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HISTOLOGICAL ASPECTS OF OOCYTE DEVELOPMENT IN OREOCHROMIS SPILURUS (GÜNTHER) REARED IN JEDDAH FISH FARMS SAUDI ARABIAN

BY

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ABSTRACT

The Oreochromis spilurus were successful cultivated and breeding in Saudi Arabian fish farms due to their ability to survive in confined environment with a low oxygen content.

Histological studies of the ovaries Oreochromis spilurus provided the information on the internal changes occurring gradually during oocyte maturation. The growth of Oreochromis spilurus oocyte under go three developmental phases including nine stages as following:

***The first growth phase** include only the immature oocyte which can be divided into four stages (chromatin nucleolus stage, early perinucleolus stage, late perinucleolus stage and yolk nucleus stage).*

***The second growth phase** (Vitellogenic Oocyte) include yolk vesicles stage, primary yolk stage, secondary yolk stage and tertiary yolk stage.*

***The third growth phase** include the ripe and ovulated stage.*

The wall of oocyte divided into three layers: Theca externa, granulose interna and zona radiate. The granulosa cells have developed certain extending processors arising from the oocyte surface extending through the second wall layer known as zona radiata and perfecting deeply into extra cellular space (pore canals) of the overlying granulosa cells.

Oogenic development of Oreochromis spilurus is asynchronous and the ripe ova discharged gradually in the breeding season which extend through May, June, August, September, November, December, January and March with decreasing temperature to 22 – 24 ^{0}c .Female Oreochromis spilurus withdraw ripe ova gradually with prolonged spawning season.

INTRODUCTION

The *Oreochromis spilurus* of the genus *Oreochromis* are economically important inhabitants of inland, fresh and brackish water as well as marine waters in many parts of the world, particularly in the tropical and subtropical region. Their increasing importance is attributed not only to their wide distribution, but also to the successful cultivation and breeding of these fish in many countries of the worlds including Saudi Arabian. *Oreochromis* species are actually considered to be ideal fishes for pond and farm breeding. The reason being their ability to survive in a confined environment with a low oxygen content (Balubid 2000).

The description of the histology of the gonads presented in this paper is intended to form the back ground for a description of the reproductive cycle of *Oreochromis spilurus* reared in Saudi Arabian fish farms. The histological studies provide a very precise information on the internal changes occurring gradually in the oocyte. The sequence through which the oocyte pass to reach the maturity is classified into phases, periods or stages according to the size, the structure and the different inclusions. The wall of the oocyte is characterized by differentiated layers according to (Zaki *et al.* 1986) which reported that the wall of oocyte can be divided into three layers: theca externa, granulose interna and zona radiata.

Due to little information's regarding the histological changes in the reproductive organs during annual reproductive cycle of *Oreochromis spilurus* reared in fish farms at Jeddah Saudi Arabia. Therefore the present study aimed to give accurate knowledge of the ovarian cycle and their functional mechanism in fishes for the successful management in fish farming. Therefore the histological structure and seasonal variations of the ovaries of *O. spilurus* were investigated.

MATERIALS AND METHODS

Samples of *Oreochromis spilurus* were collected from the commercial catch of a fresh water fish farm at Jeddah, Saudi Arabia. Sampling took place monthly from July 2000 to June 2001. Total length was measured , exceed 14 cm. After opening the abdominal cavity, the sex and stage of gonads were determined. Gonado somatic index (GSI) is determined as percentage of gonads weight to gutted weight.

The freshly separated ovaries were fixed immediately in aqueous Bouin's solution for 48 hours. They were then transferred to 70% ethyl alcohol kept until used. Embedding was done in paraffin , Sectioning was done at 7 micron thick. Staining was done by Hoematoxylin and Eosin. The stained sections were examined under a light microscope.

For electron microscope a piece of gonads was embedded in 4% gluteraldehyde in 0.1 M cacodylate buffer PH 7.2 – 7.4 at 4°c for 3 h. . washing was done in 5% sucrose in 0.05 M cacodylate Buffer overnight. Post fixation in 1% Osmium tetroxide in 0.2 M

cacodylate buffer. Rinsing and washing in buffer at PH 7.2 – 7.4. Dehydrating, embedding , sectioning and stainning with uranel acetate (saturated in 70% alcohol). Then followed examination was done by a transmission electron microscope at 80 KV.

RESULTS

Oocyte development in *O. spilurus* could be divided into three phases including nine stages as follows:

I – First growth phase which includes three stages as following :

A- Chromatin nucleolus stages:

Oocyte in this stages was small spherical cells present either solitary or in cluster of the two cells founded embedded in the ovigerous lamella. The nucleus occupied the greater part of the oocytes and was surrounded by a weakly basophilic thin layer of cytoplasm. The average diameter of oocyte ranged from 10 to 20 µ with nucleus diameter ranged from 7 to 15µ and contained nucleolus can be found in the nucleus (fig. 1,2).

