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Spermatogenesis in the rock oyster, *Saccostrea forskali* (Gmelin, 1791)

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ABSTRACT

Morphology of the differentiating spermatogenic cells of the rock oyster *Saccostrea forskali* (Bivalve: Ostreidae) was investigated by light and transmission electron microscopy. The testis is formed by several branching acini containing developing spermatogenic cells, classified into 7 stages based on nuclear characteristics, patterns of chromatin condensation and cytoplasmic contents. The spermatogonium is characterized by a euchromatic nucleus with a prominent nucleolus. The cytoplasm contains several round granulo-fibrillar dense bodies surrounded by numerous mitochondria. The round nucleus of the primary spermatocyte contains patches of electron-dense heterochromatin, numerous proacrosomal vesicles, ribosomes and mitochondria. The secondary spermatocytes contain a reticulated chromatin pattern and reduced number of proacrosomal vesicles. The early spermatids contain a small amount of euchromatin among dense patches of heterochromatin. A large single acrosomal vesicle is located in the posterior part of the cell. The middle spermatid is characterized by the migration of an acrosomal vesicle to the anterior part of the nucleus. The late spermatids contain highly condensed heterochromatin blocks and the acrosomal vesicle becomes cup-shaped and invaginated at the basal part. The spermatozoon contains a barrel-shaped head covered with the cup-like acrosome. At this stage, the subacrosomal space contains an axial rod in subacrosomal materials. Three to four transverse bands appear at the anterior region of the acrosome. The middle piece consists of spherical mitochondria surrounding the proximal and distal centrioles. The flagellum consists of 9 + 2 axonemal microtubule doublets surrounded by the plasma membrane. Our electron microscopic study of spermatogenesis in the *S. forskali* provides important new information on the mechanism of development of spermatogenesis of this species.

1. Introduction

The rock oyster, *Saccostrea forskali*, is one of nine oyster species found widely distributed along the eastern coast of the Gulf of Thailand, as well as some parts of the southern coast (Yoosukh and Duangdee, 1999). It is a commercially important species that is popular among local consumers as well as for export. The spats of this species prefer to settle throughout a wide intertidal area and shallow subtidal rocky substrata (Klinbunga et al., 2005). Culture of *S. forskali* in Thailand is small-scale, using natural spats collected from the broodstocks gathered from the sea, which is mostly established in the eastern and south provinces (Yoosukh and Duangdee, 1999). In Thailand, annual production was estimated to be approximately 10,700 tons (Department of Fisheries, 2012). Although there are many researcher investigations devoted to the study of optimal aquaculture systems for this species, basic knowledge concerning some reproductive aspects such as the histology of the testes and the production of male gamete cells are still lacking. This basic information can be very useful in the collection and production of the spermatogenic cells to be used for aquaculture of this species.

Histology of the gonad and ultrastructure of spermatogenic cells in oysters have been studied in a number of species, mainly within *Crassostrea* genus: *C. angulata* (Sousa and Oliveira, 1994), *C. gigas* (Franco et al., 2008; Kim et al., 2010a; Yurchenko et al., 2010) and *C. verginica* (Eckelbarger and Davis, 1996). According to these studies, basic morphology of spermatozoa has a similar pattern; the head contains a barrel-shaped nucleus, a cup-shaped acrosome, and subacrosomal material with an axial rod. Also, four spherical mitochondria are in the midpiece, and a flagellum that has a typical 9 + 2 microtubular pattern of axoneme organization. Morphology of spermatozoa in the *Saccostrea* genus has been studied in only two species: *S. commercialis* (glomerata) (Healy and Lester, 1991) and

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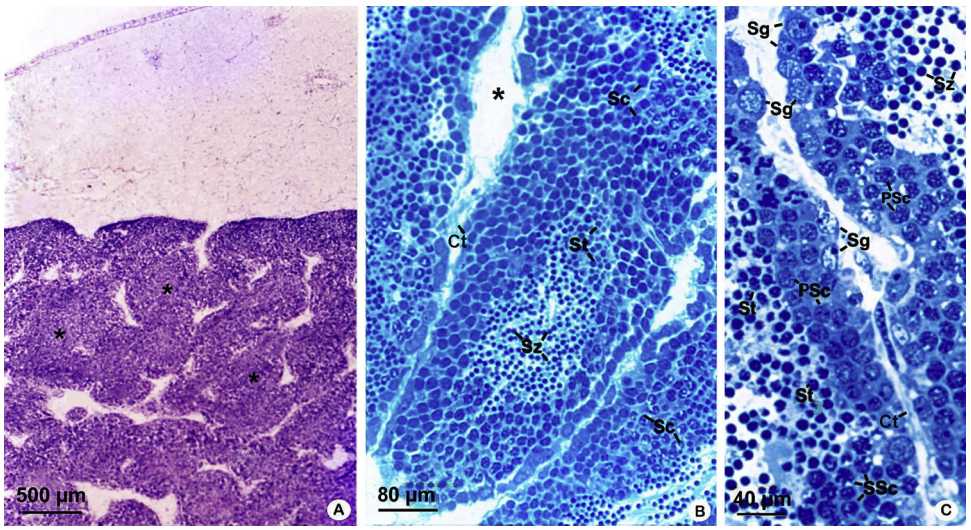

