Vol. 51, n. 3 : pp.513-522, May-June 2008
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Ovary Maturatıon Stages and Histological Investigation of Ovary of the Zebrafish (*Danio rerio*)

Nazan Deniz Koç (Yön)[1]*, Yener Aytekin[2] and Rikap Yüce[1]
[1]*Marmara University; Faculty of Art and Science; Department of Biology; İstanbul;Turkey;* [2]*Istanbul University; İstanbul Faculty of Medicine; Hist. and Embry; Department; İstanbul,Turkey.*

ABSTRACT

Microscopic ovary features of Zebrafish were analyzed. Samples were fixed in Bouin's solution, mounted in parafin and cut into 6-7 µm-thick slices which were stained with Hematoxylin and Eosin (HE), methylene blue and using Periodic-acid Schiff method (PAS). The thin sections in ultramicrotome with the thickness of approximately 0,5 – 0,7 µm were examined by using Jeol electron microscope following contrasting with Uranyl Acetate-Lead Citrate. The zebrafish was an extremely dynamic organ in which follicles underwent asynchronous development. The oocytes of Zebrafish ovary were observed in various phases. The oocyte development of zebrafish was divided into four stages (primary growth, cortical alveolus, vitellogenic and mature oocyte). Oocyte diameters were observed to vary between 0.,08 mm and 0.76 mm.

Key words: Zebrafish, gonad, oocyte, development

INTRODUCTION

The reproductive cycle must ensure a sufficient quantity of mature egg cells, which is possible only within the regular process of the oogenesis. The oogenesis is a very dynamic process in the ovaries, in which the oocyte passes through various phases of the development that are very similar in different fish species. The ovaries of the fishes have been classified into three types according to the pattern of the oocyte development (Selman and Wallace, 1989). In the case of synchronic oogenesis, all the oocytes develop at the same time, ovulation also being simultaneous. [1]The group synchronous ovary consists of at least two populations of the oocytes at different developmental stages: teleosts with this type of ovary generally spawn once a year and have a relatively short breding season. In the case of asynchronic ovulation, different development stages of the oocyte maturation and ovulation in groups may be found within the ovaries (Nagahama, 1983;Nejedli et al 2004).

The teleost oocytes as in other vertebrates are surrounded by two major cell layers as an outher thecal layer and an inner granulosa. As the oocytes grow, the follicle cells multiply and form a continuous follicular layer called the granulosa cell layer. The fish oocyte development can be divided into oocyte growth and oocyte maturation. Vitellogenesis plays an important role in the oocyte growth. Germinal vesicle migration and breakdown, coalescence of lipid droplets and yolk globules, and release of the 1st polar body are the characteristic event in the precess of maturation (Nagahama et al.1983, Yueh and Chang 2000)

Brachydanio rerio (Danio rerio) is a member of the family Cyprinidae and is native to India and Pakistan. The zebrafish, *Danio rerio*, (Hamilton-Buchanan 1822) is a widely used laboratory model

*Author for correspondence

species, especially in the developmental biology. The zebrafish model is becoming more and more popular because easy to produce. In the laboratory, zebrafish can be stimulated to breed throughout the year and the development from the fertilised egg to reproducing stage taken only about 3-4 months. Their short generation time of three months makes them an ideal candidate for the genetic studies and their susceptibility to the mutagens, carcinogens, teratogens and toxins makes them ideal environmental models. This model is also very useful for the investigation of ovarian follicle development and maturation because the zebrafish ovary contains ovary follicles at different stages of development (Maack, 1964; Garg,1998).

The structure of the ovary of zebrafish was investigated by using the histological, histomorphological, histochemical and transmission electron microscopy methods after the application of different fixatives and stains at both light and electron microcopy level.

