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Some histological and ultra structural aspects of oogenesis in *Rhabdosargus haffara* (teleostei: sparidae) from Arabian Gulf; off Dammam (K.S.A.)

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Abstract

Oogenesis was characterized histologically and ultrastructurally as well as stages of oocyte growth in the ovary of R. S during the spawning period. In the perinucleolar (previtellogenic) stage, a simple layer of flattened granulosa cells possess free ribosomes, RER together with multivesicular bodies. The cytoplasm contains plenty of free ribosomes and dense electron bodies. In the yolk vesicle stage, the vitelline envelope was observed as a single electron dense mesh pattern layer, becoming thicker during the vitellogenic stage. In early vitellogenic stage, the granulosa cells were noticed organelle rich, with elongated mitochondria, free ribosomes, dilated RER and Golgi system. The thecal cells show ultrastructural steroidogenic features including mitochondria with tubular cristae, abundant globular SER and transported vesicles. Remarkable ultrastructural changes were mentioned in vitellogenic oocyte including remarkable increase in endoplasmic reticulum proliferation of mitochondria, protrusion of the microvilli from oocyte and granulosa cells into pore canals of the vitelline membrane. The microvilli are withdrawn by the end of yolk deposition in the fully grown oocytes. After ovulation the granulosa cells dissociate proliferate and invade the empty follicle forming temporary structure.

Keywords: Histology, ultrastructure, oocytes, oogenesis

1. Introduction

In teleosts the basic pattern of oocyte growth is similar (Tyler and Sumpter, 1996). The transformations that occur in oocytes during oogenesis in fish have been divided into at least three stages, namely: 1) nuclear chromatin (premeiotic stage; 2) Perinucleolar stage and vitellogenesis. (Bruslé 1980, Cruz-Höfling and Cruz Landim 1990).

The main oocyte features used to characterize these stages are the pattern of chromatin arrangement the nucleolar organization, the presence and behaviour of electron dense bodies (nuage), the presence and maturation of yolk granules and the formation of the chorion (Brusle, 1980, Cruz- Hofling, Cruz- Landim, 1990 and 2001, Abdalla and Cruz-Landin, 2003; Srijunngam *et al.*, 2001 and 2005; Abdel Aziz, 1994 and Ramadan, 1979a).

The ultrastructure of developing oocytes in fishes made the subject of study of several authors, especially those concerned with the developmental stages of oocyte growth (Brusle 1980; Cruz-Landim and Cruz Hofling 1989 and Al-Otaibi, 2004); as well as the cellular envelopment of the developing oocytes, and the oocyte vitelline membrane during its growth and the egg maturation (Cruz-Hofling and Cruz-Landim 1990; Cruz-Landim and Cruz-Hofling 2001and Srijunngam *et al*., 2005,). Occurrence and characterization of "Nuage" make the subject of study of (Yamamoto 1963, Hamaguchi, 1985, Abdalla and Cruz-Landim 2004).

Folliculo genesis in teleost begins after the transformation of oogonia to primary oocytes and continues throughout the growth and maturation of the oocyte (M. Al- Otaibi, 2004 and Khan and Thomas, 1999).

Rhabdosargus haffara (Sparidae) is an economically important teleost, which lives in tropical and semitropical water such as the Arabian gulf and the Red Sea.

Biological information's on oogenesis in this species is still limited with the exception of few studies done in Suez – Canal (El-Boray, 1997).

The present investigation aims to study the histological and ultrastructural aspects of oogenesis in the ovary of *R.haffara* during the period of oocyte growth.

2. Materials and methods

Adult females of *R. haffara* were collected from professional fishermen in Dammam, Arabian gulf K.S.A. during the spawning season (November, December and January). The ovaries were removed and fixed in neutral formalin 4%.

