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ULTRASTRUCTURE OF VITELLOGENESIS AND VITELLOCYTES IN *LECITHOCHIRIUM GRANDID* ON *SAURIDA UNDOSQUAMIS*, TRAPPED FROM ALATAKA HARBOR, SUEZ GULF, EGYPT

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Abstract

The study gave TEM ultrastructural of vitellogenesis in *Lecithochirium grandid*, digenean intestinal parasite of *Saurida undosquamis*, four developmental phases (1, 2, 3 & 4) during vitellogenesis. Vitelline bundles were enveloped with fine fibrous layer surrounded with separ-ated circular muscle bands. Ultrastructural study showed that vitellocyte maturation comprised of increased extension of parallel cisternae of GER, increase in cell size and in the different phases increased numbers of pellets aggregation in clusters during vitellogenesis. In mature vitellocytes, there were large numbers of various aggregations such as aggregation of pellets in clusters; the important portion in construction of egg shell and a number of unsaturated lipid droplets. This agreed with different phases of oocyte development in parasitic Platyhelminthes.

Keywords: Suez Gulf*, Lecithochirium grandid*, *Saurida undosquamis*, Vitellogenesis.

Introduction

Platyhelminthes female reproductive system showed great morphological variability with significant differences in the anatomical organization and cell structure between taxa (Adiyodi and Adiyodi, 1988). Vitellocytes of parasitic are a vital portion in forming the mature eggs containing invasive larvae (Świderski and Xylander, 2000).

Platyhelminthes have been subdivided into two levels of organization, according to the female reproductive system (Falleni *et al,* 2010, Samuel *et al,* 2012; 2016). Helminths develop through egg, larval (juvenile), and adult stages. Digenea are part of Neoophora, characterized by heterocellular female gonads composed of ovary and vitellaria (vitelline gland). Each has a role in egg formation (Adiyodi and Adiyodi, 1988). Vitelline cells provide the material necessary for the formation of the eggshell and the essential nutriant material for development of future embryo (Grant *et al,* 1977; Irwin and Maguire, 1979). Trematoda oogenesis was studies by the light and electron microscope (Yosufzai 1953; Saxena 1979; Gupta *et al,* 1984; Samuel *et al,* 2016; Arafa and Salama, 2019; El-Sayed *et al,* 2019; Świderski *et al,* 2019). These studies showed the general pattern of oocyte development in parasitic platyhelminthes showed that the main pattern of oogenesis is basically similar in digeneans (Gresson, 1964; Holy and Wittrock, 1986; Orido, 1987; 1988; Poddubnaya *et al,* 2003).

The digenean parasites of genus *Lecithochirium* Lühe 1901 (Hemiuroidea, Hemiuridae, Lecithochiriinae) were reported in the stomach and intestine of marine teleost fish globally (Yamaguti 1971; Shih *et al,* 2004; Claxton *et al,* 2017; Luma *et al,* 2020).

TEM studies on vitellocyte and oocyte development proved to be taxonomic tool of control benefits (Swiderski *et al,* 2011).

The present study aimed to clarify TEM ultrastructural of different phases of oocytes development and vitellocytes during their differentiation of *Lecithochirium grandid*.

Materials and Methods

A total of 200 *Saurida undosquamis* fish of various body weights were collected from Alataka Harbor, Suez Governorate, Egypt, during the year 2022.

Fishes were examined macro- and microscopically for parasites. Digenean ones were collected alive and dissected out microscopy for processing to permanent preparations. Worms were removed, rinsed with a 0.9% NaCl solution and fixed at 4°C, 2.5% glutaraldehyde in a 0.1-M sodium cacodylate buffer at pH 7.4 and post-fixed in 1% osmi-

um tetroxide in the same buffer for an hour, dehydrated in ethanol, cleared in propylene oxide and then embedded in Epoxy resin at 60°C for a day.

Sections of 60-90nm thick were obtained by using a Reichert-Jung Ultra-microtome, placed on 200-mesh copper grids, were double-stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined under JEOL 100 CX TEM at the Electron Microscopy Unit, Faculty of Agriculture, Mansoura University, Egypt.

Ethical approval in dealing with experimental with animals in Helsinki declarations (2008) was followed.