In the present study, the oogenetic process of zebrafish was asynchronic oogenesis. The female gonad development of the zebrafish was monitored as four phases according to classification. For the zebrafish, not much literature on the gonad morphology is available but a lot of literature on toxicity in gonad of zebrafish. The report of Selman et al.(1993) illustrated the oocyte maturation and van Ree (1977) reported on the histological and histochemical studies of the zebrafish ovary. The anatomy and histology of the gonads, the thin structure of the ovary follicle (Matsuyama et al. 1991), reproduction cycle (Murua and Saorido-Rey), gonad development, reproduction and hormonal relations (Maack 1964) were investigated in different fish species. In Turkey, there have been several studies related to the anatomy and histology of the gonads in the fishes such as the reproduction model and gonad histology in *Lagos* (Gökçe et al. 2003), histological examination of thin structure of the ovary follicles and ovary steroid level alterations during the oogenesis process and gonad development in *Chalcalburnus tarichi* (Ünal et al. 1999, 2005), development of the *Liza ramada* Risso ovaries and follicle structures in their ovaries before ovulation (İşisağ, 1996), gonad anatomy and histology in gilt-head bream (Küçüktaş, 1987). There are several studies available related to the subjects including the effects of different hormones on the reproduction, reproduction physiology,reproductive toxicology and morphological development of the ovaries in Zebrafish (Maack 1964, Van Ree 1977, Garg 1998, Weber 2002, Örn 2003, Fenske 2004). There are not much studies using the histological methods on the reproduction biology of this species in the literature.

MATERIALS AND METHODS

Zebrafish were under the standardised conditions at 28^{o}C ± 1 ^{o}C. The light/ dark cycle was 14h/10h. The fishes were fed daily with *Artemia sp*. and TetraMin© Hauptfutter (Tetra Werke,Germany). For the histological analysis, the fishes were anaesthetised in the ice water and fixed as a whole Bouin's fluid for 24 h. Fixed tissue was dehydrated and embedded in the parafin wax and sectioned transversely at 6-7 µm thickness and stained with Hematoxylin Eosin and methylene blue. The Periodic Acid Schiff reaction was applied. The samples were evaluated by examining under the light microscope. The samples demonstrate the thin structure characteristics of the follicles in the ovary under microscope were prepared according to available examination procedures. The specimens were fixed in a mixture of 2.5 % glutaraldehyde and 2.5 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The ovaries were post-fixed in phosphate buffer 1% O_sO_4, dehydrated in a graded series of ethanol, followed by immersion in 1-2-propylene oxide and finally embedded in the resin. Semi-thin section were cut with a glass knife, stained with toluidine blue and examined by the light microscopy. Some of the specimens were further evaluated using the transmission electron microscopy (TEM). Ultra-thin section were cut with a diamond knife, double stained with uranyl acetate and lead citrate and examined in a Jeol TEM. For the analysis of the ovary cycle, the observed oocytes were divided into four stages according to the sizes and development stages of oocytes: primary growth , cortical alveolus (cytoplasmic and yolk growth), vitellogenic growth and mature oocyte.

RESULTS

The development of zebrafish oocytes was divided in four stages, based on morphological features. First stage was primary growth . Primary oocyte, identifiable by a few peripherally located nucleoli as well as by the small, localised areas of intense basophilia in the cytoplasm. Second stage was cortical alveolus stage. This stage was identifiable by the appearance of cortical alveoli marks (yolk vesicles) was the begining the formation of a vitellin envelope. During vitellogenic stage, the oocytes increase in size due to accumulation of the yolk. In the mature oocytes, the nucleus was dissolved and the ooplasm consisted of yolk bodies (Fig. 1).

Development stages of the oocytes

Primary growth stage

In the first growth phase**,** the multiple nucleoli were observed in the nucleus of the (germinal vesicle) oocytes (Figures 2 and 3). The oocyte diameters were found to be varying between 0.08-0.16 mm during the first growth phase of the oogenesis. There was a proportional increase observed between the oocyte growth phase in the oogenesis and the volume of the ovary or oocytes. The layers (zona radiata) around the follicle were not thick completely in the growth phase (Figures 1, 2 and 3).

Cortical alvelolus stage

In the developed cortical alveolar phase, the granular structures in the ooplasm were increased. As the oocytes grew the cortical alveoli proliferate and follicle increase in the size and the oocyte became opaque in the area that surrounded the nucleus (Figs 4 and 5). This stage was representing the oocytes with diameters of 0.16- 0.28 mm. During this stage the nucleus enlarged (Figs 4 and 5). The nucleoli were pushed from the nuclear envelope (Fig 5). In this phase the vitelline envelope (zona radiata) begin to form and the follicle epithelium became thicker (Fig 4 and 5). Invaginations of the nucleus membrane were infrequently accompanied to the irregular structure of the nucleus (Fig 5).