For histological study, fragments of the ovary were fixed in 10% natural formalin dehydrated in an

ascending series of ethanol and embedded in paraffin wax. Sections of 5 µm thickness were stained in with haematoxylin and eosin stain (Humason 1979), then examined under light microscope. For ultrastructure examination; fragments of the fresh ovary were fixed in 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer after two washes in buffer they were fixed in 1% Osmium tetroxide in the same buffer. They were then dehydrated and embedded in Epon ultra thin sections (60 nm) were stained with uranyl acetate and lead citrate and then examined and photographed under a transmition electron microscopy (TEM).

3. Results

R. haffara has asynchronous pattern of ovarian development. During the reproductive cycle the ovary usually containes oocytes at all developmental stages (Figs 1, 7); indicating a protracted spawning period.

Histological and ultra structural examination revealed that the transformation of oocytes during oogenesis in this species includes four stages; these are as follows:

1-The chromation nucleolar stage
2-Perinucleolar stage
3-Yolk vesicle stage (early vitellogenesis)
4-Vitellogenesis and Ripenning.

For light microscopy examination, semi-thin sections (one micrometer) were stained with Toludin blue (TB).

3.1. The chromatin nuclear (premeiotic) stage

Oogonia occur alone or in clusters of several small cells embedded in the ovigerous lamellae (Figures 2and5). They are lightly stained and have a large vesicular nucleus.Oogonia undergo transformation into premeiotic oocyte. The oocytes at this stage has polygonal shape and spherical nucleus with a single nucleolus or may be two large nucleoli (Figures 2,3 and 5). The cytoplasm is strongly basophilic due to accumulation of free ribosomes and electron dense bodies within it.

3.2. Perinucleolar (previtellogenic) stage

The nucleoplasmic ratio increase gradually at this stage. Numerous micronucleoli appear and are arranged under the nuclear envelop of the growing oocyte. During this stage the cytoplasm is still strongly basophilic (Figure 3) due to accumulation of free ribosomes and electron dense bodies (Figure 10). The oocyte appears to be surrounded by a thin single layer of flattened (squamous) grannulosa cells.The granulosa cells contain elongated nucleus with marginal heterochromatin blocks (Figure 10).

3.3. Yolk vesicle stage (early vitellogenesis)

Rapid increase in oocyte growth is observed during this stage. The cytoplasmic basophilia decreases gradually concomitant with increase in the number of cellular organelles (Figures 2,4 and 8).

Marginal membrane bound vesicles containing moderately electron dense granules (yolk vesicles) developed gradually at the peripheral cytoplasm (Figures 4 and 5). At this stage, the cytoplasm is loaded with polyribosomes, strands of endoplasmic reticulum(ER) with amorphous material, round mitochondria with indistinct cristae and few lysosomes (Figure 8).

An outer layer of stratified thecal cells was also detected. The two cell layer (thecal follicular and granulose) are separated by a basement membrane. Appearance of cytoplasmic processes microvilli between the oocytes and granulosa cells at the adjacent surfaces is evident (Figures 8 and 9).

Multivesicular bodies are present near the interdigitation area.

A vitelline membrane (chorion) first, deposits at the yolk vesicle stage oocyte, as a thin layer of electron dense amorphous material in the area of interdigitation of cytoplasmic processes (microvilli_ of the oocyte) and the granulosa cells (Figures 8 and 9). At this stage, the granulosa cells become differentiated from squamous to low cuboidal shape. Granulosa cells are in contact with each other by cell junctions. Well developed cisternae of the rough endoplasmic reticulum (RER) with lamellar and occasionally vesicular form (containing amorphouos material) are clearly detected, in granulosa cell cytoplasm (Figures 8and9). The nuclei of granulosa cells are ovoid in shape often with irregular outline and contain prominent nucleolus and finely granular chromatin and margin heterochromatin blocks.

The thecal cell nuclei are elongated with finely elongated granular chromatin and heterochromatin arranged under the nuclear envelope away from the nuclear pore. The thecal cell cytoplasm comprises advanced smooth endoplasmic reticulum (SER), scarce free ribosomes. Small mitochondria with lamellar and few lysosomes (Figures 8 and 9).

