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RESEARCH ARTICLE

HISTOLOGICAL, HISTOCHEMICAL AND MORPHOMETRIC PROPERTIES OF OOCYTE DEVELOPMENT IN ZEBRAFISH (*DANIO RERIO*)

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ABSTRACT

In this study, histogical, histochemical and morphometric features of ovaries from adult zebrafish (*D. rerio*) were invastigated by light microscopy. In zebrafish which has asynchronous ovary, oocyte development was observed in four main stages as primary growth stage (PGS), cortical alveolar stage (CAS), vitellogenic stage (VS) and mature oocyte stage (MOS). We analyzed presence and distribution of glycoconjugates in the different structures of the developing oocytes (cortical alveoli, yolk granules, ooplasm and zona radiata) by staining the cross sections with periodic acid Schiff's (PAS), KOH-PAS, toluidin blue (TB), alcian blue (AB) and aldehyde fuchsin (AF). Cortical alveoli, yolk granules, ooplasm and zona radiata showed glycoconjugates with glycogen and/or oxidable dioles and sialic acid residues (PAS+ and KOH/PAS +). Only zona radiata stained weakly with AF and it showed low acid sulphate glycoprotein content. AB staining was negative to all parts. Measurements showed that oocytes in PGS ranged from 26-143 µm, in CAS 145-400 µm, in VS 326-617 µm and in MOS 341-764 µm in size.

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INTRODUCTION

Oogenesis is an important process that must be well understood to reveal reproductive development of an organism. Generally, studies on reproductive biology of teleost fish focus on macroscopic classification of maturity stages of gonads, gonadosomatic index (GSI), fecundity mating and spawning behaviour (Fricke, 1980; Hunter and Macewicz, 1985; Kawase, 2003; Abascal and Medina, 2005; Branco *et al*., 2013). However, histological investigations supply more detailed data on essential morphological parameters of ooycte and ovarian development and reproductive analysis (Hunter and Macewicz, 1985; Tricas and Hiramoto, 1989).

Zebrafish *(D. rerio)*, natively live in the river of Ganj, is a frequently used model organism in developmental and genetic studies because of the advantages such as it's relatively short life cycle, small size, high reproducing potency in the laboratory conditions and transparent embryos. OECD (Organisation for Economic Co-operation and Development) and ISO (International Organisation for Standardisation) recommends zebrafish as one of the proper test materials (Maack and Segner, 2003), in addition sequencing the whole genome with significant similarity to most of human gene sequences makes it become more popular and make it important to focus on the reproduction processes of zebrafish (Deshpande and Panhratna, 2013). In the present study, oocyte developmental stages of zebrafish and their some histochemical and morphometric features were described.

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MATERIALS AND METHODS

Ten adult female zebrafish were obtained from a commercial supplier, maintained in aerated tap water at 26±2 ºC in 20 L aquaria, fed once daily with artemia and natural photoperiod was set. After the euthanasia with MS222 the ovaries were removed, fixed in Bouin's fluid for 48 hours. Routin paraffin embedding process was conducted and 5 µm serial sections stained with Hematoxylen-Eosin (H-E) for general observations; Periodic Acid Schiff's (PAS), KOH-PAS, Toluidin Blue (TB), Alcian Blue (AB) and Aldehyde Fuchsin (AF) for histochemical examinations. Sections were investigated by light microscopy and microphotographes were taken by Leica DM3000 microscope (Leica Microsystems, Wetzlar, Germany) equipped with a Leica digital camera (DFC290). Ocular micrometer was used for the measurements of oocytes and their nuclei sizes.

