Seasonal changes in the histology of the ovaries of Nile tilapia (*Oreochromis niloticus*)

El-Saba A., Abd Rabou M.I., El-Sakhawy M.A., El-Shammaa. M. A. and Hussein S.M.*

*Department of Cytology & Histology Faculty of Veterinary Medicine Cairo University

With 20 figures Received March, accepted for publication May 2013

Abstract

Basic Histology of Ovaries of Nile tilapia (*Oreochromis niloticus*) was studied. Sampling was initiated of a total 40 female sexually mature Nile tilapia fish were collected over the period from September 2009 to August 2010. Ovaries were processed by standard histological technique. Histological characteristics of ovarian tissues and oocyte stages were studied by light microscopy. It revealed different histological structure of each oocyte developmental stage: Oogonia stage; Chromatin nucleolar stage; Perinucleolar stage; Cortical alveoli formation stage; Vitellogenic (yolk) stage; Postvitellogenic (mature) stage. Yolk nucleus (Balbiani bodies) was noticed in the ooplasm of the perinucleolar stage .During breeding season the ovary was surrounded by thin tunica albuginea. The ovigerous lamellae contained oocyte in active vitellogenesis, In addition; atretic and post ovulatory follicles were increased. During winter, the tunica albuginea reached a maximuem thickness and contained oocytes in previtellogenic stages.

Keyword: tilapia, ovary, oocyte,

Introduction

Nile tilapia (*Oreochromis niloticus*) is a fish of economic importance in tropical and subtropical countries. Basic study on histology of *O. niloticus* is still limited especially in reproductive system. Some basic knowledge was mentioned that diploid ovaries from the fish of six to eight months of age contained oogonia and maturing previtellogenic and vitellogenic oocytes with irregular nuclei and vacuolated cytoplasm associated with endogenous and exogenous yolk formation (Hussain et al., 1996). Reproductive development and reproductive histology in female are well understood by

histological techniques. Histology is the most accurate method to determine the reproductive state of female fish (West, 1990). The ovarian histological pattern of teleosts was described according to the division of ovarian tissues into seven or eight stages of maturity based upon the dominant gametogenic cell type present (Crim and Glebe, 1990). The study on histology of female reproductive organ of *O. niloticus* will provide a basic knowledge of reproductive system of the fish and will be useful for further applications.

Material and methods

Specimens of Nile tilapia (*Oreochromis niloticus*) were collected monthly at the same time of the day from the River Nile at Giza. A total of 40 female sexually mature Nile tilapia fish were collected over the period from September 2009 to August 2010. Fish were transported alive to the central lab. of Cytology and Histology department, faculty of Veterinary Medicine Cairo University. Fish were physically examined to ensure that they were free from any pathological changes. The males were distinguished from the females by the examination of the urogenital area. Each Nile tilapia was weighted (700-850 gms) to ensure that all fish sexually mature as mentioned by Popma and Masser (1999). The ovaries were removed immediately after decapitation and sample were carefully separated of 1 cubic cm. Sections were taken quickly from anterior, middle and posterior parts, fixed in formol-sublimte and neutral buffered formalin for about 24hs., also Bouin′s fluid was used. The samples were dehydrated, embedded in paraplast and following that 5-6 µm. sections were cut in cross and longitudinal sections obtained for ovaries.

In Oreochromis niloticus, the spring, summer and autumn were considered breeding season (spawning season) while winter considered non breeding season (Dougbag et al., 1988c).

Section stained with Harris haematoxylin and eosin for general histological examination; Gomori's reticuline method for demonstration of reticular fibers; Periodic acid Schiff and Periodic acid Schiff - Alcian blue (PH 2.5) combination for identification and differentiation of both neutral and acid mucopolysaccharides (Drury and Wallington,1980).

Results

The histological appearance of the ovary during spring, summer and autumn seasons (from March to November)

The ovary of the Nile tilapia (*Oreochromis niloticus*) was surrounded by thin tunica albuginea which was consisted of a vascular collagenous connective tissue contained smooth muscle cells (Fig. 1). Moreover, a network of reticular fibers was present (Fig. 2). The tunica albuginea was covered externally by squamous epithelium which was the mesothelium of the visceral peritoneum. The tunica albuginea projected inside the lumen of the ovary as numerous fine strands of connective tissue folds called ovigerous lamellae (Fig. 1) that contained oogonia and oocytes in follicles in various stages of development without any arrangment (Fig. 3).

During the breeding seasons the ovigerous lamellae were thick and completely occupied the ovarian cavity (ovocoel) and the ovary was in active vitellogenesis. Oocytes in all stages of development from perinucleolar to mature stages could be identified, but late stages of vitellogenesis were dominant (Fig. 3).

The ovarian follicles of Nile tilapia passed six developmental stages according to the changes in size, nucleus, ooplasm and egg membranes of the developing ova. The stages are**:** oogonia stage, chromatin nucleolus stage, perinucleolar stage, cortical alveolar stage, yolk globule stage and mature follicles. In addition, atretic and post ovulatory follicles were observed.

1- Oogonia stage:
The oogonia, the smallest germ cells in the ovary, were found in groups or nests that embedded within the ovigerous lamellae associated with the germinal epithelium (Fig. 4). They were small spherical cells with large basophilic nuclei with a single nucleolus. Their ooplasm was scanty, faintly basophilic thin rim surround the nucleus, and was periodic acid Schiff negative. The oogonia were found to be abundant during these seasons. They divided by mitosis to give primary oocyte.

2- Chromatin nucleolus stage (Early oocytes) which were the smallest previtellogenic follicles. Each one consisted of an oocyte with a large nucleus and surrounded by a thin follicular layer. The nucleus was eccentrically located and occupied large part of the oocyte. The chromatin of the nucleus appeared thread like. The nucleoli increased in number which distributed throughout the nucleus. The ooplasm was homogenous, deeply basophilic (**Fig. 5)** and they gave periodic acid Schiff negative reaction.

3- Perinucleolar stage (Late oocytes) which is characterized by in-

creasing the size of the oocyte and its nucleus became slightly basophilic with increased number of nucleoli, arranged themselves in the peripheral part of the nucleus. The ooplasm was basophilic and surrounded by a simple follicular epithelium which made up of flattened squamous cells (Fig. 6). In the larger oocytes, the ooplasm became frothy with the appearance of few unstained vacuoles. Ooplasm contained yolk nucleus (Balbiani bodies), which appeared as juxtanuclear basophilic round mass (Fig. 7).

4- Cortical alveolar stage (vacuolated follicles) which is characterized by marked increase in size of the oocytes and nuclei. The most characteristic feature of these follicles was the appearance of large number of unstained vacuoles (yolk vesicles) in the periphery of the ooplasm. However, yolk granules appeared within the ooplasm in between the yolk vesicles (Fig. 5).