3.4. Vitellogenic stage

During this stage mature yolk (fatty yolk and protein yolk) appear in the cytoplasm of the growing oocyte. Fatty yolk droplets initially appear around the nucleus (as empty vacuoles) followed by appearance of protein yolk granules in the center of the oocyte where they form aggregates of small granules that later increase in size as they spread to the periphery (Figures 5, 6 and 7).

At the final stage of vitellogenesis, the oocyte cytoplasm becomes full of mature yolk granules and numerous free ribosomes and elongated mitochondria

with tubulo-vesicular cristae and short strands of endoplasmic reticulum (ER) clearly observed in between yolk granules (Figures 3and4). Yolk vesicles are still present in the cortical region of the oocyte by the end of vitellogenesis (Figures 5and14). The N/C ratio is now inverted, since at this stage the nucleus is small and is rarely observed in histological sections due to heavy accumulation of mature yolk granules (Figure 6).

At the vitellogenic stage, the granulosa cells increase in size and continued to be organelle rich with mitochondria, free ribosomes dilated tubular rough endoplasmic reticulum (RER) and Golgi system (Figures 10,11 and 12).

The RER cisternae contain an amorphous electron lucent material. Mitochondira are elongated, with electron dense matrix at the late vitellogenic stage. Granulosa cells are still connected with each other by cell junctions. Their euchromatic nuclei have irregular surface and thin marginal heterochromatin (Figures 10 and 11).

In the thecal layer, two types of cells appear, clearly (a) squamous fibroblast like thecal cells with poorly developed cytoplasm and (b) larger thecal cells with organelle rich cytoplasm which has the characteristics of active steroid producing cells. The cells have abundant round to oval mitochondria with tubular cristae, a globular SER and vesicles with an electron dense content (Figures 10 and 11).

Deposition of chorionic material around the microvilli, is continuous during vitellogenesis and vitelline membrane becomes thicker and perforated by pore – channels (Figures 10 and 14). At the end of vitellogenic stage, the granulose cell layer dissociate and the chromatin appear perforated by pore canals (Figure 14).

3.5. Fully grown oocyte (ripe oocyte)

Rapid increase in oocyte size takes place and together with migration of nucleus "germinal vesicle" to the animal pole. Yolk coalescence and hydration (Figure 7) were clearly observed.

Ultra structural examination of ripe ova revealed that microvilli retracted before ovulation and the chorion loses the pore canals.

Granulosa cells are dissociated and remnants of cellular debries, myelin figures, vesicles and multivesicular bodies appear in intercellular spaces (Figure 15). The proliferated granulose cells invade the empty follicle forming temporary structure.

List of Plates

Plate I

Figure (1): Light micrograph showing a transverse section of the ovary of R.haffara, containing two oocyte groups, i.e. previtellogenic oocytes (pr) and vitellogenic oocytes (v) with central nucleus (N) Hand E.

Figure (2): LH. Part of a transverse section of the ovary of R.Haffara showing nest of oogonia (og), oocytes in chromat in nucleolus stage (c) containing nucleus (N) with one nucleolus and few micronucleoli, and baso-philic cytoplasm; together with yolk vesicle stage oocyte (yv). (T.B).

Figure (3): LM. Trasverse section of the ovary of R.Haffara showing perinucleolar stage oocyte (pr) with numerous nucleoli (n) arranged under the nuclear envelop, and chromatin nucleolus stage oocyte (c).

Figure (4): LM. Yolk vesicle stage oocyte with marginal yolk vesicles (YU)and vitellogenic stage oocyte (V). The oocyte wall is formed of granulosa(G) layer and thecal cells (t). Note the presence of an oocyte in chromatin nucleolus stage and oogonia (og) CT.B.

Figure (5): LM.Part ofthe late yolk vesicle stage oocyte with marginal yolk vesicles (yv) and vitellogenic stage oocyte(V). The oocyte wall is formed of granulosa layer G and thecal cells. Note the presence of an oocyte in chromatin nucleolus stage.