Vitellogenic stage (Vitellogenesis)

The oocyte size was increased in the vitellogenic phase (Fig 1). In this stage, the vitellogenesis occures in follicles with diameters ranging between 0.28-0.74 mm. In the vitellogenic phase, the appearance of cortical vesicle, which were spherically on the periphery of the cytoplasma, was observed (Fig 6). The number and size of the yolk vesicle increased. The findings related to the protein granules and lipid accumulations were evaluated by the electron microscopy sections (Fig 7). The granular structures appeared in the cortical alveolar phase were larger and the nucleus was irregular in the shape. (Fig 6). Vitellus density in the oocyte was extended towards the center from the cortical alveolar area (Fig 6). The vitellin membrane began to develop at this stage.

Mature oocytes

The nucleus could not be observed due to the fact that the granular structures filled up the entire cytoplasm in the mature oocyte phase (Fig 8). The oocyte diameter reached 0.74-0.76 mm, which was the maximum size of the oocytes during the oogenesis. The membrane of the nucleus dissolved. The lipid and protein particles were fused and demonstrated a homogeneous appearance. The vesicles gradually joined and became larger (Figs 8 and 9). As appeared in the cortical alveolar phase and continued its development, the vitelline envelope clearly evident in this stage. Vitellin membrane which constituted the inner zone of the vitelline envelope started to disintegrate by leaving the void spaces from the outer parts (Fig 8). Outside of the membrane, the follicle epithelium cells were seen with their uniformly arranged nuclei (Fig 9). The structure of the vitelline envelope was monitored clearly by using the optic and electron microscope (Figs 8 and 9).

Atretic oocyte

In the Atretic oocyte**, the** vitelline membrane structure also started to disintegrate in accordance with the chromatin deformations in the nucleus (Fig 12). The vesicles were fused thoroughly with each other (Fig 10). The openings in the outer areas of the vitellus membrane were observed (vitelline envelope breakdown and yolk resorpsion) (Fig 12).

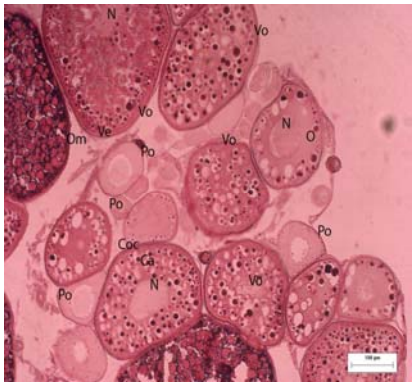

Figure 1 - General view of the ovary of the Zebrafish. Oocytes at different stages of development. Light Microscope 10x PAS. **Po**- Primary oocyte ,**Coc**- Cortical alveolus stage, **Vo**- Vitellogenic Stage,**Om**- Mature oocyte, **Ca**-Cortical alveoli, **N**-Nucleus,**ve**- Vitellin envelope, **O**- Ooplasm

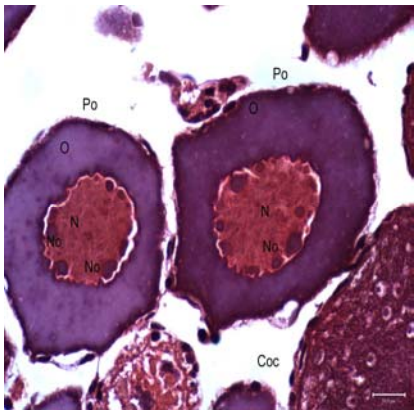

Figure 2 - Perinucleolar stage of primary oocyte growth Several nucleoli appear at the periphery of nucleus. Light microscope 100x Hematoxylin&Eosin. **Po**-Primary Oocyte ,**N**-Nucleus,**No**- Nucleoli, **Coc**-Cortical alveolus stage,**O**- Ooplasm