B – Perinucleolus stage:

1. **Early perinucleolus oocytes:**

Oocyte in this stage ranged from 30 to less than 90µ with an average 50µ in diameter. The oocyte was round to polygonal in shape and was characterized by a large nucleus occupying the greater part of the cell ranged from 15 to 60µ with an average 30µ. Nucleoli appeared bright dark and lies mostly near the nuclear envelope and varies in number from 3 to 7 and in size as their diameter range between 8µ and 20µ , respectively. The cytoplasm was highly basophilic and the nucleus was slightly eosinophilic stained (fig.1, 2,3).

2. **late perinucleolus oocytes:**

In this stage a considerable increase in size of the cytoplasm and nucleus occurred. The oocyte diameter ranged between 150 and 170µ with an average 160µ. It become more rounded than the preceding stage. The nucleus is still round in shape and ranged from 60 to 90µ with average 78µ. The nucleoli increased in number to 27 and lie close to the nuclear membrane (fig. 2, 3), The cytoplasm was homogeneous, weakly basophilic and surrounded by very thin flattened epithelial layer (fig. 4).

C-Yolk nucleus stage:

In this stage the yolk nucleus lies closer to the nuclear membrane and moving towards the periphery of the cytoplasm with the growth of the oocyte. It appeared first

near one side of the nucleus of oocyte which was about 30μ in diameter as darkly strained large granules (fig. 5). At this stage the oocyte ranged from 350 to 400μ with average about 380μ in diameter and was surrounded by very thin epithelial follicle. The nucleus being round in shape with average diameter 200μ.

II- Second growth phase: which includes four stages as follows:

A- Yolk vesicle stage:

In this stage the oocyte diameter ranges from 450 to 500μ with an average 470 ± 30μ. This stage is characterized by the presence of yolk vesicle of diameter ranging from 15 to 25μ with an average 20μ. (fig. 6) These vesicles would increase in both size and number to form several irregular rows in outer region of the cytoplasm. During this stage the nucleus increases in diameter from 190 to 210μ with an average about 200 ± 10μ. The nucleoli maintain their peripheral position, its number ranges from 11 to 20 μ.

The folliclar cells changed from flattened one layer to two layers. The first one consists of outer follicular layer known as Theca with thickness 3μ and the inner forming granulosa layer with thickness 3μ separated from each other by a basement membrane. The second layer forming zona radiata which appear as very thin homogeneous layer having mean thickness 1μ. (fig. 7,8).

B- Primary volk stage:

This stage is characterized by the appearance of fine yolk granules in the peripheral region of the cytoplasm (fig. 9). The oocytes of this stage range from 500 to 580μ in diameter with average 550 ± 50μ. The formation of the yolk granules proceeds from outside to the middle part of the ooplasm. A yolk free area around the nucleus is left. The granules are spherical in shape ranging from 2 to 4μ in diameter. The granulosa occupied by vacuoles having diameters from 9 to 30μ. The zona radiata exhibited distinct striations measuring 2μ in thickness. The outer epithelial layer (Theca layer) with thickness 3μ and inner form geranulosa increased in thickness to 8μ. The nuclei become irregular in shape and ranging between 140 to 160μ in diameter. The nucleoli laid in peripheral position (fig. 10,11).

C- Secondary volk stage:

Furthermore growth of cytoplasm is accompanied by centripetal deposition of yolk. Oocytes at this stage become oval shape with diameter ranging from 550 to 660μ and characterized by further increase in number and size of yolk vesicales and granules. Which accumulated very rapidly in the inner part of the cytoplasm and also enlarged in their size reaching about 80μ in diameter. This leads to irregularly shaped nucleus with diameter from 100 to 140μ (fig.12) The outer follicular cells increased in width to an average 4μ and the granulosa to about 8μ. The zona radiata become 3μ in width (Fig. 12,13).

D- Tertiary volk stage:

Oocyte at this stage become oval shaped with lengths of short and long axis of about 800 to 1100μ. Yolk vesicles continue to coalesce and become filled with yolk granules. The nucleus becoming small with diameter of about 150 ± 20μ. At the end of this stage, the nucleus becames amoeboid in shape and without a limited boundary and begin its migration towards the animal pole of the oocyte (fig. 14). The outer follicular layer (Theca) is 4μ thick and the inner granulosa layer is 8μ thick. The second layer forming the zona radiata appear as homogeneous layer having mean thickness 5μ (Fig. 14).

III- Third growth phase: which includes one stage:

Ripe stage:

Oocyte at this stage are generally oval in shape ranged between 1000 to 1300μ and 1300 to 1600μ as short and long axis. The nucleus with average diameter 100 ± 15μ is in its way of migration to the animal pole, the nuclear membrane seemed to disappear and no boundary being detected between ooplasm and nucleoplasm. The yolk globules tend to form yolk mass, and the zona radiata became about 6μ in thickness (fig, 15).