Figure (6): LM.Vitellogenic stage oocyte showing central nucleus (N), marginal yolk vesicles and yolk globules and three layer oocyte wall (OW), (zona radiate, granulosa layer and thecal celllayer).T.B.

Figure (7): L.M. Ovary at pre-spawning stage showing numerous vitellogenic oocytes (V) and ripe ova, note migration of germinal vesicle (gv) and yolk coalescenc~ (HandE).

Plate II

Figure (8): E.M.of a part of yolk vesicle oocyte wall showing low cuboidal granulosa cells (Gc), fibroblast like thecal cells (Tc) and deposition of vitelline envelope (arrow). Note; abundant free ribosomes @, mitochondria (m) and short "ER" strands. Free ribosomes are around interdigitated microvilli (mv).

Figure (9): E.M. showing part of yolk vesicle oocyte wall. Note, cuboidal granulaosa cells (GC), with numerous mitochondria (m), developed RER, scarce SER. Note, micro-villi coming from oocyte wall towards, follicular cells (mv) and stratified thecal cell layer and nucleus (N) with marginal heterochromatin.

Figure (10): E.M.showing part of vitellogenic oocyte wall showing microvilli (mv) extending from granulosa cells (Gc) through pore canals in chorion (Ch) towards oocyte. Also, part of perinucleolus oocyte have cytoplasm loaded with polyribosomes (R) and surrounded by flattened granulosa cells (Gc) with large elongated nucleus.

Figure (11): E.M.showing vitellogenic oocyte wall. Note, thecal cell (Tc) cytoplasm contain globular SER and free ribosomes (R) and granulosa cell cytoplasm having numerous RER cisternae, mitochondria (m) and free ribosomes (R). Basement membrane (BM), microvilli (mv), and a lysosome (L).

Figure (12): E.M.showing RER, polyribosomes (R). Globular SER, and mitochondria in granulosa cell ofvitellogenic oocyte, micro villi (mv), chorion (ch).

Figure (13): E.M. Part of cytoplasm of vitellogenic oocyte showing numerous elongated mitochondria (M).

Figure (14): E.M.of an oocyte in late vistellogenic stage showing thick vitelline envelop (ch) perforated by pores canuls and surrounded by dissociated granulosa cells. Note yolk globules (yg) and endo plasmic reticulum (ER).

Figure (15): E.M. showing retraction of microvilli (mv) and blocking of pore canals (P) in chorion and accumulation of vesicles and mylin figures (mt) between dissociated granulosa cells, in wall of ripe ovum before ovulation.

N.B. HandE = Heidenhain and Eosin T.B. = Toluidin Blue

L.M. = Light micrograph

E.M = Electron micrograph

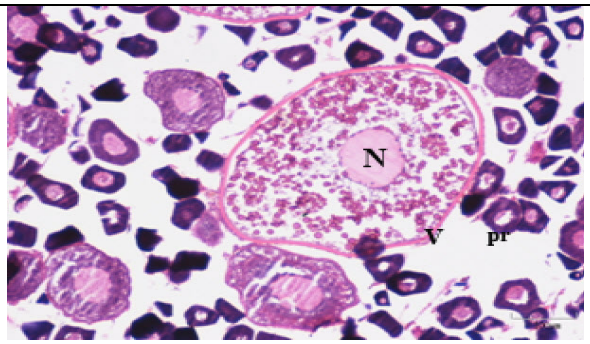
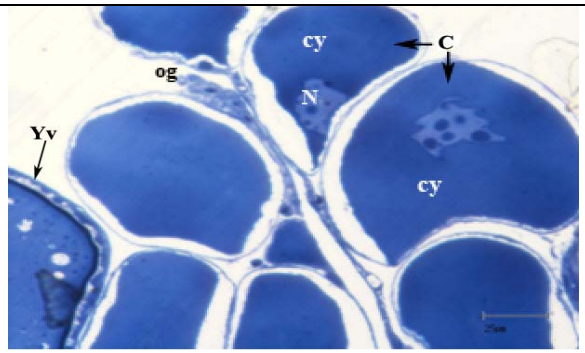


