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HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE OVARY OF THE NILE TILAPIA (OREOCHROMIS NILOTICUS) WITH REFERENCE TO THE AGE

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A B S T R A C T

This paper deals with the histological and histochemical aspects of ovarian development in the Nile Tilapia (*Oreochromis niloticus)*. A total number of 96 fish, aged from one day to six months were used in this study during the period from May to December 2010. Ovaries of Nile tilapia were located in the posterior-dorsal part of the celomic cavity, ventral to the kidneys and the swim bladder, to which it was attached by the mesovary originating from the peritoneum. Ovary is covered with very thin capsule of fibrous connective tissue, the tunica albuginea, which give rises to ovigerous lamellae. The microscopic analyses revealed five stages of ovarian development in addition to oogonia: phases I (chromatin nucleolus stage) and II (perinucleolar stage; corresponding to the immature stage of development), phases III (cortical alveoli) and IV (previtellogenic; corresponding to the maturing stage), and phase V (vitellogenic; corresponding to the mature stage) and ripening stage. Ovaries of mature females presented different phases of oocyte development, indicating the multiple spawning habit of this species. Such information would be useful for studies related to the reproductive biology of Nile Tilapia that is used for commercial aquaculture around the world.

Key Words: Ages, Developmental stages, Histology, Nile tilapia and Ovary.

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1. INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) has been widely distributed and acquired great importance in aquaculture [22]. In the tropics, tilapia fingerlings become sexually mature at about 4-5 months of age [1].

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 [4]. The Nile tilapia ovarian tissue consisted of various stages of oocyte development that contained chromatin nucleolar oocyte, perinucleolar oocyte, cortical alveolar oocyte, vitellogenic oocyte with yolk granules incorporation; the large size, ripe oocyte which indicates maturation and imminent spawning [10, 18].

The vitellogenic follicles reported to contain yolk granules and large vacuoles [6]. In the Nile tilapia fish that reached the age of 5 months, the ovaries were found to contain oocytes in all of the different stages described above [18]. However, few data are available on gametogenesis in *O. niloticus* [6, 14]**.** The aim of the present study on histology of the ovary of *O. niloticus* is to provide basic information about the reproductive patterns of the fish,

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which will be useful for further applications.

2. MATERIALS AND METHODS

A total number of 96 fish were used in this study. The fish age ranged from one to sixth months. The fish were reared under normal conditions at the department of Fish Management and Diseases at the Faculty of Veterinary Medicine of Benha University during the period from May to December 2010. At the end of two first month of age, samples of the whole larvae were obtained each second day and routinely processed for histological examination. From the third month onwards, fish samples were obtained and the ovaries were dissected and fixed in Bouin's fluid. After fixation, the specimens were dehydrated in ascending grades of ethanol, cleared in xylene, sectioned at a thickness of 5-7 µ, and picked up on clean slides. Tissue sections were stained with H&E stain for general histological observations. Crossmon's trichrome and Orcein stains were used for the differentiation between the tissue fibers. PAS stain was used for the demonstration of the neutral polysaccharides [2].

3. RESULTS

The ovary of one month old larvae was found to be sandwiched between the kidney and the swim bladder dorsally and the stomach and the intestines ventrally. It was suspended in the abdominal cavity by the mesovarian, which is attached to the peritoneum (Fig 1). The ovary was enclosed by a very thin capsule consisted of collagen fibers. From the capsule, thin trabecular ovigerous lamellae were extended to the lumen and containing the germinal epithelial cells. The germinal cells, at this stage, accommodated the oogonia and the chromatin nucleolar oocytes (Fig 2).

The oogonia were small cells with light staining cytoplasm and very small nucleus, whereas the chromatin nucleolus oocytes were relatively larger with thin dense basophilic cytoplasm and larger pale nucleus that contained one or sometimes more than one nucleoli and fine chromatin meshwork. The chromatin nucleolar follicles were surrounded by single layer of flat follicular cells. With advancing age, the number of ovarian follicles gradually increased (Fig 3).

