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# Ultrastructural investigations of vitellogenesis and oogenesis of *Proctoeces* sp. infesting *Platycephalus indicus* fishes caught from Alataka Harbor, Suez Gulf, Egypt.

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## ABSTRACT

Heavy infestations with the intestinal parasite *Proctoeces* sp. were detected in the bar-tail flathead *Platycephalus indicus* collected from Alataka harbor, Suez Gulf, Egypt. Different developmental stages of vitellocyte and oocyte maturations of this digenean parasite were studied using transmission electron microscopy. The vitelline masses are covered with a delicate fibrous layer and enclosed with segregated circular muscle bundles. The first stage(S 1) of vitellocytes maturation is distinguished by a large centrally located oval nucleus and abundant mitochondria and free ribosomes fixed at a perinuclear reside. The second stage(S 2) of vitellocyte maturation has greater cytoplasmic volume, Golgi vesicles, granular endoplasmic reticulum and the vitelline globules became more electron-dense. The third stage (S 3) is characterized by an oval nucleus, shell globules with different sizes aggregated in clusters, electron-lucent lipid droplets and osmiophilic glycogen .The cytoplasm of mature vitellocytes (S 4) was gradually degraded, involving analogous sacs of granular endoplasmic reticulum situated at the peripheral and perinuclear regions, glycogen granules, lipid droplets and packed larger globule clusters. During vitellocyte developmental stages, the nucleo-cytoplasmic ratio decreased while the synthetic activities increased. Detailed ultrastructural characteristics of four different developmental stages of oocyte maturation were briefly described.

## INTRODUCTION

The bar-tail flathead is an invasive fish inhabiting the Egyptian coasts and becomes a preferable food for millions of costal peoples. *Platycephalus indicus* is a benthic bony fish lodging intertidal zones and native to the Indian Ocean (Imamura, 2015). This economic fish has been listed in the Mediterranean Sea coasts as a migratory species through Suez Canal (Rodríguez and Suárez, 2001). Little informations are available on the ultrastructural aspects of vitellogenesis and oogenesis in digenea. Recently some studies have been carried out on spermatogenesis and vitellogenesis in digenean species (Oliva and Huaquin, 2000; Sampour, 2008; Taeleb and Mohamadein, 2013; Greani *et al.*, 2014 and Oliva *et al.*, 2018). Among 18,000 digenean species described by Bray *et al.*, (2002), vitellogenesis of only 20 species were studied (Swiderski *et al.*, 2011). Family fellodistomidae embraces relatively large sized species infecting the digestive and reproductive system of bivalves and bony fishes (Oliva and Vásquez, 1999). The digenea *Proctoeces lintoni* is a common parasite in the gonads of key-hole limpets of

the genus *Fissurella* caused a partial castration and dramatically reduced the fecundity of infected individuals (Oliva, and Huaquin 2000). *Proctoeces* sp. was recently discovered as a newly recorded species at Elataka harbor, Suez Governorate Egypt as a serious intestinal parasite of *P. indicus* (Arafa and Taelab, 2016). *Proctoeces* appears to be of doubtful value and there are at least three species in this genus. Molecular evidence confirmed that *Proctoeces humboldti* and *Proctoeces chilensis* (Digenea: Fellodistomidae) are the same species (Valdivia *et al*., 2010). Vitelline cells provide materials necessary for eggshell formation and nutritives for developing embryos (Bjorkman *et al*., 1964; Grant *et al.*, 1977 and Irwin and Maguire, 1979). Transmission electron microscopic observations on vitellocyte and oocyte development have been considered as useful tool of taxonomic and control benefits (Swiderski *et al.*, 2011). This study aimed to demonstrate the cyto-differentiation of vitellocytes and oocytes of *Proctoeces* sp. infesting the frugally important *P. indicus* to realize the relations between related species of family and their phylogeny.