Ultra structure of the Oocyte wall:

The oocyte at yolk deposition stage were examined by electron microscope to get more knowledge about wall construction.

The theca cell layer is composed of fibro blast-link cells with enlarged nuclei containing abundant large sized lipid droplet small mitochondria together with small amount of tubular smooth endoplasmic reticular (Fig. 16, 17). The nucleus has condensation of chromatin material close to the nuclear envelop. The theca layer separated by a thick basement membrane of connective tissue the granulosa layer.

Concerning the granulosa cells, the most characteristic feature was the existence of a number of elongated or spherical nuclei. On other hand they have embodied layer lipid droplet, mitochondria with lamellar cristae and extensive smooth endoplasmic reticulum which varied in size and shape (Fig. 18,19).

The granulosa cells have developed certain extending processors known microvilli arising from the oocyte surface extending through the zona radiata and projecting deeply into the extra cellular space (pore canals) of the overlying granulosa cells (Fig. 18,19).

Concomitant with growth of the oocyte, the granulosa cells develop microvillar processes; these penetrate the zona radiata to a variable distance depending on the stage of oocyte growth (Fig. 16, 18).

The zona radiata consist of outer zona radiata extrna and inner zona radiata internal (Fig. 19), but only the former is present when the oocyte is at early stage of yolk deposition .

Monthly variation in ovaries of *Oreochromis spilurus* :

The morphological characteristic in the ovaries *O. spilurus* have indicate that they pass through six successive stages of maturation throughout the year as the following: Immature stage with GSI ranging from 0.45% to 0.75% ; mature stage with GSI ranging from 1.16% to 2.05%; nearly ripe with GSI ranging from 3.05% to 4.83%; ripe with GSI ranging from 5.26% to 6.37%; spawning with GSI ranging from 4.13% to 4.73% and spent stage with GSI about 0.65%.

Most of the ovaries in the fish less than 10 cm in length belong to immature stage which were characterized by the presence of chromatin nucleolus stage and perinucleolus stage as show in (fig. 1,2).

During July most of the ovaries were representing the mature stage (fig. 1,3) or recovery stage. With mean GSI about (2.05) they were characterized by the presence of chromatin nucleolus stage, withwide spaces between the ovigorous folds; first growth phase has oocytes, mostly in the perinucleolus stage (early and late stage) and yolk vesicles stage. The ovarian wall was thick and composed of an inner circular and outer longitudinal muscular layer (fig.1).

In August, November, January and march most of the ovaries represented the maximum growth phase or gonads ripe stage with mean GSI (4.73, 5.26, 5.86 and 6.37) respectively. This stage Characterized by the presence of two growth phases. The first growth phase represented by early and late perinucleolus stage and the second growth phase represented by yolk vesicles stage in which vesicles appeared in peripheral cytoplasm beside the primary, secondary and tertiary yolk stages with many number of mature oocytes.

During September, October, December, February, April and May most of the ovaries were representing the spawning and recovering stage. They were characterized by the presence of first growth phase which has most of the oocytes especially early and late perinucleolus stage and second growth phase represented by yolk vesicles stage and many number of yolk deposition stages with empty follicles the GSI ranged from 3.83% to 4.73%.

DISCUSSION

Oogenesis in fish are known to undergo a sequence of external and internal changes. These changes in the oocytes development have been detected in various fish species. According to various authors, The course of development of oocytes have been divided into stages, phases or periods in order to differentiate the gradual changes in their peculiarities (Guraya *et al.* 1975 & Matsuyama *et al.* 1991).

The chromatin nucleolus stage (oogonia) in the present study was found in immature ovaries at the beginning of oocyte formation, When the division of oogonia were recorded. This phenomenon started from the ovigorous lamellae as it was filled with germ cells, oocytes are expanding towards the lumen of the ovary . In immature fishes, the formation of oocytes comes from the division of oogonia. This results is in accordance with that of Raina (1976).

In the present results, the perinucleolus stage undergoes a gradual increase in the oocyte size and in number of nucleoli of the nucleus. During the late perinucleolus stage a basophilic organelle appears in the cytoplasm (yolk nucleus). The yolk nucleus can be termed as, achroplasm, centrosphere, crop vitelline or Balbiani bodies (Zaki *et al* 1991).

Recent electron microscopical studies revealed that the yolk nucleus was not a homogenous structure, and it was composed of various cellular organelles such as mitochondria, Golgi bodies, smooth endoplasmic reticulum, multivesicular bodies and lipid granules (Wallace and Selman,1981).