Figure 1



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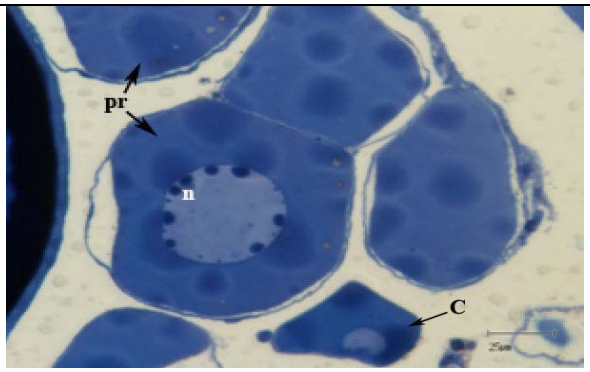

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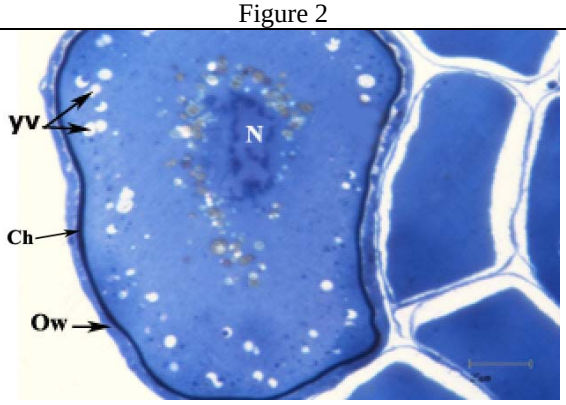

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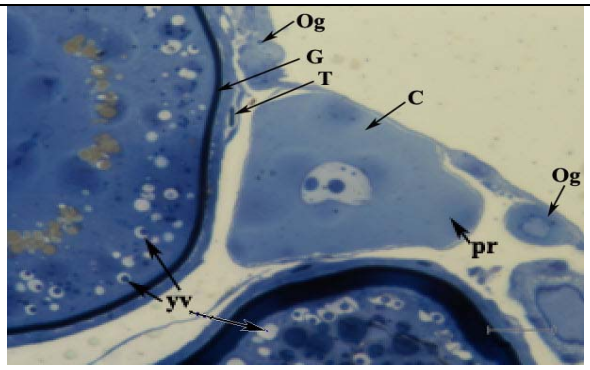

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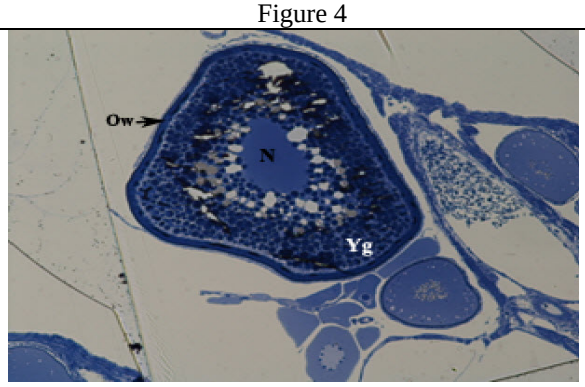

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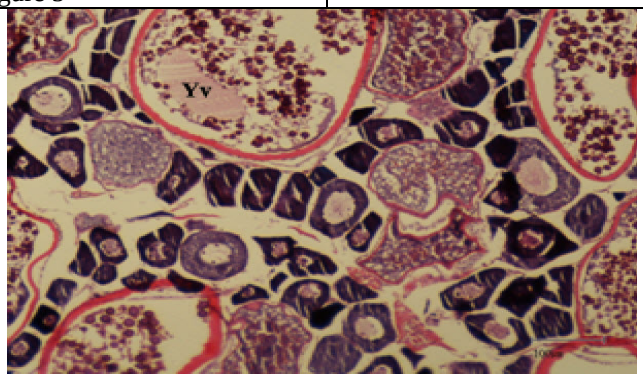


Figure 7

Plate I 1–7: Light micrographs in the ovaries of females of *R. haffara* in Saudi Arabia water.

