Available online at www.elixirpublishers.com (Elixir International Journal)

Aquaculture

Elixir Aquaculture 46 (2012) 8117-8120

ISSN: 2229-712X

Histological structure of zebrafish (*Danio rerio*, Hamilton, 1822) testicles

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ARTICLE INFO

Article history:

Received: 9 March 2012;

Received in revised form:

15 April 2012;

Accepted: 28 April 2012;

Keywords

Histology,

Zebrafish,

Testis.

ABSTRACT

In this study, histological examination of the testicles and cellular tissue changes of sperm cells in the spermatogenesis phase of zebrafish are aimed. In this respect; the microscopic structure of the testicles, the position and form of the cells belonging to spermatogenic string, tissue-generating structural cells, and these organisms are examined with the light microscope. All the phases of spermatogenesis are classified as spermatocytogenesis, meiosis and spermiogenesis taking all the phases and histological development of the cells into consideration. All the cells belonging to these phases are seen in separate groups in seminiferous tubules. It is found out that there are somatic cells around spermagony. And spermatogenic cells return into spermatogenic string cells respectively (primer spermatocyte, secondary spermatocyte, spermatide and sperm organisms) by meiosis.

Introduction

The zebrafish model is becoming more and more popular because it is easy to produce. A model organism should offer technical and practical advantages to study principal biological processes, effects and mechanisms. In addition, it needs to have traits that can be generalized, i.e. the model organism has to be representative for a larger group of organisms. Both arguments come true for the zebrafish.

It is a convenient and cost-effective species to work with from a technical and methodological point of view, and it provides conceptual insights into many aspects of vertebrate biology, genetics, toxicology and disease [1]. Whereas information on many aspects of zebrafish biology and ecology in the field is surprisingly limited [2], considerable knowledge exists with respect to optimum breeding and maintenance conditions in the laboratory [3]. The relatively short generation time of three to four months is an advantage. The principal advantages of the zebrafish model discussed above make this species also a suitable model for toxicological purposes. Zebrafish have been used as a general vertebrate toxicity model [4-5], but also as an ecotoxicological test species to determine the effects of chemicals on fish survival, growth and reproduction.

Spermatogenesis is a complex and highly coordinated process by which diploid spermatogonia produces millions of spermatozoa daily [6]. This process is fueled by spermatogonial stem cells, which have the potential for both self-renewal and for differentiating into spermatogonia committed to sperm development [7, 8]. (9) reported that zebrafish is a juvenile hermaphrodite, with all individuals having ovary-like gonads during early life and the bisexual differentiation taking place only later during development. The present study extends the observations of (9) and examines the morphological changes in gonads of juvenile zebrafish during transition from the protogynic ovary to the early testis in more detail. There are not many studies using the histological methods on the reproduction biology of this species in the literature.

Material and Methods

The preparation of histological preparates

The pieces taken from male zebrafish are determined in %10 Neutral Formalin and Bouin solutions. For the histological examinations, paraffin cubes are prepared and cut by 5 µm microtome in accordance with Luna's (10) routine preparation technique.

In order to see seminiferous tubules, testioles, connective tissues, spermatogenesis cells in seminiferous tubules (spermatogonium, primer spermatocyte, secondary spermatocyte, spermatitis, and sperms) and Leyding cells in the cuts; they were dyed with hematoxylin eosin and examined with the light microscope [10, 11, 12]. The findings are assessed and the significant parts are illustrated.

Results

It is seen in the macroscopic examinations that testicles taken from the zebrafish are surrounded by peritoneum walls. In the histological structure, seminiferous tubules, testioles, connective tissues, spermatogenesis cells in seminiferous tubules (spermatogonium, primer spermatocyte, secondary spermatocyte, spermatitis, sperms) and leyding cells in the connective tissues between seminiferous tubules are seen (*Fig 1*).