## MATERIALS AND METHODS

***Collection of Proctoeces sp. parasites.***

Specimens of *P. indicus* fishes were obtained directly from fishermen at Alataka Harbor, Suez Governorate, Egypt, during summer 2019. Fishes were transferred and dissected at parasitology laboratory, Zoology department, Faculty of Science, Zagazig University. Adult specimens of *Proctoeces* sp. were handled from the dissected intestine and washed in saline (0.9% sodium chloride) solution quickly.

***Microscopical examinations***

***Light microscopy preparations***

Mature worms were compressed, fixed in 70% ethanol, dehydrated in an ethanol series and stained with alum carmine. Specimens were cleared with clove oil, mounted and covered with cover glass using Canda balsam.

***Transmission electron microscopic preparations***

Collected worms were fixed in cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at PH 7.2. Specimens were rinsed in 0.1 M sodium cacodylate buffer at PH 7.4 and post-fixed in 1% osmium tetroxide in the same buffer for one hour. Specimens were dehydrated in ethanol, cleared in propylene oxide and embedded in Epoxy resin at $60^0$ C for a day. Ultrathin sections (60–90 nm) at the level of the ovary and vitelline follicles were cut using glass knives. Sections were placed on mesh copper grids and stained with uranyl acetate and lead citrate (Reynolds, 1963). Sections were examined under JEOL 100 CX TEM at the Electron Microscopy Unit, Faculty of Agriculture, El-Mansoura University, Egypt.

## RESULTS

**Description of adult *Proctoeces* sp.**

Adult *Proctoeces* sp. has a small perforated anterior oral sucker enclosing a rounded mouth and a large unperforated ventral sucker lies at the mid ventral region of the worm. Two rounded testes situated at the posterior third of the body behind the ventral sucker and the ovary. The cirrus sac, genital opening and convoluted uterus are found anteriorly. The excretory pore located in front of ecsoma at the posterior end of the worm (Fig. 1).

**Vitellocyte maturation**

The vitelline gland of *Proctoeces* sp**.** comprises two compact masses situated just posterior to the ventral sucker. Transmission electron microscopic studies illustrated that each gland contains vitellocytes at various stages of development. The vitelline masses are enveloped by a thin fibrous coat followed by underlying detached circular muscle bundles. Follicular vitellarium is lined internally with a thin basal lamina and imbedded within the parenchyma (Fig. 3 a). Ultrastructural examinations showed four characteristic developmental stages of vitellocyte maturation.

***Immature Vitellocyte (Stage I)***

Immature vitellocyte (Stage I) has irregular shape and concentrated at the periphery of vitelline mass. They are distinguished by large centrally located oval nuclei and uniformly distributed electron-dense chromatin throughout the nucleoplasm. Several globular mitochondria and free ribosomes concentrated at a perinuclear position were observed (Fig. 3 b).

***Stage II***

The second stage of vitellocyte maturation is characterized by decreased nucleo-cytoplasmic ratio and large nucleolus within the nucleus (Fig. 3 c). The Golgi complex is well developed with moderately electron-dense globules within vesicles. The cytoplasm is characterized by increased amounts of granular endoplasmic reticulum and electron-dense vitelline globules. Single shell inclusions were aggregated to form clusters of osmiophilic dark globules bounded by a thin membrane (Fig. 3 d).

***Stage III***

At the third stage, single shell globules with different sizes are accumulated in clusters. Clusters are formed of different numbers of coalesced globules (about 20) embedded in electron-lucent matrix. Vitellocytes have few numbers of mitochondria and granular endoplasmic reticulum. Small electron-lucent lipid droplets and glycogen particles were also observed in their cytoplasm (Fig. 3 e).

***Mature Vitellocyte (Stage IV)***

At the fourth stage of maturation, the cytoplasm of mature vitellocyte was gradually degraded and contains many shell globule clusters, few mitochondria and GER located at the peripheral and perinuclear position. Lipid droplets and large amounts of glycogen granules are scattered between the lipid droplets at the periphery of the cell (Fig. 3 f).

