Histological structure of the ovary in adult Zebra fish

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Abstract

The reproductive system in Zebra fish is intensely analyzed in research, especially in toxicological studies. Therefore, thorough knowledge about normal histology is vital. In our study we have examined 30 adult Zebra fish females with the aim of description the normal morphology and oocytes stages of development. For the gonads examination, histological samples were realized by cutting the fish in cross section or longitudinal section. Samples were fixed in Bouin, embedded in paraffin, sectioned at 5µm, H&E stained and examined at light microscope Olympus CX41. Oocytes in different stages of development were differentiated, measuredand main structures were noted. Primary oocytes were mainly disposed in clusters, had small diameter, intense basophil cytoplasm, big nucleus and multiple nucleoli, whilst the cortical alveoli oocytes had larger diameter, germinative vesicle highly irregular, with pleiomorphic and multiple nucleoli and mainly the formation of cortical alveoli with different forms and sizes. In the vitellogenic stage, the oocytes accumulate vitellogenin, a yolk-precursor protein, by endocytosis in membrane limited yolk bodies. The maturation stage of oocytes includes the fusion of the yolk bodies, the nucleus migration toward the oocyte periphery and nuclear envelope breaks down.

Keywords : *Zebra fish, Danio rerio, ovary, histology*

Introduction

Zebra fish is part of the Cyprinidae family and is originated from India and Pakistan. It's intensely used as animal model in research because of therelative facility of its maintainance, reduced development period from embryo to sexually mature adults (3-4 months) , low costs of purchase and maintainance, but most important, a remarkable similitude of zebrafish's genome with human genome and high susceptibility at mutagens, carcinogens, teratogens and toxics (1).

Gonads in adult females of zebrafish are lobated and contain a reduced quantity of stroma. The ovaries are structured in two lobes, being located in the abdominal cavity, beneath gas bladder. Considering the fact that in zebrafish the oogenesis is asynchronous, in its ovaries can be found oocytes in all the development stages. Oocytes development in fish can be divided in two stages:a stage of growth and a stage of maturation. In the growth stage, vitellogenesisis highly important, meanwhile in maturation stage can be noted the migration and break down of germinal vesicle, fusion of fat globules with yolk bodies and the release of the first polar body (2).

Materials and methods

30 females of zebrafish were examined for establishing the development stages of the oocytes. The fish were euthanised using an overdose of propofol combined with lidocaine (3). As a fixation method, we used next protocol: we sectioned the abdominal wall with a surgical scissors from the urogenital pore to the heart without penetrating the heart cavity because the blood will alter the histological results. Whole fish were introduced in a 10% formaldehide solution for one hour, after which they were cutted by cross or longitudinal (sagittal or coronal plane) sections. The obtained pieces were immersed in Bouin solution for 48 hours. Dehidration was performed by transferring the pieces in 96^{o} alcohol for 24 hours and after in 3 baths of absolute alcohol for one hour each. Samples were cleared with xylene for one hour; paraffin wax embeding was realised by introducing the pieces in 3 successive paraffin-wax baths for one hour each; then microtome

sectioned5 µm; stained with usual hematoxylin-eosin. The histological sections were examined at light microscope Olympus.

Results and discussions

The ovarian sections presented oocytes in all the development stages, differentiated bysize and morphological aspect. The oocytes diameter values was calculated for every stage.

Primary growth oocytes were distinguished through their large nuclei with multiple nucleoli and a highly intense basophilic envelope. They were disposed mainly in clusters, dividing all the oocytes from a cluster one single follicular cells layer, but in the late primary growth stage every oocyte presented a thin layer of follicular cells and thecal cells, the nuclear envelope became irregular and the nucleoli were pushed to the side, cortical alveoli begining to form and the primary follicles were scattered in the ovarian mass. The oocytes diameter was comprised betwen 10 and 80 µm (fig. 1, 2).

