Histological structure of the testis in adult Zebrafish *(Danio rerio)*

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Abstract

The aim of this study was to describe the characteristics of the different germ cells types found in testicles of Zebrafish (Danio rerio). Therefore, 30 adult male specimens were sacrificed, sampled and prepared by the usual techniques for light microscopy. The male gonads are paired organs located ventral to the gas bladder and dorsal to the liver. The functional unit of the Zebrafish testis is the spermatocyst, a cluster of clonal germ cells surrounded by cytoplasmic arms of a Sertoli cell. The seminiferous tubules organization in Zebrafish is of unrestricted type, thus spermatocysts form all along the length of the tubule, and spermatozoa are discharged into spermatic ducts. Different types of germ cells were identified into the tubules: spermatogonia spermatocytes, spermatids and spermatozoa. The biggest cells are the spermatogonia, diploid cells with slightly granular nucleus and pale cytoplasm. They pass through a series of mitotic divisions from which will result the primary spermatocytes, also diploid cells, with intensly basophilic nucleus and reduced cytoplasm. By the first meiotic division results secondary spermatocytes, smaller, haploid cells. The second meiosis produce the spermatids and by their further maturation will result the spermatozoa.

Keywords : *Zebrafish (Danio rerio), testis, histology, germ cells, spermatogenesis*

Introduction

Zebrafish *(Danio rerio)*, is a tropical fresh water fish that can grow up to 3-5 cm length as an adult, reaches sexual maturity at 3-4 months old and a female can spawn up to 400 eggs and together with the high similarity of his genome with the human genome and its important sensibility to toxics, carcinogens, theratogens and mutagens makes him an excellent model in medical and environmental research (2, 5).

Spermatogenesis is a highly organized process characterized by sequential transitions of multiple processes: self-renewal of spermatogonial stem cells (SSCs), differentiation of SSCs into differentiating diploid spermatogonia and meiotic events leading to the production of millions of spermatozoa daily (11). This process starts from spermatogonial stem cells, which have the potential for both self-renewal and for differentiating into spermatogonia committed to sperm development. Zebrafish is a juvenile hermaphrodite, with all individuals having ovary-like gonads during early life (1). During the embryonic development, the gonads in Zebrafish undergo an ovarian phase in both males and females. Only starting with the 5th week post-fertilization, in males will take place an alteration of the gonad morphology with the decrease in number and size of the perinucleolar oocytes, their irregular shape and intense basophilia and, finally, their degeneration into residual bodies (10). Increased number of gonial cells arranged in cyst-like groups appear. During the subsequent weeks, the male gonads will develop spermatogonia, spermatocytes and spermatids into the seminiferous tubules from the germ cells (3, 4, 8).

Materials and methods

30 adult Zebrafish males were taken in study. They were euthanized with overdose of propofol combined with lidocaine (14). The abdominal wall was sectioned and they were introduced in 10% neutral formalin and Bouin solutions. Cross or longitudinal (sagittal or coronal plane) sections through the fish were performed. Samples dehidration was performed by usual method with alcohol series and then cleared with xylene. Paraffin cubes were prepared and cutted

in slices of 5 µm by microtome. For histological examination, sections were stained with hematoxylin-eosin and examined with the light microscope Olympus. The seminiferous tubules with spermatogenesis cells inside (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperms) and Leyding cells were evaluated and most significant parts were illustrated.

Results and discussions

Testicles in Zebrafish are surrounded by peritoneum wall and they are structured by seminiferous tubules, germ cells (spermatogonia primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa), Sertoli cells and also Leydig cells in the connective tissue betwen seminiferous tubules. The phases of spermatogenesis are divided into spermatocytogenesis, meiosis and spermiogenesis based on the histological characteristics. The specific cells of every phase are identified as different groups of cells in the seminiferous tubules (fig. 1). Four types of spermatogonia, 2 main types of spermatocytes, 3 different types of spermatids and spermatozoa were identified in the seminiferous tubules.

Fig.1. Seminiferous tubule in cross section with all stages of germ cells H&E x400; 1- spermatogonia; 2- spermatocytes; 3- spermatid; 4- spermatozoa; 5- seminiferous tubule's lumen; 6- spermatogonial stem cells.