**Oocyte maturation**

The ovary of *Proctoeces* sp. is surrounded by a distinct fibrous basal lamina. It contains closely packed germ cells at various stages of development which are located at the periphery of the ovarian lobule. Four stages (oogonia, primary oocytes, developing oocytes, and mature oocytes) were observed during oocyte maturation of *Proctoeces* (Fig. 4 a).

***Oogonia (Stage 1):***

Oogonia are found singly or in groups near the follicular wall. These undifferentiated cells are oval shaped and show high nucleo/ cytoplasmic ratio. The cytoplasm appeared scanty and contains several mitochondria and vacuoles. They processed large ovoid nuclei in which the chromatin is reticular and marginal (Fig. 4 a).

***Primary (Previtellogenic) oocytes (Stage 2):***

Primary oocytes are oval in shape, possess a higher nucleo-cytoplasmic ratio and found near oogonia. The cytoplasm is filled with several mitochondria, rough endoplasmic reticulum, and many vacuoles concentrated in a perinuclear position.

The nucleus often has a large nucleolus and electron dense marginal chromatin materials were observed at this stage (Fig. 4 b).

***Developing oocytes (Stage 3):***

The developing oocyte is oval in shape and contains a large oval nucleus .At this stage, the oocytes continued to differentiate and their cytoplasm contains aggregates of mitochondria and several vacuoles appeared near the well-developed endoplasmic reticulum in the perinuclear region. At the same time, a number of vacuoles (secretory granules) of Golgi products are produced. Developing oocytes enter in the zygotene-pachytene stage ($1^{st}$ meiotic), as a result of the appearance of synaptonemal complexes in the nucleus. (Fig. 4 c).

***Mature oocytes (Stage 4)****:*

Mature oocytes are located in the central region of the ovary. These cells often show a triangular shape with low nucleo/ cytoplasmic ratio. In all mature oocytes, the cytoplasm and both granular endoplasmic reticulum and mitochondria are concentrated at the apical pole. Their cytoplasm incorporates several small yolk granules (Fig. 4 d).

## DISCUSSION

***Vitellogenesis***

Vitelline cells provide substances necessary for the formation of the egg shell and essential nutrients for the developing embryo (Levron *et al.,* 2010). Vitellocytes accumulate nutritive materials such as lipid droplets and glycogen particles (Martinez-Alos *et al.,* 1993). Vitellogenesis is basically the same in almost all digenean species (Erasmus, 1982). Ultrastructural studies of vitellogenesis in the digenea have little attention, as most work has been carried out on medically important species such as *Fasciola* and *Schistosoma* (Arafa and Taelab, 2016). Ultrastructural investigations in this study showed that vitelline cells maturation of *Proctoeces* sp. occurs through four stages: immature (S1); starting of the synthetic processes (S2); shell globule clusters formation (S3) and mature vitellocytes consistence (S4). The obtained results disagree with Levron *et al.*, (2010) who reported five developmental stage of maturation of vitellocytes in some digenean species. Some vitelline follicles of digenea possess interstitial cells with cytoplasmic extensions between vitellocytes (Poddubnaya *et al.*, 2012). Ultrastructural studies of vitelline follicles of *Proctoeces* showed the absence of interstitial cells between developing vitellocytes. The absence of interstitial cells in this study was in agreement with the finding of (Meepool and Sobhon, 2009). Greani *et al.,* (2012) found that vitelline follicles of *Aphallus tubarium* have interstitial cells with cytoplasmic extensions between vitellocytes. They reported that these cells concerned with the transport of materials from the parenchyma to the developing vitellocytes.

