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Ultrastructure of spermatozoa in cobia, *Rachycentron canadum* (Linnaeus, 1766)

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ABSTRACTS

Ultrastructure and development of spermatozoa in cobia, *Rachycentron canadum* are described. Sections through the testis show different developmental stages viz, Spermatocytes, spermatids and sperm. Spermatozoa of *R. canadum* exhibit the configuration of uniflagellated, anacrosomal Type I aquasperm, typical for externally fertilizing fish. Mature spermatozoon is seen with a prominent head and long cylindrical flagellum. Ultrastructure of sperm shows invaginated 'U' shaped nucleus and other organelles. The mitochondrial matrix is electron-dense with irregular arrangement of the cristae. The nucleus reveals a deep invagination (nuclear fossa) in which the centriolar complex is located. The centriolar complex lies inside the nuclear fossa and is composed of a proximal and a distal centriole. The two centrioles are placed perpendicular to each other. The flagellum has a typical eukaryotic organization (microtubule doublets 9 + 2 pattern) and measures around 36.21 ± 0.42 µm in length. This study for the first time provides a comprehensive detail on the ultrastructure and developmental process of sperm in cobia, *R. canadum*.

1. Introduction

Morphology and structure of fish sperm provide information for understanding their possible taxonomic and evolutionary relationships at family (Jamieson and Leung, 1991; Mattei, 1991), subfamily and species (Lahnsteiner and Patzner, 2008) levels. Spermatozoa structure in teleost species reveals a high diversity, mainly at the family level. In general, the spermatozoa of internal and external fertilizers differ in their organization. External fertilizers have a simpler organization, showing an ovoid or spherical nucleus and a small midpiece containing only few mitochondria, whereas species with internal fertilization have an elongated nucleus and a relatively bigger midpiece (Lahnsteiner and Patzner, 2008; Jamieson, 2009). Descriptions of sperm morphology are also important for evolution studies attempting to elucidate the adaptative significance of diverse sperm forms (Cummings and Woodall, 1985; Gage, 1998; Anderson et al., 2005) and relating sperm morphology to phylogenetic classification (Meisner et al., 2005). Cobia, *Rachycentron canadum* (Linnaeus, 1766) is the only species of the family Rachycentridae (FishBase, 2012), distributed worldwide in tropical and subtropical regions, except the eastern Pacific (Briggs, 1960). To date, research and development of cobia aquaculture has been initiated in over 23 countries and territories, half of them in the Asian-pacific region (FAO, 2009). Therefore, an attempt has been made to study the ultrastructure and sequential development of sperm in cobia, *Rachycentron canadum*.

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2. Material and methods

2.1. Animal model and collection of milt

For the present study live male cobia, *Rachycentron canadum* of a length greater than 63 cm maintained at Central Institute of Brackishwater Aquaculture (CIBA) as well as from wild (Sajeevan and Kurup, 2017) were used (n = 46). Milt was collected from oozing out conditioned male fishes using cannula (1.2 mm dia) and the sperm was saved in sterile vials placed on ice. The samples were transported to the laboratory and stored at 4 °C until further processing. Simultaneously, an attempt was made to collect milt from dead fish (Cadaveric) at landing centres (Caylor et al., 1994). Briefly, for testicular sperm collection, testis were sliced and milt was caused to flow from the sperm ducts by scraping across the cross sectional area of the testis while exerting gentle squeezing pressure. During this time, the testes were laid on crushed ice and the sperm was stored as for live collection.

2.2. Histology

For histological studies, a piece of testis was fixed in 10% neutral buffered formaldehyde solution (pH 7.0) and processed. Sections of 6–7 μm thickness were taken and stained using hematoxylin and eosin. Photomicrographs were taken at various magnifications under a bright field using Leica microscope (DM 2500) (Germany).

2.3. Scanning electron microscopy

The sperm samples were fixed in modified Karnovsky's fluid buffered with 0.1 M sodium phosphate buffer at pH 7.4. Fixation was done for 3 h at 4 °C temperature, after which the sample was washed with fresh buffer and the washing was repeated thrice in double distilled water for 15 min (Marquez and Ogasawara, 1975). The samples were then dehydrated in a graded ethanol series and subjected to critical point drying 35 °C with Co_2. The samples were mounted on metal stub and coated with gold in a sputter coater. In this same way ultrathin section (2–3 μm) of testis were processed for this study. Both sperm sample and ultrathin sections were seen under a scanning electron microscope (HITACHI-SU6600, Japan). Scanning electron micrographs were taken at various magnifications by accelerating the voltage.

2.4. Transmission electron microscopy

The sperm samples were fixed in 2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer (pH 7.4) for 4 h and postfixed in 0.1% osmium tetroxide in 0.1 M cacodylate buffer for 2 h at 4 °C. The samples were then dehydrated in a graded ethyl alcohol series followed by propylene oxide treatment twice each and embedded in low viscosity Spurr resin. Sections were made on a LKB ultramicrotome with a glass knife and stained on drops of 2% uranyl acetate followed by lead citrate for 30 min. Sections were examined in a transmission electron microscope (JEOL JEM 1400, Japan) at an accelerating voltage of 80 kV.

3. Results

The male reproductive system of cobia includes a bilobed, whitish creamy testis attached to the dorsal part of the abdominal cavity. Testes occupy nearly three quarters of the cavity and are joined posteriorly. The two lobes of the testis are more or less equal in size, but occasionally in some fish they were unequal. Testis measured around 24.5 ± 5.27 cm in length and 333.26 ± 25.1 g in weight. The lobules end blindly at the periphery of the organ and are limited by a basement membrane that separates them from the interstitial tissue. Milt is generally released during spermiation into the lobular lumen, which is continuous with the efferent duct. The milt is generally seen as highly viscous and whitish in color.

3.1. Histology of testis

Sections through the testis showed two prominent compartments that can be distinguished within the testis; viz. the germinal, composed of sertoli cells and germ cells; and the interstitial, containing connective tissues (Fig. 1A). The testis is of the unrestricted lobular type and show asynchronous development of spermatocytes (Fig. 1B). Spermatocysts containing clones of germ cells, from spermatogonia to spermatids occur throughout the entire length of lobules (Fig. 1C & D). The sections through the testis show all the stages of developing spermatozoa surrounded by seminiferous tubules (Fig. 1E). Spermatozoa are seen as dense in the centre of the seminiferous tubule, and each spermatozoon possesses a head and prominent flagellum (Fig. 1F).

Scanning electron micrographs of ultrathin sections of the testis show distinct surface topography of spermatogonia, spermatocytes, spermatids and spermatozoa (Fig. 2A). Spermatocytes are slightly larger than the spermatozoa and seen along the wall of seminiferous tubule (Fig. 2B). The spermatids are seen with emerging flagella (Fig. 2 E&F).

3.2. Morphology and ultrastructure of sperm

Spermatozoon of cobia resembles that of a typical fish sperm. With scanning electron microscopic observations the sperm are seen as uniflagellated anacrosomal Type I aquasperm, and each spermatozoon possess a prominent head and long cylindrical flagellum