At 50 day of age there was a conspicuous increase in the fibrous components of the interstitium which was predominately collagenous at the expense of the cellular components (Fig 4). Elastic fibers were also noted beneath the capsule and around the follicles (Fig 5). There was a remarkable incensement in the ovarian size due to the ovarian follicles growth and oogonial proliferation.

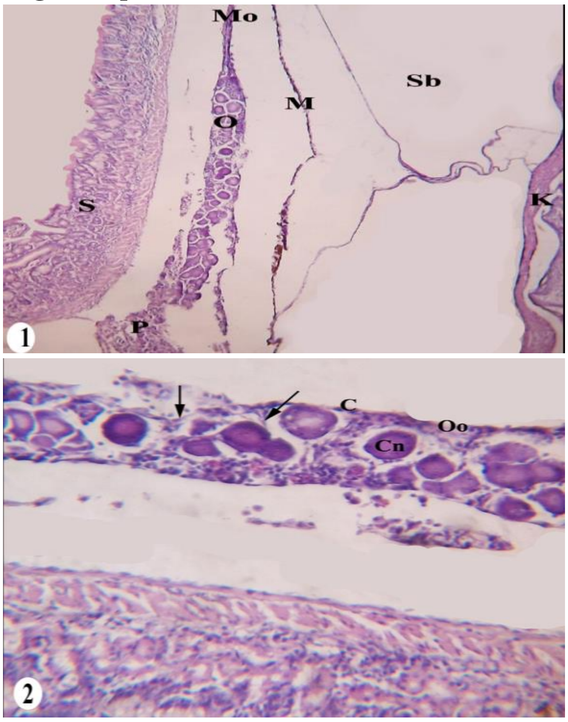


Fig. 1 One month Nile tilapia larva showed positioning of the ovary (O) between the kidney (K) and swim bladder (Sb) superiorly and the stomach (S) inferiorly. Diffuse pancreatic acini (P) are seen beside. Note; the ovary suspended in the cleome by the mesovarium (Mo) which is an extension from the mesentery (M) (H&E stain, ×40). Fig. 2 One month Nile tilapia larva Demonstrated a very thin capsule (C) and delicate lamellae (arrows) containing the ovarian follicles at the chromatin nucleolar (Cn) stage and small oogonia (Oo) next to the capsule (H&E stain, ×100).

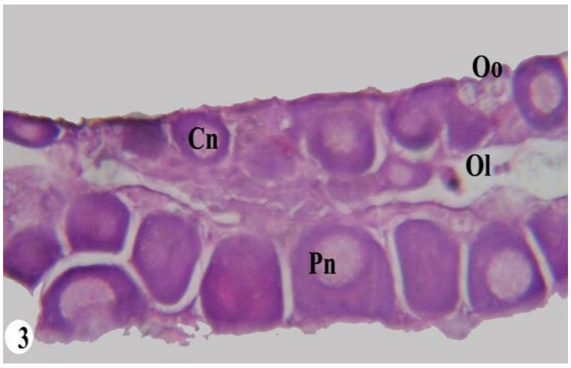


Fig. 3 Longitudinal section of the ovary at 35 days of age showed two ovarian lamellae. Ovarian lumen (Ol), chromatin nucleolar oocyte (Cn), perinucleolar oocyte (Pn), oogonia (Oo) (H&E Stain, ×400).

The oocytes at this stage reached the perinucleolar stage as was evident by the peripheral arrangement of the nucleoli at the inner side of the nuclear membrane.

At the end of the third month of age, there were no marked variations in the ovarian structure, except for progressive development of the ovarian follicles associated with looseness of the interstial tissue and appearance of adipose cells. The cortical alveoli follicles were recognized. They were characterized by the presence of small yolk vesicles at the peripheral zone of the follicles (Fig 6). With the continued development of the follicle, the numbers and sizes of these vesicles were increased.