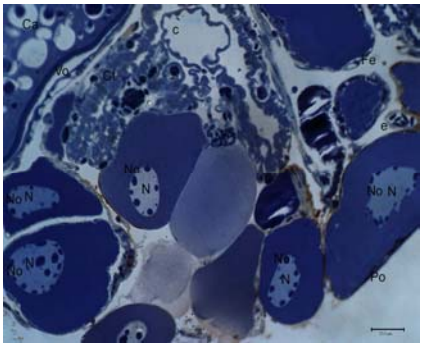

Figure 3 - Toluidine blue application to the section with the thickness of 0,7µm obtained from electron microscope block. Follicles during the first growth phase. 40x. **Po**-Primary oocyte, **N**- Nucleus ,**No**-Nucleoli ,**Vo**- Vitellogenic stage, **Ca**-Cortical alveoli, **Fe**-Follicular epithelium , **Ct**-Connective tissue, **c**- vessels,**e**- erythrocyte

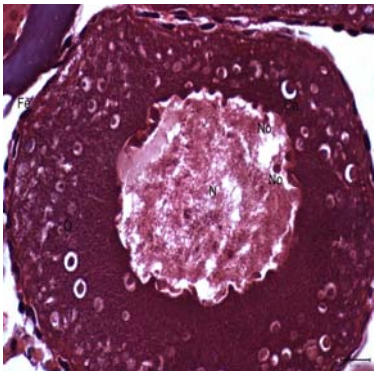

Figure 4 - Oocyte in cortical alveolus stage. Cortical alveoli fill the oocyte cytoplasm. Nucleus enlarges and becomes irregular in shape. It is notable that the chromatin material is also irregular. 100x H&E. **N**-Nucleus, **No**-Nucleoli, **Fe**- Follicular epithelium, **O**-Ooplasm, **Ca**- Cortical alveol

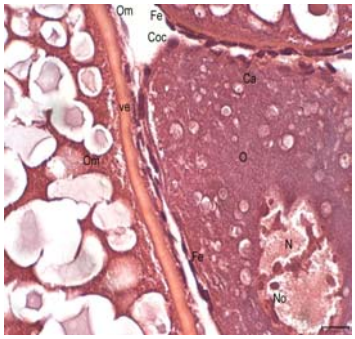

Figure 5 - Cortical alveolar phase; mature oocyte. 100x H&E. **Om**-Mature oocyte, Coc- Cortical alveoli stage, **O**-Ooplasm, **N**-Nucleus, **No**-Nucleoli, **Ca**-Cortical alveoli, ve-Vitellin Envelope, **Fe**-Follicular epitelyumium

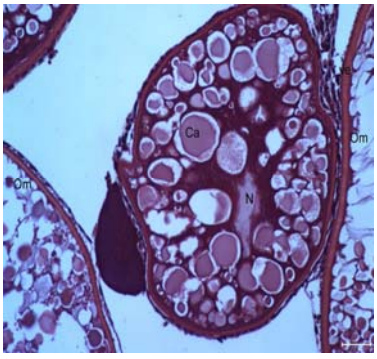

Figure 6 - Light micrograph of vitellogenic oocyte.During this growth stage the oocyte increases in size.Cortical Alveoli are progressively displaced towards periphery. 40x H&E. **Om**-Mature oocyte, **Ca**-Cortical alveoli, **N**- Nucleus

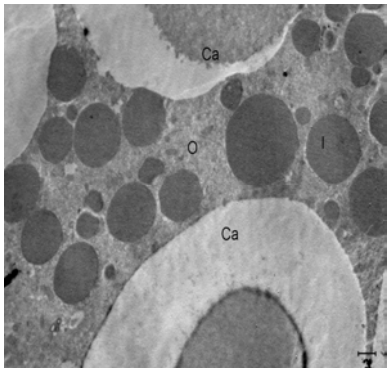

Figure 7 - Electron micrograph showing ooplasm of vitellogenic oocyte. 6000x. Rich of the protein ooplasm (**O**), **Ca**-Cortical alveoli (yolk), l- Lipid