The present study illustrated the presence of lipid droplets appeared among the Golgi apparatus, massive rough endoplasmic reticulum, and mitochondria in the cytoplasm of the early vitellogenic oocytes. These cell organelles assumed to be involved in the formation of lipid droplets through the process of endogenous autosynthetic vitellogenesis (Chung *et al*., 2005). The abundance of endoplasmic reticulum, Golgi complexes, and free ribosomes may produce high formulation activities (Irwin and Threadgold, 1970) or glycoprotein synthesis (Mlocicki, *et al.*, 2011). The large number of globules observed within the shell clusters may indicate a high rate of production needed for the shell formation (Medhat *et al.* 2014).

During vitellogenesis the shape, number, size and the clusters of shell globules are greatly varied among digeneans especially whose vitelline cells produce large amounts of nutritive reserves as *Crepidostomum metoecus* (Greani *et al* .,2016) and those whose vitellocytes contain a small amount of nutritive substances as *Halipegus eccentricus* and *Brandesia turgida* (Holy and Wittrock, 1986 and Poddubnaya *et al* ., 2013).The presence of such substances in eggs is necessary for the developing embryo or to produce eggshell substance (Gremigni and Nigro, 1983). The present study revealed that shell protein globules are attached together in the form of coalesced cluster as in *Fasciola gigantica* (Irwin, and Threadgold, 1970)). However,shell protein globules are individually grouped in clusters in vitelline cells of *Metadena depressa* (Greani *et al*., 2012).

The occurrence of lipid droplets and glycogen in fully mature vitellocytes content represent this nutrition reserves which play a role as energy sources (Smyth and Halton 1983). Glycogen particles have been also observed in many species (Greani *et al.*, 2012). Glycogen rosettes and particles were recorded in some trematodes (Meepool and Sobhon, 2009). The nucleo-cytoplasmic ratio decreases during vitellocyte maturation may be due to the synthesis of large cytoplasmic inclusions. The attendance of endoplasmic reticulum cisternae, free cytoplasmic ribosomes, and Golgi complexes in the second stage indicates a high synthetic activity (Holy and Wittrock, 1986). The lack of densely coiled endoplasmic reticulum in fully mature vitellocytes of *Crepidostomum metoecus* contributes to the conclusion that maturation ended at the fourth stage. In some other digenean species, a fifth stage presented secretion of densely coiled endoplasmic reticulum and glycogen particles that are condensed in the whole cytoplasm (Grant *et al*., 1977).

## *Oogenesis*

Ultrastructural characteristics throughout the elementary phases of oogenesis are essentially similar in the majority of digenea ( Greani *et al*., 2016). The present study showed four developmental stages during oogenesis of *Proctoeces* sp. like most other digenea. Contrarily, three stages only were described in oogenesis of some digenea (Falleni *et al.,* 2010; Greani *et al.,* 2012) and cestoda (Poddubnaya *et al.,* 2005). This study manifested that immature oogonia were observed along the wall of the ovary near the follicular wall and moved toward the center of the ovary during their development. This result was in accordance with Gresson (1964a) who reported that during maturation, oocytes migrate to the center of the ovary. Oogonia were found singly or in groups near the follicular wall and these undifferentiated cells showing a high nucleo/ cytoplasmic ratio. Oogonia are present along the wall of the ovary as for *Cryptocotyle lingua* (Cable, 1931), but do not contain a nucleolus in *Zygocotyle lunata* (Willey and Godman 1951). The present study illustrated that the developing oocyte contains several mitochondria and vacuoles and enter in the zygotene-pachytene stage. This result was in agreement with Gresson, (1964b) who documented that this process takes place in the uterus of some digenean species. In agreement with Burton,( 1963), the present study revealed a chromatoid body that transfer from nucleus to cytoplasm before the last stages of maturation . Chromatoid bodies probably contain RNA in *Gonapodasmius*, (Digenea, Didymozoidae) and this structure is often surrounded with GER and mitochondria (Justine and Mattei 1984). Results illustrated that mitochondria were uniformly distributed in the cytoplasm of oogonia and then became perinuclear in growing oocytes and finally concentrated at one pole mature oocytes. These results disagree with Yosufzai,( 1952) results observed in *C. metoecus*. In the fully mature oocytes small vesicles are randomly distributed in their cytoplasm, migrate to the cortical cytoplasm where they form a

monolayer just beneath the oolemma and this was observed in many mature digenean oocytes (Willey and Koulish , 1950 and Koulish 1965). These granules may deny polyspermy at fertilization (Meepool and Sobhon , 2009).

