EFFECT OF SEASONAL VARIATIONS ON THE HISTOLOGICAL STRUCTURES OF GONADS IN OREOCHROMIS NILOTICUS (TILAPIA NILOTICA)

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ABSTRACT

The morphological characteristics of the testes and the ovary of the Nile tilapia (Oreochromis niloticus) are presented. Testicular structure and spermatogenesis are described using histological techniques. The testes consist of capsule, seminiferous lobules (contain spermatogenic cell, Sertoli cell), interstitial tissue (leydig cell) and duct system. The coexistence in the testicular lobules of spermatozoa and spermatogenic cells indicates that this species is able to perform continuous reproduction. Seasonal trend was noticed in the number of the spermatogenic cell produced over a year period. The ovarian structure is also described using histological techniques. The ovary consist of capsule and ovarian follicles (chromatin-nucleolus, perinucleolus, cortical alveolar, vitellogenic and mature). Each follicle consists of oocyte and surrounding structure (follicular cell and zona radiata). Seasonal trend was noticed in the type of predominant follicles present over a year period.

Key word: Oreochromis niloticus, Nile tilapia, spermatogenic cell, Sertoli cell, leydig cell, ovarian follicles, follicular cell.

INTRODUCTION

Tilapias are among the most important warm water fishes used for aquaculture production and originate from Africa and the Middle East (**Fryer and Iles, 1972**).

In all vertebrates, from fish to mammals, the testis is composed of two main compartments, the tubular and the intertubular (or interstitial) compartment (**Schulz et al., 2009**). The tubular compartment comprises the seminiferous epithelium that contains only two cell types, the somatic Sertoli cells and the germ cells (spermatogenic cell), which are found at different stages of development (Matta et al., 2002). The testicular lobule (or tubule) is surrouned by a tunica propria, consists of basal lamina and peritubular myoid cells (Schulz et al., 2009). The intertubular compartment contains steroidogenic Leydig cells, blood/lymphatic vessels, macrophages, mast cells, neural and connective tissue cells. The latter being continuous with the tunica albuginea (Dougbag et al., 1988a; Grier, 1993; Le Gac and Loir, 1999; Koulish et al., 2002; Nobrega and Quagio-Grassiotto, 2007). The testicular lobules (or tubules) empty in the efferent duct system that connects these lobules to the deferent duct within the central portion of the testicular parenchyma. The testicular lobules are lined internally with Sertoli cells, whose cytoplasmic processes surround the spermatogonia and spermatocytes, forming isogenic cysts. During spermatogenesis, these cysts fill the entire width of the lobule, preventing the appearance of the typical central lumen until they open, when the mature germinal cells are released and form a lumen which anastomoses with the main spermatic ducts (**Le Gac and Loir, 1999**).

Tunica propria consists of basal lamina and peritubular myoid cells (**Schulz et al., 2009**). Peritubular myoid cells show fairly good proliferation activity in Nile tilapias. Perhaps the higher plasticity of this cell type in relation to its capacity to stretch in the tubular wall could influence its mitotic behavior (**Schulz et al., 2005**).

Sertoli cells are always located on the basal lamina that limits the testicular lobules, which separates them from the interstitial compartment. At the beginning of spermatogenesis, aggregates of Sertoli cells associated with primary spermatogonia are observed at the end of the lobule. At this time, Sertoli cells have a euchromatic and pleomorphic nucleus with a nucleolus, and a clear cytoplasm with traces of smooth endoplasmic reticulum and a few mitochondria with clear matrixes (**Sabat et al., 2009**).

Spermatogenesis is a developmental process during which a small number of diploid spermatogonial stem cells (primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes and spermatide) produce a large number of highly differentiated spermatozoa carrying a haploid, recombined genome (**Schulz et al., 2009**). Histologically, these can be identified as follow:

Primary spermatogonia: These cells are large, spherical to oval in shape. The nucleus is large, spherical, centrally located and has prominent nucleolus (Dougbag et al., 1988a). Secondary spermatogonia: These cells are nearly similar to the primary spermatogonia but smaller in size and are found in groups forming cysts of variable numbers. The individual cells in a cyst have in general similar morphological appearance. The nucleus occupies most of the cell and it is smaller and deeper in stain than that of the primary spermatogonia (Dougbag et al., 1988a). Primary spermatocytes: These cells form large cysts with prominent nuclei occupying most of the cells. Their nuclei have no clear boundaries. The darkly stained chromatin materials are found in the form of clumped granules irregularly distributed in the entire cell (Dougbag et al., 1988a). Secondary spermatocytes: They are found in cysts and have prominent darkly stained nuclei. These nuclei are slightly irregular in shape and have clumped chromatin with no clear nucleoli (Dougbag et al., 1988a). Spermatid: in general these cells are similar to the secondary spermatocytes but with small size. The chromatin is homogenous and apparently forms one clump (Dougbag et al., 1988a). Spermatozoa: They appear firstly in cysts after which they are released into the tubular lumen. The sperm head appears as darkly stained spherical body containing eccentric pale spot. Mature sperms are usually

found within the efferent ducts and the main sperm duct (**Dougbag et al., 1988a**).

