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Fine structure of spermatozoa in the common pandora (*Pagellus erythrinus* Linnaeus, 1758) (Perciformes, Sparidae)

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Summary. Scanning and transmission electron microscopy were used to investigate the fine structure of the sperm of the Sparid fish *Pagellus erythrinus* L.. The spermatozoon of pandora has a spherical head lacking an acrosome, a cone-shaped midpiece and a long tail. The midpiece houses a single mitochondrion. The centriolar complex lies inside the nuclear fossa and is composed of a proximal and a distal centriole which are arranged at right angles to each other. The flagellum is inserted medio-laterally into the head, contains the conventional 9+2 axoneme and possesses one pair of lateral fins. On the basis of its ultrastructural organization, the pandora sperm can be regarded as an evolved form of the primitive spermatozoon found in Teleosts. According to the morphological classification proposed by Mattei (1970), the sperm of pandora belongs to a "type I" designation, like that of the other Sparid fish.

Key words: Sperm ultrastructure, Fish, *Pagellus erythrinus*, Sparidae

Introduction

In contrast to other groups of vertebrates, such as snakes or mammals, fish exhibit a broad range of varying structural features that makes it impossible to depict a unique spermatic model. The pioneer work on fish sperm morphology was carried out by Geiger (1955) and Mattei (1970). More recently, some studies have been focused on morphological differences between species and have approached the issue from an evolutionary point of view (Mattei, 1988, 1991; Jamieson, 1991). In fact, the structure of fish spermatozoa may vary more or less widely even within families. For example, an ultrastructural study of the spermatozoa of seven species belonging to the family Cyprinidae revealed that each of them was characterized by a specific organization of sperm organelles within a general pattern common to the whole family (Baccetti et al., 1984). Therefore, submicroscopical features of sperm cells may be of evolutionary significance and can be used as additional characters for taxonomic classifications.

The knowledge about fish sperm has increased considerably in the last decade. Most of this work has been carried out on freshwater species, whereas investigations on marine fish sperm still remain limited, particularly as far as species valuable for aquaculture are concerned (Suquet et al., 1993; Abascal et al., 2002; Maricchiolo et al., 2002). Within the family Sparidae, the spermatozoon ultrastructure has been examined only in three species, i.e. the bogue *Boops boops* L. (Mattei, 1970), the white seabream *Diplodus sargus* L. (Lahnsteiner and Patzner, 1995) and the sharpsnout seabream *Diplodus puntazzo* Cetti (Taddei et al., 1998).

Besides its application in taxonomy, the knowledge on sperm morphology may also be useful for manipulation and preservation of gametes. In fact, Labbé et al. (1998) suggested a relationship between the number of mitochondria and motility after cryopreservation. Moreover, integrity of the nucleus and sperm plasma membrane after freezing have been suggested to be closely related with fertilizing ability and embryonic development (Leveroni Calvi et al., 1993, 1994; Lahnsteiner et al., 1996).

The aim of the present study was to investigate, by means of scanning and transmission electron microscopy, the fine structure of spermatozoa in the protogynous hermaphroditic fish *Pagellus erythrinus* L. (Sparidae), with the purpose of contributing to a better

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knowledge of Sparid spermatozoa.

Materials and methods

Semen samples from adult male *P. erythrinus* (total body length = 33.6±1.9 cm, body weight = 768±29 g, n=10) held in captivity at the facilities of the Istituto Sperimentale Talassografico of Messina (Sicily – Italy) were collected at the peak of spawning season (June), two weeks after the beginning of spermiation. Fish were anesthetized with MS222 (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), were 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.

The following morphometrical parametres of at least 50 randomly chosen spermatozoa were measured: head length, head width, midpiece length and flagellum length. All measurements were carried out on light micrographs of semen smears stained with crystal violet and rose Bengal, using a software developed in the Laboratory of Morphometry of the Institute of Ultrastructural Pathology, University of Messina (Italy).

Results

The spermatozoon of pandora is a uniflagellated cell, differentiated into a head and a tail with a short midpiece and a flagellum. The spherical head measured 1.62±0.08 mm (n=50). No acrosome was observed (Figs. 1, 2). The head was occupied almost totally by the nucleus. The nuclear chromatin appeared finely granular and homogeneously dispersed. At the base of the nucleus, the nuclear envelope invaginated, forming a depression called the nuclear fossa. The proximal and distal centrioles formed the centriolar complex, which was located inside the nuclear fossa (Fig. 3). The two centrioles, arranged at a right angle to each other, showed a conventional "9+0" pattern. The distal centriole became the basal body from which the flagellum axoneme emerged (Fig. 4).