From the beginning of the fourth month of age onward, the ovary tended to exhibited nearly the entire developmental stages characteristic of the mature ovary (Fig 7). At this stage, the nucleus of the cortical alveoli follicles started to convolute and the peripheral nuclei were still intact with the nuclear envelop (Fig 8). Later, in follicular development, the cortical alveoli follicles developed a clear zona radiata (Fig 9) associated with an increase in the height of the follicular cells till they became columnar. The theca cells were consisted of stratified squamous layer. The vitellogenic stage was more numerous at this age, and were characterized by the accumulation of yolk granules in the cytoplasm. The nuclei were still recognizable and the nucleoli were very clear. The follicular wall was trilaminar consisting of deeply eosinophilic non-cellular zona radiata, columnar to cuboidal follicular cells and a stratified thecal layer (Figs 10 & 11).

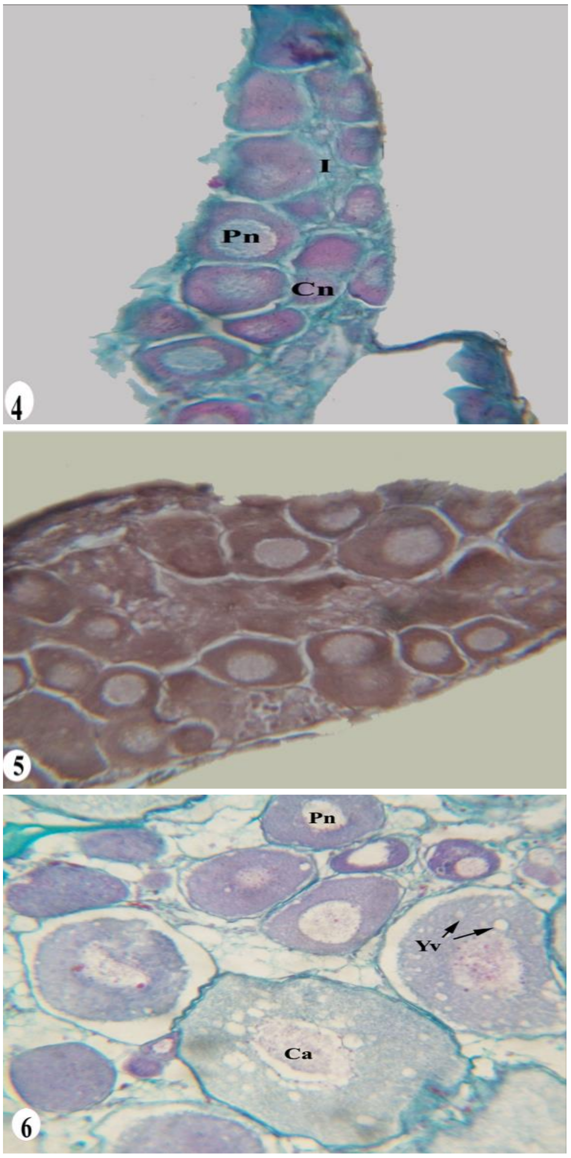
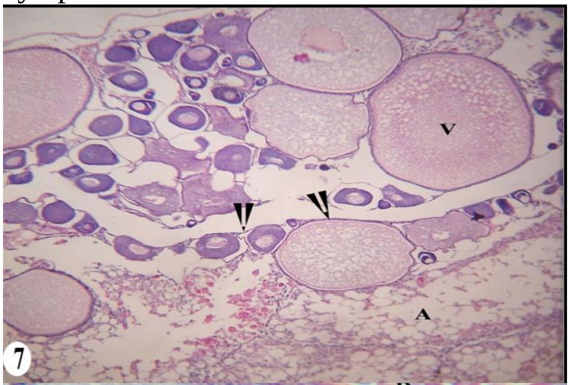
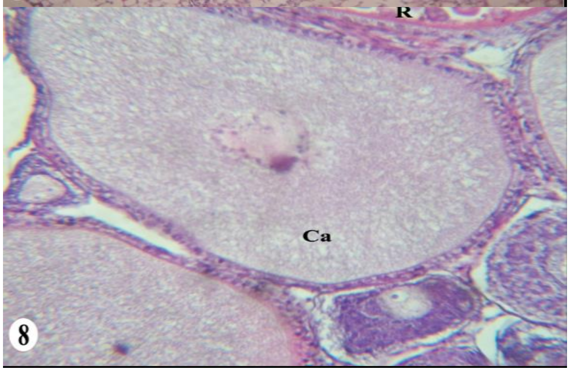


