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Research Note / Araştırma Notu

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

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Özet: ***Zebra balığında*** Danio rerio***'da (Teleostei: Cyprinidae) oosit gelişimi.*** Zebra balığı ovaryumlarında oosit gelişimi ışık mikroskobu ile araştırılmış ve histolojik özelliklere göre ayırt edilen dört esas gelişim aşaması; primer oosit evresi, kortikal alveolar evre, vitellojenik evre ve olgunluk evresi olarak tanımlanmıştır.

Anahtar Kelimeler: Zebra balığı; *Danio rerio*, oosit, vitellüs, folikül.

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.

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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 ºC 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).

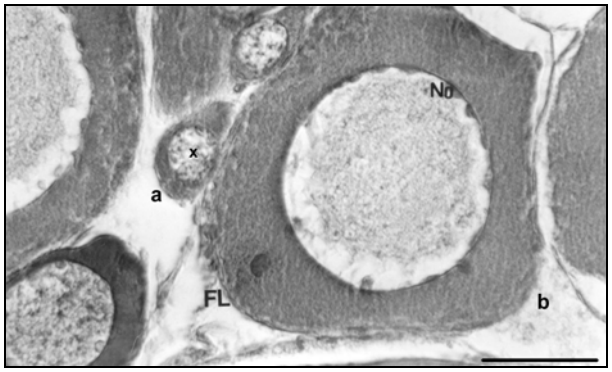

Figure 1. Early (a) and late (b) phases of primary oocyte stage. Nucleolus (No); follicle layer (FL) and chromatin material (x). Stain: H&E. Bar= 30 µm

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).

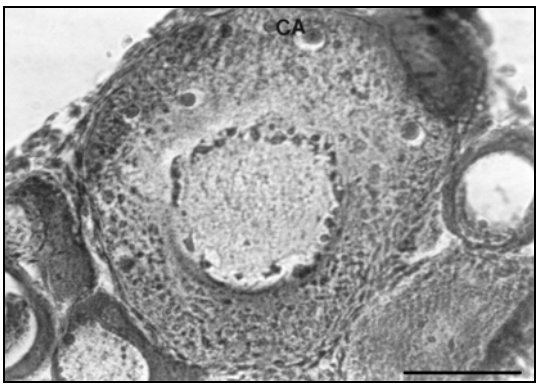

Figure 2. Early cortical-alveolar (CA) stage. Stain: H&E. Bar= 50 µm

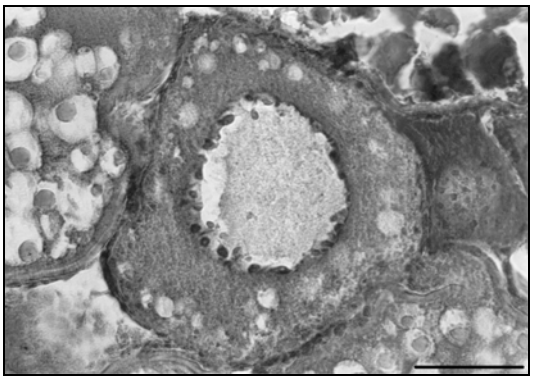

Figure 3. Progress in cortical alveolar stage. Cortical alveoli increased in number. Stain: H&E. Bar= 50 µm

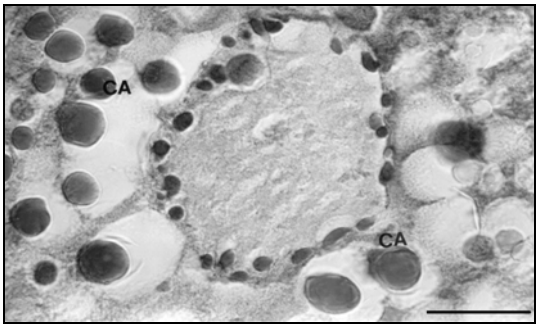

Figure 4. Late cortical alveolar stage. Ooplasm filled with cortical-alveoli (CA). Stain: H&E. Bar= 30 µm

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).

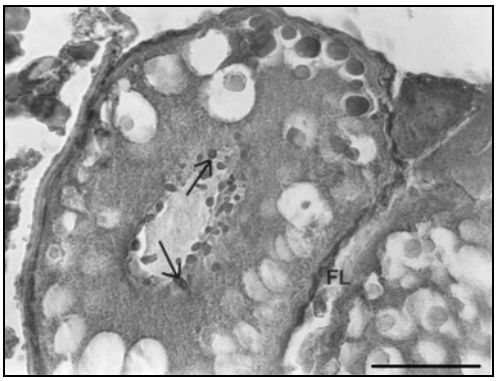

Figure 5. Cortical alveolar stage. The nucleoli (→) oriented peripherally and follicle layers (FL). Stain: H&E. Bar= 50 µm

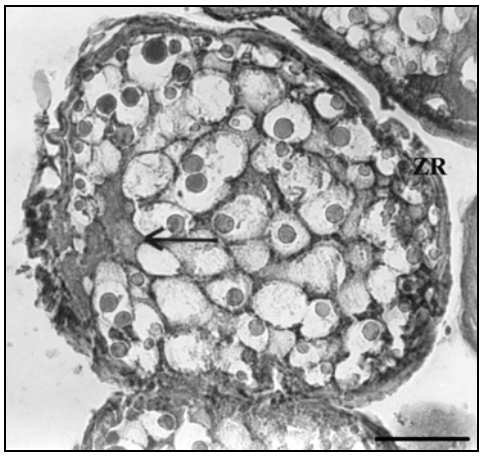

Figure 6. End of the cortical alveolar stage. Nucleus migration (→), zona radiata (ZR). Stain: H&E. Bar= 50 µm