The interstitial compartment comprises Leydig cells, blood/lymphatic vessels, macrophages, mast cells, neural and connective tissue cells (Dougbag et al., 1988a; Grier 1993; Le Gac and Loir, 1999; Koulish et al., 2002; Nobrega and Guagio-Grassiotto, 2007). The Leydig cells are polyhedral in shape and have spherical darkly stained nuclei with prominent nucleolus and vacuolated cytoplasm after H&E stain (Dougbag, 1988a).

The efferent ducts are formed by modified Sertoli cells (Grier et al., 1980; Grier, 1981; Selman and Wallace, 1986). However, the epithelial cells of the efferent ducts are morphologically very different from Sertoli cells that form germinal cysts (Porawski et al., 1997).

The vertebrate ovary is an aggregation of developing follicles enmeshed in a vascular stroma of loose connective tissue and enclosed within an envelope of gonadal epithelium. The stroma consists of collagenous, elastic, and reticular fibers and becomes greatly distended as the follicles enlarge. Only in a spent ovary when the stroma is collapsed, is it easily seen.

The ovary has connective tissue lamellae (ovigerous lamellae) projecting from the tunica albuginea into the interior of the ovary. These lamellae contain oogonia and oocytes in various stages of development which are in random arrangments (**Mousa, 1998**). Generally, four principal stages of oocyte development as out lined below have been described in several teleosts (**Selman and Wallace, 1989**). Chromatin-nucleolus stage, Perinucleolus stage, cortical alveolus stage, Vitellogenic stage and Maturation stage.

Chromatin-nucleolus phase, the oocyte is small with deeply basophilic ooplasm and the nucleus occupies the greater part of the follicle. The nucleus of the oocyte is generally spherical with multiple nucleoli in a perinuclear position adjacent to the inner layer of nuclear envelope (Guraya, 1986). Perinucleolus phase, the nucleus enlarges to form the germinal vesicle and the nuclear membrane becomes undulating. The oocyte has additionally several nucleoli that arrange at the periphery of the germinal vesicle. The oocyte is surrounded by a single layer of flattened follicular cells (Selman and Wallace, 1989). Cortical alveolus stage, this stage is characterized by the initial appearance of three components: cortical alveoli, zona pellucida (radiate), and lipid. The oocyte is surrounded by cuboidal follicular cell (Begovac and Wallace, 1988; Selman, Wallace and Player, 1991). Vitellogenic stage, the enlargement of the oocyte takes place during vitellogenesis is due largely to an accumulation of yolk protein precursors (Begovac and Wallace, 1988). At this stage, the yolk sphere increase in size while the cortical alveoli and lipid are displaced to the peripheral ooplasm. The oocyte is surrounded by columnar follicular cell (Begovac and Wallace, 1988). Mature stage (ripe stage), this stage is characterized by the enlargement of both cortical alveoli and yolk granules. The follicular cells are cuboidal or low cuboidal (Wallace and Selman, 1981; Nagahama, 1983; Kjesbu et al., 1996; Gothilf et al., 1997).

Marked seasonal variations are observed in the diameter and the frequency of these follicles in general, the follicles exhibited larger diameter during summer and smaller at winter. In summer the majority of the follicles in ovary are maturing follicles while in winter the majority are immature and no mature follicle are observed. The atretic follicles are observed throughout the year with a slight decrease in winter. In summer, the stroma is reduced by expansion of the developing follicles (**Dougbag et al., 1988b, d**).

MATERIAL AND METHODS

Samples (120 sexually mature Nile tilapia) were obtained from River Nile in Met Amer city collected at every month from January to December 2008. Fish were cut ventrally from the genital papillae to the base of the pectoral fin using a scalpel. A window on the lateral side was opened and the viscera were removed leaving gonads. The collected samples were routinely fixed for light microscope.

Small pieces $(0.5-1 \text{ cm}^3)$ of the gonads were fixed in both Bouin's and 10 % neutral buffered formalin solutions for 12 hr and 72 hr respectively. The Bouin fixed samples were extensively washed in 70% ethanol (3 X 24 hr) to get rid of the fixative before the subsequent steps of tissue processing. Formalin-fixed materials were washed for 2 hr under the running tap water before ethanol immersion. The tissue samples were dehydrated in graded series of ethanol (80%, 95% and absolute), cleared in xylene and embedded in paraffin wax. Five micron thick sections were cut by microtome and mounted on glass slides for ordinary stain. The histological processing and preparation of the gonadal slides were

carried out according to the standard histological techniques (**Patki et al., 1989; Lal, 2001**).

RESULT

Testicular structure :

The parenchyma of the Nile tilapia testis was surrounded by a thin white fibrous capsule and is divided into clear seminiferous lobules, efferent and defferent duct (Fig. 1a). The tunica albuginea is formed predominantly of collagen fibers, few elastic fibers and smooth muscle cells. Numerous fibrocytes are also recognized within these fibrous components. They were continuous with thin inconspicuous connective tissue trabeculae (septa).