## CONCLUSION

Heavy infestation rates of the intestinal parasite *Proctoeces* sp. were detected in the intestine of *Platycephalus indicus* collected from Alataka Harbor. Transmission electron microscopic studied revealed four developmental stages of vitellocyte maturation. The nucleo-cytoplasmic ratio was gradually decreased while the synthetic activities were increased during vitellogenesis. This study evidenced also four developmental stages occurred during oogenesis (oogonia, previtellogenic, developing and mature oocytes) and each phase has its own characteristics.

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## EXPLANATION OF FIGURES

Fig. 1: Photomicrograph of *Proctoeces* sp. stained with alum carmine. (Scal bar=400 μm).

*EC: ecsoma; EP: excretory pore; GO: genital opening; IC: intestinal caecum; MO: mouth; OE: oesophagus;OS: oral suckers; OV: ovary; SC: cirrus sac; TS: testes;UT:uterus; VG:vitelling gland; VS: ventral sucker*.

Fig. 2: Ultrathin section of *Proctoeces* sp. showing vitelline glands, ovary and convoluted uterus (Scal bar=400 μm). *OV: ovary; P: parenchyma; VT: vitelline gland; UT: uterus.*

Fig. 3 (a-f): Electron micrographs showing stages of vitellogenesis in *Proctoeces* sp.

(a): Section of the periphery of vitelline follicle. *Note*: The fibrous layer surrounding the follicle, adjacent parenchyma, muscle bundles and different four stages of maturation (Scale bar = 2μ).

(b): An immature vitellocyte (S 1) at the periphery of the follicle. The cell possesses a high nucleo-cytoplasmic ratio and the nucleus is filled with heterochromatin (Scale bar = 2μ).

(c): Electron micrograph of second stage (S 2) of vitelline cells maturation (Scale bar = 2μ).

(d): The cytoplasm of a cell at advanced second stage of maturation showing Golgi complexes and granular endoplasmic reticulum (Scale bar = 500 nm).

(e): The third stage(S 3) of vitellocyte maturation showing aggregation of single shell globules into a cluster surrounded by a membrane. (Scale bar = 2μ).

(f): Electron micrograph of a vitelline cell at the fourth stage (S4) of maturation filled with shell globule clusters and lipid droplets. (Scale bar = 500 nm).

*C: cytoplasm FL: fibrous layer ; GER: granular endoplasmic reticulum ;GL; glycogen granules; GV: Golgi vesicles; Hch: heterochromatin; L. lipid droplets ; N: nucleus; NU: nucleolus ; M: mitochondria; P: parenchyma ; R: ribosomes; SGC; aggregations of globules in clusters ; SV: secretory vesicles* .

Fig. 4(a-d): Electron micrographs of cross sections of the ovary showing different stages of oogenesis in *Proctoeces* flucks.

(a): Section of the peripheral region of the ovary. (Scale bar = 2μ). Note oogonia (S1) with oval shaped and scanty cytoplasm with several mitochondria and vacuoles

(b): Primary oocytes (stage 2) are oval and the cytoplasm is filled with several mitochondria, rough endoplasmic reticulum, and many vacuoles concentrated in a perinuclear position. (Scale bar = 2μ).

(c): Developing oocytes (stage 3) contain large oval nucleus and aggregates of mitochondria, vacuoles, endoplasmic reticulum which present in the perinuclear region. (Scale bar = 2μ).

(d): Mature oocytes (stage 4): triangular shaped with low nucleo/ cytoplasmic ratio, granular endoplasmic reticulum, yolk granules and mitochondria are concentrated at the apical pole. (Scale bar = 2μ).