At the end of the perinucleolus stage, and at the vesicles stage the follicular epithelium appeared surrounding the oocytes. On further growth of oocytes, these follicular epithelial cells formed a layer coating the oocytes. It was believed that the prementioned follicular cells play an important role in active transport of proteins and other nutrients from blood to oocytes during vitellogenesis as reported by Norrevang (1968). Guraya *et al.*, (1975) claimed that follicle cells and oocytes are considered to play an important role in the formation of zona radiata. Herrera *et al.*, (1988) mentioned that the cytoplasm structures was proved to synthesize sexual steroids by follicular theca envelope.In the present study the zona radiata layer contains microvillar processes pass through pore canals. The microvilli are thought to be the site of substance exchange between the follicle cells and the oocyte (Matsuyama *et al.*, 1991).

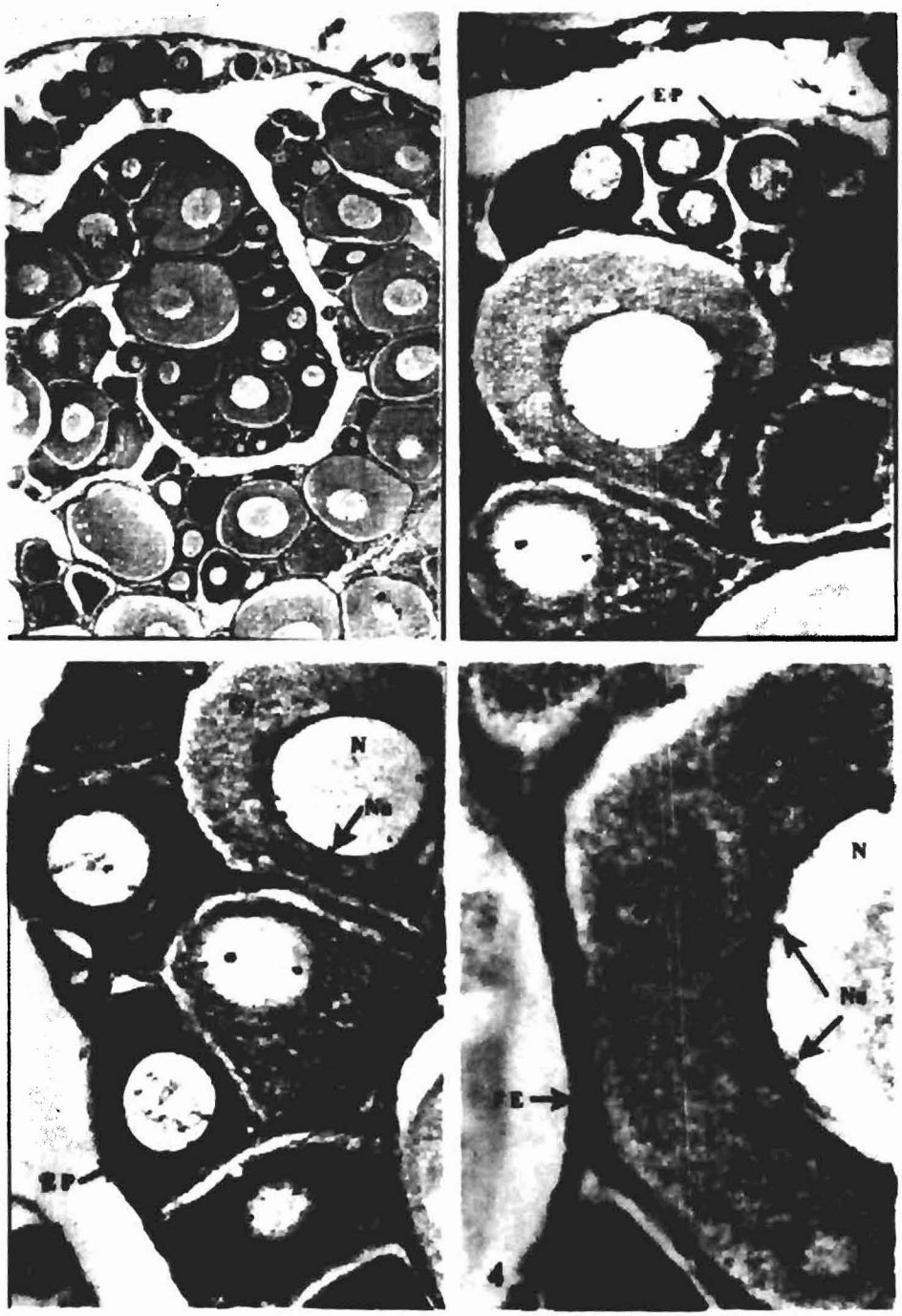
The histological and morphological characters of ovaries *O. spilurus* have indicated that the oocyte pass through nine successive stages of maturation:

Chromatin nucleolus stage; perinucleolus stage (early and late), yolk vesicles stage, olk deposition stage (1^{st} , 2^{nd} , 3^{rd}) ripe and ovulated stage, as reported by Zaki *et al* 1991).