RESULTS

Histological examinations showed four main different developmental stages in the ovary of zebrafish. In addition follicular epithelium and zona radiata were identified. In the primary growth stage (PGS) oocytes were small and spheric. The nuclei were placed in the center of the oocytes with the nucleoli in the pheripheral zone of the nuclei. Ooplasm was more intense in the primary oocytes by comparison to the other stages. In this stage, zona radiata and follicular epithelium that surround the oocyte did not observed (Fig. 1 and 2). Cortical alveolar stage (CAS) identified with the cortical alveoli that started to form periphery of the oocyte (Fig. 2, 3 and 4). These alveoli gradually growed, increased in number, migrated to the center and filled the oocyte. Some of the nucleoi oriented pheriphery of the nuclear membrane migrated to the center of the nucleus. Zona radiata was observed in this stage for the first time (Fig. 3). Yolk granules determined first in the middle of the growing oocyte in the vitellogenic stage (VS). Cortical alveoli decreased in number and occured only peripherally. Zona radiata was thickened. Because of the intense accumulation of yolk, the area of ooplasm narrowed (Fig. 5). In the mature oocyte stage (MOS), oocytes filled by vitellus droplets, cortical alveoli were at the periphery and thin ooplasm layer was examined. Yolk granules merged and became bigger than the cortical alveoli in size. In this stage, nucleus could not be identified in the most of the oocytes (Fig. 4). Atresia was determined by hypertrophy and hyperplasia in follicular cells and disarranged and dissolved appearance of zona radiata (Fig. 7). Histochemically, ooplasm of the oocytes in the early phase stained strongly while the primary, cortical alveolar and vitellogenic oocyte ooplasm stained moderately with TB (Fig. 1 and 2). Specially, cortical alveoli, yolk granules and zona radiata were observed dark pink to PAS and KOH-PAS (Fig. 3, 4 and 6). Zona radiata stained weakly with AF, however cortical alveoli, yolk granules and ooplasm did not stain. Cortical alveoli, yolk granules, ooplasm and zona radiata were negative to AB (Table 1). Cell and nucleus diameter comparison results showed that the mature oocytes were the largest cells among the other stages. Vitellogenic, cortical alveolar and primary oocytes followed, respectively. Diameters of CAS oocyte nuclei were larger than VS and PGS. Due to the fact that nuclei could not be identified in the maturation phase, diameters did not evaluated (Table 2).

DISCUSSION

Yellowish paired ovary of zebrafish position between abdominal wall and swim bladder. Oogenesis occurs asynchronously. As well as zebrafish in *Trachurus mediterraneus*, *Tilapia zillii*, *Chalcalburnus tarichi* and *Pseudotylosurus microps* asynchronous oogenesis that allows observing different stages simultaneously occur (Yön, 2006; Wallace *et al*., 1987; *et al*., 1999; Cruz-Landin and Cruz-Höfling, 2001). On the other hand, in synchonous ovaries there are only two or three different stages can be investigated concurrently (Tyler and Sumpter, 1996). Studies with various teleosts show that oogenesis can be separated into four-eight stages (Nagahama, 1983; West, 1990; Fishelson *et al*., 1996; İşisağ, 1996; Ünal *et al*., 1999; Gökçe *et al*., 2003).

Several authors reported that number of the oocyte developmental stages differed in teleosts. *Hippoglossoides platessoides* shows four (Maddock and Burton, 1999); *Pimephales promelas* and *Chirostoma humboldtianum* show five (Maddock and Burton, 1999; Cárdenas *et al*., 2008); *Chalcalburnus tarichi* shows six (Ünal *et al*., 1999) and *Tilapia zillii* shows seven stages (Cowerd and Bromage, 1998) developmental stages in the ovary.

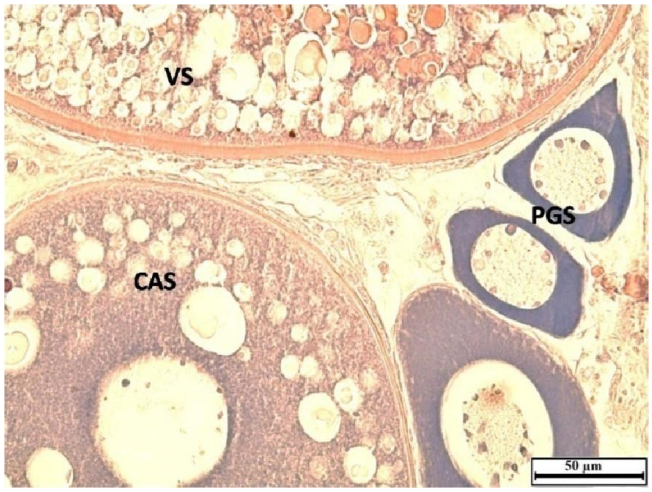

Fig. 1. Primary Growth Stage (PGS), Cortical Alveolar Stage (CAS) and Vitellogenic Stage (VS). TB