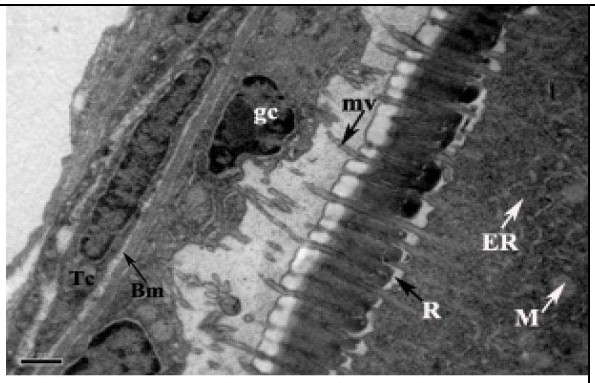


Figure 8

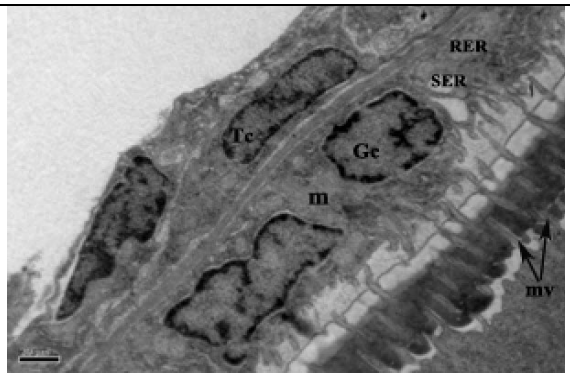
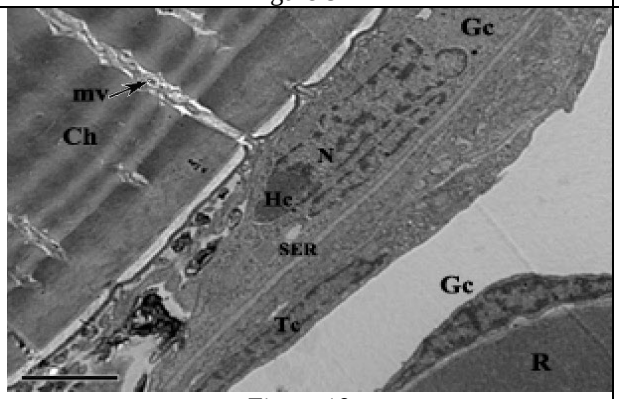


Figure 9



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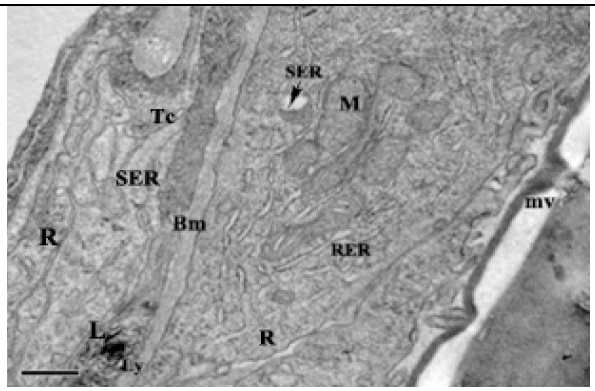

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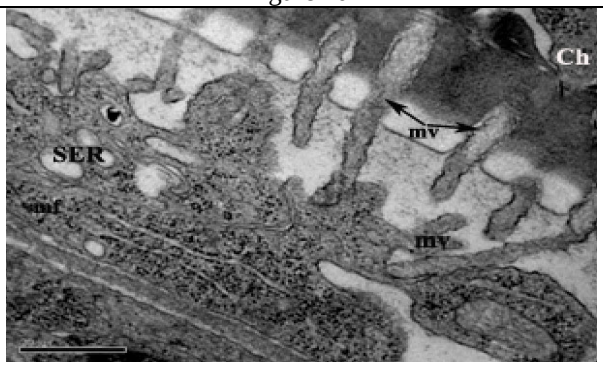