Results

Generally, the vitelline glands of *L. grandid* were follicular, with vitelline cells at various maturation phases close to each other and were diagrammatically illustrated. Immature gonial type (phase 1), an earliest (phase 2) vitellocyte, advanced vitellocyte (phase 3) and (phase 4) mature vitellocyte, with SB=2µm

Ultrastructure of vitellogenesis showed vitellocytes at different phases of maturation, progressing from immature gonial type situated usually near follicle periphery to mature vitellocytes towards center. Vitelline folicles contained four phases of vitellocytes development; 1: immature gonial type, 2: early differentiation stage, 3: advanced vitellocytes & 4: mature one. Vitelline bundles were covered by a thin fibrous coat followed by underlying separated circular muscle bands, without nurse cells in or around, but interstitial cells around developing vitelline cells. Mature cells resort to be located toward the middle of the follicle.

Phase 1 or immature gonial cells with high nucleocytoplasmic ratio and different irregular shapes. Large oval nuclei centrally located visible spherical nucleoli and heterochromatin scattered in nucleoplasm. Granular cytoplasm with few mitochondria and elongated endoplasmic reticulum lacked shell pellets' aggregation.

Phase 2 or earliest vitellocyte with rapidly increasing in size, a nucleus including electron-dense patches of chromatin, nuclei appeared large, spherical nucleoli and heterochromatin more condensed than in phase 1, with cytoplasm distinguished by granular endoplasmic reticulum, mitochondria, electron-intensive vitelline globules and GER development forming a network allover cytoplasm. Phase 2 was characterized by pellets aggregation in sets compared to phase 1 with shell pellets aggregated in clusters, proceeded with enlargement by aggregation with single shell pellets, and enclosed by endoplasmic reticulum.

Phase 3 or advanced vitellocyte characterized by more growth and differentiation of granules, increased size forming shell pellets with increased cytoplasm size and shell pellets numbers distributed allover cell. Membranes integration of numerous shell pellets forming shell pellets clusters, and heterochromatin more intensely and greater than phase 2. Glycogen granules and few mitochondria aggregated around shell pellets clusters and lipid droplets**.**

Phase 4 or mature vitellocyte characterized large cells and condensed in folliclar center, cytoplasm filled with abundant shell pellets adjacent to granular endoplasmic reticulum and lipids droplets. Shell clusters composed of various numbers globules about 47/cluster. Nuclei invisible in mature vitellocytes, endoplasmic reticulum less abundant than in phase 3, but with few residual surrounding shell pellets clusters peripherally. One or three lipid droplets were revealed in mature vitelline cells**.** Details were given in figures (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 & 12).

Discussion

Vitelline cells of trematodes and cestodes play two very important role; formation of a hard, dense and resistant egg shell, and storage and supplying of nutritive reserves for the developing embryos (Świderski and Xylander 2000). Vitellogenesis is basically the same in almost all digenean species (Erasmus, 1982). Vitellogenesis ultrastructural studies concentrated on zoonotic species such

as *Fasciola* and *Schistosoma* (Arafa and Taelab, 2016). The ultrastructural features of vitellogenesis in *L. grandid* resembled those found in different species of digenean trematodes, such as *Maritrema feliui* (Świderski *et al,* 2011), *Metadena depressa* (Samuel *et al,* 2012), *Orientocreadium batrachoides* (Taelab and Lashein, 2013), *Cainocreadium labracis* (Świderski *et al,* 2019), and *Proctoeces* sp. (Arafa and Salama, 2019).

In the present study, maturation of vitelline cells of *L. grandid* more or less agreed with Sampour (2008), who reported three vitellocyte maturation phases in *H. lateralis*, but his work on the three major phases agreed in the detailed phases of differentiation and development of vitellocytes. The interstitial cells were involved in the selection and transport of materials from parenchyma to the developing vitellocytes (Adiyodi and Adiyodi, 1988, Poddubnaya *et al,* 2007; Conn *et al,* 2009). Some vitelline follicles of digenea possess interstitial cells with cytoplasmic extensions between vitellocytes (Poddubnaya *et al,* 2012).

The present ultrastructural study showed that the vitelline glands in *L. grandid* were surrounded by interstitial cells between the developing vitelline cells. This agreed with *Metadena depressa* (Samuel *et al,* 2012) and *Aphallus tubarium* (Greani *et al,* 2016) But, this disagreed with (Taelab and Lashein, 2013; Arafa and Salama, 2019), they didn't report interstitial cells between vitellocytes.
However, Świderski *et al.* (2019) reported that vitellocytes of parasitic platyhelminthes are a key element in the mature eggs production containing the invasive larvae. They added that the β-glycogen and lipid droplets are nutritive reserves for embryogenesis

