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Fine structure of spermatozoa in the blackspot sea bream *Pagellus bogaraveo* (Brünnich, 1768) with some considerations about the centriolar complex

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ABSTRACT

Scanning and transmission electron microscopy were used to investigate the fine structure of the sperm of the sparid fish *Pagellus bogaraveo*.

The spermatozoon of *P. bogaraveo* belongs, like that of the other sparid fish, to the teleostean "type I" spermatozoon with the flagellar axis insert perpendicular to the nuclear fossa. It has an ovoidal head, a short, cylindrically shaped midpiece and a long tail region. The nucleus reveals a deep invagination (nuclear fossa), in which the centriolar complex is located, and a satellite nuclear notch shaped like a golf club. The two centrioles are perpendicular to each other and show a conventional "9+0" pattern. The distal centriole is attached to the nuclear envelope by means of basal feet and radial fibers made of electron-dense material. Below the basal plate, plasma membrane pinches in, and the necklace, a specialized connection joining axonemal doublets to the plasma membrane, is visible. The short midpiece houses one mitochondrion. The flagellum is perpendicularly and eccentrically with respect to the nucleus and contains the conventional "9+2" axoneme.

1. Introduction

Sparids (porgies or sea breams) is one of the largest Percoidei families, predominantly marine and distributed in the Atlantic, Indian and Pacific oceans (Nelson, 2006).

The family includes 33 genera with about 115 species (Nelson, 2006) and 6 subfamilies: the Boopsinae, Denticinae, Diplodinae, Pagellinae, Pagrinae and Sparinae (Smith and Smith, 1986).

Spermatozoa ultrastructure has been studied in several groups of fishes (Jamieson, 1991, 2009; Mattei, 1970, 1991) and the usefulness of this data in the identification of the phylogenetic relationships has been recognized.

In particular these studies have shown that the organization of the spermatic organelles is very conservative in the member of a same family or subfamily (Baccetti et al., 1984; Jones and Butler, 1988; Mattei, 1991; Jamieson, 1991; Burns et al., 1998; Abascal et al., 2002; Quagio-Grassiotto et al., 2003; Gusmão-Pompiani et al., 2005); therefore, ultrastructural characters of spermatozoa can be well combined with usual morphologic characters, in phylogenetic analyses.

Within the family Sparidae, sperm ultrastructure has been investigated in all subfamilies except Denticinae. In particular investigated sparids are *Acantopagrus australis* and *A. berda* (Gwo et al., 2005), *A. schlegeli* (Hong et al., 1991; Gwo et al., 1993b; Gwo and Gwo, 1993; Gwo, 1995b), *A.* (*Sparus*) *latus* (Gwo, 1995a; Dong et al., 1998), *Lithognathus mormyrus* (Mattei, 1991), *Rhabdosargus* (*Sparus*) *sarba* (Gwo et al., 2004a) and *Sparus aurata* (Maricchiolo et al., 2007) (Sparinae); *Diplodus cervinus* (Mattei, 1991; Jamieson, 2009), *D. puntazzo* (Taddei et al., 1999, 2001), *D. sargus* (Lahnsteiner and Patzner, 1995, 2007), *Lagodon rhomboides* and *Archosargus probatocephus* (Gwo et al., 2005) (Diplodinae); *Boops boops* (Mattei, 1970; Zaki et al., 2005; Lahnsteiner and Patzner, 1998) (Boopsinae); *Pagellus erythrinus* (Assem, 2003; Maricchiolo et al., 2004) (Pagellinae); and *Pagrus major* (Hara and Okiyama, 1998; Gwo et al., 2004a) (Pagrinae).

All these species have a uniflagellate anacrosomal aquasperm, as is typically found in external fertilizing fishes (Jamieson, 1991), and share some ultrastructural features, such as the location of the centriolar complex inside the nuclear fossa (characteristic of type I sperm *sensu* Mattei, 1970, 1988) and the perpendicular insertion of the flagellum with respect to the nucleus.

However, they differ in some characteristics, such as the number of mitochondria, presence of sidefins and organization of cell organelles, especially as far as the "centriolar stabilization structures" or "anchoring fiber apparatus"(*sensu* Afzelius, 1979) is concerned.

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Morphological features of centriolar stabilization structures (alar sheet, basal feet, basal plates, *etc.*) vary considerably in different species and thus may have functional and evolutionary significance.

The present paper provides a description of the ultrastructure of the mature spermatozoon of *Pagellus bogaraveo*, another member of family Sparidae (subfamily Sparinae), with particular attention on the structure of centriolar complex and its stabilization structures. On the basis of above said considerations, ultrastructural spermatozoon characters are described, adding new informations which could be useful to a better understanding of phylogenetic relationships between Sparidae.

2. Materials and methods

Semen samples from adult male *P. bogaraveo* (total body length = 30.2 ± 1.6 cm, body weight = 650 ± 15 g, $n = 10$) held in captivity at the facilities of the IAMC-CNR of Messina (Sicily, Italy) were collected at the peak of spawning season (February), two weeks after the beginning of spermiation. Fish were anesthetized with MS-222 (0.1 g/l), urine was extruded by gently squeezing the fish near the genital pore, faeces were carefully discarded, and the genital area dried. Milt was stripped from running males by gentle abdominal massage and collected in glass tubes.

