Oocyte Development in the Zebrafish, *Danio rerio* (Teleostei: Cyprinidae)*

*Özlem Çakıcı, Sema İşisa Üçüncü
Ege University, Science Faculty, Biology Department, Zoology Section, 35100 Bornova, Izmir, Turkey

*E mail: ozlem.cakici@ege.edu.tr

**Abstract:** Oocyte development in the ovary of zebrafish were investigated by light microscopy and four main developmental stages differentiated according to their histological properties were identified as primary oocyte stage, cortical-alveolar stage, vitellogenic stage, and maturation.

**Key Words:** Zebrafish; *Danio rerio*, oocyte, vitellus, follicle.

*This study is partly based on MSc. thesis of first author. Preparation of the manuscript was supported by Ege University Research Council 2000 Grant No. Fen 003.

**Introduction**
Applying of biosciences in fisheries and fish production needs to have a proper information of reproductive physiology of teleosts. Biologists have long been familiar with the maturation of the oocytes and so many studies were performed on fish ovary, oogenesis and ovarian cycle (Selman & Wallace, 1986; Kjesbu & Kryvi, 1989; Casadevall et al., 1993; Selman et al., 1993; İşisağ, 1996; Micale et al., 1999; Peixoto et al., 2003; Utoh et al., 2004; van Aerle et al., 2004; Arockiaraj et al., 2004; Simonsen & Gundersen, 2005). By the way, stages of oocyte development in the zebrafish was also described earlier (Selman et al., 1993). As a general agreement, morphological data are the main axis for physiological investigations, and fish oocytes provide an appropriate experimental system with which to investigate the molecular mechanisms controlling reproduction.

The main criteria used to determine the oocyte development is the structure of nucleus, deposition of vitellus, and the formation of acellular and cellular layers of oocytes: zona radiata, granulosa and theca. From these point of views, the aim of the present study was to describe the oocyte development stages of the asynchronous ovary of zebrafish, *Danio rerio*, in order to construct a main route for advanced researches.

**Material and Methods**
Zebrafish (*n=20*) were obtained from commercial dealers and acclimated to filtered, dechlorinated and well aerated tap water in 20 lt aquaria. They were fed once daily with *Tubifex* sp., *Daphnia* sp., and commercial fish food (Sera-San). The water temperature was maintained at 27±3 °C and the photoperiod was set at 14L/10D. Fishes were sacrificed with MS222 and the ovaries were removed and fixed in the Bouin’s fixative for 48 hours. Specimens were dehydrated in alcohol and xylol, infiltrated and embedded in paraffin. Serial sections at 6-7 µm stained with hematoxylin-eosin (H&E), and paraldehyde fuchsine (PAF) were examined by light microscope.

**Results**
According to the histological parameters, four main steps of the oocyte development described below were identified. Atretic follicles did not evaluated.

The first step of the oocyte development is named as “primary oocyte stage”. At the beginning phase of this stage, relatively small oocytes were mostly spherical in shape. Ooplasm was intensely stained while the nucleus was not (Fig. 1a). As oocytes were enlarged, the nucleoli could be easily seen to form a line oriented peripherally (Fig. 1b).

![Figure 1. Early (a) and late (b) phases of primary oocyte stage. Nucleolus (No); follicle layer (FL) and chromatin material (x). Stain: H&E. Bar= 30 μm](image-url)
Chromatin materials were clearly visible in both of the small and relatively large oocytes. Although the follicular epithelium was also observed particularly for large ones, to make a differentiation for the layers was not possible.

“Cortical-alveolar stage” is the second step of the oocyte development. While the diameter of the oocytes were increased, cortical alveoli were appeared firstly at the peripheral zone of the ooplasm (Fig. 2) and increased in number to form a peripheral row (Fig. 3), in this way, oocytes were filled with alveoli which were consequently get denser and enlarged (Fig. 4).

Nuclei were irregular in shape. Most of the nucleoli were attached to inner border of nuclear membrane (Fig. 5).

The end of the stage was characterized by the migration of the nucleus to the animal pole, just beneath the oocyte surface (Fig. 6). As shown at the same figure, follicular layers were thickened and the most prominent one was zona radiata.

In the third step which is named as “vitellogenic stage”, the oocytes were more enlarged and reached at their maximal size. When stained with PAF, the area filled by yellowish yolk droplets could be seen firstly at the central zone of the oocytes (Fig. 7).
The droplets which were heterogenous in appearance were accumulated and widened towards the peripheral zone (Fig. 8). Zona radiata was thicker, and granulosa-theca cells were easily identified with both of the staining methods (Fig. 8, 9).

Figure 8. Progress in vitellogenic stage. Accumulation and widening of heterogenous vitellus droplets (V). Stain: PAF. Bar= 50 μm

Figure 9. Follicle layers. Zona radiata (ZR), granulosa (G), and theca (T). Stain: H&E. Bar= 30 μm

Because of the continuous accumulation of yolk sac, the area occupied by ooplasm was very limited (Fig. 10) in the last step of the oocyte development which is named as “maturation stage”. Oocyte layers were folded irregularly. Spawning following the rupture of the layers would occur at the end of the stage.