*CB: chromatin body*; *CG: secretory granules; GC: germinal cells; GER: granular endoplasmic reticulum; FL: fibrous layer; M: mitochondria; N: nucleus; NU: nucleolus; OG: oogonia; PO: primary oocyte; P: parenchyma; SC: synaptonemal complex; S1: oogonia; S2: primary oocyte; S3: developing oocyte; S4: mature oocyte; V: vesicles YG: yolk granules.*

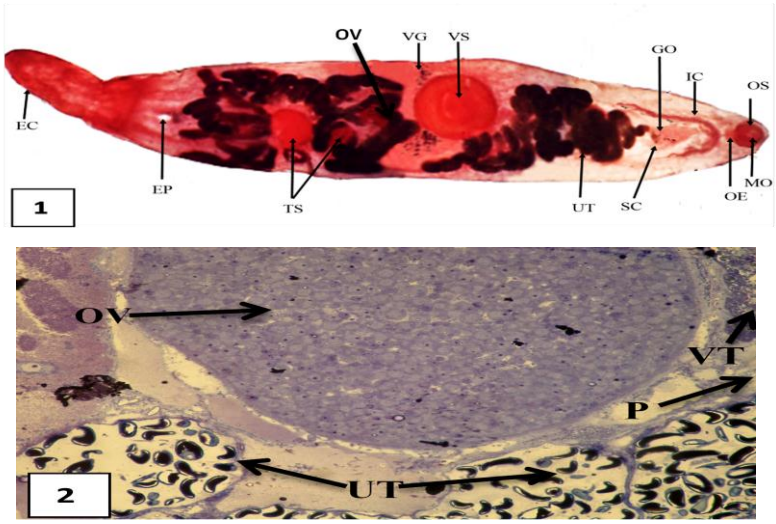



Fig. 3(a-f): Vitellogenesis

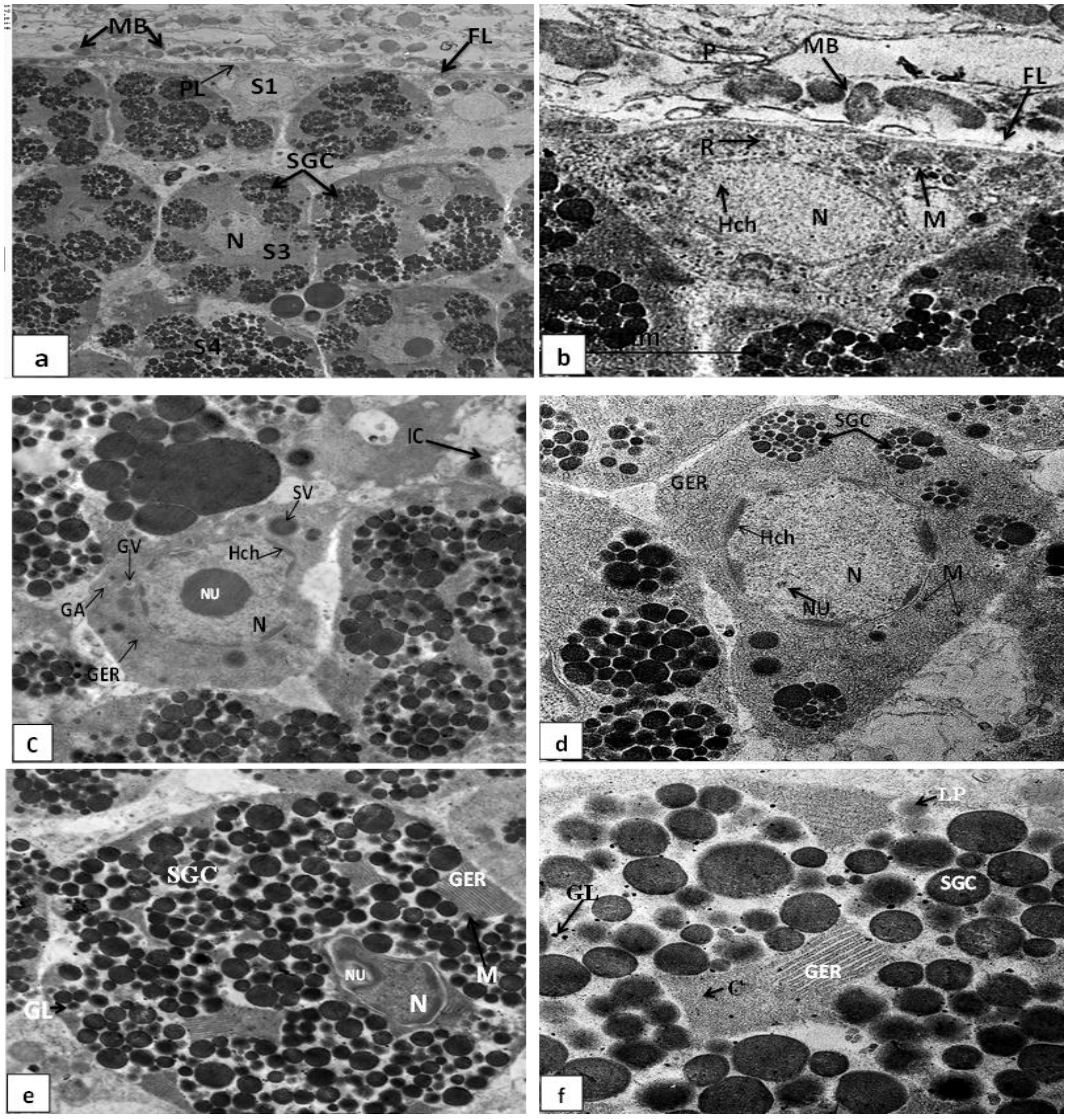
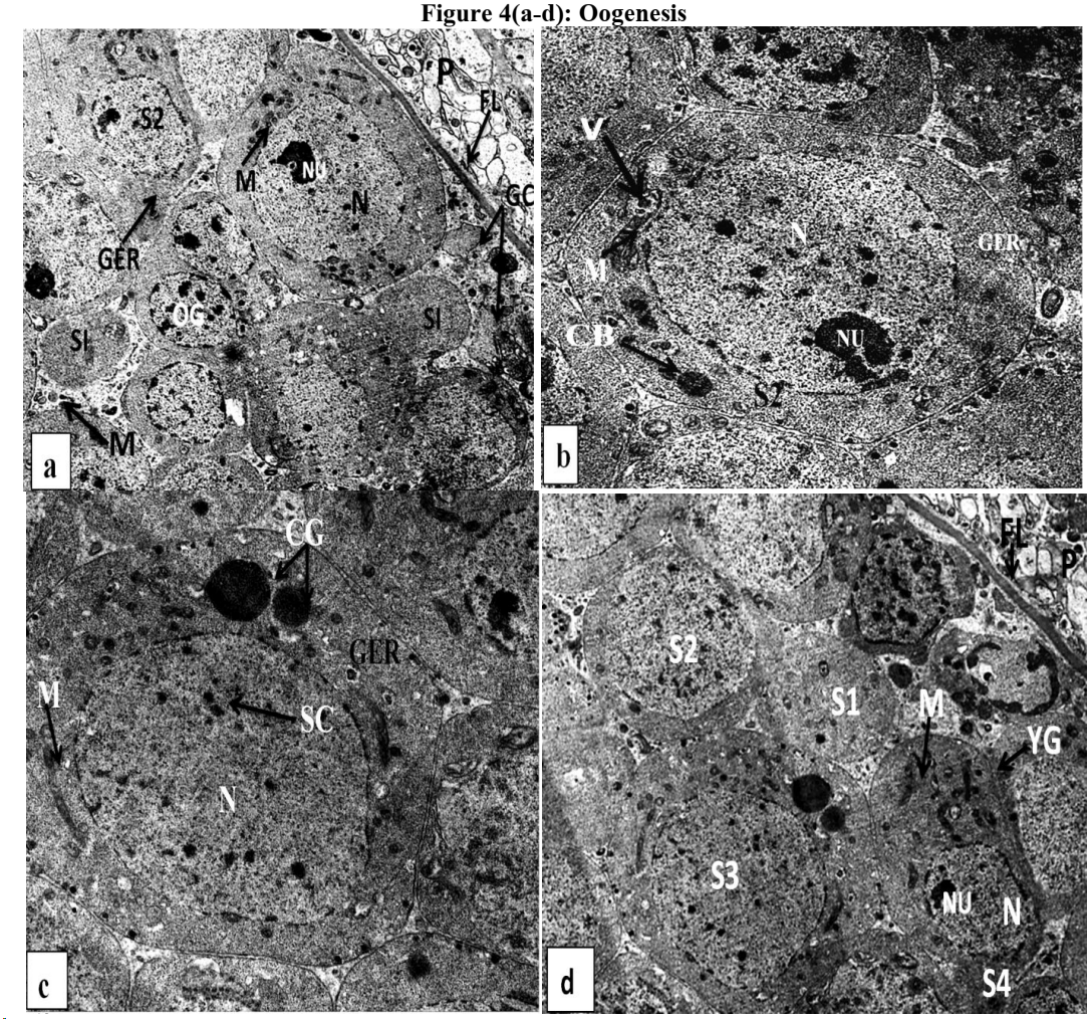