Zona pellucida (oolemma) was a cellular thin hyaline acidophilic membrane (Fig. 8). The follicular layer was formed of cuboidal cells. The stroma was formed of flat thecal cells that surrounded the follicular layer (Fig. 9).

5- Vitellogenesis (yolk globule stage) which is characterized by increasing of yolk vesicles number and size toward the center, and by yolk globules appeared toward the center of the oocyte. These yolk globules gave positive reaction with periodic acid Schiff (Figs. 10 and 11). The nuclei became relatively smaller and contained fewer numbers of nucleoli and migrated toward periphery. The zona pellucida appeared thicker and the follicular epithelium made up of cuboidal cells. Theca folliculi was divided into outer vascular collagenous connective tissue thecal layer and inner cellular theca cells. Basal lamina, periodic acid schiff positive was found between the follicular epithelium and thecal layer.

6- Mature follicle (Postvitellogenic stage), these follicles showed a marked increase in size and reached the final growth stage (Fig. 12). The most characteristic feature of these follicles was the migration of their nuclei from the center to eccentric position (Fig. 13). Their ooplasm is characterized by being full of large yolk globules. These globules were periodic acid Schiff positive (Fig. 14) and alcian blue negative. The follicular epithelium appeared as high cuboidal cells which had vacuolated, faintly stained cytoplasm and with dark stained nuclei (Fig. 15). Zona pellucida was thick and periodic acid Schiff positive only (Fig. 14). The theca folliculi were composed of squamous

cells with flat nuclei. The mature (postvitellogenic) follicles were common and abundant during these seasons of the year.

Post-ovulatory follicles:
They were formed from the collapse of the follicle after ovulation. They showed a central lumen and a wall formed of follicular cell layer and the theca (Fig. 16).

Atretic follicles:
Two types of atresia were recognized, hypertrophic atresia and cystic atresia.

The hypertrophic atresia was the most numerous type. The follicular cells were increased both in size and number invading the ooplasm. Blood cells were seen between the follicular cells and yolk. Later on, the follicle collapse and finally replaced by stromal tissue (**Fig. 17)**.

The cystic atresia showed degeneration of the oocyte. The follicular epithelium thickened and was separated from the oocyte. Later, there was a rupture of the oocyte. The follicle became a small mass of cells or a cyst-like structure. The atretic follicles were found throughout the year especially during these seasons.

The histological appearance of the ovary during winter season (from December to February)

The tunica albuginea surrounding the ovary reached a maximum thickness. Also, the stromal connective tissue was increased and contained much amount of smooth muscle fibers (Fig. 18). The ovigerous lamellae were filled with previtellogenic oocytes in oogonium stage, chromatin nucleolus stages and perinucleolar stage (Fig. 19). The lamellae were disrupted and disorganized with several empty spaces and extensive vascularization. Remnants of atretic follicles were present throughout the ovary (Fig. 20). The previtellogenic stages were abundant while mature ovarian follicles were few.

Discussion

The present work was carried out on 40 female specimens of Nile tilapia (*Oreochromis niloticus*) throughout the year, in order to observe the morphological and the histological changes in the ovaries during the different seasons of the year. The results showed that the breeding season for reproduction was from March to November, while non-breeding season was from December to February. These current findings not simulate those of Caputo et al.(2003) in *Crystallogobius linearis*;

Cinquetti and Dramis (2003) in *Padogobius martensi* and El-Hafez et al. (2009) in *Oreochromis niloticus* who mentioned that, the breeding season is between April and September, while the non-breeding season between October and march.

El-Zarka et al. (1970) observed that the spawning was more protracted-from April to August in delta Nile tilapia (*Oreochromis niloticus*). While, Trewevas (1983) mentioned that spawning occurs between April to May in Nile tilapia (*Oreochromis niloticus*).

The present study revealed that the ovary of the Nile tilapia (*Oreochromis niloticus*) was covered by tunica albuginea which was consisted of dense collagenous connective tissue, elastic fibers and network of reticular fibers. The ovarian wall was supported with smooth muscle cells and this agreed with the result of Rizkalla (1970) in *Clarias lazera*; Yoakim (1971) in *Synodontus schall*; Khallaf et al. (1991) in *Bagrus bayad*; Gaber (2000) in *Bagrus docmac* and *Bagrus bayad* and El Hafez et al. (2009) in *Oreochromis niloticus* and in other parts of the wall, there were longitudinal smooth muscle cells only as those obtained by Gaber (2000) in *Bagrus docmac* and *Bagrus bayad*.

This tunica albuginea had no uniform thickness around the year that was similar to those obtained by Gaber (2000) in *Bagrus docmac* and *Bagrus bayad* and El Hafez et al. (2009) in *Oreochromis niloticus*. As the tunica albuginea reached a maximum thickness during winter (resting season) and decreased during the following breeding seasons (spring, summer and autumn).

Numerous ovigerous lamellae projected from the tunica albuginea into the interior of the ovary. Each lamella acted as a unit and contained oogonia and various developmental stages of the follicles. These findings were similar to Van den Hurk and Peute (1979) in rainbow trout; Dougbag et al. (1988c) in *Oreochromis niloticus*; Ismail (1992) in *Clarias lazera;* Mousa (1998) and El-Gohary (2001) in *Oreochromis niloticus*; Dutta and Maxwell (2003) in *Lepomis macrochirus* and El-Hafez et al. (2009) in *Oreochromis niloticus*.

The early germ cells were first found on the edge of the lamellae, and as the oocytes matured and increased in size, they were pushed deeper into the stroma and when matured they were ovulated into the lumen. According to the classification of Wallace and Selman (1981), the ovary of the Oreochromis niloticus showed an asynchronous mode,

since not all the oocytes were at the same stage of development at any time so; the Oreochromis niloticus had a prolonged spawning period. This observation agreed with Moustafa (1984) in Clarias lazera; Zaki et al. (1986a) in Clarias gariepinus and El-Zoghby et al. (2009) in Clarias lazera.

Ovaries of tilapia were of cystovarian type, as the ovarian cavity is connected directly with the oviduct (Alka′abi, 1996), subsequently the *Oreochromis niloticus* don′t release their mature ova into coelemic cavity. The overall pattern of oocytes development in Oreochromis niloticus was the same as in other teleost species (Coward and Bromage, 1998).

Van den Hurk and Peute (1979) in rainbow trout (*Salmo gairdneri*) divided into five stages: 1- previtellogenic, 2- vitellogenic, 3- mature, 4- postovulatory and 5-atretic follicles. On the other hand, Dougbag et al. (1988c) observed eight stages of oocyes development in *Oreochromis niloticus*.