Fig. 4 Ovary of 50 days old fry showed an increase in the amount of the fibrous interstitial tissue (I); predominately collagenous. Germinal epithelium consists of chromatin nucleolus oocytes (Cn), perinucleolar oocyte (Pn) (Crossmon's trichrome, ×400). Fig 5 Longitudinal section though the ovary of about two months old fry showed the distribution of elastic fibers below the capsule and around the follicles (Orcein stain, ×100). Fig. 6 Ovary at the end of the third month showed perinucleolar follicles (Pn) with numerous peripheral nucleoli and cortical alveoli follicles (Ca) with large yolk vesicles (Yv) (Crossmon's trichrome stain, ×400).

By the end of the fourth month of age, few ripe follicles were evident and their cytoplasm contained large yolk globules. The follicular cells consisted of low columnar or cuboidal cells, and a mono-layered theca cells. With the development of the vitellogenic follicles to ripe or mature follicles, the yolk granules enlarged and aggregated in rosette shape, filling the cytoplasm of the follicle.





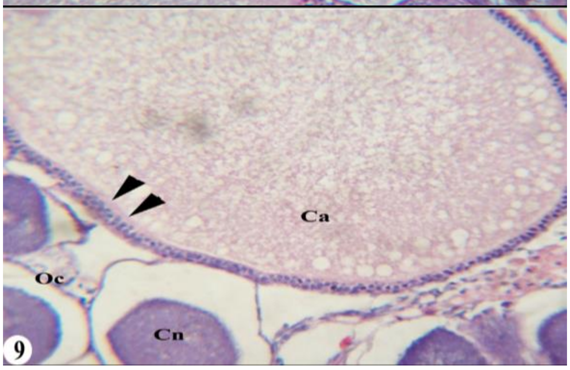
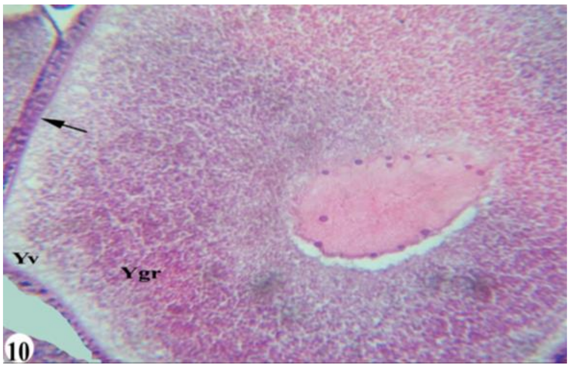
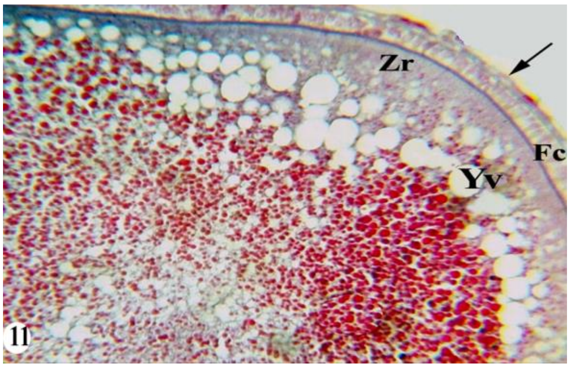


Fig. 7 Ovary at the 4th month showed ovarian lamellae (Arrow heads) and vitellogenic follicles (V). Note: adipose tissue (A) (H&E stain, ×100). Fig. 8 Ovary at the end of the fourth month of age showed all stages of follicular developmental, cortical alveoli stage (Ca) with corrugated nucleus and peripheral nucleoli and trilaminar follicular wall. Ripe stage (R) with yolk globules (H&E stain, ×400). Fig. 9 Cortical alveoli follicle (Ca). The follicular wall starts to develop zona radiata (head arrows). Many chromatin nucleolar (Cn) follicles are present in addition to oogonial cyst (Oc) (H&E stain, ×400).