Most of the testicular tissue was made up of the seminiferous lobules, where the spermatozoa are formed. These lobules are basically two-ended loops, with one end opening into the efferent duct. The seminiferous lobules were arranged in the form of cystes were enclosed by a distinct lamina propria (basal lamina and myoid cell) and contained two distinct cell types; Sertoli cells and spermatogenic cells (Fig. 1b).

The Sertoli cells were easily identified. They were large irregular with flattened elongated dense nuclei that were often deeply infolded or invaginated and contained large central nucleoli. They were located located in contact with the basal lamina and their cytoplasmic processes shared in formation of germinal cysts and were extending between the spermatogonia (Fig. 1c).

The Spermatogenic cells were differentiated into four morphologically different groups,

i.e., spermatogonia, spermatocytes, spermatids, and spermatozoa.

The undifferentiated spermatogonia were a single, large spherical cell (diameter10.60 \pm 0.21 μ m) with clear centrally located nucleus (Fig. 1c). They were not found inside cyst and mostly seen under connective tissue capsule close to Sertoli cells. They were mainly located at the periphery of seminiferous lobules beside cyst which contain other spermatogenic cells.

The primary spermatogonia with a mean diameter 8.87 \pm 0.298 μ m. found in pairs or small groups. They had cytoplasm and a large prominent nucleus. Occur inside cysts (Fig. 1c).

The secondary spermatogonia were found in groups and are enclosed in a cyst. Their mean diameter of $5.23 \pm 0.177 \,\mu$ m. The nucleus is spherical and central in location. The nucleus occupied most of cell and cytoplasm was deeply stained (Fig. 1d). In general the spermatogonia are predominant during winter season.

The primary spermatocytes had a mean diameter of 4.80 \pm 0.130 μ m. Their nuclei were spherical with deeply stained chromatin (Fig. 1d).

The secondary spermatocytes had a mean diameter $4.07 \pm 0.135 \ \mu$ m. They were characterized by their large nuclei that were occupying most of the cells. The nuclei that were surrounded with thin rim of cytoplasm. They were found in large nests extending into the lobular lumen (Fig. 1e). Spermatocytes were

predominant during autumn season.

The spermatids had a mean diameter of 2.70 \pm 0.119 μ m. They had a sparse cytoplasm with spherical dense nuclei (Fig. 1d). Some cysts of spermatids ruptured and the spermatozoa released into the lobular lumen.

The spermatozoa had a mean diameter of $1.93 \pm 0.117 \mu m$. They were present in the lumen of testicular lobules, and were characterized by deeply stained rounded head with very fine protoplasmic tail that was barely visible under the light microscope. They concentrate in the lumen of seminiferous lobules after breaking through the cyst wall. They were encountered all over the year (Fig. 1d).

The interstitial cells (Leydig cells) were seen either singly or in groups in the interlobular space. There were polygonal in shape, with ill -definite cell boundaries and centrally located spherical nuclei (Fig. 1f).

The Sertoli cells appeared to be hypertrophied and remain on the basement membrane. The basal lamina open and works as an efferent duct. That links system links the seminiferous tubules to the deferent duct (Fig. 1a). That stored and releases the spermatozoa into common duct that leads to a genital pore. There were not accessory glands, or seminal vesicle.

Ovarian structure :

The ovaries were surrounded by a single layer germinal epithelium, which enspheres a thick tunica albuginea which consisted of a dense collagenous connective tissue with smooth muscle cell and also contained

reticular fibers and few fine elastic fibers (Fig. 2a). Five stages of ovarian follicles were identified.

The chromatin-nucleolus stage had a very minute polygonal oocytes distributed in the ovary (diameter $31.17 \pm 1.42 \mu$ m). The nucleus was spherical the cytoplasm showed a strongly basophilic zone around the nucleus. One or two nucleoli scatter towards center of the nucleus (Fig. 2b). A follicle layer in the wall of young oocyte is not distinguished. These follicles were common in winter and autumn seasons and less abundant during spring and summer seasons.

The perinucleolus stage had an oocytes were polygonal in shape and increase in size (diameter $126.93 \pm 4.66 \ \mu$ m). The cytoplasm was acidophilic. The nucleus was spherical and contained an average of 8 basophilic nucleoli arranged peripherally of nuclear membrane. The oocyte was surrounded by a simple layer of flattened squamous (diameter 1.47 \pm 0.133 μ m). These follicles were common in winter and autumn seasons and less abundant during spring and summer seasons (Fig. 2c).

The cortical alveolar stage had an oocytes were characterized by increasing of its size (diameter $366.67 \pm 11.57 \mu$ m) and their nucleui which became slightly basophilic with increased number of nucleoli but, still adjacent to nuclear membrane. The ooplasm is basophilic and surrounded by a simple follicular epithelium which was made up low cuboidal cells (diameter $3.47 \pm 0.19 \mu$ m). Some minute vacuoles are located towards the outwards of cytoplasm (cortical alveoli) and appeared as large vacuoles around the nucleus (lipid vacuole). The zona radiate was firstly appeared between the follicular cells and oocytes (diameter 1.27 \pm 0.12 μ m), and was PAS positive

layer. The ooplasm was PAS negative. These follicles were abundant during spring and summer, but decreased during autumn and rarely observed during winter Fig. 2d).