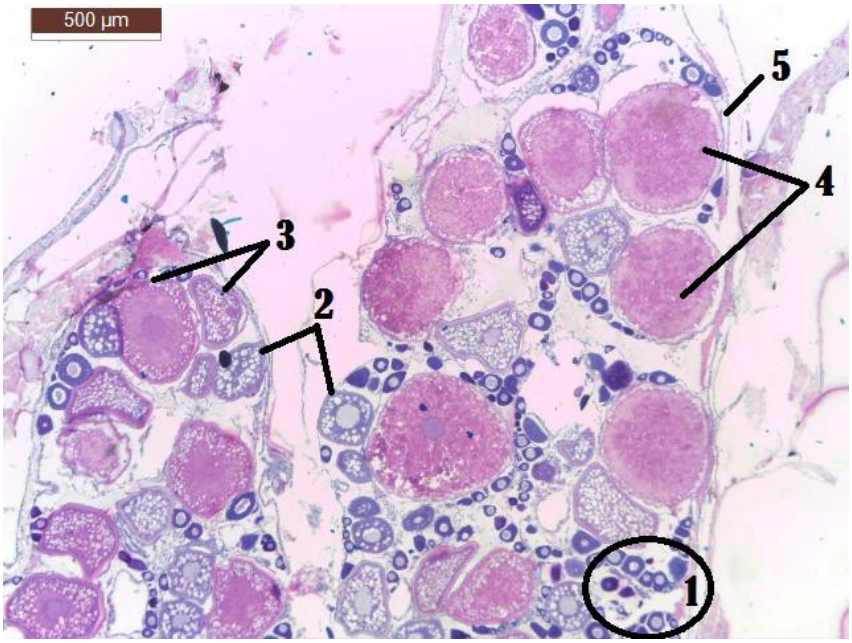

Fig. 1. Ovary in cross section x40. All stages oocytes can be observed.
1- primary oocytes; 2- cortical-alveoli oocytes; 3-vitellogenic oocytes;
4- mature oocytes; 5- ovarian albugineea , H&E.

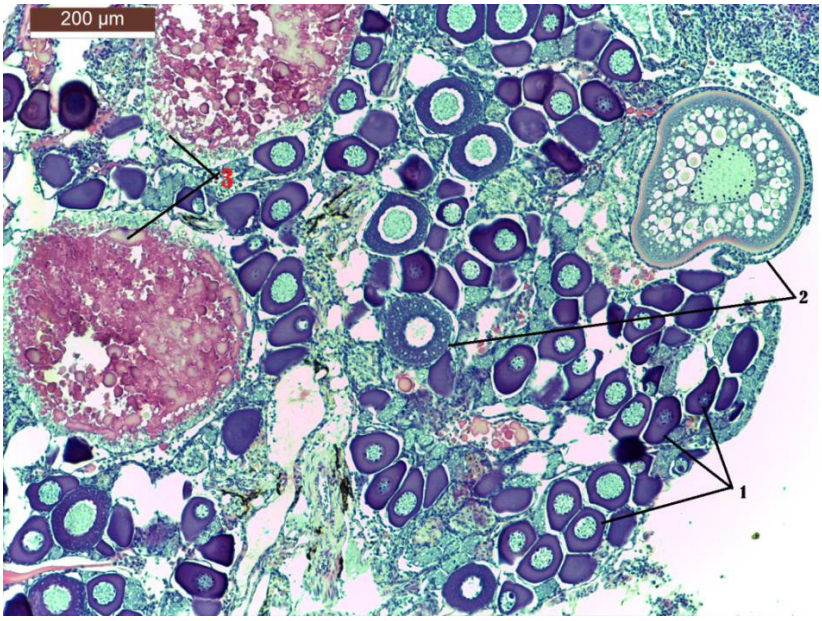

Fig. 2. Ovary in cross section x100. All stages oocytes can be observed.
1- primary oocytes; 2- cortical-alveoli oocytes;
3- oocytes filled with vitellogenic granules, H&E.

The cortical alveolae stage begins when the accumulation of cortical alveolae starts. In this phase the oocytes increase their volume due to cortical alveolae deposits loaded in polysaccharides and proteins, becoming several times larger during this stage. Therefore, their diameter had values betwen 80-220 µm (fig.3).