All the phases of Spermatogenesis are recognized considering histological structures as spermatocytogenesis, meiosis, and spermiogenesis. All the cells belonging to these groups are seen as different groups in seminiferous tubules; the connective tissues between seminiferous tubules and the cells in spermatogenesis phases are also monitored (*Fig 2*).

Fig 1. Microscopic image of general testicals, seminiferous tubules (H.E x10)

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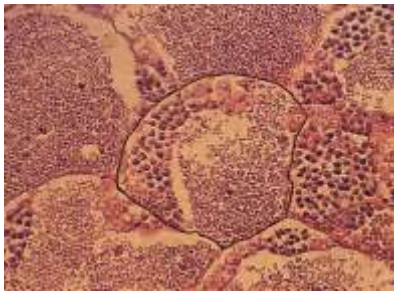

Fig 2. Microscopic image of seminiferous tubule (H.E x40)

Spermatocytogenesis

It is found out that in this phase spermatogonium cells have large, pale and homogeny cytoplasm and a very large nucleus. It is determined that the ones with the vague nucleus are A spermatogonium and the ones with darker and clear nucleus are B spermatogonium cells. Spermatogoniums have mitotic division thus multiply and turn into spermatocytes. In the histological cuts spermatocyte cells in this phase are smaller, have denser cytoplasm than spermatogoniums. As a preparation to meiosis, the cytoplasm is covered with chromatin; thus the nucleus could not be seen clearly. It is clearly seen that the cells belonging to each phase form groups (*Fig 3, 4*).

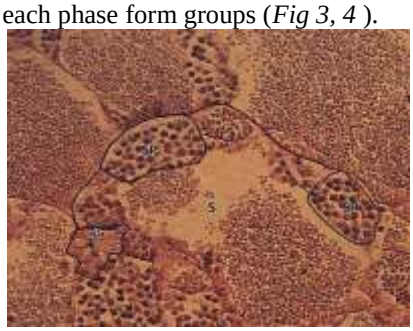

Fig 3. The cells in seminiferus tubule. SG; spermtogonium cells, SP; spermatocyte cells, S; sperm cells, arrow; type B spermatogonium cells (H.E x40)

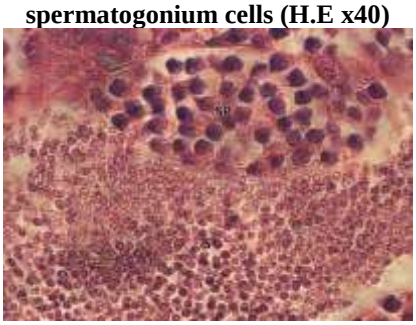

Fig 4. Cells in seminiferus tubule. SP; spermatocyte cells, S; sperm cells (H.E x 100)

Meiosis

It is determined that in this phase, spermatocyte cells are divided into two as primer and secondary spermatocyte. It is seen that primer spermatocyte cells are ovoid and have dense cytoplasm. In the first phase of meisosis, it is seen that primer spermatocyte cells covered with dense chromatin have vague cell cores; and even though secondary spermatocytes have similar shape with primer spermatocyte, they are smaller and rounder (*Fig 5*). It is also determined that spermatites constituted after the meiosis of secondary spermatocytes are the smallest and roundest cells in semifinerous tubule.

Spermiogenesis

In this final phase it is seen that spermatites become smaller and have sperm cells with tail-like structures (*Fig 6*). All the spermatogenesis phases in seminiferous tubules are observed by using different magnification ratios (*Fig 7, 8, 9, 10*).

It is determined that while all the spermatogenesis cells except sperms are generally found in the basal part of seminiferous tubule; sperms are found in the lumen of semifinerous tubule. Even though leyding cells are seen in connecting tissues in seminiferous tubules; there is no track of sertoli cells.