The vitellogenic stage had an oocyte increased in size due to growth and accumulation of yolk granules in inner part of cytoplasm (diameter 538 \pm 20.34 μ m). The oocyte became irregular in shape. The nucleus became irregular in shape and zona radiata layer increased in thickness. The yolk granules intermingled with lipid vesicles. The nucleus began to migrate to the animal pole. The follicular cells were cuboidal epithelial cells. The zona radiate became thicker (diameter 1.47 \pm 0.133 μm). The follicular cell become tall cuboidal (diameter 5.20 \pm 0.174 μ m). These follicles were abundant during spring and summer, but decreased during autumn and rarely observed during winter (Fig. 2e).

The ripe stage (mature stage) had an oocyte was irregular in shape, this oocyte is the largest one (diameter $1346.93 \pm 29.83 \ \mu$ m). The nucleus migrate into animal pole. The yolk enlarge form yolk sphere and follicular cell became low cuboidal (diameter $4 \pm 0.33 \ \mu$ m). Their ooplasm was characterized by being full of large yolk globules. The zona radiate showed its maximum thickness (diameter $6 \pm 0.33 \ \mu$ m) and gave PAS positive reaction. These follicles were common during spring season and few in summer but, rarely observed during autumn and winter (Fig. 2f).

DISCUSSION

The present study demonstrated that the testis of Oreochromis niloticus was covered by tunica albuginea, hundreds of smooth muscle cells and blood vessels, which send out septa to the inner part of the organ, forming lobes that are filled with seminiferous lobules. The seminiferous lobules are composed of cysts, which are defined by cytoplasmatic projections of Sertoli cells. The spermatogenic cells in each cyst are in a stage of development. Similar findinges were previously by **Santos et al. (2006) in** Oligosarcus hepsetus.

The testicular lobules of Oreochromis niloticus were surrounded by boundary cells. These finding were consistend with **Marshall and Lofts (1956)** in Labeo fish, Lofts and **Marshall (1957)** in Esox fish and **Moser (1967)** in S. Paucispinis. On the other hand the lobules of other species were surrounded by fusiform shaped myoid cells as a contractile element around the lobules **Rosenblum et al. (1987)** in bullhead catfish, I.nebulosus).

The present studies clarified that the individual lobule contained many different germinal cysts and each cyst had the same spermatogenic stage. The germinal cysts were surrounded with the cytoplasmic processes of the Sertoli cells that shared with very thin collagen fibers in the formation of the wall of cysts. This resembled the results of **Abraham et al. (1980)** in A.dispar, **Saad and Billard (1987)** in C.carpio, **Arenas et al. (1995)** in **G.affinis and Nakaghi et al. (2003)** in C. Macropomum.

The undifferentiated spermatogonia of the

Oreochromis niloticus were the largest cells and could be differentiated, into two type; primary spermatogonia and secondary spermatogonia which were similar to each other but, the secondary spermatogonia were smaller in size. This result was supported by **Gaber** (2000) in Bagrus species.

Apparently the males of Oreochromis niloticus are able to reproduce at any time of the year, because of the presence of spermatogonia and spermatozoa inside the seminiferous tubules all over the year. However, the reproductive phase can be associated with the female ovarian maturation period, according to **Santos et al. (1995)**.

The primary spermatocytes were not the largest germ cells but, they were smaller than spermatogonia and slightly bigger than secondary spermatocytes. These findings were similar to those of **Rizkalla (1970)** in C. Lazera, **Rosenblum et al. (1987)** in I.nebulosus, **Iiadou and Fishelson (1995)** in catfish, and **Oteme et al. (1996)** in African clariid catfish and Heterobranchus longifilis.

The secondary spermatocytes of the Oreochromis niloticus were characterized by their large dense nuclei that were occupying most of the cells and were surrounded with a thin rim of cytoplasm. Numerous mitotic divisions were seen in the primary spermatocytes. Both types of spermatocytes were found throughout the year, but they were abundant during the spring, summer and autumn seasons with increased toward the spawning seasons.the aforementioned findinges were in agreement with result of **Nayyar and Sundararaj (1970)** in **H. fossili, Rosenblum et al. (1987)** in **I.nebulosus and Gaber (2000)** in Bagrus species.

The spermatids of the Oreochromis niloticus were smaller than the previous germ cells and appeared as small cells of indistinct outline with scanty cytoplasm and dense spherical nuclei. This finding was supported by **Iiadou and Fishelson (1995)** in **P. aristotelis, and Oteme et al. (1996)** in H. lonifilis.

The spermatozoa of the Oreochromis niloticus were the smallest germ cell and characterized by deeply stained rounded heads with very fine protoplasmic tails. They were present in the lumen of the testicular lobules without any arrangement. These findings were in harmony with those of **Rosenblum et al.** (1987), Gaber (2000), Nakaghi et al. (2003) and Rutaisire et al. (2003) in other fish. On the contrary, James (1946) found the spermatozoa of L.macrochirus in compact masses.