Fig. 1. Light micrographs of the testis of the rock oyster, *Saccostrea forskali*. A. The localization of testis under the mantle epithelium showing numerous acini (indicated by asterisks). B. Subcompartments showing spermatogenic cells distributed in a centripetal pattern from the acinus wall toward the lumen. Sc, spermatocytes; St, spermatids and Sz, spermatozoa; asterisk, hemocoel. C. Part of acini showing various stages of developing male sex cells. Sg, spermatogonia; PSc, primary spermatocyte; SSc, secondary spermatocyte; St, spermatid; Sz, spermatozoa.

S. cf. mordax (Yurchenko, 2012). The ultrastructure of spermatogenic cells was studied within the *Crassostrea* genus: *C. angulata* (Sousa and Oliveira, 1994), *C. gigas* (Franco et al., 2008; Kim et al., 2010a; Yurchenko et al., 2010) and *C. verginica* (Eckelbarger and Davis, 1996), but has never been explored in any *Saccostrea* genus. The aim of this work was to study the morphology of the male *S. forskali* gonad by investigation of ultrastructural characteristics of the spermatogenic cells and spermatozoa, and then compare this with other studied species.

2. Materials and methods

2.1. Animals

Twenty adult male *S. forskali* with the shell length of 4.5–5 cm and body weight of 10–13 g were collected from farms located in the eastern coast of the Gulf of Thailand in Chonburi province during March to April.

2.2. Light microscopy

S. forskali were anaesthetized in 5% magnesium chloride ($MgCl_2$). The testes were dissected out and fixed in Bouin's solution overnight. The tissue samples were washed in 70% ethyl alcohol, dehydrated in a graded series of ethyl alcohol (70–100%) for 45 min each, and cleared with two changes of dioxane. Finally, they were infiltrated and embedded in paraffin wax. Five-micrometer thick sections were cut and stained with hematoxylin and eosin (H&E), then observed and photographed under an Olympus light microscope.

2.3. Transmission electron microscopy

Testes were diced into very small pieces and fixed in a solution of 4% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.8, at 4 °C overnight. The tissue samples were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, at 4 °C, for 2 h. Then, the tissue samples were dehydrated in a graded series of ethanol (50–100%) for 30 min each, cleared in two changes of propylene oxide, and infiltrated in a mixture of propylene oxide and Araldite 502 resin at the ratios of 3:1 for 1 h, 2:1 for 2 h, and 1:2 overnight. They were then infiltrated in pure Araldite 502 resin for 6 h, which was finally polymerized at 30 °C, 45 °C, 60 °C for 24, 48 and 48 h, respectively. One micrometer plastic sections were cut in an MT-2 ultramicrotome and stained with methylene blue for light microscopic observations. The ultrathin sections were cut at 50–60 nm thickness, stained with uranyl acetate and lead citrate, and then examined under a Hitachi H-300 Transmission electron microscopy at 75 kV. The ultrathin sections obtained from seven stages of sex cells were identified: spermatogonia, primary and secondary spermatocytes, early, middle and late spermatids and spermatozoa. At least twenty cells of each stage were measured. Cell diameter was calculated using Image J software analysis. A measurement of cell size is presented as mean and standard deviation ($\overline{X} \pm$ S.D.).

3. Results

The testis of *S. forskali* is a diffuse organ that consists of numerous acini and located between mantle and digestive gland (Fig. 1A). Each acinus is surrounded by a connective tissue compartment containing a fluid-filled space called the hemocoel and is subdivided into various subcompartments that partially isolate groups of maturing spermatogenic cells (Fig. 1B). Spermatogonia closely adhere to the inner wall of the acinus. Spermatocytes and spermatids are located above spermatogonia, close to the acinus lumen, while spermatozoa are completely detached and concentrate in the central lumen of the acinus (Fig. 1B and C).

Population of sex cells in the *S. forskali* testis can be classified into 7 stages (Table 1) based on nuclear characteristics; patterns of chromatin condensation and cytoplasmic contents are described as follows.

The spermatogonium is a spherical-shaped cell 5.6 ± 0.3 µm in diameter. It contains a euchromatic nucleus with a thin rim of heterochromatin along the nuclear envelope and small blocks of heterochromatin scattered throughout the central area (Fig. 2A–C). The nucleolus is peripherally located and high electron density (Fig. 2B). The cytoplasm contains several round granulo-fibrillar dense bodies surrounded by group of mitochondria that are peripherally located in the cell (Fig. 2B and D).

The primary spermatocyte is a round-shaped cell with a diameter of 4.3 ± 0.2 µm. It contains a round nucleus that shows patches