The Natural History Journal of Chulalongkorn University 1(1): 53-59, August 2001


# Histological structures of Nile Tilapia *Oreochromis niloticus* Linn. Ovary

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**ABSTRACT.– Basic Histology of female reproductive organ of Nile tilapia *Oreochromis niloticus* Linn. was studied. Sampling was initiated at the age of 4 months until the age of 8 months. The data on relative ovary weight showed that the GSI of *O. niloticus* increased in the initial phase and then became stable at the later period. 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: Chromatin nucleolar stage; Perinucleolar stage; Cortical alveoli formation stage; Vitellogenic (yolk) stage; Ripe (mature) stage. Ovarian interstitial tissues were found to consist of interstitial cells, adipose cells, yolk granules and blood capillaries. Oogonial cyst was noted in ovarian tissues of the fish at the age of 5 months. Postovulatory structure, corpora lutea was also observed in the ovary at the age above 6 months. Intraoocytic and extraoocytic deposition of yolk granules and yolk materials was always seen at the age above 6 months. The ovary of the fish that reached the age of 5 months contained all oocytes of different stages.**

**KEY WORDS**: ovarian histology; oocyte developmental stage; Nile tilapia

## INTRODUCTION

Nile tilapia *Oreochromis niloticus* Linn. is a well known fresh water fish of the big order Perciformes, family Cichlidae. It originated exclusively from the African continent and from Palestine. Introduction of tilapias outside Africa was begun since 1939. Now tilapia occur in natural waters throughout the tropics, even in Australia (Philippart and Ruwet, 1982). They are fish of economic importance in tropical and subtropical countries. *Oreochromis niloticus* was first introduced to Thailand in March 1965 by His Imperial Majesty Akihito, the Emperor of Japan. Consequently, they were given to the Department of Fisheries for further development of culturing by His Majesty the King of Thailand (Phumipat, 1981). Nowadays, Nile tilapia is an essential food fish, widely cultured in many areas throughout Thailand.

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 technique. Histology is the most accurate

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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.

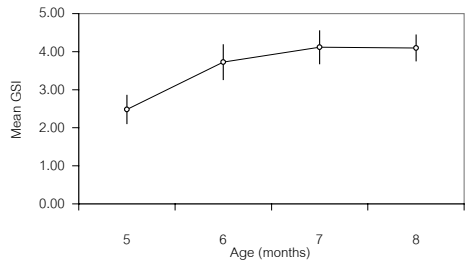



FIGURE 1. Mean (±SE) gonadosomatic indices (GSI) of *O. niloticus* between the age of 5 and 8 months.