The efferent duct system links the seminiferous tubules to the deferent duct. A number of authors agree that these efferent ducts are formed by modified Sertoli cells (**Grier et al., 1980; Grier, 1981; Selman & Wallace, 1986**).

The ovary of the Oreochromis niloticus was covered by tunica albuginea which consisted of dense collagenous connective tissue, elastic fibers and network of reticular fibers. The ovarian wall was supported with smooth muscle cells. This result agreed with the result of **Rizkalla (1970)** in C. lazera, **Yoakim (1971)** in S.shall, **Khallaf et al. (1991) and Gaber (2000)** in B. bayad. The oocytes in the chromatin-nucleolus stage were small with deeply basophilic ooplasm and nuclei occupied the greater part of the follicle. This stage corresponds to the prematuration period of **Zaki et al.** (1986) in Claries gariepinus, **Zaki and El-Gharabawy** (1991) in Mugil capito, **Assem** (1992, 1995) in Solea species. They were found throughout the year, but were common in the autumn and winter and less abundant during spring and summer.

The perinucleolus stage was characterized by increase of size in oocytes. The nucleoli increased in number and were mostly located toward the periphery of nuclear membrane. During this stage, the wall of the oocyte is composed of one thin layer of flattened follicular cells. This result resembles that described by **Abdo (1996)** in Dicentrachus labrax, **El-Gahary (1996)** in O. niloticus, **Zaki et al.** (1996) in Siganus rivulatus, **Moustafa and El-Boray (1999)** in Rhabdosargus hoffara.

The cortical alveolar stage had less basophilic and frothy ooplasm, they were found throughout the year, but were common in the autumn and winter and less abundant during spring and summer. This result was consistent with those of **Ismail (1992)** in C.lazera and **Jirarach Srijunngam (2000)** in Oreochromis niloticus.

The vitellogenic follicles cytoplasm became acidophilic duo to the deposition of vitellogenin into the oocytes which characterized by the appearance of the yolk granules. These granules appeared firstly at the periphery of ooplasm then aggregated to ward the center of the oocytes. This finding was similar to the result of **Yoakim** (1971) in S.schall and **Gaber** (2000) in Bagrus species. These vitellogenic follicles were decreased during autumn but, abundant during spring and summer in order to turn rapidly into mature follicles.

The mature or ripe follicles characterized by migration of their nuclei toward the animal pole, with presence of large yolk globules. This finding was similar to the result of **Yoakim (1971)** in S.schall and **Gaber (2000)** in Bagrus species.

Fig.1: testicular structure

- **a)** General structure of testis of Nile tilapia showing tunica albuginea (TA), seminiferous lobules (curved arrow), septa (arrow), efferent duct(ED) and defferent duct (DD). H&E (X 10).
- **b**) Testis of Nile tilapia showing basal lamina (BL), primary spermatogonia (PSG), spermatide (SD) and spermatozoa (SZ). PAS (X40).
- **c)** Transverse section of testis of Nile tilapia showing undifferentiated spermatogonia (SG), primary spermatogonia (PSG) and Sertoli cells (arrow head). H&E(X 100).
- **d**) Testis of Nile tilapia showing seminiferous lobules contains undifferentiated spermatogonia (SG), primary spermatogonia (PSG), secondary spermatogonia (SSG),, spermatozoa (SZ) and leydig cell (L). H&E(X 100).
- e) Testis of Nile tilapia showing undifferentiated spermatogonia (SG), primary spermato-

gonia (PSG), primary spermatocytes (PSC), secondary spermatocytes(SSC), spermatozoa (SZ) and leydig cell (L). H&E(X 100).

f) Transverse section of testis of Nile tilapia showing seminiferous lobules contains undifferentiated spermatogonia (arrow head), primary spermatogonia (PSG), secondary spermatogonia (SSG), spermatozoa (SZ) and leydig cell (arrow). H&E(X 100).

Fig. 2 : ovarian structure

- **a)** General structure of ovary showing, tunica albuginea (TA), ovigerous lamellae (OL) contains different stages of ovarian follicles (OF).H&E(X4).
- **b)** Ovary of Nile tilapia showing, chromatinenucleolus stage (CH). H&E(X100).
- c) ovary of Nile tilapia showing, perinucleolus follicle (P) with follicular cell (arrow), nucleus (N), nucleolus (arrow head) and connective tissue septa (CT).H&E(X40).
- **d)** Ovary of Nile tilapia showing, corticalalveolar follicle(C) with cortical alveoli (arrow head), follicular cell (arrow), nucleus (N) and appear zona radiata (curved arrow). H&E(X100).
- e) Ovary of Nile tilapia showing, vitellogenic stage (V) with cortical alveoli (arrow head), follicular cell (arrow), cortical alveoli (arrow head) nucleus (N) and appear zona radiata (curved arrow) and yolk (Y). H&E (X100).
- **f)** Ovary of Nile tilapia showing, mature follicle (M), and yolk sphere (Y) and connective tissue septa (CT).H&E(X40).





Fig 1(c)



Fig 1(f)

Fig 1(e)



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REFERENCES

Abdo, M. A. A. (1996) : Reproductive biology and induced spawning of Dicentrachus Ladrax. Ph. D. Thesis, Faculty of Science, Alexandria University, Alexandria, Egypt.