Table 1. Histochemistry of the *D. rerio* oocytes

	Cortical Alveoli	Yolk Granules	Ooplasm	Zona Radiata
PAS	+++	+++	+++	+++
KOH-PAS	+++	+++	++	++
TB	-	-	+++	-
AB (pH 2.5)	-	-	-	-
AF	-	-	-	+

Intensity of reaction: -: negative; +: weak; ++: moderate; +++: strong

Table 2. Sizes of the oocytes and nuclei in the different stages (µm) (Mean±Standart Deviation)

	PGS	CAS	VS	MOS
Nucleus Size (µm)	44.3900±12.79508	98.8800±33.46431	97.4667±31.07564	-
Cell Size (µm)	85.7500±28.51352	277.4400±77.94821	454.4000±69.81690	565.2000±109.13753
Nucleus-Cell Ratio (µm)	0.5476±0.13213	0 .3508±0.07302	0.2150±0.06381	-

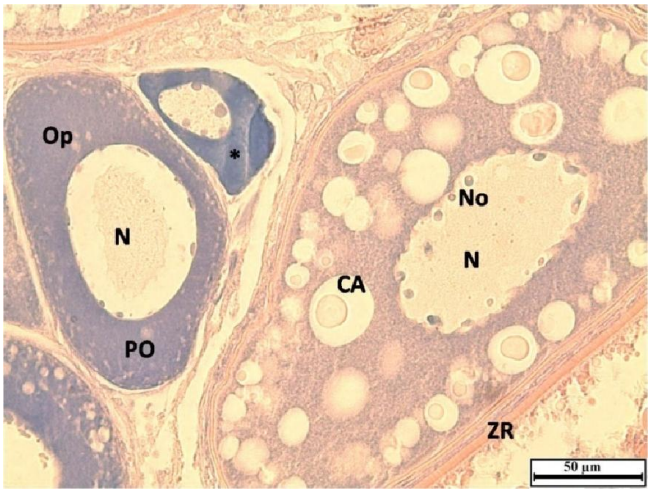

Fig. 2. Primery Oocyt in the Beginning Phase (*), Primary Oocyte (PO), Nucleus (N), Ooplasm (Op), Cortical Alveoli (CA), Nucleolus (No), Zona Radiata (ZR). TB

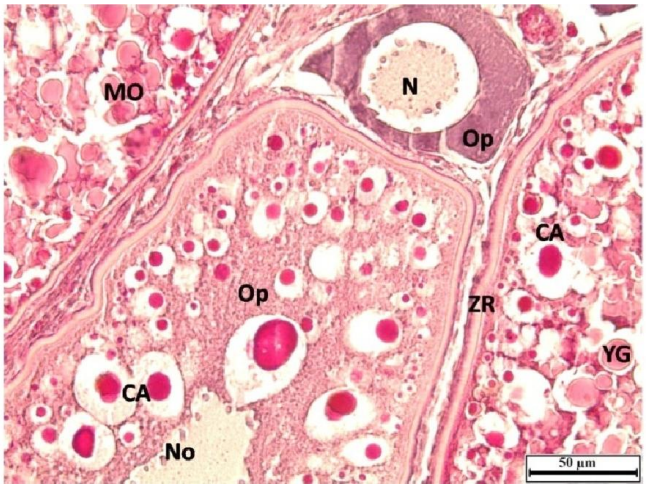

Fig. 3. Mature Oocyte (MO), Ooplasm (Op), Nucleus (N), Nucleolus (No), Cortical Alveol (CA), Yolk Granule (YG), Zona Radiata (ZR). PAS.

However, oocyte development in teleosts are similar basically (Brandäo *et al*., 2003).