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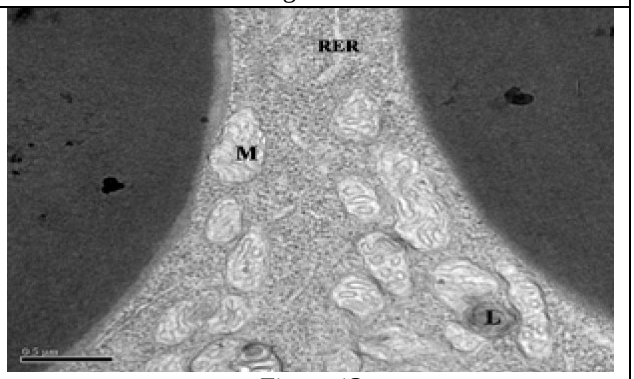

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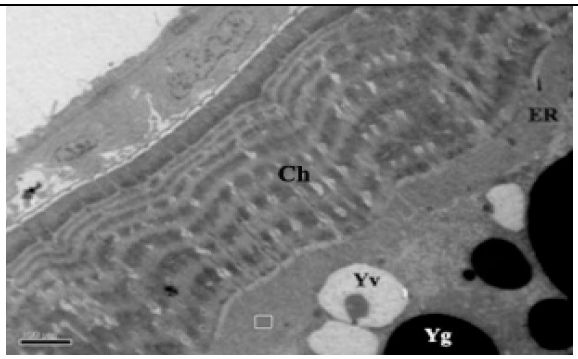

Figure 14

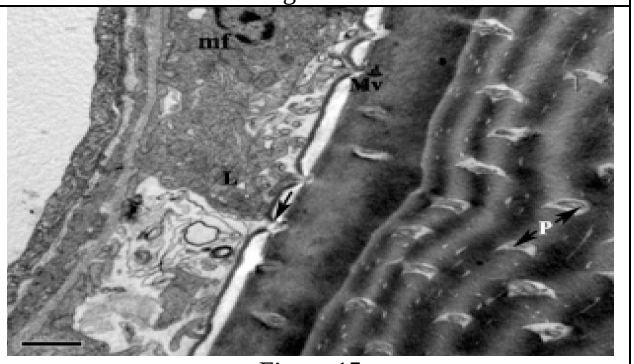

Figure 15

Plate II 8 - 15: Electron micrographs in the ovaries of females of *R. haffara* in Saudi Arabia water.

4. Discussion

The ovaries of adult *R. haffara* are of the asynchronous type. The coexistence of oocytes in the initial stages of development together with mature oocytes was clearly observed. The oogonial nests appear located adjacent to the ovigerous lamellae. Oocyte development in this species does not differ from that reported for other marine teleosts (Guraya *et al*., 1975, Guraya, 1965 and Gaber, 2003).

Various authors divided the developmental stages of oocytes into two phases, namely, early growth phase and second growth phase. In the present study we followed the system given by (West, 1990) in his description of developmental stages of oocytes.

In the nuclear chromatin stage, the oocyte is characterized by having a nucleus with a single large nucleolus and basophilic cytoplasm. This has been described by various authors in marine and freshwater teleosts.

The perinucleolar oocyte is characterized by the occurrence of a large nucleus with numerous marginal micro nucleoli, and basophilic cytoplasm because of the presence of polyribosomes. Some authors subdivided this stage into two phases, i.e. early perinucleolar stage and late (alveolar) stage; (West, 1990).

Abdalla and Cruz-landim (2004) stated that the material of the micro nucleoli which are present on the periphery of the nucleus, are transferred to the cytoplasm, where they form what they called Balbiani's bodies. This material, from our point of view, seems to diffuse into the cytoplasm, and might share in the yolk formation.