Abraham, M.; Ahamim, E.; Tibika, H. and Golense, E. (1980) : The blood testis barrier in Aphanius dispar (Telostei). Cell Tissue Res., 211: 207 -214.

Arenas, M. I.; Fraile, B.; Paz De Miguel, M. and Paniagua, R. (1995) : Cytoskeleton in Sertoli cells of Mosquito fish, Gambusia affinis holbrooki. Anat. Rec., 241: 225 - 234.

Assem, S. (1992) : Reproductive biology and physiology of one species of family Sparidae in Mediterranean Sea. M. Sc. Thesis, Faculty of Science, Alexandria University, Alexandria, Egypt.

Assem, S. (1995) : Reproductive physiology and induced spawning of Solea species. Ph. D. Thesis, Faculty of Science, Alexandria University, Alexandria, Egypt.

Begovac, P. S. and Wallace, R. A. (1988) : Stages of oocyte development in the pipfish, Syngnathus scovelli. J. Morph., 193:1139-1163.

Dougbag, A.; El-Gazzawy, E.; Kassem, A.; El-Shewemi, S.; Abd El-Aziz, M. and Amin, M. (1988a) : Histological studies on the testis of Tilapia niloticus. I. Basic structures. Alex. J. Vet. ScL, 4: 49 - 58.

Dougbag, A.; El-Gazzawy, E.; Kassem,

A.; El-Shewemi, S.; Abd El-Aziz, M. and Amin, M. (1988b) : Histological and histochemical studies on the testis of Tilapia niloticus. II. Seasonal variations. Alex.J. Vet. Sci, 4:59 - 69.

Dougbag, A.; El-Gazzawy, E.; Kassem, A.; El-Shewemi, S.; Abd El-Aziz, M. and Amin, M. (1988d): Histological and histochemical studies oh the ovary of Tilapia niloticus. II. Seasonal variations.Alex. J. Vet. Sci, 4: 39-48.

El-Gohary, N. M. A. (1996) : Effect of temperature on the histology, histochemistry and biological activities of the gonads of Oreochromis niloticus. M. Sc. Thesis, Faculty of Science, Ain Shams University, Egypt.

Fryer, F. E. and Iles, T. D. (1972) : The Cichlid Fishes of the Great Lakes of Africa: Their biology and evolution. Oliver and Boyd, (Ed.). Edinburgh.

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 University.

Gothilf, Y.; Meiri, I.; Elizur, A. and Zohar, Y. (1997) : Preovulatory changes in the levels of three gonadotropin-releasing hormone-encoding messenger ribonucleic acids (mRNAs), gonadotropin β -subunit mRNAs, plasma gonadotropin, and steroids in the female gilthead seabream, Sparus aurata. Biol. Reprod. 57:1145-1154.

Grier, H. J. (1981) : Cellular organization of the testis and spermatogenesis in fishes. AM. Zool., 21:345-357.

Grier, H. J. (1993) : Comparative organization of Sertoli cells including the Sertoli cell barrier. In: Russell LD, Griswold MD (eds) The Sertoli cell. Cache River Press, Clearwater, FL, pp 704-739.

Grier, H. J.; Linton, J. R.; Leatherland, J. F. and Devalming, V. L. (1980) : Structural evidence for two different testicular types in teleost fishes. Amer. J. Anat, 159 : 331 -345.

Guraya, S. S. (1986) : The cell and molecular biology of fish oogenesis.(Karger: Basel). In: G., West, Methods of assessing ovarian development in fishes. J. Mar. Freshwat. Res., 41: 199 - 222.

liadou, K. F. L. and Fishelson, L. (**1995**) : Histology and cytology of testes of the catfish, Parasilurus aristotelis (Siluridae: Teleostei), from Greece. Jp. J. Ichthyol., 41 : 447 - 454.

Ismail, R. S. (1992) : Physiological study on spawning in some fishes (Glorias lazerd). M.V.Sc. Thesis. Animal physiology. Zagazig University (Benha branch).

James, M. (1946) : Histology of gonadal changes in the bluegill, Lepomis macrochirus, and largemouth bass, Huro salmoides. J. Morph.,79:63-92.

Jirarach Srijunngami; Noppadon Kitana, I.A.N. P. Callard and Kingkaew

Wattanasirmkit (2000) : Ultrastructural Changes in the Ovarian Follicular Wall During Oocyte Growth in the Nile Tilapia, Oreochromis niloticus Linn. The Natural History Journal of Chulalongkorn University 5(1): 21-30.

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

Kjesbu, O. S.; Kryvi, H. and Norberg, B. (**1996**) : Oocyte size and structure in relation to blood plasma steroid hormones in individually monitored, spawning Atlantic cod.J.Fish Biol., 49:1197-1215.

Koulish, S.; Kramer, C. R. and Grier, H. J. (2002) : Organization of the male gonad in a protogynous fish, Thalassoma bifasciatum (Teleostei: Labridae). J. Morphol. 254, 292-311.