In agreement with Alka′abi (1996) and El-Hafez et al. (2009), the process of oogenesis in *Oreochromis niloticus* was classified according to the changes in size, nucleus, cytoplasm and egg membranes of the developing ova into six stages beginning with oogonia. These stages were oogonia, chromatin nucleolus stages, perinucleolar stage, yolk vesicle stage, yolk globule stage and mature stage. Coward and Bromage (1998) recorded nine stages for oogenesis process in *Tilapia zilli*. However, Essa (2011) in *Oreochromis niloticus* stated that the developmental stages of oocytes were classified into five stages according to West (1990), chromatin nucleolus stages, perinucleolar stage, cortical alveoli formation stage, vitellogenic stage and mature stage.

A characteristic Balbiani bodies similar to that seen by Yoakim (1971) in *schilbe mystus*; Van den Hurk and Peute (1979) in the rainbow trout (*Salmo gairdneri*); Alka′abi (1996) in *Oreochromis niloticus*; Hamdoon and Zayed (1998) in *Oreochromis niloticus*; Bardakci et al. (2000) in teleost and El-Hafez et al. (2009) in *Oreochromis niloticus* was present in our investigation. Norrevang (1968) and Guraya (1979) revealed that most teleost oocytes accumulated a small juxtanuclear basophilic mass, which was termed yolk nucleus or Balbiani bodies. They were composed of various cellular organelles such as mitochondria, Golgi bodies, smooth endoplasmic reticulum, multivesivular bodies and lipid granules in *Salmo gairdneri* (Beams and Kessel, 1973) and in teleosts (Wallace and Selman, 1981).

Guraya (1979) stated that, although the role of Balbiani bodies was yet not clear, it had been considered that the yolk nucleus function as a center for the formation of organells within the oocytes.

The oogonia, small spherical cells with large nuclei, were arranged in nests. This finding was similar to Yoakim (1971) in *Synodontus schall* ; Dougbag et al. (1988c) in *Oreochromis niloticus*; Gaber (2000) *Bagrus docmac* and *Bagrus bayad* and El-Ghohary (2001) in *Oreochromis niloticus*. The oocytes in the previtellogenic stages had basophilic cytoplasm and did not have yet yolk granules. They were classified into two phases; a) early oocytes which were small oocytes with homogenous deeply basophilic ooplasm and b) late oocytes which were larger and had less basophilic and frothy ooplasm. They were found throughout the year, but were common in the non-breeding season (winter) and less abundant during spring, summer and autumn (spawning seasons). This result come in agreement with that revealed by Yoakim (1971) in *Synodontus schall*; Dougbag et al. (1988c) in *Oreochromis niloticus*; Salem (1991) in *Lethrinus bungus*; Ismail (1992) in *Clarias lazera* and El Hafez et al. (2009) in *Oreochromis niloticus*.

The vitellogenic follicles cytoplasm became acidophilic due to the deposition of vitellogenin into the oocytes (vitellogenesis). They were characterized by the appearance of unstained yolk vesicles in the periphery of the ooplasm and later the appearance of the extravesicular small eosinophilic yolk granules. These granules appeared firstly at the periphery of the ooplasm then aggregated toward the center of the oocytes. This finding was similar to results of Yoakim (1971) in *Synodontus schall* and Gaber (2000) in *Bagrus docmac* and *Bagrus bayad*. These vitellogenic follicles were decreased during winter as it was resting season but, abundant during spring, summer and autumn (spawning American plaice contained oocytes undergoing vitellogenesis indicate spawning activity.

Yolk globule stage was the most important phase of oocyte development; since it was during this phase, vitellogenesis occured, resulting in an extensive oocyte growth. Coward and Bromage (1998) in *Tilapia zillii* and Chmilevskii and Kameneva (2003) *in Tilapia mossambica* stated that oocytes were enlarged chiefly by rapid incorporation of large amounts of exogenous hepatically derived vitellogenin. While Patino (1997); Arockiaraj et al. (2004) reported that growing ovarian follicles

produced steroid hormones. This steroid left the follicle via blood vessels supplying the theca cell layer and was transported to the liver where it induced the production of vitellogenin. Vitellogenin was transferred to the ovary via circulation, where it taken up by the oocyte and was deposited as yolk protein which serves as building and energy material after fertilization.

The mature or post-vitellogenic follicles characterized by migration of their nuclei toward the animal pole, with presence of large yolk globules. This finding was similar to the results of Yoakim (1971) in *Synodontus schall*; Van den Hurk and Peute (1979) in the rainbow trout (*Salmo gairdneri*) and Gaber (2000) in *Bagrus docmac* and *Bagrus bayad*. The postvitellogenic follicles were common and abundant during spring as they were in the beginning of the spawning and ready to spawn and ovulate, while less abundant during winter season, as most of them were already spawned.

The egg wall was consisted of zona pellucida, follicular epithelium and theca folliculi. Where zona pellucida was considered as secretory product from the follicular cells, its appearance for first time could vary among fish species. In this study, it was firstly seen in larger late oocytes. This observation was similar to those of Ismail (1992) in *Clarias lazera,* Latif and Saady (1973) in *Oreochromis niloticus* and Davis (1977) in *Tilapia tandanus*. On the other hand, it appeared at the end of the yolk vesicle stage by Fahmy (1997) in *Clarias rueppllii* but appeared at early vacuolated oocytes as reported by Gaber (2000) in *Bagrus docmac* and *Bagrus bayad*.

The thickness of zona pellucida was not the same throughout the different stages, but it began in larger late oocytes as very thin acidophilic membrane, then increased at both early and late vacuolated oocytes and reached its maximum thickness at mature follicles. In our study, the zona pollicida had a strongly periodic acid schiff (PAS) positive reaction that indicated presence of mucpolysaccharides. This was similar to those results of Yoakim (1971) in *Synodontus schall*; Alves et al. (1983) in *Oreochromis niloticus;* Van den Hurk and Peute (1985) in *Clarias gariepinus*; Emel (1992) in the channel catfish and Gaber (2000) in *Bagrus docmac* and *Bagrus bayad*.

The follicular epithelium differed among the different stages where it appeared in the previtellogenic follicles as flattened squamous cells and reached its high columnar cells in the mature follicles which ap-

peared to have both neutral and acidic mucopolysaccharides as it gave positive periodic acid Schiff (PAS) and alcian blue reaction, a result that was supported by Alves et al. (1983) in *Oreochromis niloticus*. The follicular epithelium was covered by thin theca folliculi. This similar to those recorded by Davis (1977) in *Tandanus tandanus*; Hussein and Abbas (1985) in two species of genus Morone; Van den Hurk and Peute (1985) in *Clarias gariepinus*; Khallaf et al. (1991) in *Bagrus bayad* and Gaber (2000) in *Bagrus docmac* and *Bagrus bayad.*

In agreement with Braekevelt and Memillan (1967) in *Eucolia inconstans;* Dougbag et al. (1988c) and Essa (2011) in *Oreochromis niloticus* two types of atresia, hypertrophic and cystic were present in our study. El-Hafez et al. (2009) in *Oreochromis niloticus* stated that the presence of follicular atresia seem to be a very common phenomenon of the teleost ovary. The atretic follicles were usually seen at any stage. The atretic follicles were present throughout the year but, they were abundant during winter (resting ovaries). These atretic follicles indicated the spawned individuals.