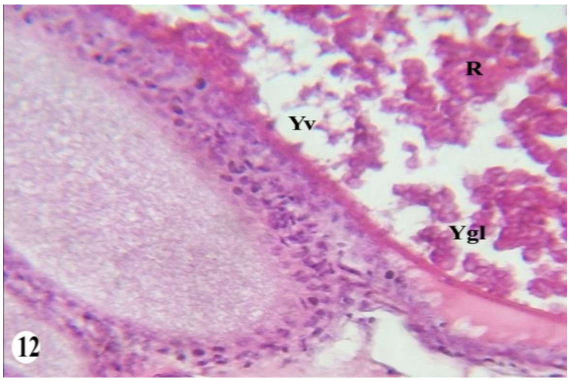


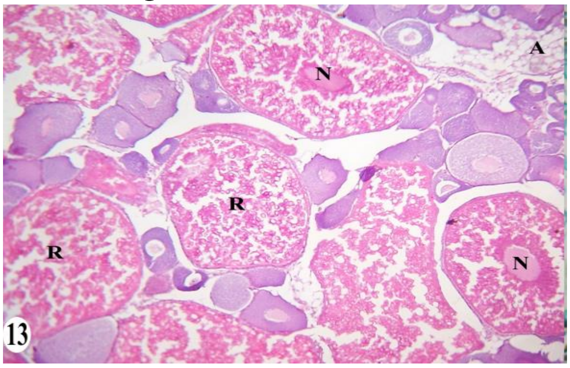
Fig. 10 Vitellogenic follicle with the yolk granules (Ygr) incorporating the cytoplasm. Note: the peripheral zone of the yolk vesicles (Yv) and the follicular wall (arrow) with a thin zona radiata and cuboidal follicular cells (H&E stain, ×400). Fig. 11 Vitellogenic follicle showed the centripetal distribution of the yolk granules associated with decrease in the granules size from outward to inward. Yolk vesicles (Yv), zona radiata (Zr), follicular cells (Fc) and theca cells (Arrows) (Crossmon'a trichrome stain, ×400). Fig. 12 Ripe follicle (R) with rosette-shaped aggregates of large yolk globules (Ygl) intermingled with the coalescent yolk vesicles (Yv) (H&E stain, ×400).

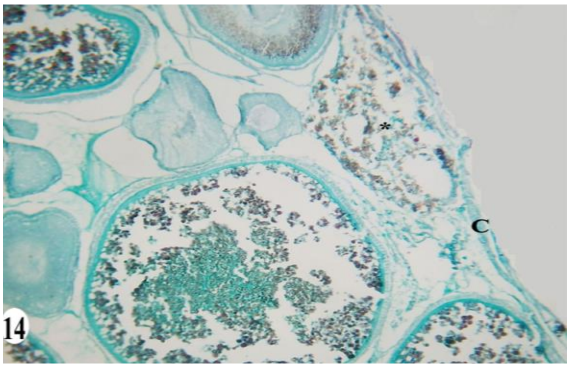
At the same time the yolk vesicle coalesced forming large cavities incorporating the cytoplasm (Fig 12). The ovarian capsule remained thin during this period, and the interstitial cells were numerous and were arranged either in short cords or in small groups.

At five months of age, the ovary size was relatively enlarged and it was found to contain numerous ripe follicles with dark eosinophilic yolk globules. The nuclei of the follicles were gradually disappeared although some of them were still remarkable. The interstitial tissue was predominately adipose tissue (Fig 13). The capsule was very thin and was firmly attached to the interstitium. With progressing development, the amount of the adipose tissue, and the extra-follicular yolk granules were increased (Fig 14). The number of mature follicles also increased with noticeable enlargement of the yolk vesicles and their fusion forming large cavities in the cytoplasm. The follicular wall of the ripe follicle showed a decrease in height of the follicular cells that became cuboidal or even low cuboidal. On the other hand, the number of the theca cells was reduced into a single layer of flattened cells. However, the infiltration of the interstitial tissue with the yolk granules was progressively increased (Fig 15). The ripe follicles of the ovary at five months of age and above showed strong PAS reactivity. The reaction was more obvious in the yolk globules and zona radiata. The zona radiata and the yolk granules of the vitellogenic follicles possessed fairly moderate reactivity, whereas the follicular cells of both follicles and the interstitial tissue were negatively reacted to PAS stain (Fig 16). The chromatin nucleolus and perinucleolar follicles strongly reacted with the PAS. The reaction was confined to the cytoplasm. The cortical alveoli follicles possessed positive reactivity exclusively in the follicular wall. The oogonia and the interstitial tissue showed moderate reactivity to PAS stain (Fig 17).

