Anatomical Ultrastructure of Spermatozoa of *Platichthys stellatus* (Pleuronectidae, Pleuronectiformes) from Korea

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**ABSTRACT** The spermatozoa of *Platichthys stellatus* is relatively simple cells composed of a spherical head, a short midpiece, and a tail, as in most Pleuronectiformes. The ultrastructure is characterized by the following features: a round nucleus with a deep nuclear fossa, the centriolar complex located at a right angle to each other, a short midpiece, a tail with paired lateral ribbon and no acrosome. However there are some minor morphological differences, including the appearance and number of the mitochondria, the shape and size of the nuclear fossa and the structure of the basal body. Especially the basal body structure consisting of a basal foot, a rootlet and nine alar sheets structures varies considerably in different species. It can be used as indicator of relationships in Pleuronectiformes because minute morphological differences might have functional and evolutionary significance. In conclusion, the spermatozoa of *P. stellatus* show a certain structural homogeneity and provide support for the concept that ultrastructural features of spermatozoa can be useful in taxonomic studies of Pleuronectiformes.

**Key words**: Spermatozoa, ultrastructure, Pleuronectiformes

**INTRODUCTION**


Fish show great variations in their organization. In general within the divergency of type a more specialized and complicated organization is found in spermatozoa of internal fertilizing fish in comparison to external fertilizing one (Billard, 1986). The spermatozoa of external fertilizing fish have the simple cells composed of a spherical head devoid of an acrosome, a short midpiece and an elongated tail. This simplified structure is close to that of the gamete of aquatic invertebrate with external fertilization (Mattei, 1991).

In previous survey, we reported that the spermatozoa of a bastard halibut, *Paralichthys olivaceus* (Pleuronectiformes), possesses some features, satellite rays and satellite lamellae, which may be useful in systematic studies (Kim *et al.*, 2011). However, these structures have been described only rarely in fish spermatozoa except in some species of supposedly advanced Neopterygii, i.e., order Perciformes and Atheriniformes (Mattei and Mattei, 1976; Jamieson, 1989). Afzelius (1979) proposed that a system of satellite rays is typical of invertebrate spermatozoa but is reduced in size in higher phyla.

The present report provides a description of the ultrastructure of the mature spermatozoa of stary flounder *Platichthys stellatus*, another member of Pleuronectiformes, with special reference to the basal body containing a basal foot, a rootlet and alar sheets structures that have been described only rarely in teleosts and also compares it those of other teleosts.

**MATERIALS AND METHODS**

During their spawning period lasting from the beginn-
ing to the end of April (2009), male stary flounder were collected in the east sea of Uljin (Korea). The fish was identified and kept in the fish collection of laboratory.

For transmission electron microscopy (TEM), semen and pieces of testis were dissected and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer. Then they were dehydrated in a graded ethanol series and embedded in Epon 812. The samples were sectioned with an ultramicrotome (RMC, MTXL, Germany), stained in 4% aqueous uranyl acetate, poststained with lead citrate, and examined using TEM (Hitachi, H-7500, Japan).

For scanning electron microscopy (SEM), species were fixed in glutaraldehyde and postfixed in O,O₄ as described above. Following dehydration in a graded ethyl alcohol series, sample were critical-point dried, coated with gold, and observed with Hitachi S-4800.

Figures 1 to 11 Platichthys stellatus spermatozoa.

Fig. 1. A scanning electron micrograph of spermatozoa showing a spherical head, short midpiece and a long tail. Arrows indicate lateral ribbon (bar: 0.5 μm).

Fig. 2. Longitudinal section of a spermatozoon showing the bell shaped nuclear fossa. Arrows indicate the nuclear envelope and the plasma membrane Note alar sheet and basal foot (bar: 0.5 μm).

Fig. 3. Transverse section through the region of nuclear fossa including distal centriole (bar: 0.5 μm).


RESULTS

The spermatozoa of P. stellatus is a relatively simple and elongated cell composed of a round head, a short midpiece and a tail (Fig. 1). There is no acrosome. The nucleus is spherical, measuring about 1.6 μm in diameter and covered by a nuclear envelope (Fig. 2). The nuclear envelope and the plasma membrane are undulated through the length of the spermatozoon (Figs. 2, 3). The chromatin is highly electron-dense with irregular small clear lacunae. The base of the nucleus is indented by a nuclear fossa, the length of which is about two-fifth of the length of the nucleus. The nuclear fossa contains the centriolar complex and is bell shaped in longitudinal section (Fig. 2). A series of transverse sections reveal it to be circular (Fig. 3). The two centrioles lie within the deep nuclear fossa and are connected to each other by osmiophilic filaments (Figs. 2, 4). The proximal and distal centrioles are located at a right angle to each other (Figs. 2, 5) and display characteristic nine triplet pattern. The fibrous materials are located within the hollows in upper sides of the proximal centriole (Fig. 5) and give rise to short electron-dense projections which connect the dense material to...
the proximal centriole and anchor the proximal centriole to the nucleus as well (Figs. 1, 2). The distal centriole, which forms the basal body of the axoneme, extends from the level of the anterior end of the cytoplasmic canal to the basal nuclear fossa (Figs. 2, 4, 5). In a cross section the basal body resembles a cartwheel (Fig. 6). The basal feet extend laterally from the basal body and the basal body is flanked by the rootlet (Figs. 2, 4, 5). In posterior region alar sheets connect the basal body to the plasma membrane by a Y shaped bridge (Figs. 2, 7).

