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Pre-spermiogenic initiation of flagellar growth and correlative ultrastructural observations on nuage, nuclear and mitochondrial developmental morphology in the zebrafish *Danio rerio*

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ABSTRACT

The microstructural and ultrastructural changes of germ cells during spermatogenesis of zebrafish (*Danio rerio*) were examined using light microscopy (LM) and transmission electron microscopy (TEM). Generally the process of spermatogenesis in zebrafish is similar to that of other teleosts, however, here we describe some peculiar features of zebrafish spermatogenic cells which have a limited report in this species. (1) The basic events of spermiogenesis are asynchronous, location of flagellum finished in initial stage, while chromatin condensation sharply occurred in intermediate stage and elimination of excess cytoplasm mainly taken place in final stages. (2) Surprisingly, the cilia or initial flagellae are created in spermatocytes, approach toward the nucleus of early stage spermatids, and then the centrioles depress into nuclear fossa and change their orientation to each other from right angle to obtuse angle about 125°. (3) During spermatogenesis, the chromatin compaction performs in a distinctive pattern, condensed heterogeneously from granular into chromatin clumps with central electron-lucent areas, round or long, which diminished to small nuclear vacuoles in spermatozoa. This finding demonstrates the origin of nuclear vacuoles in zebrafish spermatozoa for the first time. (4) Nuages are observed in both spermatogonia and spermatocytes. They are connected with the mitochondria and nuclear membrane, and are even located in the perinuclear spaces of spermatogonia nuclei. (5) Mitochondrial morphology and distribution shows diversity in different germ cells. The condensed mitochondria appear in pachytene spermatocytes, and mitochondria including membrane conglomerate exist in both spermatocytes and spermatids. This study was undertaken in order to disclose specific spermatogenic cells features in zebrafish that could be helpful for understanding the correlative function in this model species.

1. Introduction

The zebrafish (Teleostei, Cyprinidae) is a model organism in developmental, physiological, toxicological, and genetic studies because it offers several advantages to researchers in these fields (Briggs, 2002; Hill, 2005; Norton and Bally-Cuif, 2010; Traver et al., 2003). For example, the transparent embryos have been invaluable for developmental studies, and the short reproductive and developmental cycles allow for rapid and efficient data collection. Spermatogenesis in zebrafish has been studied by relatively few authors (Leal et al., 2009; Rupik et al., 2011; Schulz et al., 2010). Leal et al. (2009) distinguished the types and the generation of germ cells, and divided spermiogenesis into three stages based on increasing nuclear compaction, Rupik et al. (2011) presented the basic ultrastructural characteristics of spermiogenesis of zebrafish and, in contrast to Leal et al. (2009), divided spermiogenesis into four stages.

Although some aspects are now well described, there are no reports providing complete ultrastructural descriptions of the processes of spermatogenesis, proliferation and meiosis. There are also some important uncertainties regarding primary events, such as when the initial flagellum forms, how the chromatin becomes concentrated within spermatids and so on. These are related to the final structure of the spermatozoa, the dynamics of gene expression and

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the mechanisms underlying loss of optimal functionality, such as the reduced motility that occurs when zebrafish embryos develop in the presence of environmental toxicants (McAllister and Kime, 2003).

The present study addresses the fine structural changes in the spermatogenic cells of zebrafish, with particular focus on flagellar formation, chromatin condensation, appearance of nuclear vesicles, distribution of nuages, and the change and arrangements of mitochondria. These results will be helpful for understanding the correlative functional and structural observations in this model species.

2. Materials and methods

Sexually mature male zebrafish were collected from the Shanghai Laboratory Animal Research Center. They were reared under controlled photoperiod (daily range: 14–10 h LD), temperature (daily range: 28–30 °C) and pH (7.0–7.8) in a recirculating aquaculture system. They were fed twice daily with dry food pellets (protein > 38.5%). The fishes were taken at random from the tank, and euthanized with an overdose of the anesthetic MS-222 (tricaine methane sulfonate, 150 mg/L). (Ethics Statement: All efforts were made to minimize animal's suffering. The slaughtering and sampling procedures were approved by the Nanjing Agricultural Veterinary College Experimental Animal ethics committee. The approval ID is SYXK (SU) 2010-0005.)

Three male zebrafish, removed heads and tails, were fixed in Bouin's solution overnight, embedded in paraffin wax, and the entire fishes serially sectioned (at 3 μm). The sections were stained with hematoxylin and eosin for light microscopic observation, using an Olympus microscope (BX53), camera (Olympus DP73). For transmission electron microscopy (TEM), five pairs of contralateral testes were removed, cut into small blocks, then fixed in a mixture of 2.5% (v/v) glutaraldehyde in phosphate buffered saline (PBS; 4 °C, pH 7.4, 0.1 M) for 24 h, post-fixed in 1% (w/v) osmium tetroxide in the same buffer for an hour, dehydrated in ascending concentrations of ethyl alcohol, and infiltrated with a propylene oxide-Araldite mixture for embedding in Araldite (Zhang et al., 2007). The blocks were sectioned, and the ultrathin sections (50 nm) were mounted on Formvar-coated grids, stained with uranyl acetate and lead citrate for 20 min per step. The sections were examined using a HITACHI H-7650 transmission electron microscope.