The spermatocytogenesis includes the mitotic transformations of the germinative epithelium. The spermatogonia types differ by their nuclear form, the extent of the cromatin condensation, the number of nucleoli and by cells size. They were the largest germ cells from the seminiferous tubules and they were diploid.Type A undifferentiated spermatogonia were isolated

cells scattered through germinative epithelium with irregular nuclear envelope and elongated, slightly basophilic and low heterochromatic nucleus (fig. 3). They had rich and pale cytoplasm. The type A differentiated spermatogonial cells differed from the undifferentiated ones by the fact that they could be found in groups of two to eight germ cells in a cyst and by their round or oval nucleus with regular envelope. Type B spermatogonia formed groups 16 or more cells arranged in cysts and they had a darker and clearer nucleus than type A spermatogonia due to the high amount of heterochromatin (fig. 4, 5) .

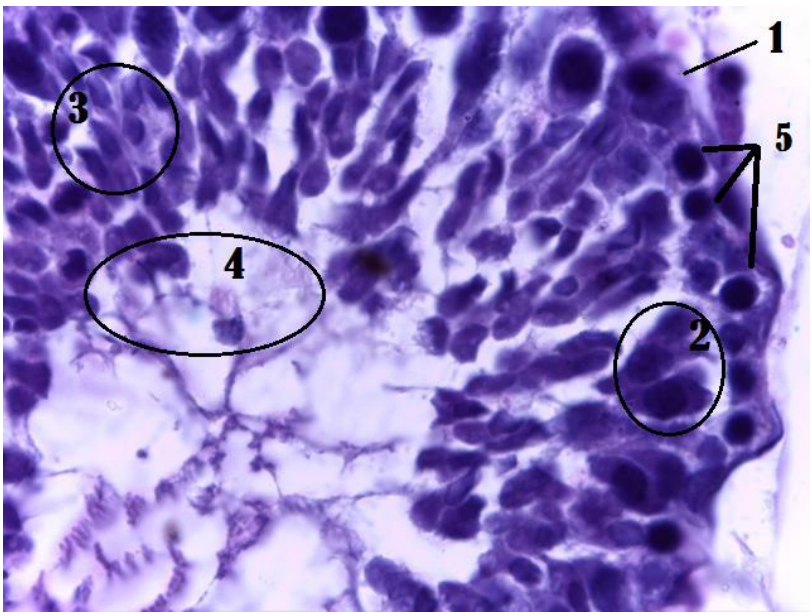

Fig.2. Seminiferous tubule in cross section with all stages of germ cells H&E x900; 1- type A undifferentiated spermatogonia; 2- type B spermatogonia; 3- spermatocytes; 4- spermatozoa; 5- spermatogonial stem cells.

The meiotic phase starts after the type B spermatogonia turn into primary spermatocytes. In this stage that is also called spermatocitary phase spermatocytes in all meiotic phases were founded (fig. 3, 4). At the end of the first meiotic division secondary spermatocytes can be observed. After they pass through another meiotic division, spermatids are formed and spermiogenesis starts.