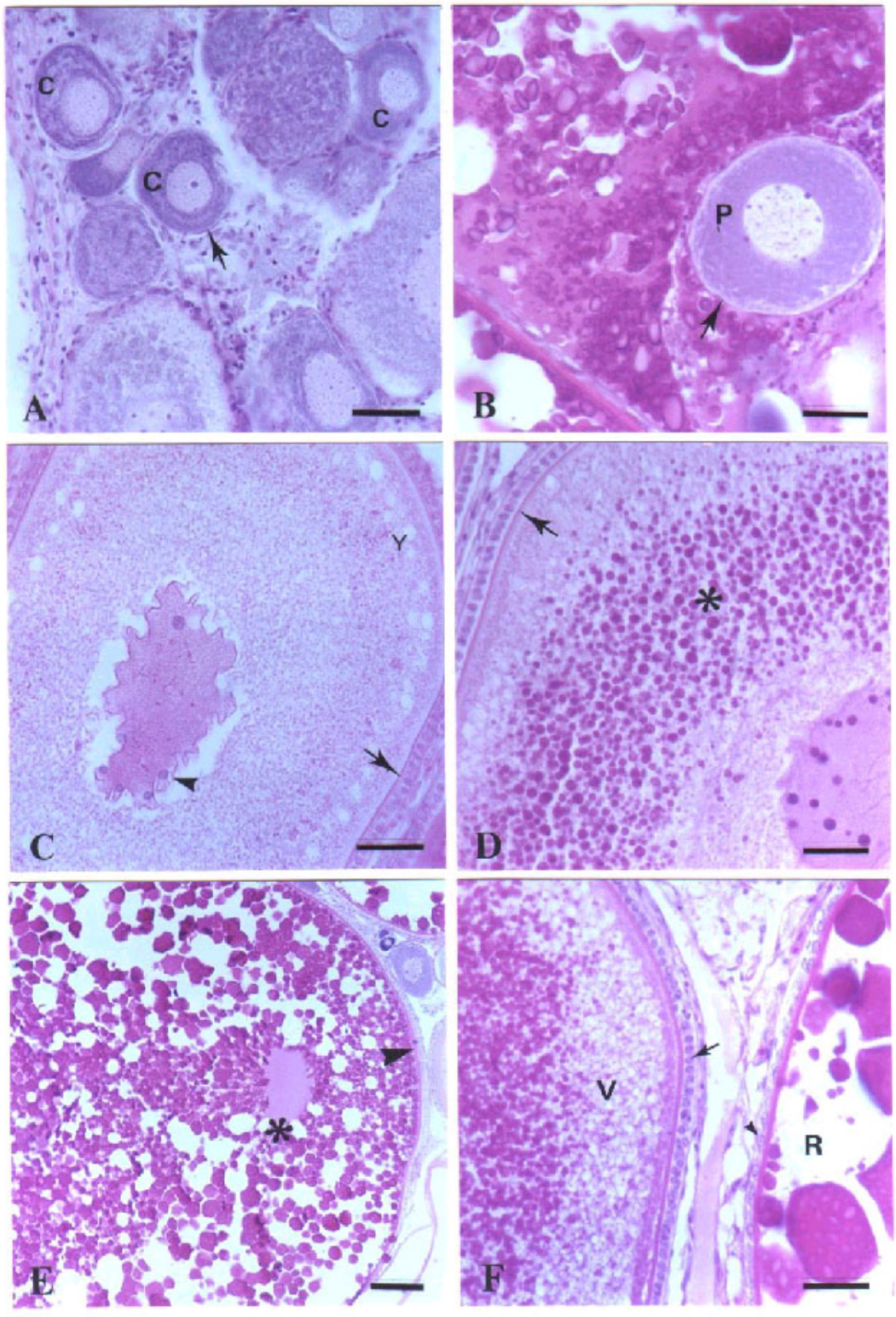
## MATERIALS AND METHODS

The *O. niloticus* broodstock used in this study were obtained from Pathumtani Breeding Station, Department of Fisheries, the Ministry of Agriculture and Cooperatives of Thailand. The fry (at the age of 2 days) were reared in 325-L glass aquaria in static water renewal system. The fish was fed commercial pellets (CP Company) twice daily at approximate 3-5 % body weight throughout the study. Water temperature ranged between 24 and 27 °C. Dissolved oxygen ranged between 7 and 7.4 mg/l and hardness between 66 and 112 mg/l as $CaCO_3$. The pH value ranged between 5.4 and 5.9.

The fish were sampled (n= 20) begin from the age of 4 months because the ovaries were obviously recognized and possibly dissected. Sampling was initiated by dipnetting randomly selected fish from each aquaria every month until the age of 8 months, the age in consideration of sexual maturation. The length (cm) and weight (g) of each fish was measured. Then, they were cool shocked at 0 °C. The left and right ovaries were removed and weighted and then fixed in Bouin's fixative for 48 hours and preserved in 70% ethanol. The relative gonad weight or gonadosomatic index (GSI) of female fish were calculated by dividing the ovaries weight by the whole body weight and multiplying by 100. The female reproductive maturity were determined by analysis of the GSI. The ovaries were processed according to standard histological techniques (Humason, 1979; Preece, 1972). All tissue blocks were sectioned at 7 μm and stained with hematoxylin and eosin. Histological study were performed by light microscopy.