Figure 10. Mature oocyte filled by vitellus droplets (V), cortical alveoli (CA), and ooplasm (Op). Stain: PAF. Bar= 50 μm

Discussion

Gonadal development and reproductive strategy have been described in many teleost species in an effort to understand the physiological mechanisms of reproduction. Many investigations have been carried out on the development of germ cells in fish (e.g., Wallace & Selman, 1981; Arockiaraj et al., 2004). Oocyte maturation follows a similar pattern in most teleosts (Casadevall et al., 1993; Kayaba et al., 2001, Brandão et al., 2003, Carrason & Bau, 2003). In general terms, zebrafish oocyte development stages are also in accordance with other teleosts (Selman et al., 1993).

Adult zebrafishes spawn several times in a month, most teleost species with asynchronous ovary have protracted spawning seasons with multiple spawning (Selman et al., 1993, Carrason & Bau, 2003). There is no doubt that this kind of reproductive strategy may present some important advantage for achievement to a high productive capacity.

As might be expected, there is not a consensus on the terminology of oocyte maturation stages; especially first development stages of oocytes. For example, this stage of oocyte maturation is named previously as “first and second stage” for Blennius pholis (Shackley & King, 1977); “immature oocyte stage” for Barbus luteus (Al-Daham et al., 1979); “primary growth stage” for Gadus morhua (Kjesbu & Kryvi, 1989) and Brachydanio rerio (Selman et al., 1993); “previtellogenesis stage” for Ophidion barbatum (Casadevall et al., 1993); “chromatine-nucleolus and perinucleolar stage” for Labeo capensis (van Der Merwe et al., 1988); Thunnus thynnus (Corriero et al., 2003) and Aidablennius sphynx (Carrason & Bau, 2003); “perinucleolar stage” for Pimaphelus promelas (van Aarle et al., 2004) and Farfantepenaeus paulensis (Peixoto et al., 2003). In this article, the term “primary oocyte stage” is preferred. Although this situation results in marked confusion, it is clear that all of the main histological aspects of maturation stages described in above-mentioned papers are similar.

Three different forms of vitellus of the teleost oocyte are oil droplets, yolk vesicles and yolk globules. However, the first one is absent in zebrafish (Leung et al., 2000). Despite the general agreements on the function of droplets for buoyancy and energy supply, little is known about the structural functions of its. Based on the conclusion of Kayaba et al. (2001), it is plausible that the phospholipidic content of the droplets are consumpted in the organelles such as mitochondria and Golgi apparatus. At this point, a critical question arises: What are the main sources which are used for construction of organelles in zebrafish eggs which have not oil droplets? It is strongly possible that the main sources of structural organization are maintained from not only the contents of oil droplets, but also the contents of vesicles and/or globules.

Kayaba et al. (2001) stated that cortical alveoli (yolk vesicles) which are initially observed at the periphery of the ooplasm then increase in number and form a peripheral row. According to same authors, as the size and number of cortical
alveoli is small, and the accumulation of yolk globules start immediately after the appearance of cortical alveoli, it is difficult to distinguish them by H&E stain in Japanese eel. However, this is not the case for zebrafish and the other teleosts except Japanese eel.

Ultrastructural observations indicated that cortical alveoli are closely associated with golgi bodies and the endoplasmic reticulum (Kayaba et al. 2001). Two different types of cortical alveoli, those containing filamentous and those containing latticed material, were distinguished in B. pholis (Shackley & King, 1977) and A. japonica (Kayaba et al. 2001). On the other hand, cortical alveoli contained only flocculent material in F. heteroclitus was also reported by Anderson (1968).

There is no doubt that these statements cannot be confirmed by light microscopy.

Vitellus globules are known to be formed by accumulation of small vesicles (Anderson, 1968; Shackley & King, 1977). It is observed that most parts of the oocyte is filled with large number of heterogenous globules in this study. Nagahama (1983) was reported that vitellus vesicles of zebrafish have had a crystalin structure surrounded with a superficial layer and outer membrane. This kind of crystallization is also observed in Gadus morhua (Kjesbu & Kryvi, 1989). Selman et al (1993) were noted that yolk globules would lose their crystalline interior and become homogeneous through maturation. The transparency of the zebrafish eggs may be due to this process.

Vitellogenesis is one of the most important reproductive phenomenons in egg-laying animals and it is well known that many proteins and lipids are actively synthesized and transported into the oocyte during vitellogenic processes. In this regard, the most important protein is probably vitellogenin, which is synthesized in the liver and bounded to vitellogenin receptor located in the oolemma (Wallace, 1985; Selman et al., 1983). Selman & Wallace (1986) named it as secondary membrane.

According to Suzuki et al. (2000) the pores of zona radiata are to maintain the resistance to abrasion while granulosa layer that is related to adhesiveness. On the other hand, Brandão et al. (2003) noted that the “grooves” of zona radiata were covered by filaments and related to adhesiveness. Kayaba et al. (2001) were performed that the microvillar processes extending from both the oocyte cytoplasm and the follicle cells increased in number and length during egg maturation of Japanese eel, Anguilla japonica, and also noted that the formation of such a reticular network is in a general agreement with observations in other teleosts. Undoubtly; vitellogenesis, egg maturation and hatching are very complex processes which need active synthesis and transport of many proteins and lipids. As might be predicted, it is not possible to give a conclusion for transporting mechanisms of precursor materials by light microscopy. This study should only assist in developing improved methods for further researches.

References