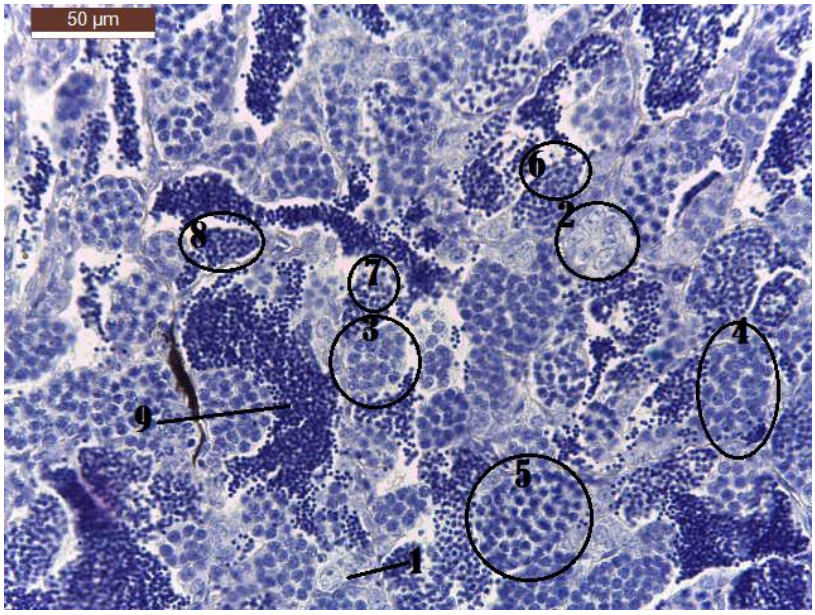

Fig.3. Seminiferous tubule in cross section with all stages germ cells H&E x400; 1-type A undifferentiated spermatogonia; 2- type A differentiated spermatogonia; 3- type B spermatogonia; 4- primary spermatocytes; 5- secondary spermatocytes; 6- initial spermatids; 7- intermediate spermatids; 8- final spermatids; 9- spermatozoa, H&E.

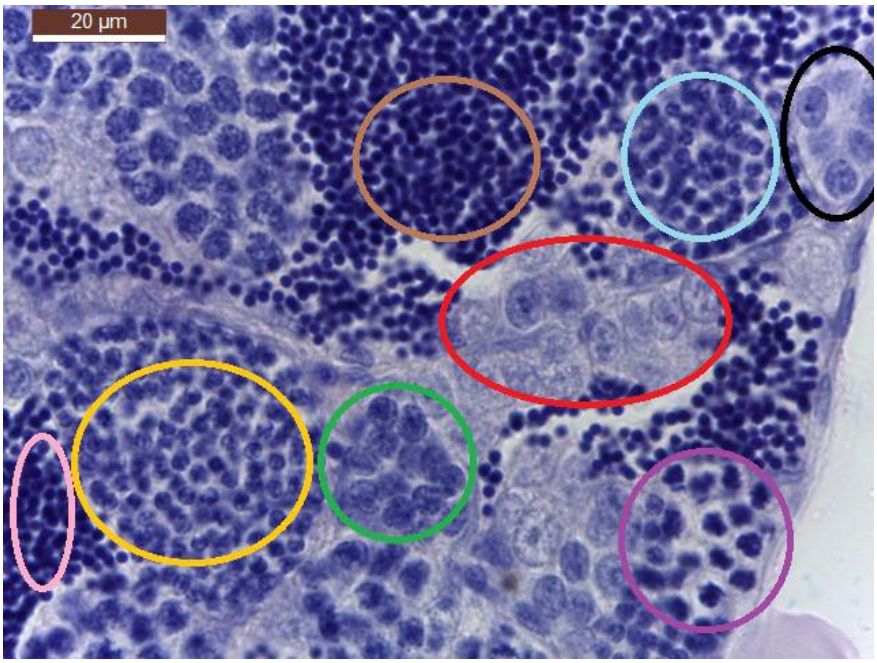

Fig.4. Seminiferous tubule in cross section with all stages of germ cells H&E x900; black circle - type A differentiated spermatogonia; red circle –type B spermatogonia; green circle –primary spermatocytes; purple circle –secondary spermatocytes; yellow circle – initial spermatids; blue circle – intermediate circle; brown circle – final spermatids; pink circle- spermatozoa.