During this stage the formation of flattened squamous granulosa cells with elongated nucleus takes place: the cytoplasm of the granulosa cells contains few organelles (Srijusnngam *et al*., 2005). Thecal cells are also formed. Cytoplasmic processes between oocytes and granulosa cells can be also detected at the end of perinuleolar stage.

In yolk vesicle stage, cytoplasmic basophilia decreases gradually concomitant with increase in cytoplasmic organelles. The cortical alveoli are formed before the yolk granules and are concentrated exclusively at the periphery. According to the observations given by Abdalla and Cruz Landim 2003 these granules persisted in the mature oocytes and may have the same function as in other vertebrates, participitating in the hardening of the chorion or zona radiata after ovulation thereby preventing polyspermia. This observation is contrary to the observations given by Cruz-Landim and Cruz- Hofling (1989), where cortical granules were distributed in the cytoplasm.

The vitelline envelop (Zona radiata) become evident once the oocyte enters the yolk vesicle stage. At early vitellogenesis, according to the present results, electron dense amorphous material is formed at the interdigitation of cytoplasmic processes (microvilli) of the oocyte and the granulosa cells. This is in accordance with Abdalla and Cruz Landim (2003), who cited that the chorion starts to be formed by the end of the perinucleolar stage.

The electron dense material which are found between cells of follicular epithelium, seems to originate from substance intaken in the basal side of thecal cells and which travel to the apical ones (Cruz-Landim and C. Hofling, 2001).

These last authors postulated that this material might be of vitellogenic nature.

However, Srijunngam *et al*., (2005), stated that the origin of this material (electron dense component of vitelline envelop) is not clear.

This electron dense material in the vitelline envelop has been identified in several species of vertebrates (Cruz-Landim and Cruz Hofling, 1989; Eddy, 1975)

The structure of the vitelline envelop differs between different species. Thus in the common carp, it is composed of four layers (Linhart *et al*., 1995), while in *Pagrus major* (marine teleost) the vitelline envelop is composed of two major layers (Matsuyama *et al*. 1991). In Chionodraco it is composed of several concentric layers (Baldacci *et al*., 2001)

In *Oreochromis niloticus* it is composed of two layers (Srijunngam *et al*., 2005). In the present study, the vitelline envelop consists of two layers, an outer electron dense and a slightly striated inner one.

In the vitellogenic oocyte, the cytoplasm becomes full of organelles; mitochondria, ribosomes, RES and SER, together with some lysosoms and yolk granules.

This feature was also recorded by various authors (Cruz Landim and Cruz Hofling, 2005and Alotaibi, 2004; Guraya, 1965).

Srijunngam *et al*. (2005) postulated that differences in vitelline envelop could be due to differences in habitat or in diet or both.

The flattened squamus epithelial cells of the granulosa layer during perinucleolar stage turns out to be cuboidal in the vitellogenic stage.

We should note that, the presence of electron dense material and well developed tubular RER, free ribosomes together with the Golgi system, show active protein synthesis in granulosa cells. This observation was given before in fish ovarian follicles (Matsuyama *et al*., 1991; Srijunngam *et al*., 2005).

The presence of SER and mitochondria with tubular cristae in the thecal cells is in accordance with what was cited before by Srijunngam (2005) in the developing ovum of *Oreochromis niloticus*.

According to Srijunngm, 2005, the granulosa cells and thecal cells play different roles in steroidogenesis. Thus the thecal cells produce androgens (steroid precursors), while the granulosa cells convert androgens to estradiol 17 B. He cocluded that thecal cells have a streroidogenic capacity. This result is in accordance with the observation given by various

authors (Yamamoto 1963, Matsnuyama *et al*., 1991; Guraya, 1986 and Al - Dtaibi, 2004).

The present results reveal what happens to the wall of the ovum at spawning, No much work has been done on this problem, but it seems that the follicular cells, undergo destruction in order to liberate the new ovum.

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