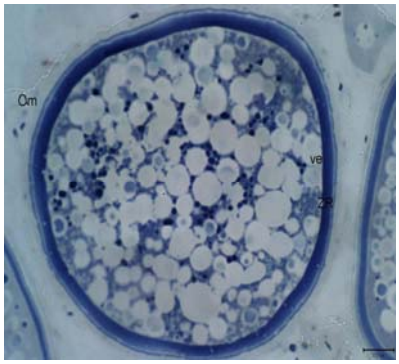

Figure 8 - Electron micromicrograph of mature oocyte. Vesicles in oocyte plasma form bigger vesicle by integration 40x Toluidin blue. **Po**- Primer ooctye, **Om**- Mature oocyte **ve**-Vitellin envelope, **ZR**- Zona radiata, **Ca**- Cortical alveoli

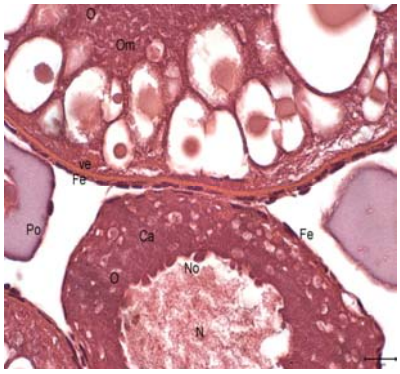

Figure 9 - Mature oocyte and cortical alveoli stage.100x H&E. **Po**-Primer folliculer. Nucleus with dispersed and irregular chromatin. **N**-Nucleus, **No**-Nucleoli, **O**-Ooplasm, **Ca**-Cortical alveoli, **ve**-Vitellin envelope, **Fe**- Folliculer epithelium, **Om**- Mature ooctye

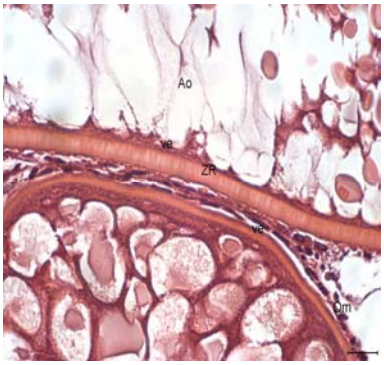

Figure 10 - Light micrograph of atretic oocyte stage.100x H&E. Oocyte in two different phases. Line formed by microvilli in Zona radiata. **Ao**-Atretic oocyte, **Om**-Mature oocyte, **ve**- Vitellin envelope, **ZR**- Zona radiata

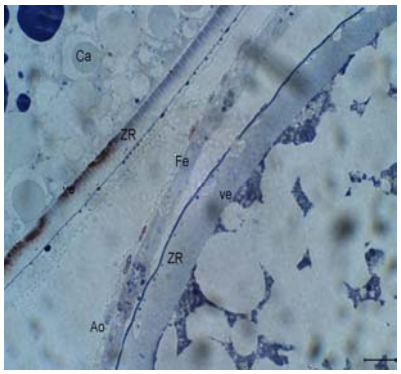

Figure 11 - Toluidine blue application to the sections with the thickness of 1µ obtained from the blocks prepared for electron microscopy. Different ooplasm content correspond to two different oocyte and related alterations in Zona radiata are notable. 100x Toluidin blue. **Ao**-Atretic oocyte, **Fe**-Follicular epithelum, ve-Vitellin envelope, **Ca**-Cortical alveoli, **ZR**-Zona radiata

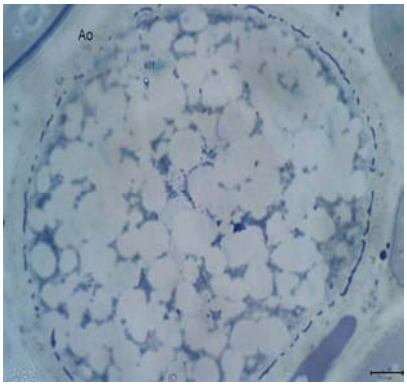

Figure 12 - Toluidine blue application to the sections with the thickness of 1µ obtained from the blocks prepared for electron microscopy. 40x. Fused vesicles in the follicle in Atretic oocyte phase. **Ao**-Atretic oocyte