In our investigation it was apparent that during breeding seasons (spring, summer and autumn) the ovaries were filled with vitellogenic and mature follicles, while in non-breeding seasons (winter), the ovaries revealed predominance of previtellogenic stages.

References

Alka′abi, N.A.O. (1996): Histologi cal and histochemical comparative studies on gonads of Grouper fish in Arabian Gulf and Tilapia fish in aquacultures. Ph.D. Department of Zoology, Collage of Science for Girls. Dammam- KSA.

Alves, M.M., Leme Dos Santos, H.S., Lopes, R.A., Petenusci, S.O. and Haiyashi, C. (1983): Rhythm of development in the oocyte of the tilapia *Oreochromis niloticus* L. (Pisces: Cichlidae); a morphometric and histochemical study. Gegenbaurs Morphol Jahrb., 129**:** 575 - 592.

Arockiaraj, A.J.; Haniffa, M.A.; Seetharaman, S. and Singh, S. (2004): Cyclic changes in gonadal maturation and histological observations of threatened fresh water catfish "Narikeliru" (Jerdon, 1849). J. Acta Icth. Et Pisc. (34) 2: 253 – 266.

Bardakci, F.; Ozansoy, U and Koptagel, E. (2000): A comparison of oogenesis under constant and fluctuating temperatures in Doctor

fish, Garra Rufa Heckel, 1843 (Teleostei: Cyprinidae).

Beams, H. W. and Kessel, R. G. (1973): Oocyte structure and early vitellogenesis in the trout, *Salmo gairdneri*. Am.J.Anat. 136: 105-122.

Braekevelt, C.R. and Memillan, D.B. (1967): Cyclic changes in the ovary of brook stickleback, *Eucolia inconstans*. J.Morph.,123:369 - 373.

Caputo, V.; Mesa, M. L.; Candi, G. and Cerioni, P. N. (2003): The reproductive biology of the crystal goby with a comparison to that of the transparent goby. Journal of Fish Biology (6) 2: 375 – 385.

Chmilevskii, D.A. and Kameneva, T.O. (2003): Oogenesis of *Tilapia mossambica*. IV. Yolk formation. J. Tsitologiia (45) 1: 5 – 13.

Cinquetti, R. and Dramis, L. (2003): Histological, histochemichal, enzyme histochemical and ultrasastructural investigations of the testis of *Padogobius martensi* between annual breeding seasons. J. Fish Biol., 63: 1402 - 1428.

Coward, K. and Bromage, N.R. (1998): Histological classification of oocyte growth and the dynamics of ovarian recrudescence in *Tilapia zilli*. Journal of Fish Biology 53: 285–302.

Crim, L. W. and B. D. Glebe.(1990): Reproduction. In: C. B. Schreck and P. B. Moyle (eds.), Methods for Fish Biology, pp. 529-553. American Fisheries Society,Bethesda.

Davis, T.L.O. (1977): Reproductive biology of the fresh water cartfsh, *Tandanus tandanus*, in the Gwydir River, Autralia. I. Structure of the gonad. Aust. J. Mar. Fresh water Res., 28: 139 - 158.

Dougbag, A.; El-Gazzawy, E.; Kassem, A.; El-Shewemi, S.; Abd El-Aziz, M. and Amin, M. (1988c): Histological and histochemical studies on the ovary of Tilapia niloticus. I. Basic structures. Alex. J. Vet. Sci., 4: 26 - 38.

Drury, R. A. B. and Wallington, E. A. (1980): Carleton's histological technique. Fourth Edition Oxford University Press, New York, Toronto.

Dutta, H. M. and Maxwell, L. (2003): Histological examination of sublethal effects of Diazinon on ovary of bluegill, Lepomis macrochirus. Environ. Poll. 121, 95–102.

El-Gohary, N.M.A. (2001): The effect of water quality on the reproductive biology of the Nile tilapia, Oreochromis niloticus in Lake Man-

zalah. Ph.D. Thesis. Faculty of Science. Ain Shams University.

El-Hafez, E.A.A.; Mahmoud, D.M. M. ; Ahmed, SH.M. and Hassan, A.H.S (2009): Histomorphological changes in the ovaries of *Oreochromis niloticus* during breeding and non-breeding seasons. Assiut Vet. Med. J. 55: 227 - 253.

EL-Zarka, S.; Shaheen, A.H. and EL-Aleem, A.A. (1970): Reproduction of *Tilapia nilotica***.** Bulletin of the institute of Oceanography and fisheries, Cairo 1: 182 – 192.

El-Zoghby, I. M. A.; Bakry, H. H.; Ghallab, A. M. and Emam, M. A. (2009): Histological studies on the gonads of the catfish during different seasons. Lucrari Stiintifice USAMV Lasi, Medicina Veterinara, vol 52(11): 352 – 362.

Essa, O.M. (2011): Histology of gonads of tilapia fish (tilapia nilototicus). Ph.D. Faculty of Veterinary Medicin. Cairo University.

Fahmy, A.F.A. (1997): Some aspects of the reproductive biology of *Chrysichthys rueppelli*. M.Sc.Thesis. Faculty of Science. Zagazig Univeristy.

Gaber, S.A.O. (2000): Biological, histological and histochemical studies on the reproductive organs and pituitary gland of *Bagrus docmac* and *Bagrus bayad* in the Nile water, with special reference to the Ultrastructure of supporting tissues. Ph. D.Thesis. Faculty of Science, Zagazig Univeristy.

Guraya, S.S. (1979): Recent advances in the morphology, cytochemistry and function of Balbiani's vitelline body in animal oocytes. Znt. Reu. Cytol. 59: 249-321.

Hamdoon, N.T. and Zayed, A.E. (1998): Feminization of Nile tilapia *Oreochromis niloticus* by oral administration of sex reversal hormone "Diethylstilbsterol". J. Assuit Vet. Med. (39) 78: 117 – 129.

Hussein, Kh. and Abbas, A. (1985): The state of gonads of two species of genus Morone in Lake Manzalah. Egypt. J. Histol., 8: 33 - 36.

Hussain, M. G., D. J. Penman and B. J. McAndrew.(1996): Effects of triploidy on sexual maturation and reproduction in Nile tilapia, Oreochromis niloticus L. Proceedings of the Third International Symposium on Tilapia in Aquaculture 3: 320-325.

Ismail, R.S. (1992): Physiological study on spawning in some fishes

(*Clarias lazera*). M.V.Sc. Thesis. Animal physiology. Zagazig University (Benha branch).