By the mid of the fifth month of age, the ovary contained all the follicular developmental stages, with the predominance of the late stages of follicular development, mainly the cortical alveoli, vitellogenic and the ripe, mature follicles.

The number of the ripe follicle continued to increase and their cytoplasm was distended with the yolk globules and consequently the follicular wall was thinner and the follicular cells became cuboidal or low cuboidal with a marked reduction in the thickness of zona radiata and the thecal layer became single layer. The interstitium was almost highly vascularized adipose tissue (Fig 18). The extra follicular deposition of the yolk was continued throughout this period. The tunica albuginea was thin.





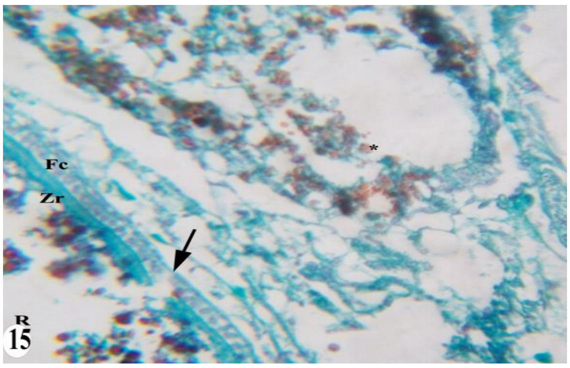


Fig. 13 Ovary at 5 month of age showed ripened follicles (R) possesses nuclei (N), distended yolk globules and an increased amount of adipose tissue (A) (H&E stain, × 100). Fig. 14 Five months old Nile tilapia showed very thin capsule (C). Note: yolk deposition in the interstitial tissue (*) (Crossmon's trichrome stain, × 100). Fig. 15 Ripe follicle (R) with yolk globules, thick zona radiata (Zr), cuboidal follicular cells (Fc) and squamous thecal cells (arrow). Note: profuse extra follicular deposition of the yolk granules (*) (Crossmon's trichrome stain, ×100).

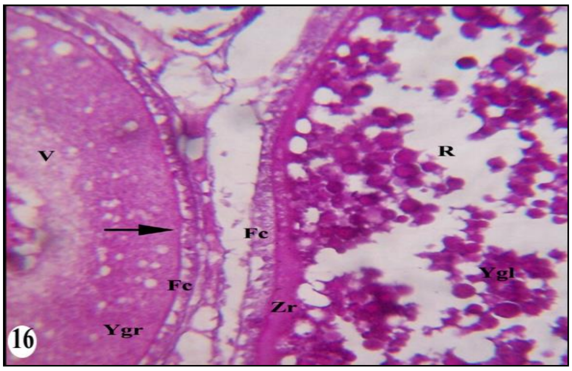
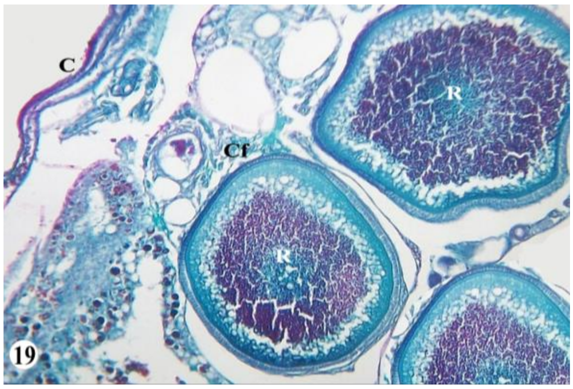


Fig. 16 Strong PAS positive reaction in the yolk globules (Ygl) and the zona radiata (Zr) of a ripe follicle (R), whereas the yolk granules (Ygr) and zona radiata (arrow) of the vitellogenic follicle (V) showing moderate reactivity. The follicular cells (Fc) of both follicles and the interstitial tissue are negatively reacted (PAS stain, ×400).