FIGURE 2. Photomicrograph of *O. niloticus* ovaries showing oocytes in different stages of development (H&E stain).

A. Ovary of *O. niloticus* shows chromatin nucleolar stage of oocyte (C) with a big nucleolus in nucleus and deeply basophilic cytoplasm. Follicular monolayer is seen as simple squamous lining (⇔). Bar scale = 30 μm.
B. Ovary of *O. niloticus* shows perinucleolar stage of oocyte (P) with several small nucleoli attached to nuclear membrane. Follicular monolayer is seen as simple squamous lining (⇔). Bar scale = 30 μm.
C. Ovary of *O. niloticus* shows cortical alveolar stage of oocyte with perinucleoli attached to the convoluted nuclear membrane (™) and cortical alveoli or yolk vesicles (Y) formed at the periphery of the oocyte. Thin acidophilic *zona radiata* (⇔) and columnar follicular cells surrounded by squamous thecal cells are seen. Bar scale = 30 μm.
D. Ovary of *O. niloticus* shows vitellogenic stage of oocyte with acidophilic yolk granules incorporated in the cytoplasm (*). Follicular trilayer consists of *zona radiata* (⇔), simple culumnar follicular cell layer and stratified squamous thecal cell layer. Bar scale = 30 μm.
E. Ovary of *O. niloticus* shows ripe stage of oocyte with migratory nucleus (*). Follicular trilayer consists of thick acidophilic *zona radiata* (™), cuboidal follicular cell layer and stratified squamous thecal cell layer. Bar scale = 100 μm.
F. Ovary of *O. niloticus* shows vitellogenic oocyte (V) comparing with ripe oocyte (R). The follicular cells of the vitellogenic oocyte are columnar cells (⇔) whereas that of the ripe oocyte are low cuboidal cells (™). Disappearance of the thecal layer of the ripe oocyte is noticed. Bar scale = 30 μm.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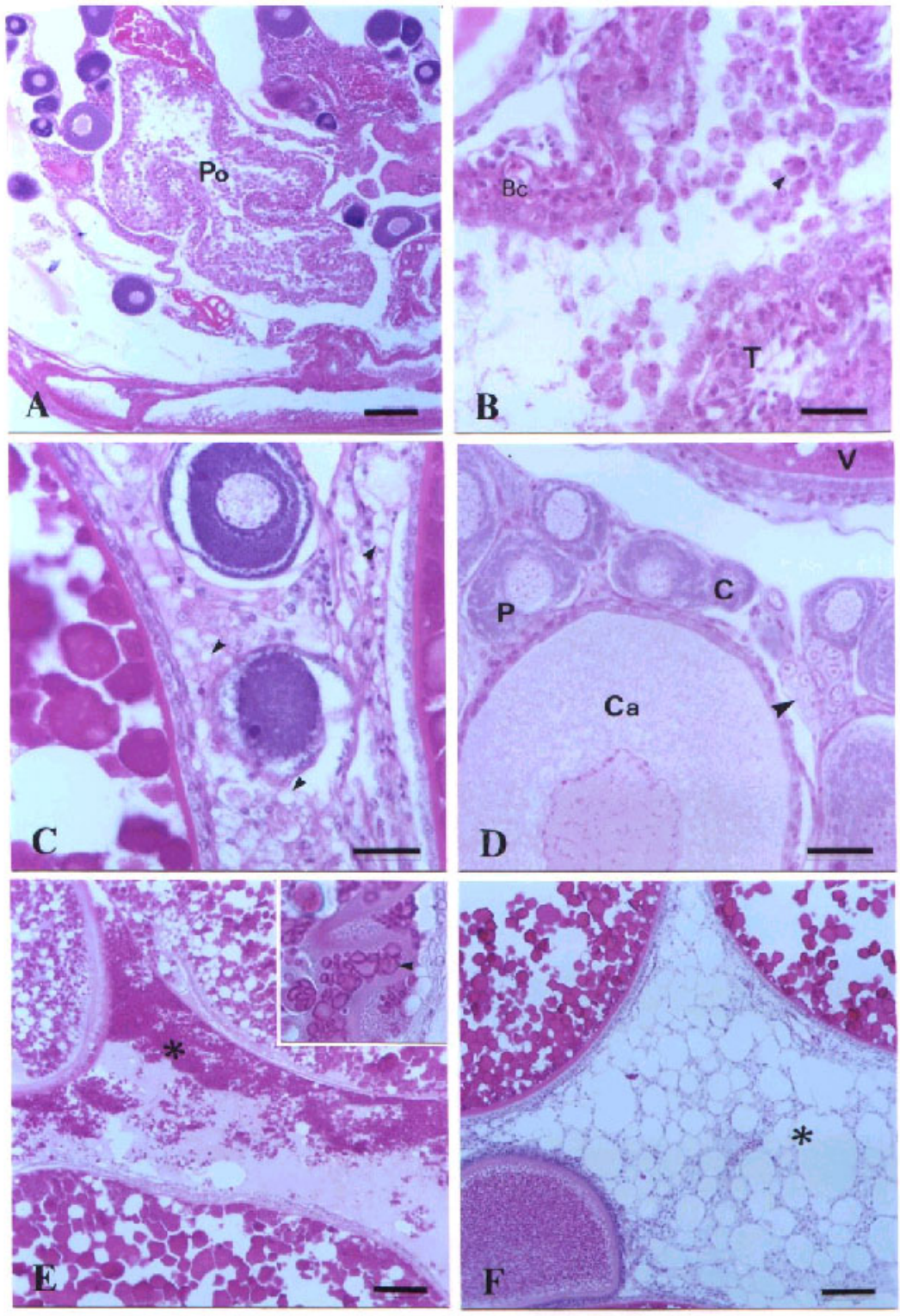
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## RESULTS