The cone-shaped midpiece had a length of 1.73±0.28 µm (n=50) (Fig. 2). It contained a single, large, round mitochondrion with tubular cristae. At the midpiece level, the axoneme was separated from the plasmatic membrane by a narrow cytoplasmatic canal which was formed by an invagination of the membrane itself (Fig. 3).

The flagellum had a typical eukaryotic organization (9+2 pattern) (Fig.5). The length of the flagellum, which was inserted medio-laterally into the nuclear fossa, perpendicularly to the base of the nucleus (Fig.3), was 53.94±10.32 mm (n=50). The flagellum had a cylindrical shape throughout its length and showed one unpaired sidefin, which represented an evagination of the plasmalemma (Fig.1).

Discussion

The spermatozoon of *P. erythrinus* exhibited the configuration of the uniflagellate anacrosomal aquasperm, which is typically found in external fertilizing fish (Jamieson, 1991). On the basis of its ultrastructural organization, the spermatozoon of *P. erythrinus* could be regarded as a "type I" sperm as defined by Mattei (1970). Such a sperm type is characterized by a centriolar complex that lies inside the nuclear fossa and a flagellar axis that is perpendicular to the nuclear base as a result of rotation of the spermatid nucleus during the spermiogenesis. The "type I" sperm occurs in only 12 out of the 41 families of the perciforms studied so far (Mattei, 1991). From an evolutionary viewpoint, the spermatozoon of *P. erythrinus* could be regarded as an evolved form of the primitive spermatozoon, as defined by Mattei (1988), in which one single, large mitochondrion is present as a result of a number of mitochondria fusing during spermiogenesis. Such an evolved type of gamete has been reported in five families; namely Blennidae, Centracanthidae, Clinidae, Gobiidae and Sparidae (Mattei, 1991). ATP produced by mitochondrial respiration is the main energy source for sperm motility. ATP is used by the dynein arms, which give origin to a self-oscillatory bending behaviour of the flagellar axoneme. The presence of only one mitochondrion might indicate a lower energy delivering capacity compared to those species possessing more mitochondria (Lahnsteiner and Patzner, 1995). Thus, the number of mitochondria might be a factor contributing to a more or less successful gamete preservation. In fact, Labbé et al. (1998) observed that turbot sperm, which possesses 8 to 10 mitochondria, retains a good motility after cryopreservation, contrarily to rainbow trout sperm, which possesses only one mitochondrion.

The spermatozoa of *P. erythrinus* were very similar to those of the three sparid species studied so far (Mattei, 1970; Lahnsteiner and Patzner, 1995; Taddei et al.,

1998). An acrosome-less head, such as that of *P. erythrinus* spermatozoon, is a common feature of all teleosts, related to the presence of a micropyle in the egg. The micropyle is an opening that permits penetration of the spermatozoon into the egg at the time of fertilization (Amanze and Iyvengar, 1990). The shape