The present results indicate that perinucleolus oocyte of *O. spilurus* are enclosed in a single layer of follicular epithelial cells, With progressive development of the oocyte the boundary envelope is composed of two layers; an inner granulosa cell layer and an outer theca cell layer. It seems that both theca and granulosa cells are the major cellular sites of steroid synthesis in the ovaries of *O. spilurus* fish as described in *oncorhynchus rhodurus* (Kagawa, 1985); *Oreochromic mossabicus* (Smith and Haley, 1987) and *Pagrus major* (Matsuyama *et al*., 1991)

This boundary envelope have exhibited both quantitative and qualitative variation concomitant with the successive maturity stages of oogeneses. These feature were taken as an indication in the process of steroidoogeneses accompanying the development of the ovaries and formation of ripe oocyte , these results conform with those presented by Matsuyana *et al* (1991) in *Pagrus major*.

From the monthly variation of gonadosomatic index and histological examination of the ovaries it is cleared that the breeding season of *O. spilurus* reared in Saudi Arabia fish farm takes place during May, June, August, September, November, December, January and March. This result show that the female withdraw the ripe ova gradually through the breeding season with prolonged spawning.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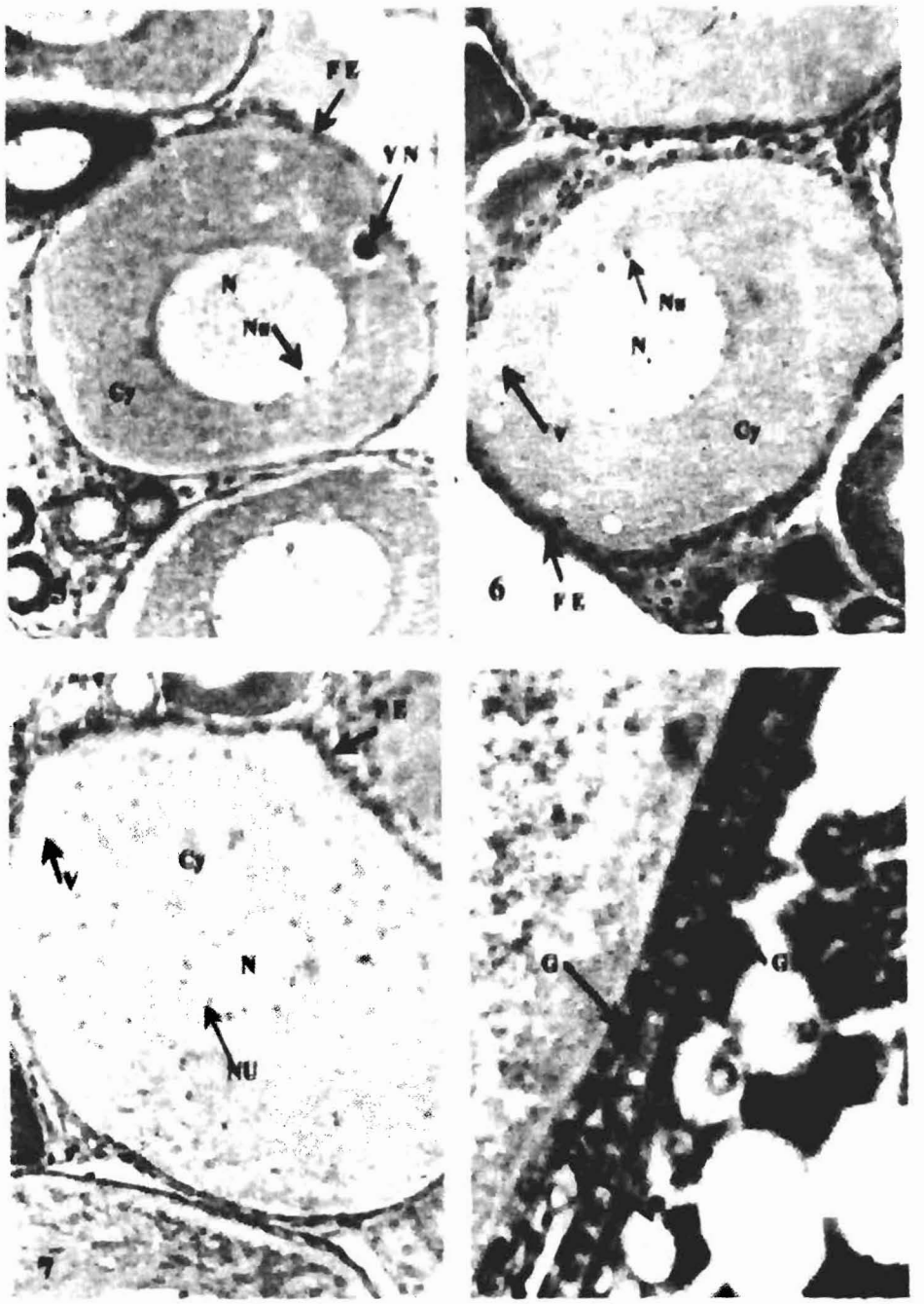
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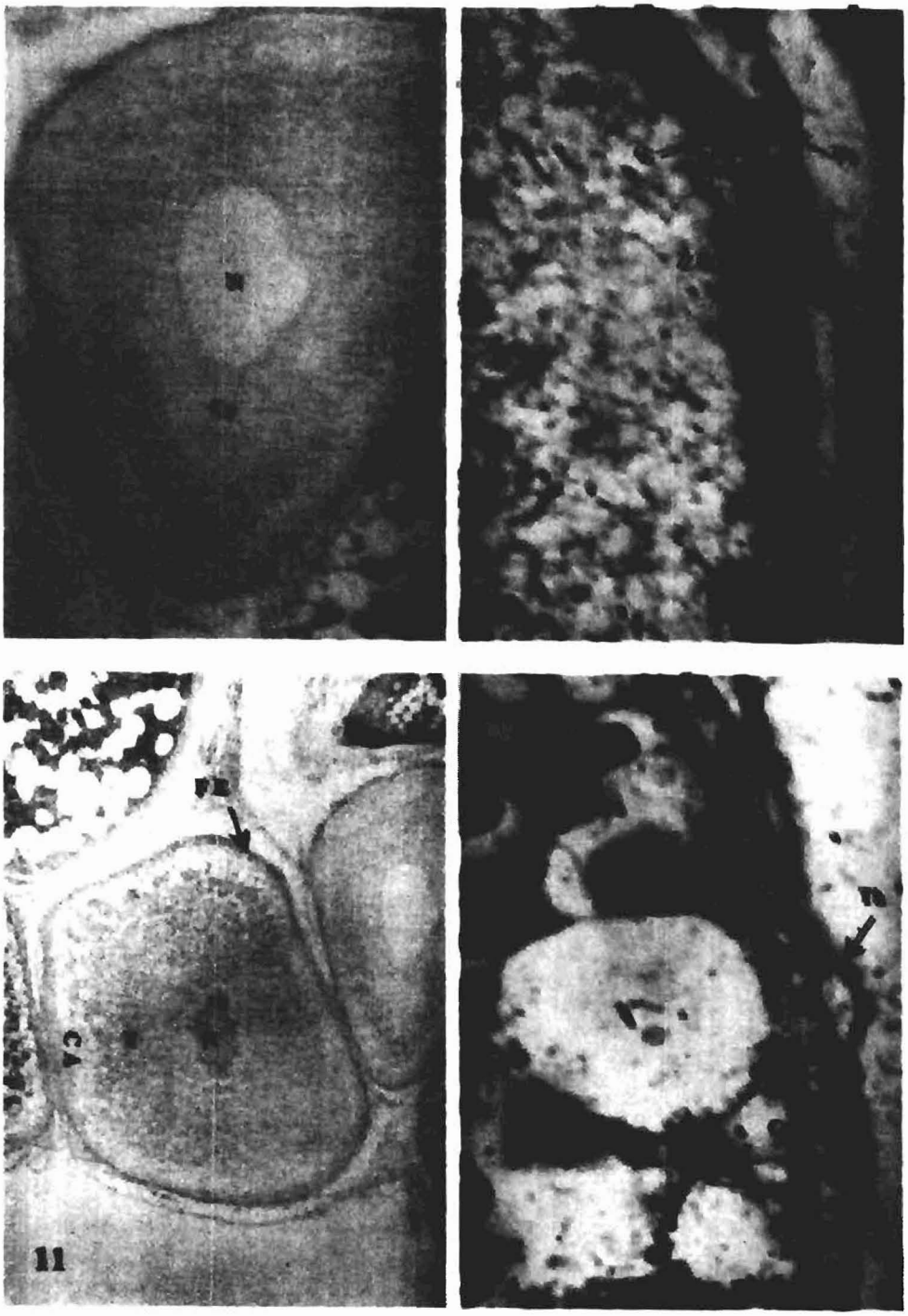
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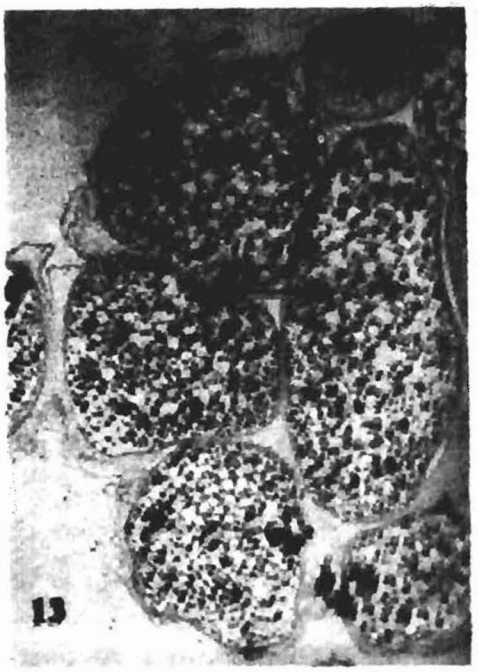