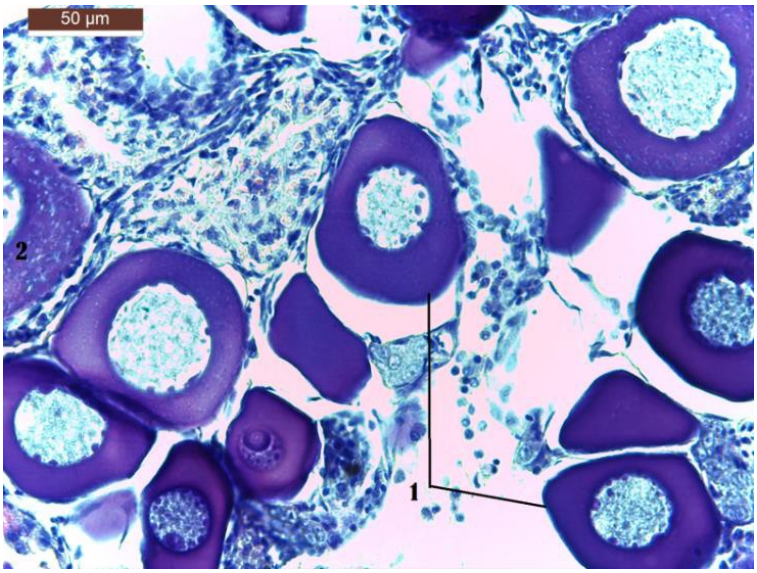

Fig. 3. Primary growth oocytes x400. 1- primary oocytes;
2- oocyte that begin to form cortical-alveolae, H&E.

Nevertheless, the most important growth of the oocytes takes place in the vitellogenic stage when they acumulate vitellogenic granules which contain a protein sinthesized by the liver, vitelogenin, and transported in the ovary by endocytosis. The vitellogenic granules are eosinophilic, accumulate in the center of the oocyte and they push to the periphery the cortical alveoli. The nuclear envelope is irregular. The vitellogenic oocytes can measure up to 400 µm (Fig. 4, 5).

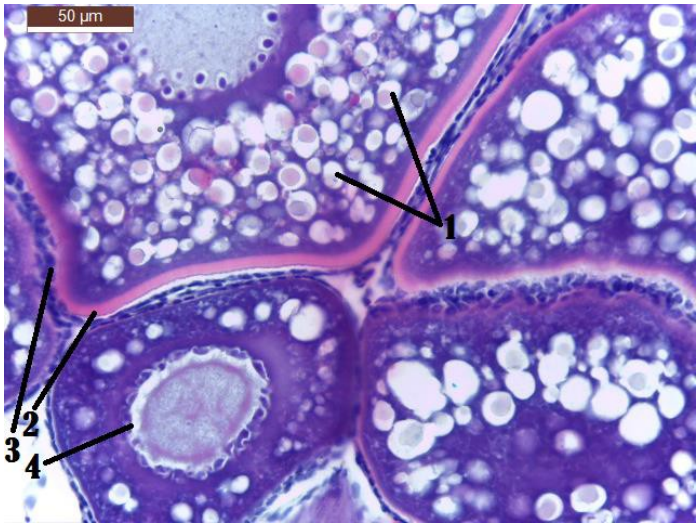

Fig. 4. Oocytes with cortical alveolae x40. 1- vitellogenic granules; 2- thickening of the vitelline envelope; 3- follicular cells layer, 4-nucleoli, H&E.