In the present study, lipid droplets and glycogen granules were observed in advanced and mature vitelline cells of *L. grandid*. They are generally considered as important energy reserves, although this may not be the case for all trematodes (Taelab and Lashein, 2013). This finding was also recorded in the advanced and mature vitelline cells of *M. feliui* (Świderski *et al,* 2011), *C. labracis* (Świderski *et al,* 2019) and *Proctoeces* sp. (Arafa and Salama, 2019).
The shape, number, size and the clusters of shell pellets during vitellogenesis greatly varied among digeneans especially whose vitelline cells produce large amounts of nutritive reserves as *Crepidostomum metoecus* (Greani *et al,* 2016). Shell globule clusters in the vitellocytes of *O. batrachoides* consist of loosely packed electron-dense shell globules of irregular size situated in a moderately electron-lucent matrix (Taelab and Lashein, 2013). The present study proved that shell clusters were attached together as in *Proctoeces* sp. (Arafa and Salama, 2019) and *C. labracis* (Świderski *et al,* 2019).

Conclusion

The TEM showed four developmental phases of vitelline cells during vitellogenesis. Vitellocytes *Lecithochirium grandid* process are characterized by a heavy accumulation shell pellets clusters and cytoplasm showed increase in formation of numerous large, parallel profiles of granular endoplasmic reticulum, interstitial cells. Lipid droplets and glycogen granules appeared in the advanced and mature vitellocytes.

The authors declared that they neither have conflict of interest nor received any fu-nds and both equally shared in the study

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Explanation of figures

Fig.1: Diagram of developmental phases of vitelline cells in *Lecithochirium grandid,* 1, 2, 3 & 4 SB=2µm.Note: numerous islands of heterochromatin (Hch), granular endoplasmic reticulum (GER), several batches of aggregation of shell pellets (ap), aggregation of shell pellets in clusters (apc), Lipid droplet (L),Mitochondria (M),Glycogen granules (G), membrane of shell pellets (mb), lucent matrix (LM),nucleus (N) and Nucleolus (Nu).

Figs. 2 & 3: TEM of vitelline follicle of *L. grandid* showed various developmental phases within follicle. 1- an immature phase, 2- early phase of vitellocyte, 3- phase of vitellogenesis (advanced), & 4- mature vitellocyte; (apc) aggregation of shell pellets in clusters, granular endoplasmic reticulum, heterochromatin, interstitial cells, fibrous layer, muscle bands, nucleus and nucleolus.

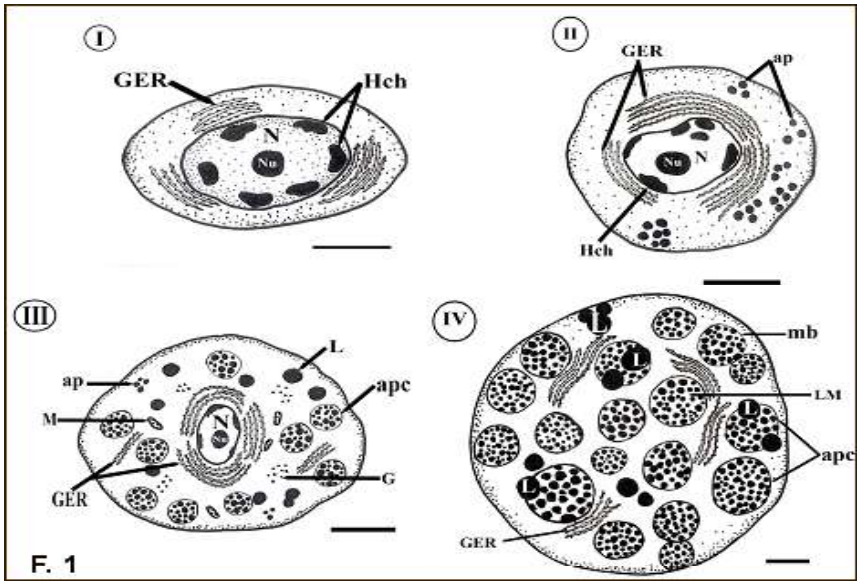
Fig. 4: Magnified TEM of first 3 phases of vitellogenesis on follicles sides, an immature phase 1 with large nucleus with heterochromatin and endoplasmic reticulum * 2: cytoplasm of 2nd & 3rd showed granular endoplasmic reticulum * in parallel around nucleus and numerous aggregation of shell pellets in clusters, several groups of shell pellets, interstitial cells, fibrous layer.