DISCUSSION

Reproductive studies of the fishes require knowledge of the stage of the gonad development in the teleosts. The structural alterations were observed in the zebrafish oocytes during the oocyte development in the histological studies performed. In this study, the oocyte development of the zebrafish was divided to four stages. In the majority of teleost fishes, the process of oogenesis may be divided to five, six or eight stages (Fishelson et al. 1996, Nagahama 1983, Ünal et al. 1999, West 1990, Gökçe et al.2003, İşisağ 1996). Arockıaraj et al. (2004) described the morphological changes in the gonad of *Mystus montanus* and histologically divided into five stages. Ovaries of *Trigla lyra* contain oocytes in different synchronous groups of development which are discharged as they mature. In addition to the postovulatory follicles and atretic oocytes, seven stages of development are described based on the histological and ultrastructural characteristics of the oocytes (Munoz et al 2002). According to Nejedli et al. (2004), the oogenesis in Sardines is manifested by a series of changes in the oocytes, which makes their division into four basic group. According to Fishelson et. al (1996) and West (1990), the nucleus was large in the primary growth phase, with 2-4 nucleolus situated in the centre of the nucleoplasma. Yolk vesicles were seen on the cytoplasma at the cortical alveolus phase. The nucleus membrane dissolved and the peripheral migration of the nucleus started in the mature oocyte phase. In the present study, all the stages were identified in similar manner. Because of the sizes of the oocytes, the number of nucleolus may vary between the species (Fishelson et. al 1996). In this study, which was performed related to ooctyes development, two inclusion formations were considered as significant such as lipid and protein formation in vitellogenesis phase of the oocytes. The amounts and sizes of the protein granules and the lipid droplets were increased. The inclusion particles were fused and formed spaces. The lipid droplets begin to accumulate in the cytoplasm during the secondarty growth phase in *Trigla lyra* described by Munoz (2002). According to Yueh and Chang (2000), the morphological changes of the oocytes of black porgy during maturation were similar to those of other teleost fish. Vitellogenic oocytes proceed through final maturation with coalescence of the yolk globules and oil droplets. The oocyte diameter gradually increases. The sizes of the oocytes are evenly distributed in the range of oocyte diameter from 0 to 400 µm in the late vitellogenic *Acanthopagrus schlegeli*. There is a close relationship between the amount of vitellogenesis and oocyte size. The vitellin membran appears commonly at the yolk vesicle stages and sometimes at the late perinucleolus or at the of the yolk vesicle stages (West 1990, Ünal et al. 1999,2005). In this study, the vitellin membrane began to develop at the vitellogenic growth stage. In another study the macro- and microscopic ovary features of *Hemiodus microlepis*, *H. ternetzi* and *H. unimaculatus* were analyzed by Brandao et al. (2003). The microscopic analysis indicated a group-synchronous oocyte development, common to the three species that were characterized as iteroparous synchronous spawners with a total spawning. The remarkable thickness of the vitelline membrane layer and the large size of the vitellogenic oocytes of *Hemiodus ternetzi* distinguished this species from the others. The oocyte development of these three species was very similar. The increase in the number of the oocytes in the cortical alveolus stage and vitellogenic stages of zebrafish was also accompanied by an increase in their diameter (Van ree 1977, Garg 1998), which was characteristic of the oogenesis of the fish, as described in other fishes (Selman and Wallace 1989, Ünal et al. 1999, Gökçe et al.2003, Pina 2003, Ünver and Saraydın Ünver 2004). The appearance of the yolk vesicle within the oocytes was an indication of the process maturation. According to Weber at al. (2002), increased ovarian follicle atresia has also been reported in the studies where zebrafish have been developmentally exposed to the estrogenic chemicals. The treatment of the zebrafish during the period of the gonadal differentiation with either the non-steroidal aromatase inhibitor fadrozole or 17α-methyltestosterone changed the gonad morphological differantation described by Fenske and Segner (2004). The period of gonad transformation and to evaluate the impact of the estrogenic androgenic model substances on the sex differentiation and vitellogenin induction in the juvenile zebrafish was analysised by Örn et al. 2003. the degeneration was characterized by the granulation of the cytoplasm, appearance of large vacuoles and irregularity in the shape of the