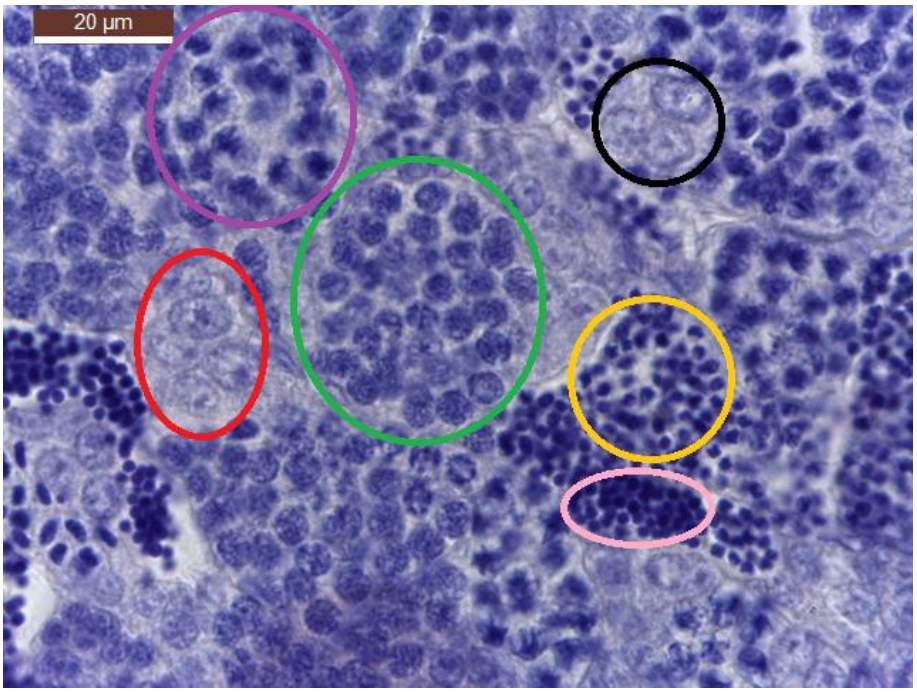

Fig. 5. Seminiferous tubule in cross section with all stages germ cells H&E x900; black circle - type A differentiated spermatogonia; red circle – type B spermatogonia; green circle – primary spermatocytes; purple circle – secondary spermatocytes; yellow circle – initial spermatids; pink circle- spermatozoa

Spermiogenesis is the final phase and it also may be called differentiation phase. At this point 3 types of spermatids were founded: initial, intermediate and final, according to the nuclear condensation, cytoplasmic reduction and cell size reduction, increase of the space betwen the cells inside the cyst due to cytoplasmic elimination and flagellum development (fig. 4). The initial spermatids were smaller than the anterior germ cells and more concentrated in the cyst and had a reduced, rounder and condensed nucleus. Intermediate spermatids were even smaller with more reduced and condensed nucleus, scanty cytoplasm and bigger space betwen them. The smallest cells from the spermiogenesis are the final spermatids that have even more concentrated cytoplasm that will form only a reduced strip around the more condensed nucleus. Larger spaces betwen final spermatids appear. The completion of the flagellum development, cytoplasmic residues elimination and final spermatids maturation leads to sperm formation. The spermatozoa were the smallest from all the germ cells and were found only in the tubular lumen (fig. 1, 2, 4).

Maack et al. (2003) observed that the alterations of gonadal morphology in some of the 5–11 week-old post fertilization zebrafish are interpreted as a transformation of early ovary-type gonads into testes and that sexual differentiation of developing gonads in fish is considered to be under the control of steroid hormones.

Schulz et al. (2010) has examined the development of spermatogenesis and the testicle structure of zebrafish both with electron and light microscope. He noted that the Sertoli cells could be seen with electron microscope, but not with light microscope and we consider that this observation is related to the fact that we could not see in our sections examined at light microscope the sertoli cells. Thereby, our findings are similar to the data determined in this study.

Leal at al. (2009) found that undifferentiated type A spermatogonia in zebrafish were distributed along the entire germinal compartment, and so they concluded that zebrafish testis belongs to the unrestricted type. Moreover, they noted that zebrafish testes contain anastomosing tubules, which is another primitive feature. Another observation was that type-A differentiated spermatogonia are originated from previous ones and showed cytoplasmic bridges among them, due to incomplete cytokinesis during mitosis process.

Conclusions

The testicles in Zebrafish have a different histological structure than mammals. The dividing germ cells are held together by intercellular junctions forming clusters of clonal and synchronously developing cells, structuring the functional unit of the Zebrafish testis, the spermatocyst. This cyst has also in his structure the cytoplasmic processes of Sertoli cells. As the spermiogenesis reaches its end and the spermatozoa are formed, the spermiation process will degenerate the spermatocyst and the sperm will continue their way through the seminiferous lumen, spermatic duct, urogenital sinus and ultimately are released through the urogenital pore (9, 13).

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