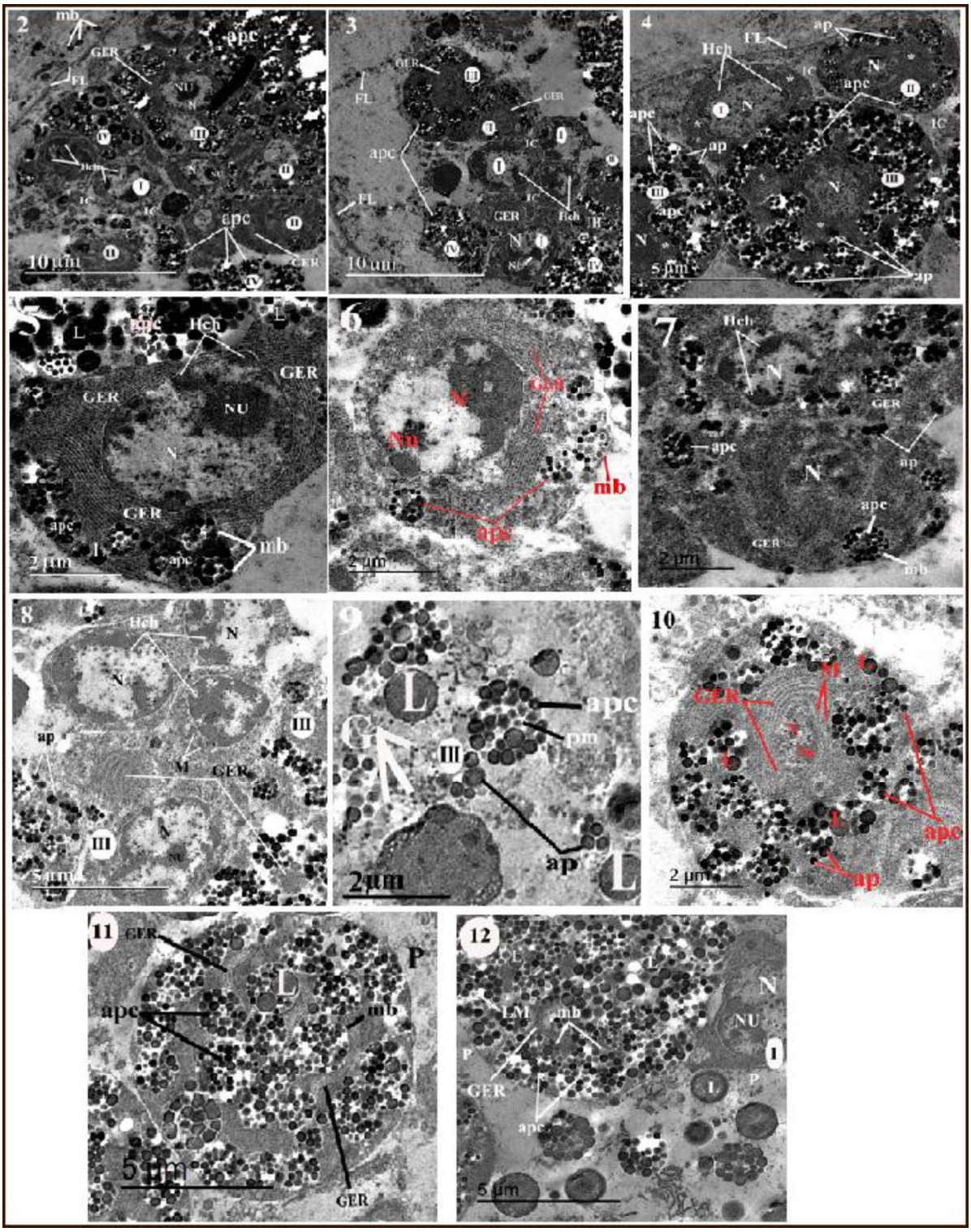
Figs. 5, 6 &7: TEM of 2nd maturation phase showed some aggregation of shell pellets in clusters, aggregation of shell pellets, membrane of shell pellets, lipid droplets, very large nuclei with nucleoli & many heterochromatin islands adjacent to well-developed in parallel cisternae.

Figs. 8 & 9: TEM of 3rd advanced vitellocyte showed shell pellets in clusters, aggregation of shell pellets and pellets matrix. Eight parts showed high magnification of shell pellets in clusters and glycogen granules surrounding shell pellets clusters and lipid droplets, granular endoplasmic reticulum, mitochondria, heterochromatin, nucleus and nucleolus.

Fig. 10: TEM of 3rd vitellocyte showed aggregation of shell pellets in clusters, several lipid droplets and aggregation of shell pellets, granular endoplasmic reticulum, mitochondria, nucleus and nucleolus.

Figs. 11 & 12: TEM of 4th mature vitellocyte showed aggregation of shell pellets in clusters, several lipid droplets and aggregation of shell pellets; membrane of pellets; lucent matrix, granular endoplasmic reticulum; parenchyma, phase 1, nucleus and nucleolus.

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