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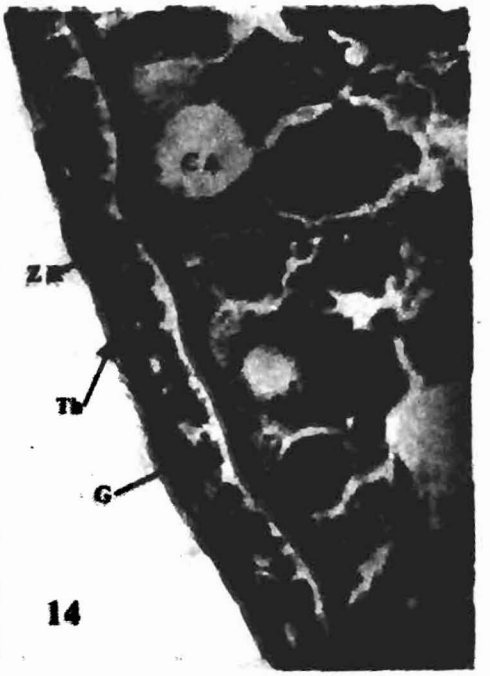

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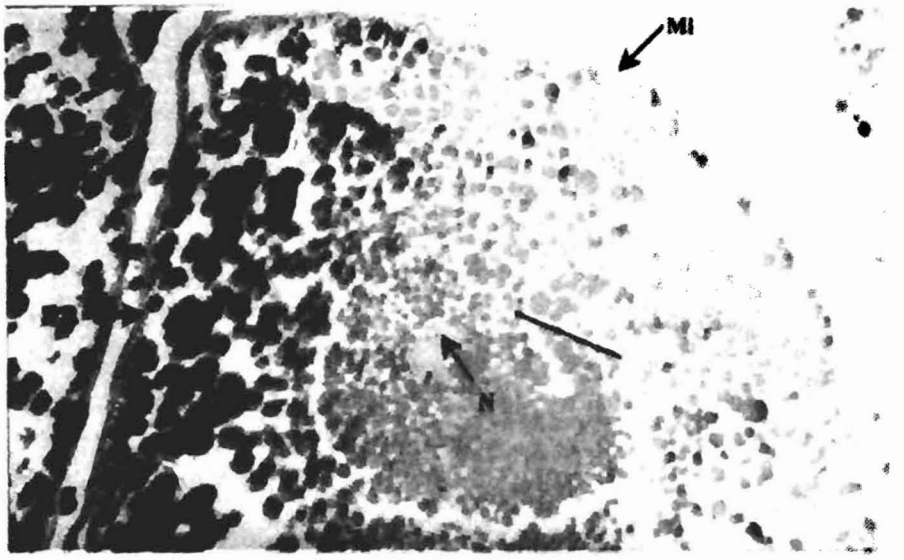