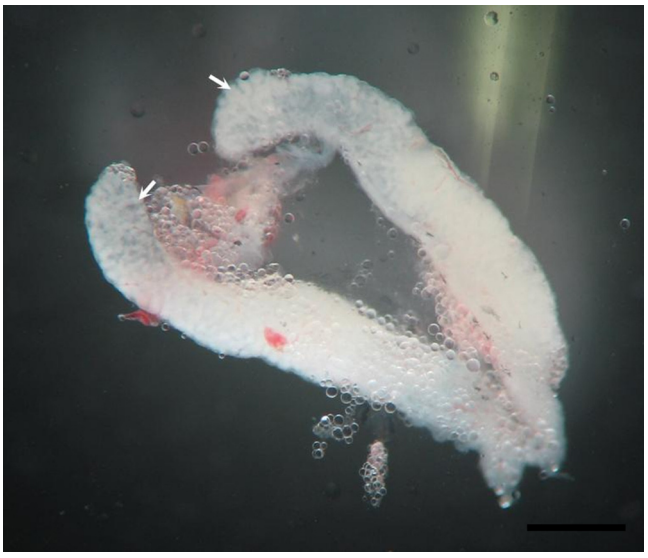

Fig. 1. The testis of zebrafish composed of numerous milky and tortuous seminiferous tubules (arrow), viewed under the dissecting microscope. Scale bar: 1 mm.

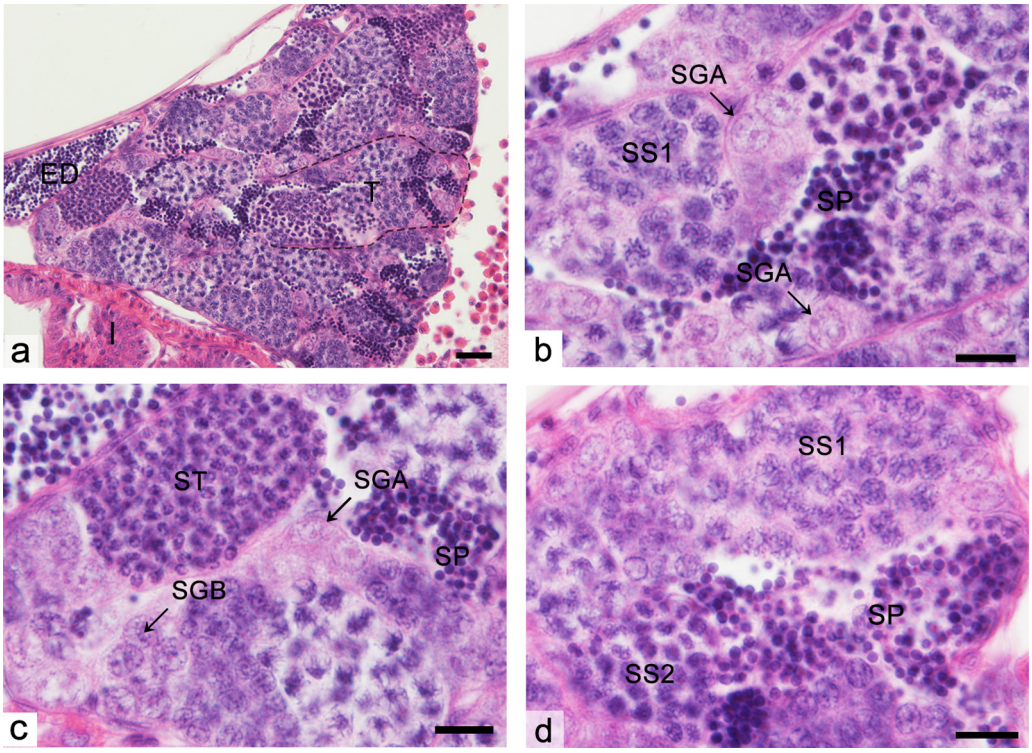

Fig. 2. The histological structure of testis in zebrafish, H&E stain. (a) Transverse section of the testis. (b–d) Spermatocysts containing different germ cells. *Abbreviations*: ED, efferent ducts; I, intestine; SGA, type A spermatogonia; SGB, type B spermatogonia; SS1, primary spermatocytes; SS2, secondary spermatocytes; SP, spermatozoa; ST, spermatids; T, seminiferous tubule. Scale bars: 20 μm (a) and 10 μm (b–d).