At the sixth month of age,

The ovarian capsule was thin and made up of collagenous fibers. Large blood vessels were present in the interstitium just beneath the capsule (Fig 19). All the follicular stages of development were clearly identified (Fig 20).

Fig. 17 Strong PAS positive material in the cytoplasm of the chromatin nucleolar (Cn), perinucleolar follicles (Pn) and in the follicular wall of the cortical alveoli follicles (Ca). Note: oogonial cyst (Oc) with moderate reactivity (PAS stain, ×100). Fig. 18 Ripe follicle (R) distended with yolk globules (Ygl). The follicular wall consists of thin zona radiata (arrow) and low cuboidal follicular cells (Fc) with the absence of the thecal layer. Note the highly vascularized interstitial tissue with large adipocytes (A) (H&E stain, ×400).



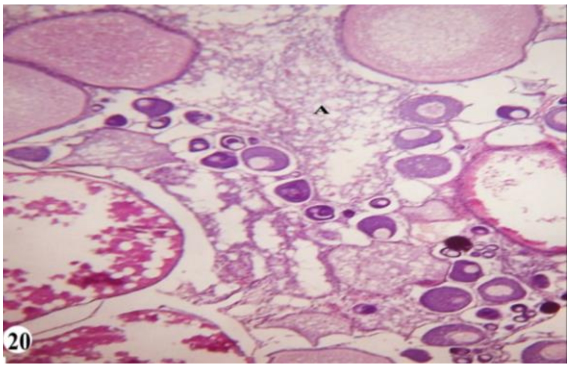


Fig. 19 Ovary of 6 month old Nile tilapia showed the ovarian capsule (C) and the underlying interstitial tissue consisting of collagen fibers (Cf). Ripe follicles (R) (Crossmon's trichrome stain, ×400). Fig. 20 Tilapia ovary at 6 months of age showed all the developmental stages of the germinal epithelium embedded in a stroma with abundant adipose tissue (A) (H&E stain, ×100).

4. DISCUSSION

Previous studies concerning ovarian development and folliculogenesis have been made for many species of teleost fish [9, 13, 15]. These studies have shown that the presence of gonadal structures (i.e. formation of ovarian cavity), difference in germ cells number, and female earlier meiosis are valid criteria to determine gonadal sex at early phases in development.

The position of the developed ovary in the peritoneal cavity dorsal to the gut and ventral to the developing kidney and its attachment to the mesentery through the meso-ovarian has been noticed in many fish species including *Cichlasoma dimerus* [13] and rosy barb [3]. Nile tilapia in the present study was no exception. In *Cyprinus caprio* the developing ovary remains compact for the first 90 days post fertilization, then small invaginations start to form along its ventral region to form the ovigerous lamellae [12]. Ovigerous lamellae in developing Nile tilapia ovary in this study were seen earlier by the end of the first month.

The grouper ovary has been divided into three developmental ovarian-phases, which were Ov-1 with the formation of the ovarian lumen; Ov-2 with the occurrence of gonial meiosis concomitantly with the development of ovarian lumen; and Ov-3 with the development of cortical alveolus stage oocytes [11]. With the application of the previous classification on Nile tilapia in the present investigation, the first stage was completed by the end of the first month, the second stage could be equivalent to the period up to the middle of the third month, and the third stage from nearly the end of the third month were cortical alveoli were evident for the first time.

The establishment of ovarian follicles in medaka was noted by the 20 day post fertilization [17]**.** This in agreement with the present study in that the chromatin nucleolus follicles in tilapia were distinguished earlier before the completion of the first month of age when the chromatin nucleolus oocytes were surrounded by a single layer of follicular cells. The cytoplasmic basophilia of this stage has been attributed to the accumulation of cytoplasmic mRNA [16].

Later, in tilapia development, at about 50 days post hatching, the chromatin nucleolus follicles reached the perinucleolar stage in which multiple nucleoli in the peripheral region of the oocyte nucleus were clearly observed. In *Odontesthes bonariensis* [19] and in *Cichlasoma dimerus* [13], the presence of perinucleolar follicles were obvious after 119 and 100 day post hatching, respectively; whereas in bluegill sunfish [7], they were noticed earlier, about 60 days post hatching. The appearance of multiple peripheral nucleoli of the oocyte nucleus indicates an intense transcription of ribosomal mRNA [17].