Lal, S. S. (2001) : "A Text Book of Practical Zoology Vertebrate". Seventh Revised Edition. 'Gangotri' Shivaji Road, Meerut-250002, Publ. Rastogi publications: 138-148.

Le Gac, F. and Loir, M. (1999) : Male reproductive system, fish. In: Knobil E, Neill JD (eds) Encyclopedia of reproduction, vol 3. Academic Press, San Diego, pp 20-30.

Lofts, B. and Marshall, A. (1957) : Cyclical changes in the distribution of the testis lipids of a teleost fish, Esox hicius. Quart. J. Micr. Sci., 98: 79 -88.

Marshall, A. and Lofts, B. (1956) : The

Leydig cell homoluge in certain teleost fishes. Nature, 177: 704 - 707.

Matta, S. L. P.; Vilela, D. A. R.; Godinho, H. P. and França, L. R. (2002) : The goitrogen 6-n-propyl-2-thiouracil (PTU) given during testis development increases Sertoli and germ cell numbers per cyst in fish: the tilapia (Oreochromis niloticus) model. Endocrinology; 143: 970-978.

Moser, G. (1967) : Seasonal histological changes in the gonads of Sebastodes paucispinis Ayres, an ovoviviparous teleost (Family : Scorpaenidae). Ibid., 123 : 329 - 354.

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.

Nagahama Y. (1983) : The functional morphology of teleost gonads. In WS Hoar. DJ Randall, EM Donaldson, eds. Fish physiology. Vol.9. part a. endocrine tissue and hormones. New York; academic press, pp.223-275.

Nakaghi, L. S. O.; Mitsuiki, D.; Santos, H. S. L.; Pacheco, M. R. and Ganeco, L. N. (2003) : Morphometry and morphology of nucleus of the Sertoli and interstitial cells of the tambaqui Colossoma macropomum (Cuvier, 1881) (Pisces: Characidae) during the reproductive cycle. Braz. J. BioL, 63.

Nayyar, S. K. and Sundararaj, B. I. (1970) : Seasonal reproductive activity in the testes and seminal vesicles of the catfish, Het-

eopneustes fossilis (Bloch). J. Morph, 130: 207 - 225.

Nobrega, R. H. and Guagio-Grassiotto, I. (2007) : Morphofunctional changes in Leydig cells throughout the continuous spermatogenesis of the freshwater teleost fish, Serrasalmus spilopleura (Characiformes, Characidae): an ultrastructural and enzyme study. Cell Tissue Res 329(2):339-349.

Oteme, Z. J.; Rodriguez, J. N.; Kouassi, C. K; Hen, S. and Agnese, J. F. (1996) : Testicular structure, spermatogenesis and sperm cryopreservation in the African clariid catfish, Heterobranchus longifilis (Vaenciennes, 1840). Aquaculture Research, 31 : 805 - 813.

Patki, L. R.; Bhalchandra, B. L. and Jeevaji, I. H. (1989) : An Introduction to microtechnique. S. Chand & Company, Ltd. Ram Nagar, New Delhi-110055. 28-78 pp.

Porawski, M.; Wassermann, G. F. and Achaval, M. (1997) : Functional morphology of male gonad of kingfish. Rev. Bras. Biol., 57: 151-159.

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

Rosenblum, P. M.; Pundy, J. and Callard, I. P. (1987) : Gonadal morphology, enzyme histochemistry and plasma steroid levels during the annual reproductive cycle of male and female brown bullhead catfish, Ictalurus nebulosus. J. Fish. BioL., 31: 325 - 341. **Rutaisire, J.; Muwazi, R. T. and Booth, A. J. (2003) :** Structure and cytology of the testes of Labeo victorianus (Pisces: Cyprinidae). African Zoology, 38: 119 - 126.

Saad, A. and Billard, R. (1987) : Spermatozoa production and volume of semen collected after hormone stimulation in the carp, Cyprinus carpio. Aquaculture, 65: 67 - 77.

Sabat, M.; Fabiana Lo Nostro; Margarida Casadevall and Marta Munoz (2009) : A Light and Electron Microscopic Study on the Organization of the Testis and the Semicystic Spermatogenesis of the Genus Scorpaena (Teleostei, Scorpaenidae) Journal of Morphology.

Santos, A. A.; Matta, S. L. P. And Andrade, D. R. (1995) : Época e tipo de desova de lambari Oligosarcus argenteus. Anais do Encontro Brasileiro de Ictiologia, G4-G4.

Santos. J. E; G.E. Padilha; O. Boncompagni Junior; G. B. Santos; E. Rizzo & N. Bazzoli. (2006) : oocyte growth and follicular envelope development in the cat Iheringichthys labrosus (Siluriformes: Pimelobibae). Tissue and Cell 38 (5): 303-310.

Schuiz, R. W.; Menting, S.; Bogerd, J.; Franca, L. R.; Vilela, D. A. R. and Godinho, H. P. (2005) : Sertoli cell proliferation in the adult testis- evidence from two fish species belonging to different orders. Biol. Reprod, 73: 891 - 898.