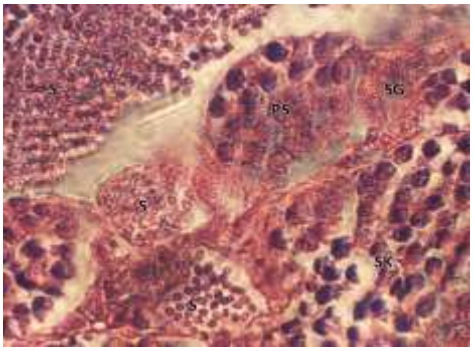

Fig 5. The cells in the semifinerous tubule. SG; spermatogonium cells, PS; primer spermatocyte, SK; secondary spermatosyte cells, S; sperm cells. (H.E x100).

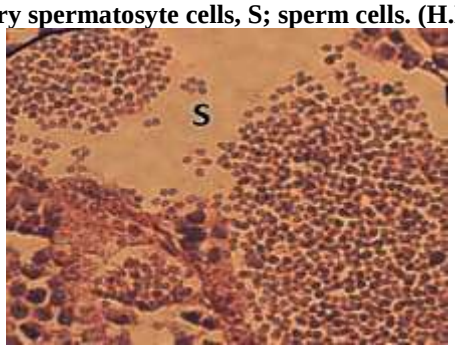

Fig 6. The cell structures of changing sperms and tail-like structures. S; sperm cells 400 µm

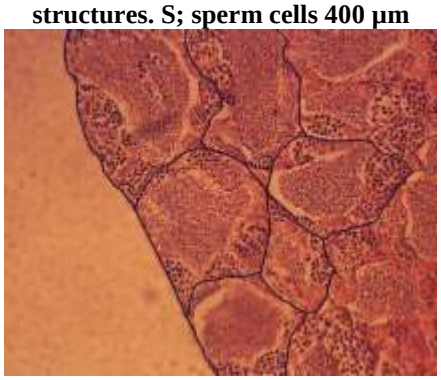

Fig 7. General testicular structure and seminiferous tubules (H.E x20)

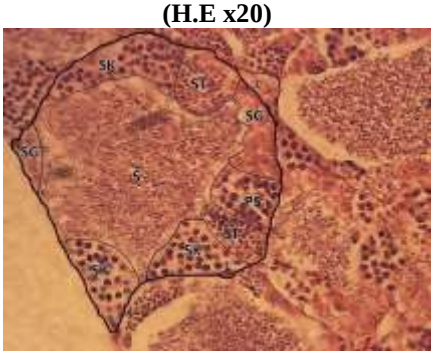

Fig 8. The general view of the cells in seminiferous tubule. SG; spermatogonium cells, PS; primer spermatocyte, SK; secondary spermatocyte cells, ST; spermatite cells, S; sperm cells, L; leyding cells, (H.E x40)

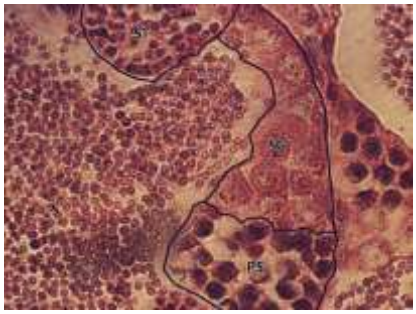

Fig 9. The general view of the cells in spermatogenesis phase. SG; spermatogonium cells, PS; primer spermatocyte, ST; spermatite cells, S; sperm cells (H.E x100)

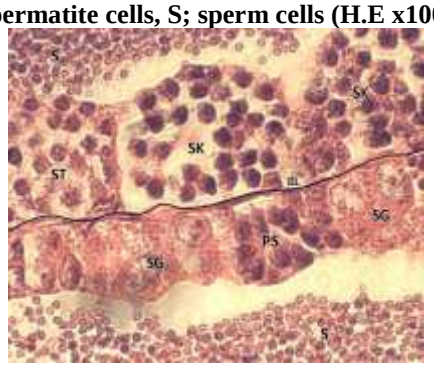

Fig 10. The cells between the borders of two semifinerous tubule. SG; spermatogonium cells, PS; primer spermatocyte, SK; secondary spermatocyte cells, ST; spermatite cells, S; sperm cells, BL; basal lamina (H.E x100)