*1. Gonadosomatic index (GSI)*

The GSI of *O. niloticus* increased in the initial phase (age: 5-6 months) and became stable at the later period (age: 7-8 months). The GSI ranged from 2.48±0.37 at the beginning of sampling period to 4.10±0.34 at the end (Figure 1).

*2. Basic histology of O. niloticus ovary*

Histological characteristics of ovarian tissues of the fish at the age of 4 to 8 months were investigated. Developmental stages of oocytes were determined upon the histological characteristics according to the stages classified by West (1990) and were modified as followed.

1. Chromatin nucleolar stage
2. Perinucleolar stage
3. Cortical alveoli formation stage
4. Vitellogenic (yolk) stage
5. Ripe (mature) stage

The observation via light microscopy in this study revealed different histological structure of each oocyte developmental stage.

Chromatin nucleolar stage: the oocyte was small spherical cell containing a central nucleus. The nucleus contained one to four nucleoli together with chromatin network. Cytoplasm was thin layer and strongly basophilic. Follicular cell was difficult to see (Figure 2A). It was predominantly found at the age of 4 months.

Perinucleolar stage: the number of nucleoli increased and arranged along the inner side of nuclear membrane. Nucleus was large and surrounded by increased mass of cytoplasm which appeared less basophilic. Follicular cells was monolayer of simple squamous lining surrounded the oocyte (Figure 2B).

Cortical alveoli formation stage: this stage is characterized by the appearance of clear vesicles (cortical alveoli) in the cytoplasm. The vesicle was begun to accumulate from the periphery of the oocyte. The nuclei were still perinucleolar. The nuclear membrane began to be convoluted. In this stage, a thin acidophilic *zona radiata* or primary envelope became visible for the first time. Follicular layers were also seen at the first time to consist of simple cuboidal or columnar layer surrounded with stratified squamous thecal layer (Figure 2C).