Mitochondria appear circular in longitudinal section (Fig. 8). The mitochondria number is six (Fig. 9). They are separated from the axoneme by the cytoplasmic canal. Its matrix is rather loose, and the internal membranes or cristae are difficult to distinguish. The distribution of the mitochondria is shown the level of the basal nucleus and arranged one layer around the axoneme (Figs. 8, 9).

The flagellum is composed of a typical 9+2 microtubular doublet construction and has paired lateral ribbon (Fig. 10). The lateral ribbons are in line with the two centriole tubules and the cytoplasmic matrix within these lateral ribbons is more electron-dense than the rest of the flagellum. The lateral ribbons disappear proceeding posteriorly (Fig. 11).

**DISCUSSION**

The ultrastructure of the spermatozoa of *P. stellatus* resembles that of other Pleuronectiformes; *Pegusa tricipitalis*, *Paralichthys olivaceus*, *Platichthys flesus*, *Oryzias latipes* (Mattei, 1970; Grier, 1976; Jones and Butler, 1988; Kim *et al.*, 2011). They are characterized by a round nucleus, a deep nuclear fossa, the centriolar complex located at a right angle to each other, a short midpiece, a tail with paired lateral ribbon and no acrosome. However there are some minor morphological differences, including the appearance and number of the mitochondria, the shape and size of the nuclear fossa and the structure of the basal body.

The nucleus of *P. stellatus* has a deep nuclear fossa at its base. The deep nuclear fossa is also found in other teleost species (Jamieson, 1991; Gwo and Gwo, 1993; Gwo *et al.*, 1996). According to Jamieson (1991) the deep nuclear fossa is considered to be a apomorphic as compared with the shallow nuclear fossa in teleost fish.

The relative position of the two centriole in fish spermatozoa varies considerably among species. The angle of the proximal centriole to the distal centriole is species-specific among Cyprinidae species (Baccetti *et al.*, 1984; Kwon *et al.*, 1998). The proximal and distal centrioles are serially coaxial in Tetraodontiformes (Mattei, 1970; Grier, 1976; Jones and Butler, 1988). The proximal and distal centrioles are parallel and each forms a flagellum (biflagellate) in Batrachoidiformes (Casas *et al.*, 1981), Polyteriformes (Mattei, 1970) and Gobiesociformes (Mattei and Mattei, 1978). Baccetti *et al.* (1984) suggested that the centriolar geometry is correlated to both nuclear and flagellar positions. Jamieson (1991) reported that a proximal centriole perpendicular to a distal centriole is a plesiomorphic feature in fish spermatozoa. The proximal and distal centrioles of *P. stellatus* are located at a right angle to each other.

The midpiece of *P. stellatus* is short and contains several mitochondria, as in most Pleuronectiformes. Midpiece length may be related to fertilization strategy that internal fertilization is associated with a long midpiece and external fertilization is associated with a short midpiece (Idelman, 1967). The mitochondria are various in their numbers and distribution in teleost sperm. In Pleuronectiformes the mitochondrial number also varies, but is frequently five or six (Mattei, 1970; Grier, 1976; Jones and Butler, 1988; Kim *et al.*, 2011). Baccetti *et al.* (1984) suggested that mitochondrial number, the specific characteristics of the cyprinid spermatozoa, is a good characteristics from a phylogenetic point of view. The mitochondria
of *P. stellatus* are similar to those of other Pleuronectiformes in number, layer and distribution, but differ in appearance. The mitochondria in *P. stellatus* dispose closely to the nucleus and surround the initial region of flagellum, and arrange only one row with six mitochondria. The location of mitochondria encircling the flagellum and separating from it by the cytoplasmic canal in *P. stellatus* been also reported in the Oncorhynchus masou formsosamus (Gwo et al., 1996), Acanthopagrus lactus (Gwo, 1995), Epinephelus malabaricus and Plectropomus leopardus (Gwo et al., 1994a) and Chanos chanos (Gwo et al., 1995). The separation of the mitochondria from the flagellum by the cytoplasmic channel is uniform in fish spermatozoa and implies the transfer of ATP from the energy producing structures to be energy using system (Lahnsteiner et al., 1991). The mitochondria matrix in *P. stellatus* unlike in Pleuronectiformes rather loose and the cristae are difficult to distinguish.