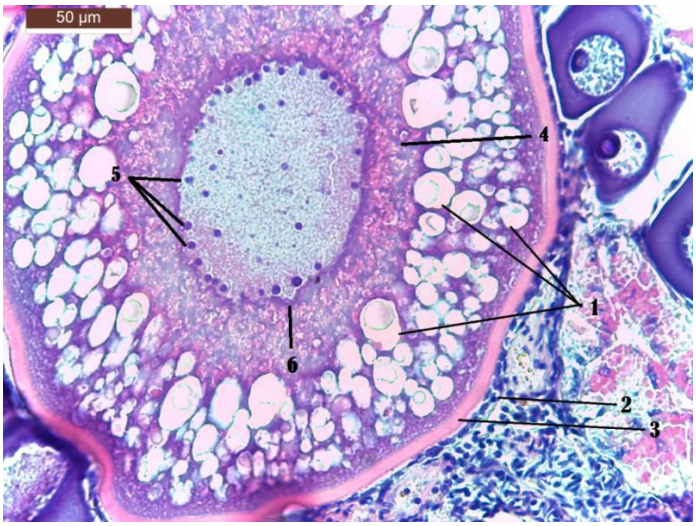

Fig. 5. Oocyte in vitellogenic stage with cortical alveolae and vitellogenic granules x400. 1-cortical alveolae; 2- follicular cells layer; 3- thickening of the vitelline envelope; 4- small vitellogenic granules; 5-nucleoli; 6- indented nuclear envelope, H&E.

Fig. 6. Primary and mature oocytes x 100;
1- oocytes in primary growth stage; 2- mature oocytes, H&E.

In the maturation stage, the oocytes were filled with vitellogenic granules. The oocyte in the maturation process is surrounded by a granulosa cells layer. The nucleus los its central position due to nuclear envelope break down and will be located at the periphery, but it is barely observed because of the abundant vitellogenic granules and the sectioning process (fig.6). Also, it is difficult to differentiate oocytes in the late vitellogenic stage of the oocytes that had just entered the maturation stage due to the fact that there are no structural histological markers to indicate this differentiation. At this stage, oocytes with a diameter of up to 500 µm are observed.

The final phase is the maturation stage, when the follicle loses the follicular cells layer and is released into the ovary lumen and subsequently into the oviduct and through the urogenital pore, located posterior to the anus, and finally in the external environment.

The literature describes 4 or 5 stages of oocytes growth in Zebrafish. Thus, Selman et al. (1993) divides the development of the ovocytes in five stages: the primary growth stage subdivided into a stage where the oocytes are clustered in nests and a stage in which the oocyte is sheltered by a definitive follicle and enlarges its size; IIndstage reveals the appearance of cortical alveolae of different sizes and a thickening of the vitelline membrane; IIIrdstage, of vitellogenesis, is marked by the accumulation of a precursor protein of yolk, vitellogenin, in the form of vitellogenic bodies delimited by their own membrane. At this stage there is an important development of oocytes. IVth stage is those of maturation, in which the meiosis is resumed, the germinal vesicle migrates to the periphery of the oocyte, the nuclear membrane breaks, the first meiotic division take place, and the chromosomes move towards the second meiotic metaphase where they stop - at this point the oocyte become an egg. Vth stage is those of mature egg, ovulation and release into the ovarian lumen. The same results were highlighted in our study.

Kaviani et al. (2013) describe as the first stage the immature oocyte when it measures between 40 and 60 μm, it is spherical or oval and the ooplasm is best colored by the basic dyes. IIndstage is the primary growth stage, when the nucleus is oval or spherical, located centrally, large and with many nucleoli attached to the inner part of the membrane. Oocytes have an average diameter of 78 μm and are surrounded by a thin layer of follicular cells. In the third stage, that of the cortical alveolae, the average oocyte diameter is 215 μm. The nucleoli grow in volume, but they are numerically reduced, the nucleus is large and oval, located centrally. There are large vacuoles near the oolem. The fourth stage of vitelogenesis, with a mean diameter of 295 μm, is characterized by numerous acidophilic vitelogene globules in the ooplasm and migration of the nucleus to the periphery. The last stage, (Vth), involves an average diameter of 415 μm, the nucleus disappearance, the fusion of the vitelogenic globules and the reduction of zona radiata. The same results have been observed in our study. Özlem Çakıcı et al. (2007) claim the existence of 4 stages: primary growth stage, cortical alveolae stage, vitelogenesis stage and the stage of oocyte maturation. Unlike other authors, they note the migration of the nucleus as occurring at the end of the second stage.

Conclusions

The ovary of Zebrafish is bilobated, covered by albuginea, and oocytes four stages of development were identified. Dimmensions of follicles varies from 10 to 500 μm. The oocyte in the maturation process is surrounded by a granulosa cells layer.

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