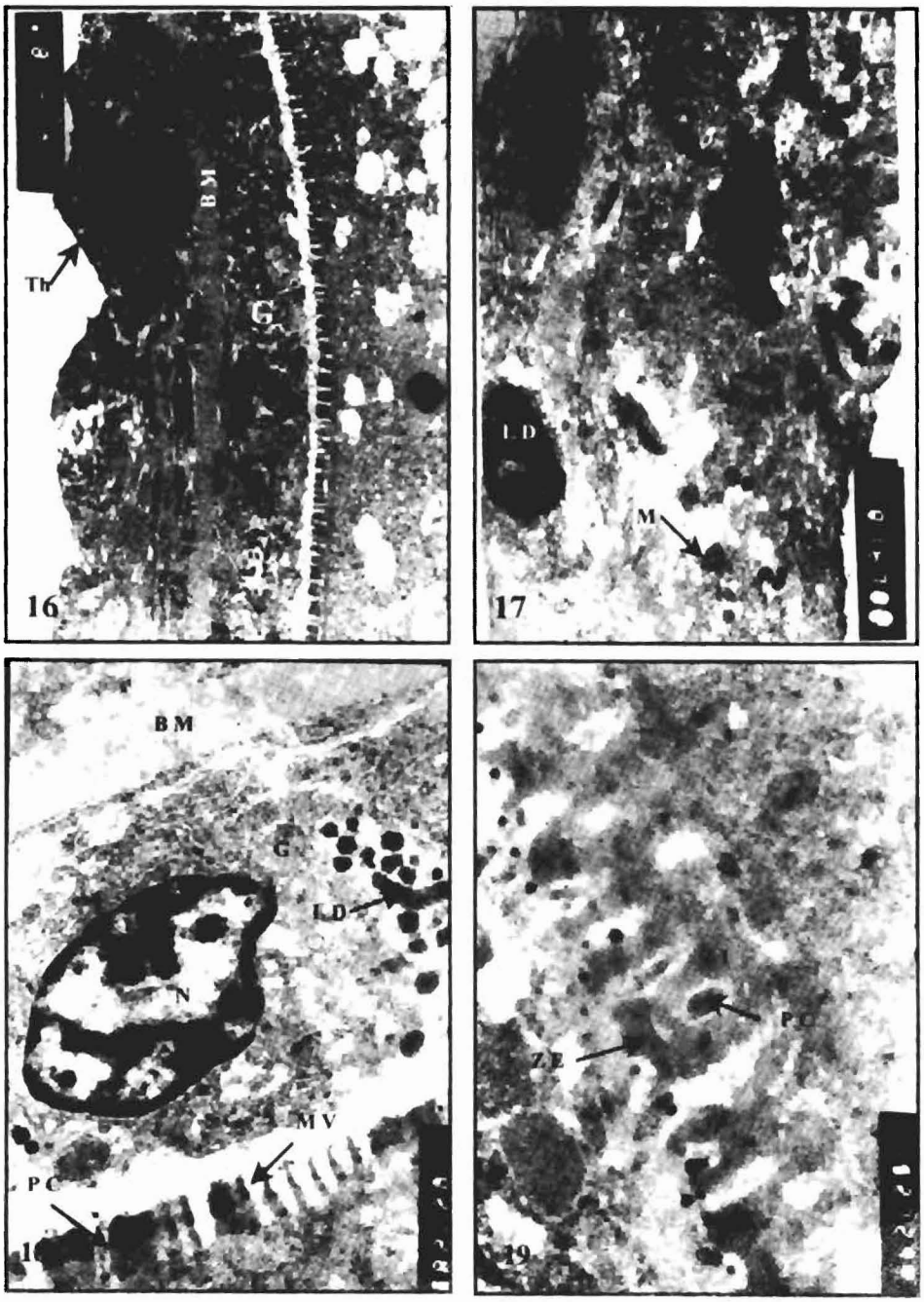
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Fig. (9): Part of transverse section of *O.spilurus* ovary showing oocyte at primary yolk deposition stage bounded by a distinct basophilic wall (W) with clear yolk granule (Y G) dispersed in easinophilic cytoplasm (Cy) and easinophilic nucleus (N). (x25).

Fig. (10): A magnified part of the wall of primary yolk oocyte showing the theca (TH); granulosa (G) and very thin layer of zona radiata (Z R). (x1000).

Fig. (11): Part of transverse section of *O. spilurus* ovary showing oocytes at secondary yolk deposition stage bounded by basophilic wall (W) with strong easinophilic cytoplasm contains yolk granules (Y G) and cortical alveoli (C A) at the margin of cytoplasm beside, easinophilic oval nucleus (N).

Fig.(12): A magnified part of the wall of secondary yolk oocyte showing the theca (TH); granulosa (G) zona radiata (Z R).

Fig.(13): Transverse section of *O. spilurus* ovary at ripe stage showing spherical tertiary yolk deposition stage oocyte (T Y) and oval ripe oocyte (R O) bounded by a distinct basophilic wall (W) (x40).

Fig.(14): A magnified portion of the tertiary yolk oocyte revealing the oocyte wall composed of theca externa (TH), granulose (G) and zona radiata (Z R) layer. Beside strongly easinophilic cytoplasm contains yolk vesicles (Y V) and cortical alveoli (C A) at the margin of cytoplasm (x1000).

Fig.(15): A magnified portion of ripe ova, showing migration of nucleus to animal pole 400x.

Fig.(16): Electron micrograph of follicular cells and zona radiata of secondary yolk stage oocyte. The zona radiata external (Z E). A basement membrane (B M) clearly separates the granulose and theca layers. Microvilli (arrow) from the oocyte protrude into the zona radiata.

Fig. (17): Electron micrograph of theca layer of yolk stage oocyte. **Note** : mitochondria (M) .

Fig.(18): Electron micrograph of the granulose layer of yolk stage oocyte. **Note:** Microvilli extending from the oocyte and granulosa cells (G) protrude into the zona radiata (Z R).

Fig. (19): Electron micrograph of the granulose layer of yolk stage oocyte.

Note: pore canal (P C) containing processors from both granulosa (G) and zona radiata extern (Z E) and zona radiata interna (ZI).

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