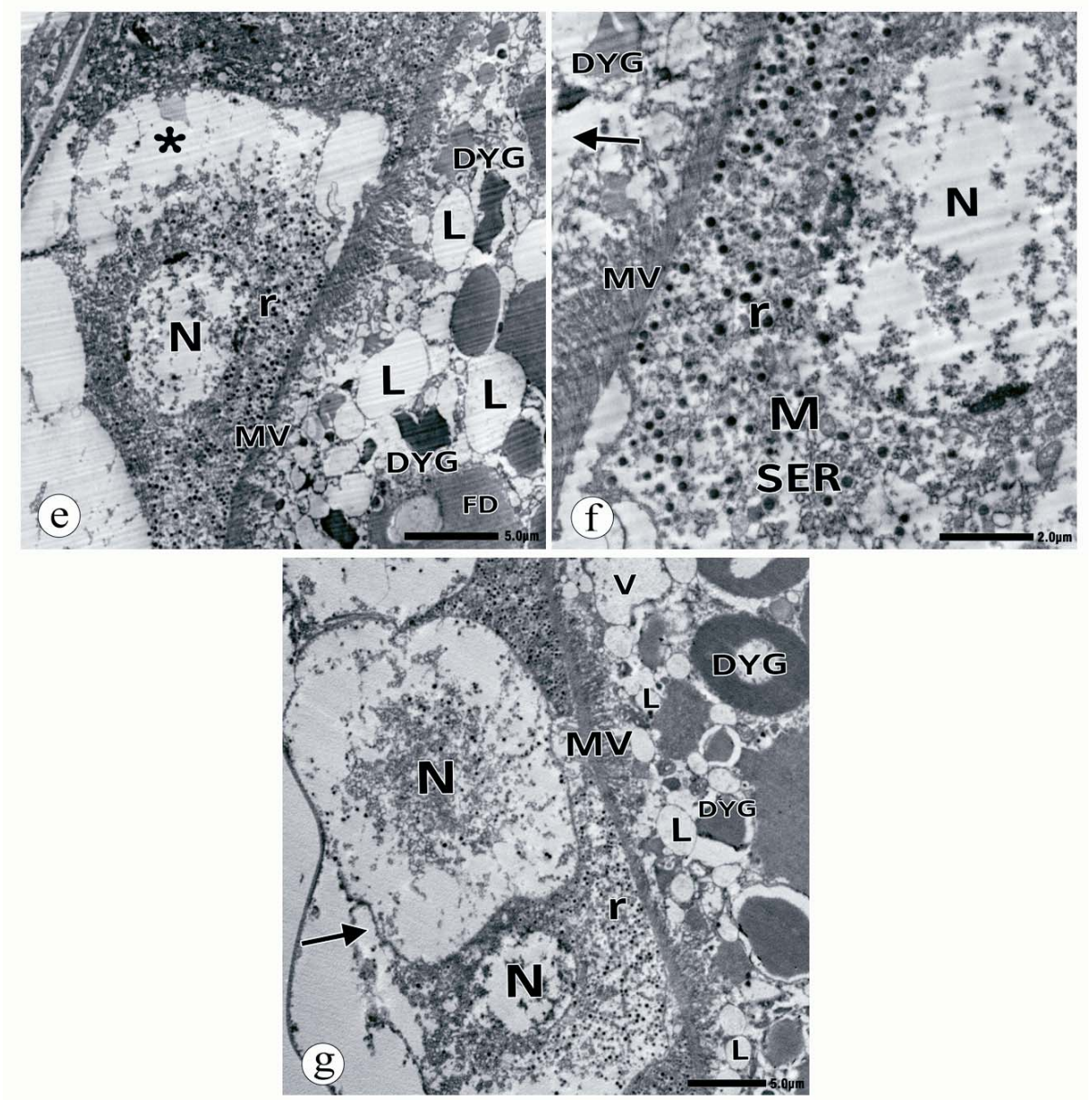

Fig. 9 continued (e-g) – e, f & g – vitellogenic oocyte with necrotic follicular epithelial cells (FEC). Note: karyolysed nucleus (N), lysis of cytoplasm (arrow), distorted microvilli (Mv), lysis of ooplasm (double head arrow) and degenerated yolk granules (DYG). Mitochondria (M), smooth endoplasmic reticulum (SER), free ribosomes (r), lysosomes (L), fat droplets (FD), pinosomes (Pi), vacuoles (V).

& SHONOUDA (2017) who discovered severe ultrastructure alterations in the ovarian cells of *B. polycresta* inhabiting a polluted soil.

Overall, our present study should increase the perception of environmental pollution resulting from the plastic industry, which may affect reproduction.

Conclusion

Industrialization leads to a high risk of exposure to heavy metals. Heavy metals cause many biological ramifications, particularly disturbances in reproduction. Insects are efficient biomarkers in detecting environmental pollution. An effort is needed in order to reduce the hazards resulting from the plastic industry. In our opinion, studying oogenesis alterations in a biomarker such as an insect enables a perfect explanation for reproduction retardation in humans.

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Compliance with Ethics Requirements

All Institutional and National Guidelines for the care and use of animals (insects) were followed.

Author Contributions

Research concept and design: D.A.M.K., L.M.E-S.; Collection and/or assembly of data: D.A.M.K., L.M.E-S.; Data analysis and interpretation: D.A.M.K., L.M.E-S.; Writing the article: D.A.M.K.; Critical revision of the article: D.A.M.K., L.M.E-S.; Final approval of article: D.A.M.K., L.M.E-S.

Conflict of Interest

The authors declare no conflict of interest.

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