oocytes. Surrounding follicular epithelial cells contained the degradation products. Although the follicle epithelium cells became more distinctive according to the growth of the egg, their existence in the primary growth phase were also observed. When there was no formation between the oocyte and the follicle cells surrounding the oocyte in the primary growth phase (previtellogenic phase), the microvilluses developed in the oocyte membrane extended towards the follicle cells and these formations were observed in entire oocyte surface. In this phase, the mitochondrion was seen distributed all over the cytoplasm. This appeared in the first phases of the primary follicle of the vitelline envelope in the zebrafish. In *C. tarichi*, the microvilli began to form on the oocyte surface in cortical alveolus phase (Ünal 2005). The vitelline envelope was striated in early vitellogenesis in *Liza aurata*. In the vitelline envelope of L. aurata, each striated line represented a canal with pores opening at both ends described by Shabanipour and Heidari (2004). In addition, a perivitelline space was noted between the vitelline envelope and oolemma, as also seen in *Crenicichla johanna* (Cruz-Höfling and Cruz-Landim 1993). Its development was completed in the vitellogenic phase and its dissolution was observed in the atretic oocyte phase. The development phases of the follicle epithelium cells were found in accordance with the follicle alterations as well as vitelline envelope in zebrafish.

In conclusion, the oocytes growth was similar in the teleosts. In most of the teleosts, the progress of oogenesis might be in four, five, six and eight stages. The oocytes development in the zebrafish was manifested in a series of changes, which their division into four stages. During the oocytes development, the oocyte enlarged due to hydration and preteolysis of the yolk protein. The vitelline envelope began to form in the cortical alveolus stages and develop in the vitellogenic stages and was clearly observed in the mature oocytes. Results of the present study hopefully would contribute knowledge to the research on the process of the oogenesis of the zebrafish.

REFERENCES

Arockıaraj, A., Hanıffa, M., Seetharaman, S., Sıngh, S. (2004), Cyclıc Changes In Gonadal Maturation and Hıstologıcal Observations of Threatened Freshwater Catfish "Narikeliru" *Mystus montanus* (Jerdon, 1849). *Acta Ichthyologıca Et Piscatoria*, **34(2)**, 253-266.

Brandao, C.A., Valentim, A. F.M., Pellegrini-Caramaschi, E. (2003), Ovary Maturation Stages and Oocyte Features in Three Species of the Neotropical Fish *Hemiodus* (Müller, 1842). Brazilian *Archives of Biology and Technology*, Vol.**46:3**, 433-441.

Cruz-Höfling, M.A., Cruz-Landim, C. (1993), Differantiation of the Follicular Epithelium During Oocyte Growth in the Amazonian Fish, *Crenicichla johanna* (Teleostei:Cichlidae). *Zool. Jb.Anat*. **23**, 59-74.

Fenske, M., Segner, H. (2004), Aromatase Modulation Allters Gonadal Differantation in Developing Zebrafish (*Danio rerio*).*Aquatic Toxicology*, **67**, 105-126.

Fishelson, L.; Goren, M.; Van Vuren, J.; Manelis, R. (1996), Some aspects of the reproductive biology of *Barbus spp*., *Capoeta damascina* and their hybrids (Cyprinidae, Teleostei) in Israel. *Hydrobiologia* **317**, 79-88.

Garg,R.A. (1998), *Cloning and mRNA expression of the Activin TypeIIB Receptor and Role of Activin in Final Oocyte Maturation in Zebrafish,Danio rerio*. York University, Department of Biology,Toronto.

Gökçe, M. A.; Cengizler, İ.; Özak, A. A.(2003), Gonad Histology and Spawning Pattern of the White Grouper (*Epinephelus aeneus*) from İskenderun Bay (Turkey). *Turk J. Vet. Anim. Sci*. **27**, 957 – 964.

İşisağ, S. (1996), *Liza ramada* Risso (1826) (Mugilidae, Teleostei) ovaryumlarının gelişimi üzerine histolojik çalışmalar. *Journal of Fisheries and Aquatic Sciences***. 13**, 3-4.

Küçüktaş, H. (1987), Çipura Balığında (*Sparus auratus*) Gonadların Anatomik ve Histolojik Yapıları Üzerinde Bir Araştırma. PhD Thesis. Akdeniz University, Isparta-Turkey.