The presence of the cortical alveoli stage in the ovary of Nile tilapia were evident late in development in fish older than three months. This finding was in accordance with that documented in *Odontesthes bonariensis* [19] in which the cortical alveoli were not recognizable until after 140 day post hatching. in zepra fish both perinucleolar oocytes and cortical alveolar oocytes at 50 day old onward [20].

Many terms have been applied to the structures that accumulate in oocyte cytoplasm at the cortical alveoli stage: intra-vesicular yolk, endogenous yolk, cortical vesicles, and cortical alveoli [16, 21]. It has been established that somatic cells from the ovarian stroma of the common snook [8] and the swamp eel [15] become distributed around follicle cells and form the theca.

The accumulation of yolk granules in the cytoplasm of the oocyte indicating the beginning of the vitellogenic stage [5]. The yolk materials are of hepatic origin and are transported via blood circulation and formed into yolk granules in the inter-follicular spaces, and then into the oocyte by pinocytotic uptake [18]. This could provide an explanation for the accumulation of the yolk material in the ovarian interstitial tissue and the centripetal distribution of the yolk granules in the ooplasm noted in the present study.

The present study on ovarian histology of *O. niloticus* revealed the basic histological architecture and identified the oocytes found within the ovary during its development from one to sixth month of age. It provided a basic knowledge regarding the development of the ovary in the Nile tilapia. This information would be useful for other studies in reproductive biology of this popular fish which is used for commercial aquaculture around the world.

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دراسات نسيجية ونسجوكيميائية علي مبيض البلطي النيلي في الاعمار المختلفة

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الملخص العربى

الدراسات النسجيه لتركيب الاعضاء التناسليه انحصرت علي الانواع التجاريه من الاسماك مثل اسماك السالمون، البلطي و المبروك. تمت دراسة المراحل المختلفه لتطور الاعضاء التناسليه في الاسماك لمعرفة الية وتنظيم تكون البويضات. في هذا البحث تمت دراسة نسيجية ونسجوكيميائية لتطور مبيض البلطي النيلي. البلطي النيلي هو النوع الرئيسي في جنس البلطي والذي يوجد في افريقيا. حديثا" انتشر البلطي النيلي بصوره كبيره واكتسب اهميه كبيره في الاستزراع السمكي الذي يعتمد بصورة اساسيه علي الاداء التناسلي للاسماك. تم استخدام عدد 96 من اسماك البلطي النيلي في هذه الدراسه تراوحت اعمارها مابين شهر الي سبعة اشهر. تمت رعاية الاسماك بصوره طبيعيه في قسم رعاية وامراض الاسماك في كلية الطب البيطري بجامعة بنها خلال الفتره من مايو الي ديسمبر 2009. يقع المبيض في الجزء الخلفي الظهري من من التجويف البطني، أسفل للكليه والمثانة الهوائيه والتي يتصل المبيض بها عن طريق الرباط المبيضي الذي ينشأ من البريتون. يحاط المبيض بطبقه رقيقه من الغلاله البيضاء والتي تمتد لتكون الصفاحات المبيضيه. اظهرت الدراسه النسجية وجود خمسه مراحل لتطور الجريبات المبيضيه هي طور كروماتين النويه وطور حول النويه وطور الحويصلات القشريه (هذه المراحل الثلاث تمثل المراحل ماقبل البلوغ) وطور الحويصلات المحيه (تمثل بداية مرحلة البلوغ) وطور النضوج (ويمثل اكتمال مرحلة البلوغ) بالاضافه الي طور الحويصلات الفارغه والتي تنتج من عملية التبويض. يحتوي المبيض في الاناث البالغه علي كل اطوار نمو الحويصلات المبيضيه دلاله علي طبيعة التبويض المتعدد لهذا النوع من الاسماك.

(مجلة بنها للعلوم الطبية البيطرية: عدد 23(2)، ديسمبر 2012: 61-70)