Khallaf, E.A; El-Saadany, M.M. and Authman, M. (1991): Oogenesis of *Bagrus bayad* (Forsk.). J. Egypt. Ger. Soc. Zool., 4: 1 - 4.

Latif, A.F.A. and Rashid, M.M. (1972): [Cited in Trewavas (1983); not available for checking]. Studies on *Tilapia nilotica* from Lake Nasser. I.Macroscopic characters of gonads.Bulletin of Institute of Oceanography and Fisheries. Cairo: pp 215-238.

Latif, A.F.A. and Saady, B.E. (1973): Oogenesis in the Nile bolti, *Tilapia nilotica* L. Bull. Inst. Ocean. Fish, 13:183 - Ovarian development and related condition changes in American plaice. J. Fish Biol., 53: 928 - 944.

Mousa, A. M. (1998): Immunocytochemical and Histological studies on the reproductive endocrine glands of the Nile tilapia, *Oreochromis niloticus* (Teleostei, cichlidae). J. Egypt.Ger.soc.zool., 27: 109 - 134.

Moustafa, Z.A. (1984): Biological studies of the catfish, *Clarias lazera*, with special references to the histological changes in the ovarian cycle and the peculiarities of the fecundity. M.Sc. Thesis. Faculty of Science. Zagazig Univeristy.

Norrevang, A. (1968): Electron microscopic morphology of oogenesis. Znt. Reo. Cytol. 23: 113-186.

Patino, R. (1997): Manipulation of the reproductive system of fishes by means of exogenous chemicals. The Progressive Fish Culturist 59: 118 – 128.

Popma, T. and Masser, M. (1999): Tilapia life history and biology. SRAC Publication No. 283

Rizkalla, W. (1970): Studies on the gonads of the teleost Nile catfish, *Claria lazera* (C. and V.), with special reference to their endocrine tissue. Acta Veterinaria, 20:1- 12.

Salem, S.A. (1991): On the sexual cycle of *Lethrinus bungus* (Ehrenb.) in the Egyptian Red Sea coast. I. Microscopic Peculiarities of the ovary. Egypt. J. Histol., 14: 55 -62.

Trewevas, E. (1983): Tilapia Fishes of the Genera Sarotherodon, *Oreochromis and Danakilia*. British Museum of Natural History, Publ. Num. 878.Comstock. Comstock Publishing Associates. Ithica, New York, pp: 583.

Van den Hurk. R. and Peute, J. (1979): Cyclic changes in the ovary of the rainbow trout, *Salmo gairdneri*, with special reference to sites of steroidogenesis. Cell Tissue Res. 199: 289 – 306.

Van den Hurk, R. and Peute, J. (1985): Functional aspects of the post-ovulatory follicle in the ovary of the African catfish, *Clarias gariepinus* after induced ovulation. An ultrastructural and enzyme histochemical study. Cell. Tiss. Res., 240 : 199 - 208.

Wallace, R.A. and Selman, K. (1981): Cellular and dynamic aspect of oocyte growth in teleosts. Amer. Zool., 21: 325 - 343.

West, G. (1990): Methods of assessing ovarian development in fishes: a review. Aust. J. Mar.Freshwater Res. 41: 199-222.

Yoakim, E.G. (1971): Seasonal variations in the pituitary gland and gonads of the Nile catfish (*Synodontus schall*) in relation to its reproductive cycle. PhD. Thesis. Faculty of Science. Ain Shams University.

Zaki, M.I.; Dowidar, M.N. and abdala, A. (1986a): Reproductive biology of *Clarias gariepinus* (Syn, lazera) Burchell (clariidae) in lake Manzalah, Egypt. I. Structure of the ovaries. Folia Morphologica, 34: 301 - 306.

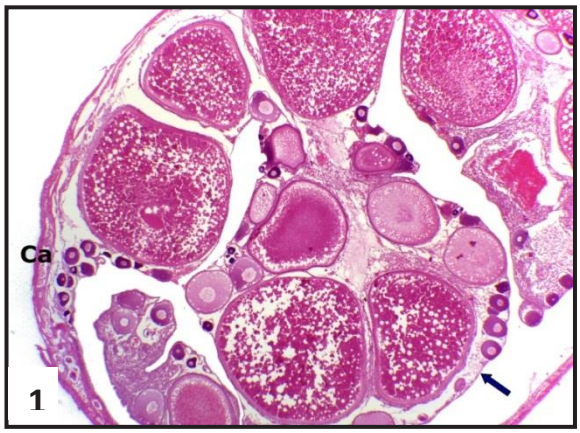

Fig (1): Section of ovary of Nile tilapia during spring showing: Capsule (Ca) and Ovigerous lamellae (arrow). H&E, X 40

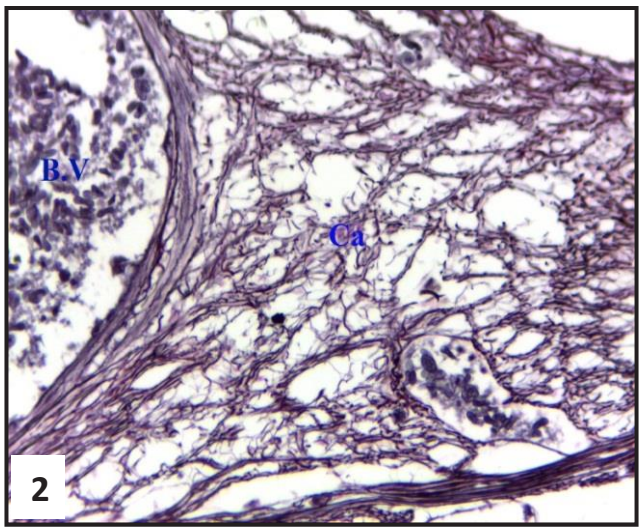

Fig (2): Section of ovary of Nile tilapia during summer showing: Capsule (Ca) and Blood vessels (B.V). Gomeri's reticuline stain, X 400

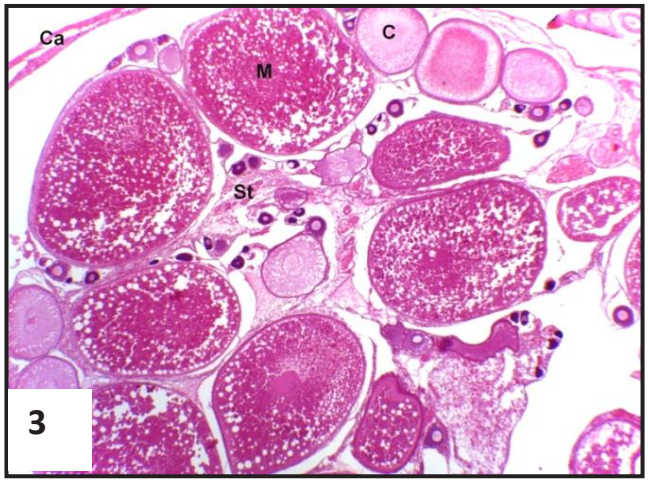

Fig (3): Section of ovary of Nile tilapia during spring showing: - Capsule (Ca); Stroma (St); Mature follicle (M) and Cortical alveolar stage (C). H&E, X 40