Vitellogenic (yolk) stage: the oocyte size increased. Small yolk granules were visible as a ring of deep eosinophilic in the cytoplasm and later incorporated the whole cytoplasmic area. The nucleus was still convoluted. The zona radiata was clearly visible as a noncellular deep eosinophilic band. Follicular layers were well-developed simple cuboidal or columnar layer surrounded by stratified squamous thecal layer (Figure 2D and 2F).

Ripe (mature) stage: the stage was characterized by the enlargement of both cortical alveoli and yolk granules. The oocyte size markedly increased. The peripheral migration of the nucleus was observed. The zona radiata was clearly visible. Follicular cells were cuboidal or low cuboidal surrounded by thin thecal layer (Figure 2E and 2F).

FIGURE 3. Photomicrograph of *O. niloticus* ovaries showing postovulatory structure and ovarian interstitial tissues (H&E stain).

A. Ovary of *O. niloticus* contains postovulatory follicle, corpora lutea (Po). Bar scale = 100 μm.
B. Higher magnification of the *corpora lutea* shows swollen granulosa cells with pyknotic nuclei (™) at the inner part, thecal layer (T) at the outer part. Blood capillary (Bc) is seen near the irregular lining of the thecal layer. Bar scale = 30 μm.
C. Ovary of *O. niloticus* in high magnification shows interstitial tissue consisting of interstitial cells with fat cells (™). Bar scale = 30 μm.
D. Ovary of *O. niloticus* contains chromatin nucleolar oocyte (C), perinucleolar oocytes (P) cortical alveolar oocyte (Ca) and vitellogenic oocyte (V) at the upper right corner. Oogonial cysts are seen consisting of numerous nuclei (™). Bar scale = 30 μm.
E. Ovary of *O. niloticus* shows yolk materials accumulated in the interfollicular space (*). Inset shows pinocytotic uptake of yolk granules (™) on the ovarian follicle. Bar scale = 100 μm.
F. Ovary of *O. niloticus* shows adipose tissues with large fat cells (*) in the follicular space. Bar scale = 100 μm.

Ovarian interstitial tissues were found to consist of interstitial cells, adipose cells, yolk granules and blood capillaries (Figure 3). Oogonial cyst, cyst of early meiotic oocytes, was noted in ovarian tissues of the fish at the age of 5 months (Figure 3D). Postovulatory structure, corpora lutea was also observed in the ovary of the fish at the age above 6 months. The convoluted structure was found to consist of swollen granulosa cells at the inner most, surrounded with thecal cells and blood capillaries (Figure 3A and 3B). Intraoocytic and extraoocytic deposition of yolk granules and yolk materials was always seen in fish at the age above 6 months (Figure 3E).

The overall histological architecture of *O. niloticus* ovary consisted of different stages of oocytes embeded in ovarian interstitial tissues (Figure 4A). The observation by light microscopy through a number of ovarian sections revealed that the ovary of *O. niloticus* contains all stages of oocytes after they reached the age of 5 months. The oocytes and interstitial tissues are encapsulated by a connective tissue capsule consisting of germinal epithelium and tunica albuginea with blood vessels (Figure 4B).