Discussions

The testicles in zebrafish are organs in the shape of anastomosis tubule in the ventral cavity in dorsolateral position. According to the order of spermatogenesis cells, testicles are determined to be in the order of unlimited spermatogonial string. Spermatogenesis steps in different phases are observed at the end of examinations on histological preparates stained by hematoxylen-eosin. All the cells of spermatogenesis phases are examined. Spermatononium cells are determined to be the largest cells with eminent cores. It is clearly seen that up to the sperms in the final phase, the size of the cells gets gradually smaller. Even though sertoli cells could not be observed, leyding cells were clearly seen.

Schulz et al. (13) has examined the development of spermatogenesis both in mammals and fish in his studies. He monitorized the testicle structure of zebrafish both with electron and light microscope. Although he could see the sertoli cells with electron microscope; they were invisible when he used light microscope. Thus they verified that it is not possible to monitorize sertoli cells with light microscope. It is thought that the reason we could not see sertoli cells in our study is that we used light microscope in our examinations. Our findings are parallel to the data determined in this study.

Parenti and Grier (14) determined in their study on the gonads of teleostei that the fish in the *Megalops atlanticus*, *Abbotina rivularis*, *Oncorhynchus mykiss* and *Exos lucius* family have anastomosis tubular testicles. They observed that spermagonial order in the lobular testicles has a form of limited or unlimited distribution. It is determined that in the light microscope images of *Fundulus grandis*, *Mugil cephalus*, *Hemiramphus brasiliensis* family, testicles have limited distribution while spermatogonial order of *Percopsis omiscomaycus* and *Abbotine rivularis* family has unlimited distribution. Also, they observed the anastomosis tubular structure of zebrafish. Spermatogonial orders found in this study have similarities with the findings in our research. Furthermore, anastomosis tubular testicle structure is found to be similar to the findings in our study.

Dziewulska and Domagala (15) have determined in their studies examining the testicle structure with light microscope that spermatogonial order is unlimited. Also, they have seen that sertoli cells only form a cystic organism around spermatogonium cells. As the findings of this study are similar to ours, we can say that the histology of salmon fish testicles is similar to the histology of zebrafish testicle.

Grassiotto and Carvalho (16) have examined the structure of testicles of *Sorubim lima* fish with electron microscope. They monitorized that the testicles are lobular with electron microscope. They determined that sertoli cells are in basal lamina of seminiferous tubules. They have seen that spermatogenesis cells are surrounded by sertoli cell cytoplasm. It is seen that spermatogenesis cells are inside of cystic structures formed by sertoli cells. The results of this study are also similar to ours. Thus we can say that the spermatogonial order of *Sorubim lima* fish are similar to zebrafish.

It is determined in Hyder (17) study of *Tilapia leucosticta* that spermatogonial order has unlimited distribution. In the light of this fact; even though there are similarities between the spermatogonial order of zebrafish and fish belonging to *Tilapia* family, some differences are seen as the sertoli and connective tissue cells could not be observed. Testicle structure of *Parasilurus aristoteles* has similarities with the structure of zebrafish testicles in terms of the fact that the density of spermatogenesis cells in the tubular structure and seminiferous tubular is not equal. However, it shows difference because it has limited spermatogonial order [18]. It is determined that although the fish in the *Thunnus alalunga* family have lobular testicles, there are similarities among spermatocyte, spermatite and sperm cells in our study on zebrafish which has testicular testicles [19]. It is determined in the studies of (20) on the testicles of *Fundulus heteroclitus* that spermatogonial order has unlimited distribution.

References

1. Segner H. Zebrafish (*Danio rerio*, Hamilton,1822) as a model organism for investigating endocrine disruption. Comp Biochem Physiol. 2009; 149: 187-195.