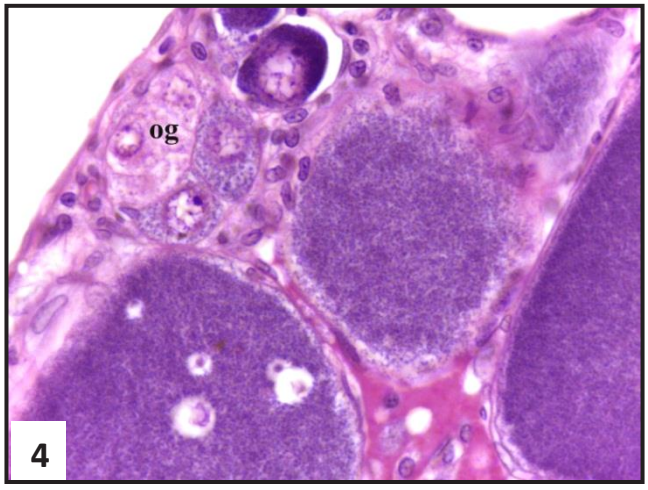

Fig (4): Section of ovary of Nile tilapia during autumn showing: Oogonia (og). H&E, X 400

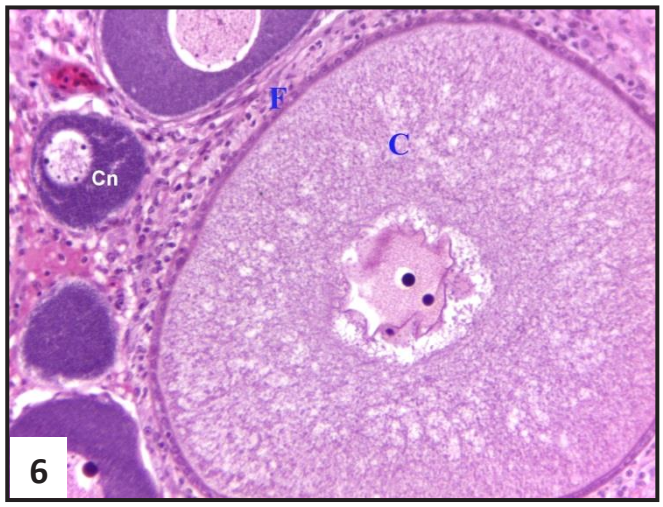

Fig (5): Section of ovary of Nile tilapia during summer showing: Cortical alveolar stage (C); Follicular cells (F) and Chromatin nucleolus stage (Cn). H&E, X 400

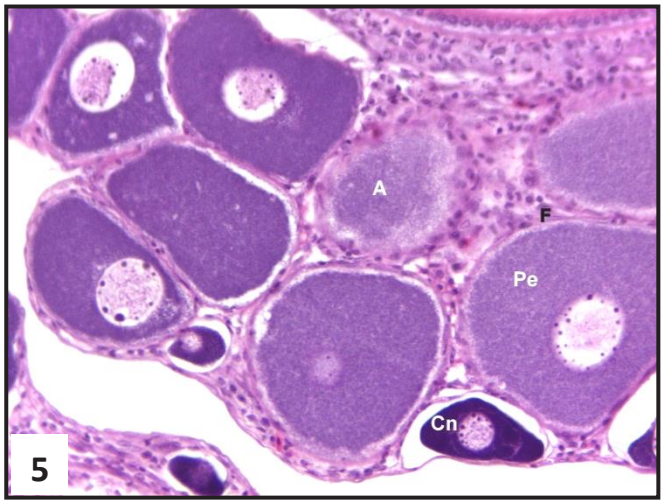

Fig (6): Section of ovary of Nile tilapia during spring showing: Atretic follicles (A); Chromatin nucleolus stage (Cn); Perinucleolar stage (Pe) and Follicular cells (F). H&E, X 100

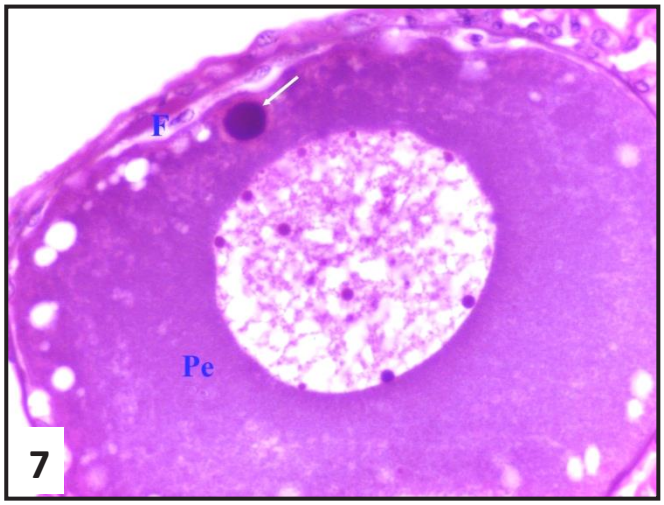

Fig (7): Section of ovary of Nile tilapia during summer showing: Follicular cells (F); Perinucleolar stage (Pe) and Balbiani body (Arrow). H&E, X 400

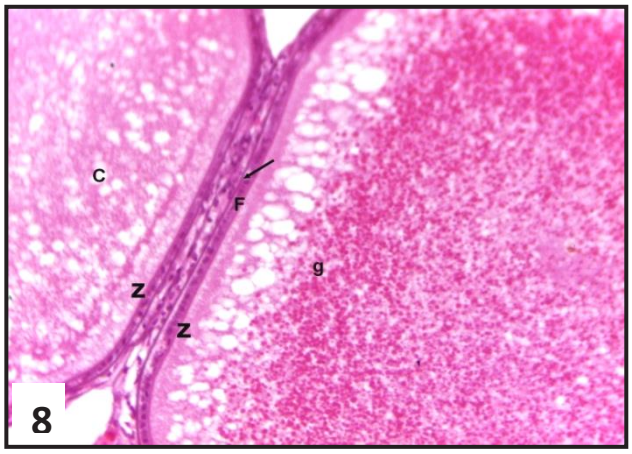

Fig (8): Section of ovary of Nile tilapia during summer showing: Follicular cells (F); Yolk granules (g); Cortical alveolar stage follicle (C) and Zona pellucida (Z). H&E, X 400