Schulz, R. W.; Luiz Renato de França; Jean-Jacques Lareyre; Florence LeGac; Helio Chiarini-Garcia; Rafael Henrique Nobre**ga and Takeshi Miura (2009) :** Spermatogenesis in fish. General and Comparative Endocrinology.

Selman, K., and Wallace, R. A. (1989) : Cellular aspects of oocyte growth in teleosts.Zoolog. Sci. 6 211-231.

Selman, K.; Wallace, R. A. and Barr, V. (1986) : Oogenesis in Fundulus heteroclitus.IV.Yolk vesicle formation. J. Exper.Zool., 239: 277-288.

Selman, K.; Wallace, R. A. and Player, D. (1991) : Ovary of Seahorse, Hippocampus erectus. J.Morphol., 209: 285-304.

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

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

Zaki, M. I.; Dowidar, M. N. and Abdala, A. (1986b) : Reproductive biology of Glorias gariepinus (Syn, lazera) Burchell (clariidae) in lake Manzalah, Egypt. II. Structure of the testes. Folia Morphologica, 43: 307-313.

Zaki, M. I.; Massoud, A. A.; Madkour, G. A. and El-Mesiry, G. E. (1996) : The cyclic changes in the pituitary gland and gonads of Siganus rivulatus from the Red Sea (Hurghada Area). J. KAU. Mar. Sci., Vol.7, special Issue: Symp. Red Sea Mar. Environ. Jeddah, PP. 271-287.

أجريت هذه الدراسة على عدد ١٢٠ من سمك البلطى النيلى من كلا الجنسين لتوضيح التركيب الهستولوجي للمناسل في المواسم المختلفة . ١- الخصية

وجد أن خصية البلطى النيلى محاطة بطبقة من الرداء الأبيض والتى تعطى فواصل من النسيج الضام تقسم الخصية الى عدد كبير من الفصيصات الخصوية المسافة بينها كانت ممتلئة بالنسيج البينى الذى يحتوى على خلايا بينية وغنى بالشعيرات الدموية وتبطن هذه الفصيصات الخصوية بخلايا سيرتولى وأنواع أخرى من خلايا مختلفة لتكوين الحيوانات المنوية وهى : أمهات المنى التى تنقسم بعد ذلك الى أمهات المنى الابتدائية و الثانوية،الخلايا المنوية الابتدائية و الثانوية والطلائع المنوية والحيوانات المنوية.

وتترتب هذه الخلايا داخل كل فصيص فى صورة حويصلات حيث تحتوى كل حويصلة على طور واحد من مراحل تكوين الحيوانات المنوية. وقد أظهرت خصية البلطى النيلى تغيرات موسمية فى تركيبها. ففى موسم الشتاء ظهرت الفصيصات صغيرة الحجم مقارنة بالمواسم التالية وكذلك كل مراحل تكوين الحيونات المنوية كانت موجودة وكانت أعداد أمهات المنى كبيرة وتعتبر الخصية فى هذا الموسم فى حالة راحة أو عدم نشاط.

وقد زاد حجم الفصيفصات في موسم الربيع ووصل أقصاه في الصيف حيث قمة موسم التزاوج ووجدت ممتلئة بكل مراحل تكوين الحيونات المنوية وقد ظهرت بعض الفصيفصات ممتلئة بالحيونات المنوية .

بينما في موسم الخريف قل نشاط الخصية عما كانت علية في مواسم التزاوج السابقة فظهرت بعض الفصيفصات فارغة تماما والبعض الآخر إحتوى على بعض الحيونات المنوية.

فلذلك تعتبر الخصية في هذا الموسم في موسم ما بعد التزاوج.

۲– المبيض

يحاط مبيض البلطى النيلى بطبقة من الرداء الأبيض ويحتوى على أمهات البيض وحويصلات في مراحل تطويرية مختلفة . وتظهر أمهات البيض كخلايا دائرية صغيرة في صورة تجمعات أو أعشاش .

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تشتمل مرحلة ما بعد تكوين المح على الحويصلات الناضجة والتي تتميز بهجرة نواتها إلى الطرف وكذلك إمتلاء سيتوبلازمها بكريات المح الكبيرة .

ووجد نوع أخر من الحويصلات والتي تميزت بتضخم خلاياها الحويصلية وتدمير محها وقد عرفت هذه الحويصلات بالحويصلات المرتوقة .

وقد أظهر مبيض البلطى النيلى تغيرات موسمية في تركيبة . ففي موسم الشتاء ظهر الرداء الأبيض سميك جدا . مع سيادة الخلايا الكروماتينية و الخلايا البيضية . فلهذا يمكن إعتبار الشتاء كموسم راحة أو عدم نشاط للمبيض .

وقد قل سمك الردا ـ الأبيض في كل من الربيع والصيف مع سيادة الحويصلات ذات التجاويف الحويصلات الناضجة لذلك فيعتبر المبيض في موسم التزاوج .

بينما في فصل الخريف . كان الرداء الأبيض سميك نسبيا عما كان عليه في مواسم التزاوج. وكانت الحويصلات المرتوقة التي تدل على إقمام التزاوج هي الطور السائد في هذا الموسم لذلك فيعتبر هذا الموسم (موسم ما بعد التزاوج) .