2. Spence R, Gerlach G, Lawrence C, Smith C. The behaviour and ecology of the zebrafish, (*Danio rerio*, Hamilton,1822). Biol Rev. 2008; 83: 13-34.

3. Westerfield M. The zebrafish book. A guide for the laboratory use of the zebrafish (*Brachydanio rerio*). 3rd edition. University of Oregon Press, Eugene, USA 2000.

4. Maack G, Segner H. Morphological development of the gonads in zebrafish. J Fish Biol. 2003; 62: 895-906.

5. Maack G, Segner H, Tyler CR. Ontogeny of sexual differentiation in different strains of zebrafish (*Danio rerio*). Fish Physio Biochem. 2004; 28 (1-4):125-128.

6. Hess RA, Franc ALR. Structure of the sertoli cell. In: Skinner MK, Griswold MD (eds.), Sertoli Cell Biology. San Diego, CA: Elsevier Academic Press; pp 19-40, 2005.

7. De Rooij DG, Russel LD. All you wanted to know about spermatogonia but were afraid to ask. J Androl. 2000; 21: 776-798.

8. Ehmcke J, Wistuba J, Schlatt S. Spermatogonial stem cells: questions, models and perspectives. Hum Reprod Update. 2006; 12: 275-282.

9. Takahashi H. Juvenile hermaphroditism in the zebrafish, (Brachydanio rerio). Hokkaido University Bulletin of the Faculty of Fisheries.1997; 28, 57-65.
10. Luna LG. Manual of histologic staining methods of the armed forces institue of pathology. McGraw Hill Book Company, New York, 1968.
11. Ural M, Özdemir Y. The morphological characteristic and ovarium histological structure of freswater chub (*L.cephalus*) in the Keban Dam Lake. University of Fırat, Journal of Science Engineer. 2004; 12(1): 313-321.
12. Ural M, Özdemir Y. Water quality from Koçkale and Pertek regions of Keban Dam Lake (Elazığ/TURKEY) on ovarium tissue of *Capoeta trutta* (Heckel,1843) histopathological effect. Aust J Basic Appl Sci. 2011; 5(8): 1096-1105.
13. Schulz RW, França LD, Lareyne JJ, Legac F, Garcia HC, Nobrega RH, Miura T. Spermatogenesis in fish. General and Comp Endocri. 2010; 165(3): 390-411.
14. Parenti LR, Grier HJ. Evolution and phylogeny of gonad morphology in bony fishes.
Integr Comp Biol.2004; 44: 333-348.
15. Dziewulska K, Domagala J. Histology of Salmonid Testes During Maturation. Reproduc Biol.2002; 3(1): 47-61.
16. Grassiotto IQ, Carvalho ED. The ultrastructure of *Sorubim lima* (Teleostei, Siluriformes, Pimelodidae) Spermatogenesis: Premeiotic and Meiotic Periods. Tissue and Cell. 1999; 31 (6): 561-567.
17. Hyder M. Histological studies on the testis of *Tilapia leucosticta* and other species of the Genus *Tilapia* (Pisces: Teleostei). Trans Amer Microsc Soc. 1969; 88(2): 211-231.
18. Iliadou K, Fishelson L. Histology and cytology of the catfish *Parasilurus aristotelis*
(Siluridae, Teleostei) From Greece. Japan J Ichthyol. 1995; 41 (4): 447-454.
19. Ratty FJ, Laurs RM, Kelly RM. Gonad morphology, histology, and spermatogenesis in South Pacific Albacore Tuna *Thunnus alalunga* (Scombridae). Fishery Bulletin. 1990; 88(1): 207-216.
20. Pickford GE, Lofts B, Bara G, Atz JW. Testis stimulation in hypophysectomized male killifish, *Fundulus heteroclitus*, treated with mammalian growth hormone and/or luteinizing hormone. Biology of Reproduction. 1972; 7 (3): 370-386.