**Figure 4(a-d): Oogenesis**



## ARABIC SUMMARY

### فحوصات تركيبيه دقيقه لتخليق الخلايا المحيه وبويضات الطفيل المعوي من جنس *Proctoeces* sp. الذي يصيب أسماك الراقده *Platycephalus indicus* المصطاده من ميناء الأتكه، خليج السويس ،مصر

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رصدت اصابات شديده بطفيل من جنس *Proctoeces* بداخل أمعاء الأسماك مفلطحه الرأس *Platycephalus indicus* التي جمعت من ميناء الأتكه بخليج السويس بمصر. تم دراسه المراحل المختلفه لتكوين الخلايا المحيه والخلايا البيضيه لهذا الطفيل ثنائي العائل بإستخدام المجهر الإليكتروني الثاقب. تغطي الكتله المحيه بطبقه ليفيه رقيقه وتحاط أيضا بحزم من عضلات دائريه منفصله. المرحله الاولي لتكوين الخليه المحيه تتميز بوجود نواه بيضاويه مركزيه وعدد وفير من الميتوكوندريا والريبوسومات الحره التي تحيط بالنواه. تمتاز المرحله الثانيه بزياده في حجم السيتوبلازم وحويصلات جولجي والشبكه الاندوبلازميه وكريات محيه باهته. في المرحله الثالثه للنمو تكون النواه بيضاويه وتتجمع كريات القشره في مجموعات مختلفه الاحجام كما تتوافر كريات دهنيه شفافه وحبيبات الجلوبيولين داكنه اللون. يضمحل سيوبلازم المرحله الرابعه للخليه المحيه الناضجه ويمتاز بوجود أكياس متوازيه للشبكه الاندوبلازميه والتي تتركز علي حافه الخليه وحول النواه. تتواجد أيضا بوفره حبيبات الجليكوجين وقطيرات الدهن وتجمعات كرويه متزاحمه . يمككنا استخلاص انه اثناء تطور الخلايا المحيه تتناقص النسبه بين النواه والسيتوبلازم بينما تذداد الأنشطه التخليقيه. تم في هذه الدراسه أيضا وصفا دقيقا للمراحل الأربع المختلفه لتكوين البويضات.