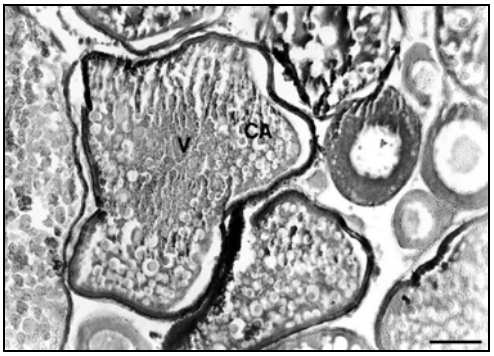

Figure 7. Beginning of the vitellogenic stage. Vitellus droplets (V) at the central zone and cortical alveoli (CA) at the peripheral zone. Stain: PAF. Bar= 50 µm

The droplets which were heterogenous in appearance were accumulated and widened towards to 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).

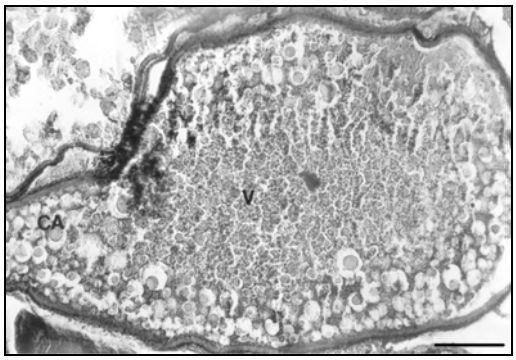

Figure 8. Progress in vitellogenic stage. Accumulation and widening of heterogenous vitellus droplets (V). Stain: PAF. Bar= 50 µm

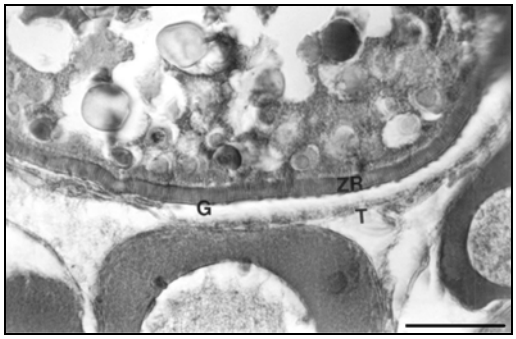

Figure 9. Follicle layers. Zona radiata (ZR), granulosa (G), and theca (T). Stain: H&E. Bar= 30 µm

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.

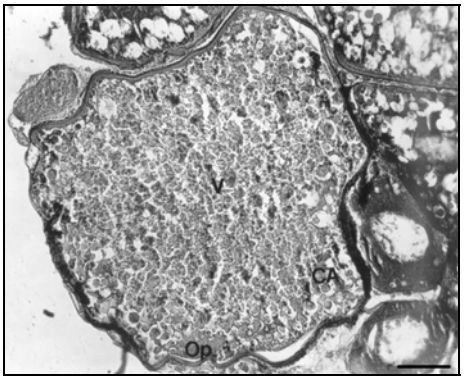

Figure 10. Mature oocyte filled by vitellus droplets (V), cortical alveoli (CA), and ooplasm (Op). Stain: PAF. Bar= 50 µm

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, Brandäo *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 thinnus* (Corriero et al., 2003) and *Aidablennius sphynx* (Carrason & Bau, 2003); "perinucleolar stage" for *Pimaphelas promelas* (van Aerle *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 phospolipidic 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 & Wallace, 1986; Tyler *et al.,* 1990). Furthermore, detailed histochemical findings about the relationships between follicles and sex steroid hormones were revealed by Young *et al.,* 1983; Selman *et al*. (1993) and Afonso *et al*. (1996).

Leaving physiological mechanisms involved in vitellogenesis for further studies, the vitellin membrane, zona radiata deserves special mentioning at least histologically. It is 7-8 µm and 30 µm in *Carassius auratus* and *Salmo gairdneri* (Nagahama, 1983). In zebrafish, we have measured it as 5-6 µm. These differences are possibly due to the various ecological conditions, as noted by Brandäo *et al*., (2003); Guraya (1986) and Suzuki *et al*. (2000) for demersal eggs which are often subjected to abrasive forces, generally developes thick envelopes with complex lamellae. The differences of the thickness of zona radiata of the oocytes regarding *Hemiodus* species are seemed to be related to the resistance of their oocytes (Brandäo *et al*., 2003). The thicker zona radiata can provide a mechanic protection against the abrasion of the bottom, as noted by *S. gairdneri* (Nagahama, 1983).

The origin and denomination of zona radiata is a contraversial issue. Selman & Wallace (1986) named it as primary membrane in *Hippocampus erectus* and *Syngnathus fuscus*, anticipating that it is originated by the oocyte. However, connecting the formation process to follicular cells, Wourms & Sheldon (1976) named it as secondary membrane. Based on its radially striated appearance by light microscope, which is due to the transport function, the term “zona radiata” was preferred at this study.

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, Brandäo *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 hatcing 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.

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