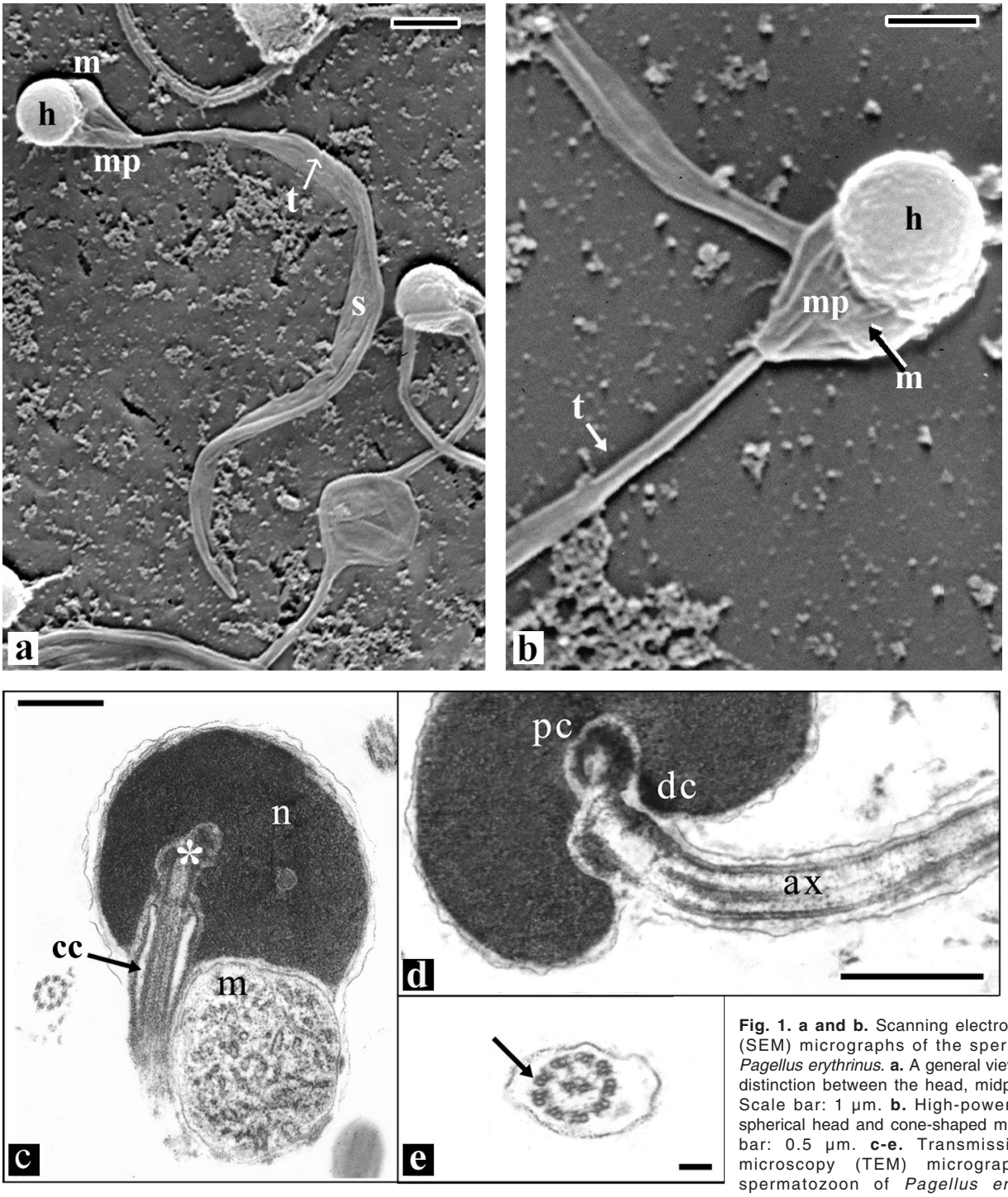

Fig. 1. a and b. Scanning electron microscopy (SEM) micrographs of the spermatozoon of *Pagellus erythrinus*. **a.** A general view showing the distinction between the head, midpiece and tail. Scale bar: 1 µm. **b.** High-power view of the spherical head and cone-shaped midpiece. Scale bar: 0.5 µm. **c-e.** Transmission electron microscopy (TEM) micrographs of the spermatozoon of *Pagellus erythrinus*. **c.** Longitudinal section through the sperm showing the nuclear fossa (asterisk) and the medio-lateral inserting flagellum. Scale bar: 0.5 µm. **d.** Longitudinal section through the centriolar complex. Scale bar: 0.5 µm. **e.** Cross-section of the flagellum showing the 9+2 axonemal pattern (arrow). Scale bar: 0.1 µm. ax: axoneme; cc: cytoplasmic canal ; dc: distal centriole; h: head; m: mitochondrion; mp: midpiece; n: nucleus; pc: proximal centriole; s: sidefin; t: tail.

of the head is highly variable between teleosts. A spherical head, such as that of *P. erythrinus*, is the result of a simple spermiogenesis (Grier, 1981), whereas a more or less elongated head region derives from a more complex spermiogenic process and is considered an advanced morphological feature (Jamieson, 1991). A short midpiece, such as that observed in *P. erythrinus*, is common in teleosts with external fertilization (Nicander, 1970), while internal fertilization is linked to elongation and complexity of the gamete (Jamieson, 1991).

The presence of sidefins is one of the possible variations the spermatozoon may show at the level of the flagellar apparatus. Although the functional significance of sidefins is still unclear, their presence might be related to the swimming behaviour of the sperm. However, early hypotheses giving sidefins a key role in spermatozoal cynetics through improving the efficiency of flagellar propulsion (Nicander, 1970; Stoss, 1983) have been questioned by more recent computer-assisted cell-motion analysis studies (Lahnsteiner et al., 1995). The presence of sidefins is not family-specific, as shown by Baccetti et al. (1984) in Cyprinidae and Thiaw et al. (1986) in Cyprinodontidae. Such a diversity seems to occur also in the family Sparidae. In fact, in *P. erythrinus* we observed one unpaired sidefin, as in *D. sargus* (Lahnsteiner and Patzner, 1995), whereas no sidefins were reported in *B. boops* and *D. puntazzo* (Mattei, 1970; Taddei et al., 1998).

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References

Abascal F.J., Medina A., Megina C. and Calzada A. (2002). Ultrastructure of *Thunnus thynnus* and *Euthynnus alletteratus* spermatozoa. J. Fish Biol. 60, 147-153.

Amanze D. and Iyvengar A. (1990). The micropyle: a sperm guidance system in teleost fertilization. Development 109, 495-500.