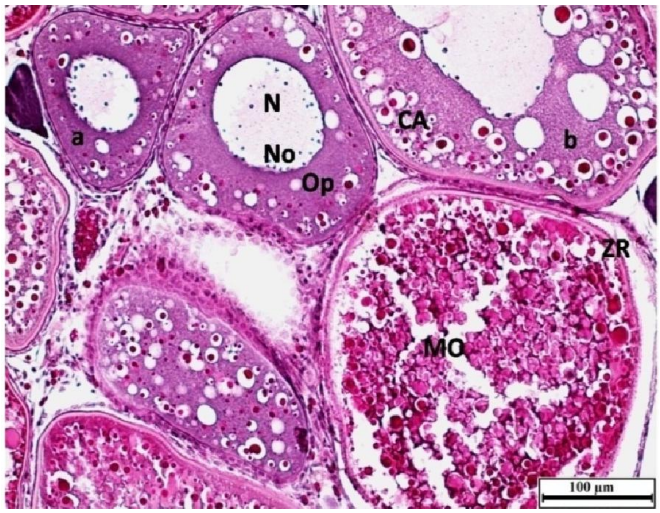

Fig. 4. Early Cortical Alveolar Oocyte (a), Late Cortikal Alveolar Oocyte (b), Nucleus (N), Nucleolus (No), Ooplasm (Op), Cortical Alveoli (CA), Mature Oocyte (MO), Zona Radiata (ZR). PAS

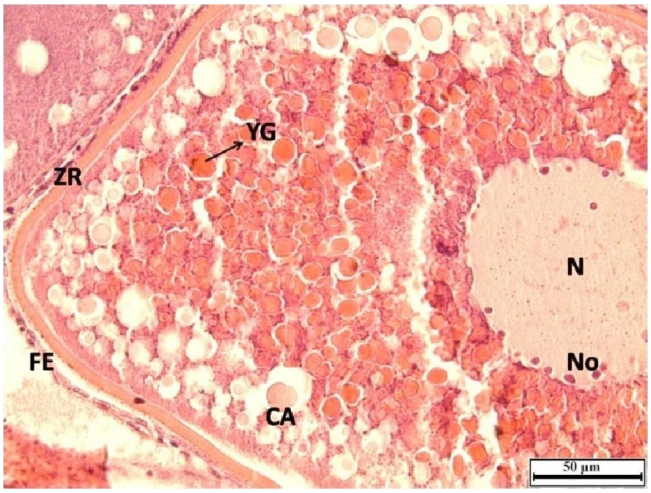

Fig. 5. Vitellogenic Oocyte. Yolk Granule (YG), Nucleus (N), Nucleolus (No), Cortical Alveoli (CA), Folicular Epithelium (FE), Zona Radiata (ZR). H-E

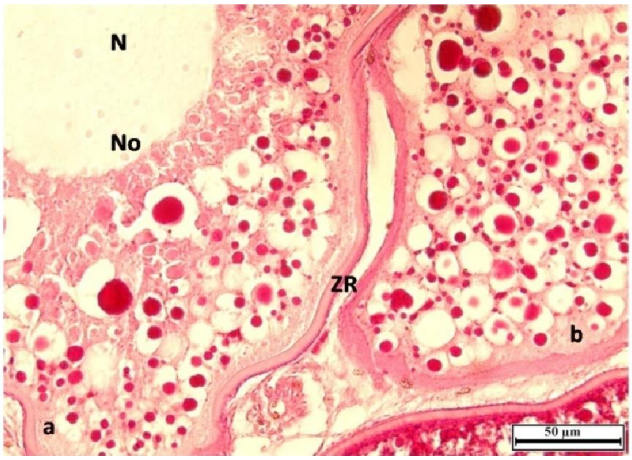

Fig. 6. Cortical Alveolar Stage (a), Late Cortical Alveolar Stage (b), Nucleus (N), Nucleolus (No), Zona Radiata (ZR). KOH-PAS

When several authors noted that there were four different developmental stages in the ovary of zebrafish (Üçüncü and Çakıcı, 2009; Koç (Yön) and Akbulut, 2012), Selman *et al*., (1993) identified five stages.