Samples were fixed in 0.1 M cacodylate buffer (pH 7.5) containing 4.5% paraformaldehyde, 2.2% glutaraldehyde and 5% sucrose for 2 h in ice bath, postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer with 5% sucrose for 1 h in ice bath and centrifugated at 900 × *g* for 10 min. The sperm samples were then processed for transmission (TEM) or scanning (SEM) electron microscopy. For TEM, sperm pellets were encapsulated in agar (Glauert, 1975), dehydrated in an ethanol series and embedded in Araldite. Ultrathin sections were cut using an ultramicrotome (Ultracut-E, Reichert-Jung), stained with uranyl acetate and lead citrate, and were examined under a Jeol Jem 100SX transmission electron microscope. For SEM, sperm pellets were glued on poly-L-lysine-coated coverslips (Scala and Pasquinelli, 1987). After dehydration through an ascending ethanol series, samples were critical point dried using liquid argon (Balzers CPD 030), coated with 20 nm gold–palladium in an SCD050 sputter coater (BAL-TEC) and examined under a Cambridge Stereoscan 240 SEM operating at 20 kV.

Measurements were performed on both SEM and TEM electron micrographs and are expressed as mean ± standard deviation.

3. Results

The spermatozoon of *P. bogaraveo* is a uniflagellate cell, differentiated into an acrosome-less head, a short midpiece, and a long cylindrical tail (Fig. 1a and b).

The head is ovoid with the lateral axis greater than longitudinal axis.

The nucleus shows a homogenous, electron-dense chromatin compact in texture (Fig. 1b).

At the base of the nucleus, the nuclear envelope invaginates, forming a deep depression, called the nuclear fossa, containing the centriolar complex. The nuclear fossa appears deep and bell-shaped in a sagittal longitudinal section. It appears smaller at the apical end and enlarging posteriorly (Fig. 1c). The anterior region of the nuclear fossa is occupied by the proximal centriole, whereas the distal centriole (which serves as the basal body of the flagellum) is located in the posterior region.

A peculiar satellite nuclear notch shaped like a golf club is visible in cross-section, originating from the nuclear fossa, at the level of the distal centriole; some electron-dense material occurs inside the notch (Fig. 1d and f).

The two centrioles are hollow, cylindrical structures with the conventional "9+0" microtubular pattern lie in the same axis and are oriented perpendicularly to each other (Figs. 1c and 2a).

The proximal centriole is connected by osmiophilic filaments (Fig. 2a and b) to an osmiophilic ring surrounding the anterior end of the distal centriole.

Two conical-shaped basal feet are visible at the apical level of the distal centriole connecting it to the nuclear envelope; at the posterior end, the distal centriole is traversed by a basal plate (Fig. 2b).

Radial alar sheets project peripherally from the distal basal plate (Figs. 1e and 2b), connecting the caudal part of the centriole to the plasma membrane. Alar sheets are clearly visible, in a cross-section, with the distal centriole resembling a cartwheel (Fig. 1e).

Below the basal plate electron-dense particles, termed the necklace, are leaned against the membrane (Fig. 2b). The neck of flagellum cross-sectioned, through the necklace region, shows Y-shaped bridges, a champagne glass-like structure, consisting in a short stem and a cup linking the doublets to plasma membrane (Fig. 1e).

The midpiece is short and cylindrically shaped (Fig. 1a) and contains numerous electron-clear vesicles in the cytoplasm. One spherical mitochondrion can be seen in the midpiece region (Fig. 1a and b). The mitochondrion contains an electron-dense matrix and has irregularly arranged cristae; it is separated from the axoneme by a narrow cytoplasmic canal, which is formed by an invagination of the plasma membrane. A second membrane is located underneath the invaginated portion of the plasmalemma extending along the cytoplasmic canal (Fig. 2b).

The flagellum, which has a cylindrical shape throughout its length, measures 50.64 ± 4.82 μm ($n = 50$) and is inserted perpendicularly and eccentrically with respect to lateral and longitudinal axis of ovoid nucleus (Fig. 2b).

The axoneme shows a typical eukaryotic organization, consisting of nine outer doublet microtubules and a central pair of singlet microtubules (9+2 pattern).

Transverse sections at different levels of the flagellum show radial spokes, tubules A and B of outer doublets of microtubules and dynein arms (Fig. 2d).

Neither intratubular differentiations (ITD, microtubules A and B of axonemal doublets are both hollow) nor lateral extension of the membrane (sidefins) is present in the flagellum.

4. Discussion

The teleostean *uniflagellate anacrosomal aquasperm*, which is typical of many external fertilizing fish (Jamieson, 1991), is characterized by a spherical or ovoid nucleus, a short midpiece with a few spherical mitochondria and a flagellar tail with the usual 9+2 microtubular pattern. Although very simple in morphology, the *anacrosomal aquasperm* can adopt a wide range of structural variations that may bear phylogenetic implications and prove valuable in taxonomy (Baccetti et al., 1984; Jamieson, 1991; Mattei, 1991).

Mattei (1970) classified the *simple anacrosomal aquasperm* in two distinct categories on the basis of the spermiogenesis features. In particular, Mattei (1970, 1988) suggested that spermiogenesis in Teleostei may result in two basic spermatozoon types: type I and type II, with an intermediate type between them. In the type I spermatozoon a nuclear rotation occurs during spermiogenesis that makes the flagellar axis perpendicular to the base of the nucleus; conversely in type II spermatozoon this rotation does not take place and the flagellum remains parallel to the nucleus. Nuclear rotation may also be incomplete and the spermatozoon is an intermediate between two types.