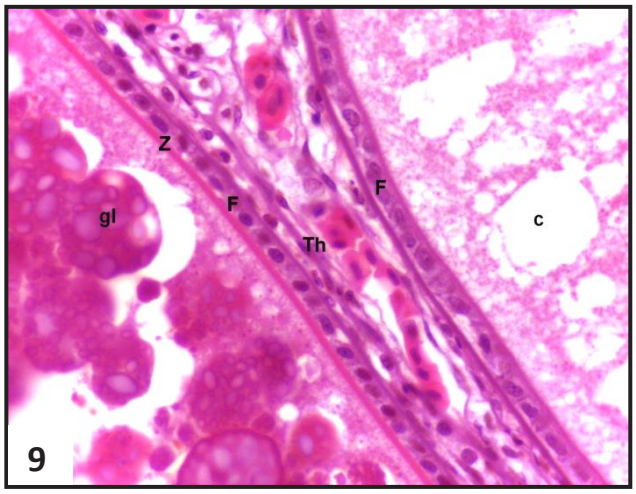

Fig (9): Section of ovary of Nile tilapia during spring showing: Cortical alveoli (C); Follicular cells (F); Thecal layer (Th); Zona pellucida (Z) and Yolk globules in mature follicle (gl). H&E, X 1000

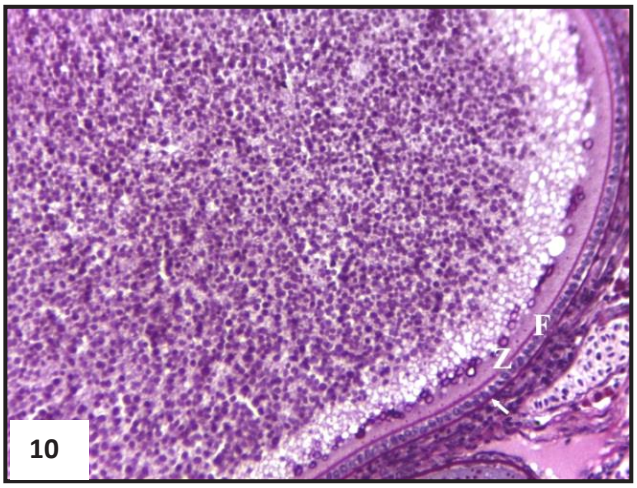

Fig (10): Section of ovary of Nile tilapia during spring showing yolk globule stage. Note: Follicular cells (F); Zona pellucida (Z) and Basement membrane periodic acid Schiff positive (arrow), periodic acid Schiff (PAS) and Alcian blue (AB) (PH 2.5), X400

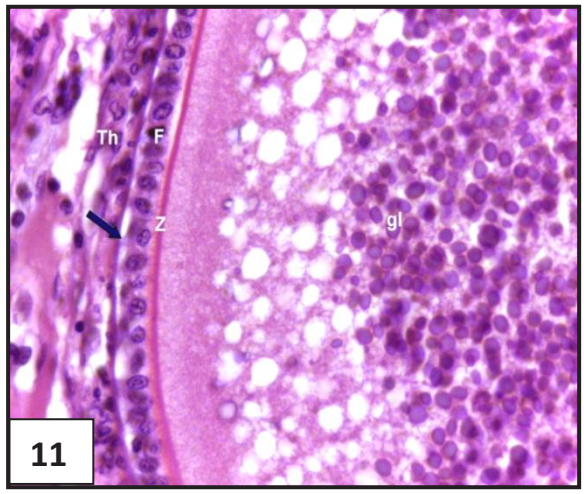

Fig (11): Section of ovary of Nile tilapia during spring show*ing yolk globule stage. Note:* Follicular cells (F); Zona pellucida (Z); Yolk globules (gl); Thecal layer (Th); Basement membrane periodic acid Schiff positive (arrow). periodic acid Schiff (PAS) and alcian blue (AB) (PH 2.5), X1000

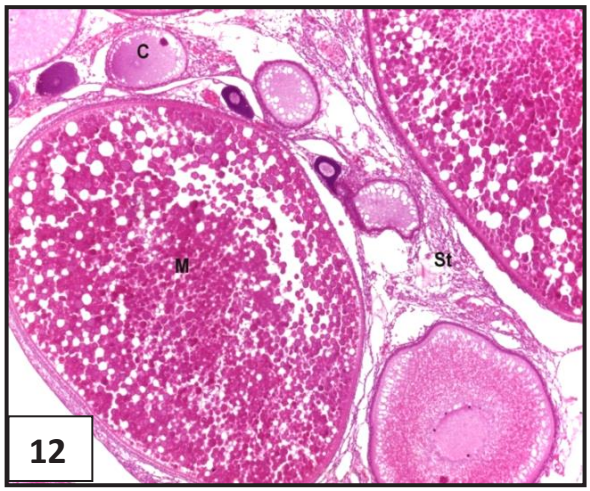

Fig (12): Section of ovary of Nile tilapia during summer showing: Mature follicles (M); Cortical alveolar stage (C); Stroma (St). H&E, X 100

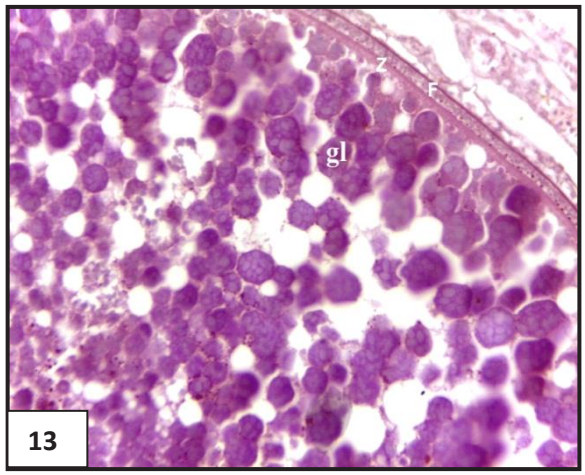

Fig (13): Section of ovary of Nile tilapia during summer showing mature follicle: Migratory nucleus in the mature follicles (N). H&E, X 100

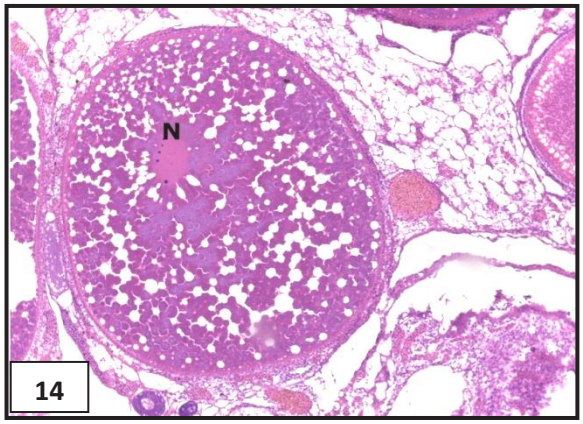

Fig (14): Section of ovary of Nile tilapia during summer showing mature follicle. Note: Follicular cells (F); Zona pellucida (Z) and Yolk globules (gl). Periodic acid Schiff (PAS), X400