Baccetti B., Burrini A.G., Callaini G., Gilbertini G., Mazzini M. and Zerunian S. (1984). Fish germinal cells. I. Comparative spermatology of seven cyprinid species. Gamete Res. 10, 373-396.

Geiger N. (1955). Electron microscopy studies on salmonid spermatozoa. Revue Suisse Zool. 62, 325-334.

Glauert A.M. (1975). Fixation methods. In: Fixation, dehydration and embedding of biological specimens. Pratical methods in electron microscopy. Glauert A.M. (ed). Elsevier, North-Holland Biomedical Press. pp 73-110.

Grier H.J. (1981). Cellular organization of the testis and spermatogenesis in fishes. Am. Zool. 21, 345-357.

Jamieson B.G.M. (1991). Fish Evolution and systematics: Evidence from spermatozoa. Cambridge University Press. Cambridge. pp. 1-319.

Labbé C., Maisse G. and Billard R. (1998). Fish sperm cryopreservation: what has to be improved. In: New species for mediterranean aquaculture. Enne G. and Greppi G.F. (eds). Elsevier. Paris. pp 97-110.

Lahnsteiner F. and Patzner R.A. (1995). Fine structure of spermatozoa of two marine teleost fishes, the red mullet, *Mullus barbatus* (Mullidae) and the white sea bream, *Diplodus sargus* (Sparidae). J. Submicrosc. Cytol. Pathol. 27, 259-266.

Lahnsteiner F., Berger., Weissman T. and Parzner R. (1995). Fine structure and motility of spermatozoa and composition of the seminal plasma in the perch. J. Fish Biol. 47, 492-508.

Lahnsteiner F., Berger B., Weissman T. and Patzner R.A. (1996). Motility of spermatozoa of *A. alburnus* (Cyprinidae) and its relationship to seminal plasma composition and sperm metabolism. Fish Physiol. Biochem. 15,167-179.

Leveroni Calvi S., Zoccarato I., Gasco L. and Andrione A. (1993). Effetto del diluitore e delle modalità di congelamento e scongelamento sulla motilità del seme crioconservato di carpa (*Cyprinus carpio* L.). Riv. Ital. Acquacol. 28, 187-195.

Leveroni Calvi S., Zoccarato I., Gasco L. and Andrione A. (1994). Effetto dell'aggiunta di trealosio e/o albumina e della concentrazione di metanolo sulla motilità del seme di carpa (*Cyprinus carpio* L.). Riv. Ital. Acquacol. 29, 45-51.

Maricchiolo G., Genovese L., Laurà R., Micale V. and Muglia U. (2002). The ultrastructure of amberjack (*Seriola dumerilii*) sperm. Eur. J. Morphol. 40, 289-292.

Mattei X. (1970). Spermiogenèse comparée des poissons. In: Comparative spermatology. Baccetti B. (ed). Academic Press. New York. pp 55-70.

Mattei X. (1988). The flagellar apparatus of spermatozoa in fish. Ultrastructure and evolution. Biol. Cell 63, 151-158.

Mattei X. (1991). Spermatozoon ultrastructure and its systematic implication in fish. Can. J. Zool. 69, 3038-3055.

Nicander L. (1970). Comparative studies on the fine structure of vertebrate spermatozoa. In: Comparative spermatology. Baccetti B. (ed). Academic Press. New York. pp 47-56.

Scala C. and Pasquinelli G. (1987). Gli elettroni secondari. In: Microscopia Elettronica a Scansione in Biologia. CLUEB. Bologna. pp. 59-181.

Stoss J. (1983). Fish gamete preservation and spermatozoan physiology. In: Fish physiology. Vol. IX B. Hoar W.R., Randall J.D. and Donaldson E.M. (eds). Academic Press. New York. pp 605-650.

Suquet M., Dorange G., Omnes M.H., Normant Y., Le Roux A. and Fauvel C. (1993). Composition of the seminal fluid and ultrastructure of the spermatozoon of turbot (*Scophthalmus maximus*). J. Fish Biol. 42, 509-516.

Taddei A.R., Abelli L., Baldacci A., Barbato F., Fausto A.M. and Mazzini M. (1998). Ultrastructure of spermatozoa of marine teleosts *Umbrina cirrosa* L. and *Diplodus puntazzo* (Cetti), and preliminary observation on effects of cryopreservation. XXXIII International Symposium on New Species for Mediterranean Aquaculture. Alghero, April 22-24, pp. 407-413.

Thiaw O., Mattei X., Romand R. and Marchand B. (1986). Reinvestigation of spermatic flagella structure: the teleostean Cyprinodontidae. J. Ultrastr. Mol. Str. Res. 97, 109-118.

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