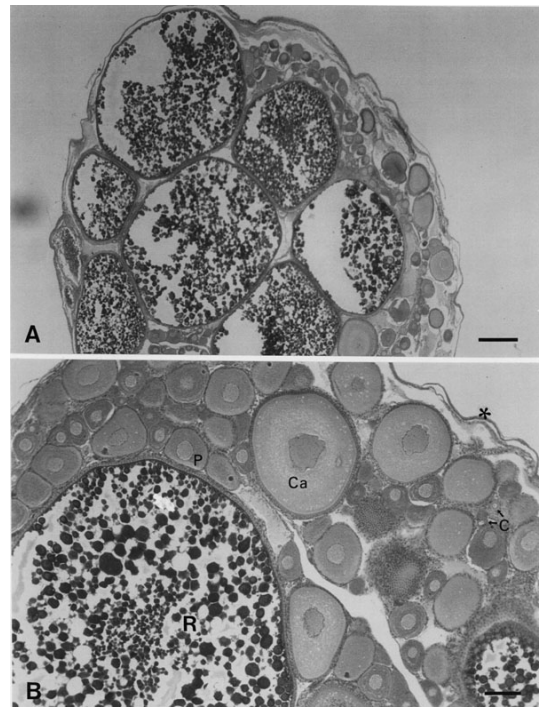


FIGURE 4. Photomicrograph of *O. niloticus* ovary at the age of 5 months showing overall histological structures (H&E Stain).

A. Ovary of *O. niloticus* contains different stages of oocytes embeded in ovarian interstitial tissue. Bar scale = 300 μm.

B. Higher magnification of the ovary shows chromatin nucleolar oocytes (C), perinucleolar oocytes (P), cortical alveolar oocytes (Ca) and ripe oocytes (R). The ovary is encapsulated by connective tissue capsule (*). Bar scale = 100 μm.

## DISCUSSION AND CONCLUSION

Female reproductive maturity was commonly quantified by the GSI. Determination by sizing is low accurate because the size to which the fish grow, and at which they mature, varied greatly (Lowe-McConnell, 1982). In general, correlated with the results of the present study (Figure 1), the GSI of fertile (2n) female tilapia *O. niloticus* was gradually increased throughout the successive age of maturity between 4 to 10 months (Hussain et al., 1996). However, determination of reproductive maturity using only the GSI is not enough because the structures within the ovary, such as oocytes at different stages, interstitial tissue with accumulation of yolk materials, can not be interpreted by weight. Direct observation of histological architecture is the most accurate method to let us know exactly the stage of maturation at which the ovary is undergoing.

Ovarian tissue of Nile tilapia consisted of various stages of oocyte development including the small size, chromatin nucleolar oocyte and perinucleolar oocyte; the medium size, cortical alveolar oocyte and vitellogenic oocyte with yolk granules incorporation; the large size, ripe oocyte which indicates maturation and imminent spawning. The result correlated with previous histological study that the ovaries of *O. niloticus* (6-8 months) contained oogonia, maturing previtellogenic and matured vitellogenic oocytes (Hussain et al., 1996). In other teleosts such as the walking catfish, *Clarias*

*batrachus*, the ovaries contained 6 stages of oocytes with similar histological characteristics with those of *O. niloticus* (Limsuwan et al.,1987). The ovaries of the fish that reached the age of 5 months were found to contain all of these oocytes in different stages. This result implies that the oocyte maturation process was complete at this age. Accumulation of yolk granules was found in both intraoocytic and extraoocytic spaces. As indirect evidences in support of the hepatic origin of fish vitellogenin was provided by a number of studies, it is generally accepted that the liver are stimulated to synthesize and secrete vitellogenin (Ho, 1987). The yolk materials are transported via blood circulation and formed into yolk granules in the interfollicular spaces, as evident in this study. The yolk granules are taken into the oocyte by pinocytotic uptake. Postovulatory follicle, corpora lutea is seen at the age above 6 months indicated spawning.

The present study on ovarian histology of *O. niloticus* revealed the basic histological architecture and identified the oocytes found within the ovary. It provides a basic knowledge for other studies such as reproductive biology, reproductive toxicoloy and histopathology of this animal.

However, sexual maturation and development of fish is also influenced from other parameters such as season, holding space, holding conditions and capture. The age of maturation and developmental process of ovary in laboratory condition might be unable to represent that of natural environment and should be carefully interpreted.

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*Accepted: 17 August 2001*