Maack G. (1964), *Estrogen Related Alterations of Gonad Development and of Reproduction in the Zebrafish, Danio rerio*, Ham. Buc. Hamburg

Matsuyama, M.; Nagahama, Y.; Matsuura, S. (1991), Observetaions on Ovarian Follicle Ultra Structure in the Marine Teleost, *Pagrus major*, during Vitellogenesis and Oocyte Maturation. *Aquaculture*, **92**, 67 – 82.

Munoz, M.,Sabat,M., Mallol, S., Casadevall, M. (2002),Gonadal Structure and Gametogenesis of *Triglia lyra* (Pisces:Triglidae). *Zoological Studies*, **41(4)**, 412-420.

Murua, H.; Saorido-Rey, F., Female Reproductive Strategies of Marine Fish Species of the North Atlantic J. Northw. Atl. *Fish. Sci*. **33**, 23 – 31.

Nagahama, Y. (1983), The functional morphology of teleost gonads.In: Hoar W.S., Randall D.J., Donaldson E.M. (eds), *Fish Physiology*. Academic Press, New York, pp. 233-275.

Nejedli, S., Petrinec, S., Ku·Ir,E., Srebocan,E. (2004), Annual Oscillation of Ovarian Morphology in European Pilchard (*Sardina pilchardus* Walbaum) in the Northern Adriatic Sea. *Veterinarski Arhıv*, **74(2),** 97-106.

Örn, S.,Holbech, H., Madsen, T., Norrgren, L., Peterson, G. (2003), Gonad Development and Vitellogenin Production in Zebrafish (Danio rerio) Exposed to Ethinylestradiol and Methyltestosterone, *Aquatic Toxicology*, **65(4)**, 397-411.

Pina, T., Esteves,E., Andrade, J.P. (2003), Gross and Histological Observations of Ovarian Development in Twaite Shad, *Alosa fallax fallax*, from the River Miraand Guadiana (Portugal). *Scı. Mar*., **67(3**), 313-322.

Selman,K; Wallace, R.A. (1989),"Cellular aspects of oocyte growth in Teleosts". *Zoological Science*,**6**, 211-231.

Shabanipour, N., Heidari, B. (2004), A Histological Study of the Zona Radiata During Late Oocyte Developmental Stages in the Caspian Sea Mugilid, *Liza aurata* (Risso 1810). *Braz.J.Morphol.Sci*.,**21(4**), 191-195.

Ünal, G.; Çetinkaya, O.; Elp, M. (1999), Histological Investigation of Gonad Development of *Chalcalburnus tarichi* (p., 1811). *Tr. J. Of Zoology*, **23-1**, 329 – 338.

Ünal, G.; Karakişi, H.; Elp M. (2005), Ovarian Follicle Ultrastructure and Changes in Levels of Ovarian Steroids during Oogenesis in *Chalcalburnus tarichi* Palla, 1811. *Turk J. Vet. Anim. Sci*., **29**, 645 – 653

Ünver, B., Saraydın Ünver, S. (2004), Histological Examination of Ovarium Development of Shemaya *Chalcalburnus chalcoides* living in Lake Tödürge (Sivas/Turkey). *Folia Zool*., **53 (1)**, 99-106.

Van Ree, G.E. (1977), İn vitro induction in nuclear breakdown in ooctye of the zebrafish, *Brachydanio rerio* Ham.Buc., Effects of the composition of the medium and of protein and steroid hormones." *Proc. Kon. Ned. Akad. Wetensch. Series C.*, **80**, 353-371.

Weber, L.P.,Hill, R.L., Janz, D.M. (2002), Developmental Estrogenic Exposure in Zebrafish (*Danio rerio*): II.Histological Evaluation of Gametogenesis and Organ Toxicity. *Aquatic Toxicology***, 62**, 431-446.

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

Yueh, W., Chang,C. (2000), Morphological Changes and Competence of Maturing Oocytes in the Protandrous Black Porgy, *Acanthopagrus schlegeli. Zoological Studies* , **39(2),** 114-122.

Received: September 09, 2005;
Revised: August 10, 2006;
Accepted: March 24, 2008.