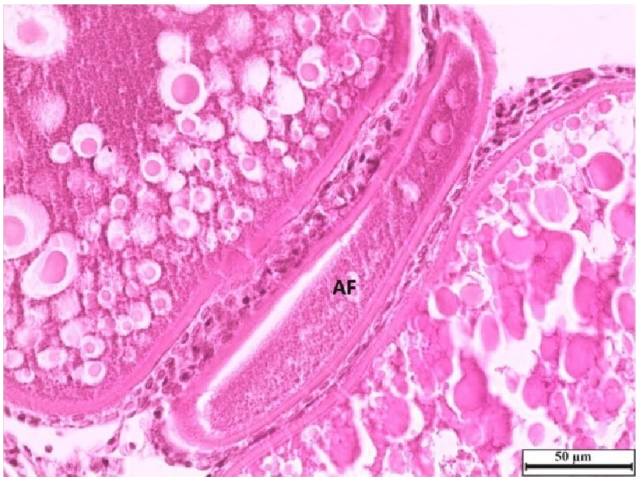

Fig. 7. Atretic Follicule (AF). H-E

In this study, histological observations showed four main different stages as primary oocyte, cortical alveolar,

vitellogenic and maturation stages. Variations in oogenesis phases of different fish species are due to one or more egg breeding, periods of the phases and involvement to the phase (Matsuyama *et al*., 1987; De Vlaming, 1983). Examinations in point of size Leino *et al*. (2005) reported that the oogonia of *P. promelas* were 12-20 µm; early primary oocytes 12-35 µm, late primary oocytes 35-170 µm; cortical alveolar oocytes 170-425 µm and vitellogenic oocytes 425-1070 µm in size. 5-214 µm previtellogenic oocytes in the ovary of *T. zilli* became 964 µm in vitellogenesis (Cowerd and Bromage, 1998). It was noted that diameters of *C. humboldtianum* early oocytes 10-25 µm; primary growth oocytes 25-270 µm; cortical alveolar oocytes 270-380 µm; vitellogenic oocytes 380-650 µm and mature oocytes 650-1150 µm (Cárdenas *et al*., 2008). Oogonia of zebrafish ranged from 9-11 µm; newly transformed oocytes 24-40 µm; primary oocytes 40-140 µm; oocytes at the end of vitellogenesis 550-690 µm in size (Deshpande and Pancharatna, 2013). In this study, we measured PGS oocytes 26-143 µm; CAS oocytes 145-400 µm; VS oocytes 326-617 µm and MOS oocytes 341-764 µm. In paralel with yolk accumulation the oocyte size increases. On the contrary, diameters of nuclei diminish gradually. Nuclei of PGS oocytes measured as 21-79 µm; CAS oocytes 45-169 µm and VS oocytes 40-149 µm. Nuclei disappeared in the mature oocytes.

Deshpande and Pancharatna (2013) stained zebrafish ovary with AB, PAS, Mercury Bromophenol Blue, Sudan Black B, Oil Red O and Nile Blue. Histochemically, vitellogenic follicle yolk granules stained with all methods mentioned above, however previtellogenic oocytes did not stain with PAS, Oil Red, Nile Blue and Sudan B, and stained slightly with Bromophenol Blue and Alcian Blue. The authors indicated that the content of the yolk granules composed of protein, carbohydrate and lipids. Cruz-Landin and Cruz-Höfling (2001) reported that the substance in the intracellular area of follicular cells did not stain with AB, AF and TB, however stained with PAS. In this study, only zona radiata stained weakly by AF and AB staining was negative. Strongly staining of cortical alveoli and yolk granules with PAS and KOH-PAS pointed their glycogene and sialic acid residues component. Ooplasm of early primary oocytes stained strongly with TB, late primary oocytes, CAS and VS oocytes faintly stained. It shows anionic glycoconjugate content decreases following the vitellogenesis. Beside being a popular aquarium fish, zebrafish is a model organism for developmental biology, toxicology and more disciplines. Therefore zebrafish is continually bred. Reproduction processes of this fish must be elucidated comprehensively to guide other studies and the database should be enlarged. For this purpose, histological, histochemical and morphometric properties of zebrafish ovary was investigated in this study.

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