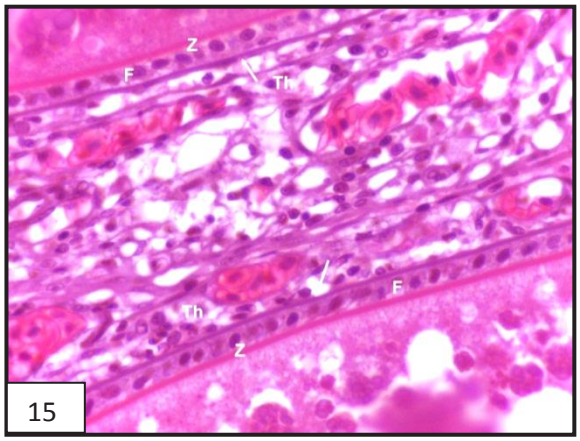

Fig (15): Section of ovary of Nile tilapia during spring showing: Follicular cells (F); Zona pellucida (Z); Basement membrane (arrow) and Thecal layer (Th). H&E, X1000

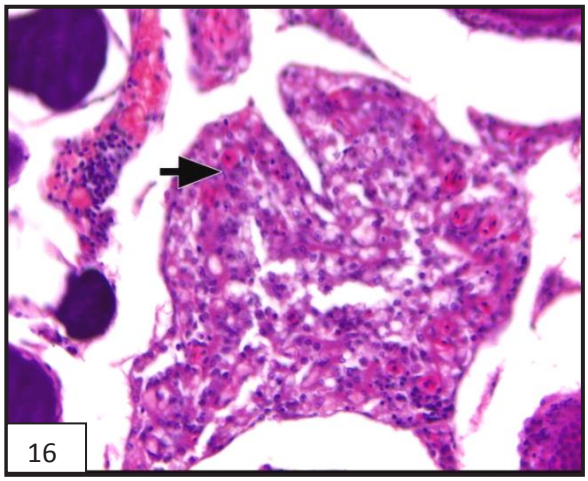

Fig (16): Section of ovary of Nile tilapia during spring showing: Post ovulatory follicle (arrow). H & E, X 100

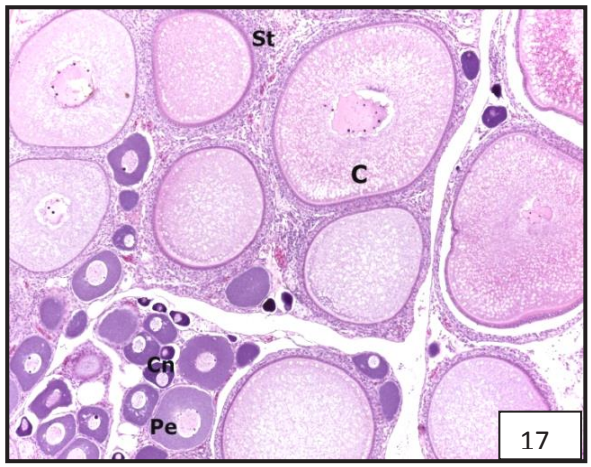

Fig (17): Section of ovary of Nile tilapia during autumn showing: Atretic follicles (A). H&E, X400

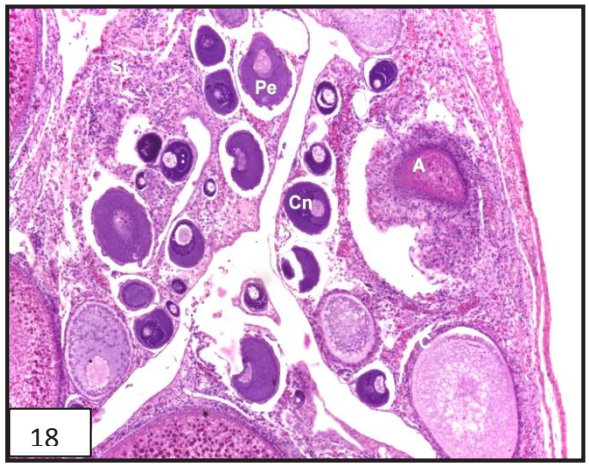

Fig (18): Section of ovary of Nile tilapia during winter showing: Capsule (Ca) and Circular muscle layer (arrow). H&E, X 400

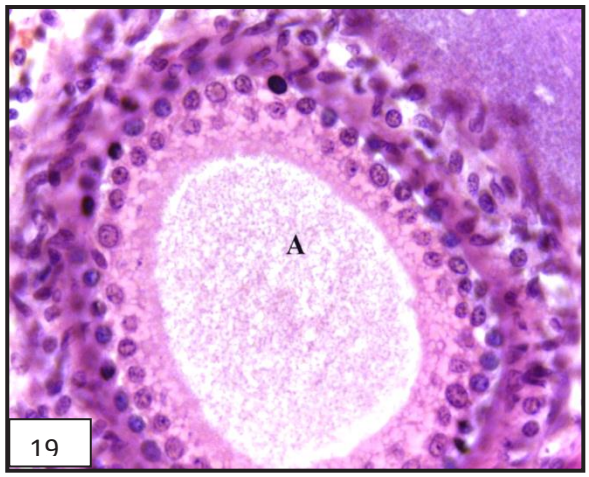

Fig (19): Section of ovary of Nile tilapia during winter showing: Chromatin nucleolus stage (Cn); Perinucleolar stage (Pe); Cortical alveolar stage (C) and Thick stroma (St). H&E, X40

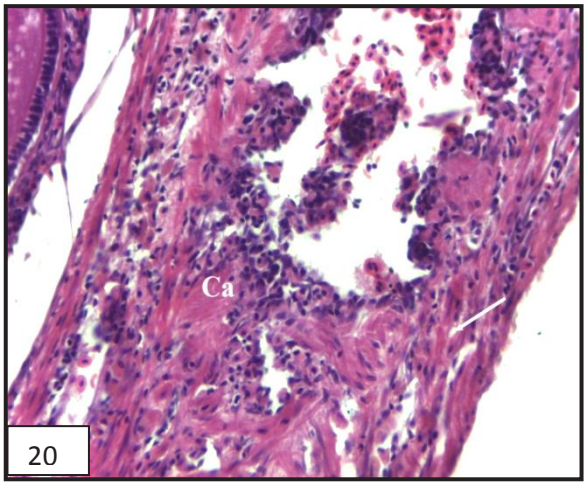

Fig (20): Section of ovary of Nile tilapia during winter showing: Chromatin nucleolus stage (Cn); Perinucleolar stage (Pe); Cortical alveolar stage (C); Atretic follicles (A) and Thick stroma (St). Stain: H&E, X40