The structure of the spermatozoal flagellar apparatus is diverse among animal species (Afzelius, 1982). The lateral ribbons are very common in many fish species (Afzelius, 1979; Matteri, 1991). But the lateral ribbons vary in size and number, and their presence is independent of reproductive mode, type of spermatozoa, and number of flagellum (Mattei, 1991). The flagellum of *P. stellatus* owns two lateral ribbons. Two lateral ribbons are very common in the spermatozoa of fish (Billard, 1970; Stein, 1981; Kim et al., 2011). But some of the flagella have three lateral ribbons in rainbow trout (Billard, 1973) and Epiplatys bifasciatus (Thiaw et al., 1986). The lateral ribbons may be regarded as structures to enable a more economic movement of the spermatozoon (Afzelius, 1982).

To establish the spatial relationship between the nucleus and centriolar complex or flagellum and to increase the strength and rigidity of the system, the anchoring apparatus of fish spermatozoa involve various different structure among species. These structures include basal foot, rootlet, alar sheet, microtubule, satellite, electron-dense body and Y-shaped link (Grier, 1973; Billard, 1973; Jones and Butler, 1988; Lahnsteiner et al., 1991; Gwo and Gwo, 1993; Gwo et al., 1993, 1994b). Especially the structure of the basal body of the *P. stellatus* correspons in part to that of the basal bodies of other vertebrate spermatozoa described by both Anderson (1972) and Wheatley (1982) and that of the basal bodies of Perciformes, Acanthopagrus latus by Gwo (1995). It consists of a basal foot, a rootlet and nine alas sheets structures that are rarely described in teleostan spermatozoon.

The basal foot attached to the distal centriole of *P. stellatus* was also observed in the spermatozoa of Pegusa triephalus, Paralichthys olivaceus and Platichthys flesus (Mattei, 1970; Grier, 1976; Jones and Butler, 1988; Kim et al., 2011) of Pleuronectiformes, and Micropogonias undulatus of Perciform (Gwo and Arnold, 1992) and two Serranidae species (Gwo et al., 1994b).

The rootlet consisting of osmiophilic disks found in *P. stellatus* spermatozoa is similar in location and appearance to that of the striated rootlet of Perciformes, Salmoniformes (Gwo, 1995; Gwo et al., 1996). A striated rootlet linking the distal centrioles to the adjacent plasma membrane was also described in Cypriniformes, Leuciscus souffia (Baccetti et al., 1984). But the striated rootlet was not observed in *Paralichthys olivaceus*, *Platichthys flesus*, *Oryzias latipes* (Grier, 1976; Jones and Butler, 1988; Kim et al., 2011).

The alar sheets form a nine pointed star which apparently keeps the distal centriole anchored to the plasma membrane (Afzelius, 1979). However, this structure has been described only rarely in fish spermatozoa except in some species of supposedly advanced Neopterygii (Jamieson, 1989). The alar sheets are very common in lower metazoan sperm (Afzelius, 1979) and the large anchoring fiber apparatus is gradually lost toward the higher phyla. The alar sheets are also responsible for the stabilization of the spatial relationship between the nucleus and the centriolar complex in fish spermatozoa. Summers (1972) proposed that the alar sheets may contain a contractile protein and they possibly also have a function in transporting ATP from the mitochondria to the flagellum.

The basal foot, striated rootlet, and alar sheet appear to fasten the centriole to the plasma membrane and are also responsible for the stabilization of the spatial relationship between the nucleus and the centriolar complex in fish spermatozoa, which is important during sperm tail movement (Gwo et al., 1996). Some other cytoplasmic structure are retained in mature spermatozoa for attaching either the flagellum or centriolar complex to the nucleus. These include a striated satellite in Poecilia latipinnia (Grier, 1973), electron dense material and microtubules in *Oryzias latipes* (Grier, 1976), microtubules in *Thymallus thymallus* (Lahnsteiner et al., 1991) and rainbow trout (Billard, 1973), and two centriolar caps in *Icterus punctatus* (Poirier and Nicholson, 1982) and one centriolar caps in *Cymatogaster aggregata* (Gardiner, 1978). For unknown reason, the morphological features of alar sheets, basal feet and rootlets vary considerably in different species (Vorobjev and Nadezhdina, 1987). This fact justifies the investigation of the ultrastructural details of the basal body in various species, since minute morphological differences might have functional and evolutionary significance. Afzelius (1979) indicated that the anchoring fiber apparatus, mitochondria and centriolar complex can be used as indicator of relationships among metazoan phyla.

The fibrous materials, located in upper sides of the nuclear fossa in *P. stellatus*, resemble those of the centriolar appendage in the spermatozoa of Salmonidae (Gwo et al., 1996), Sparidae (Gwo, 1995) *Paralichthys olivaceus*, *Platichthys flesus* and *Oryzias latipes* in Pleuron-
ectiformes (Mattei, 1970; Grier, 1976; Jones and Butler, 1988; Kim et al., 2011). This structure is connected to the nucleus and proximal centriole.

The spermatozoa of *P. stellatus* examined to date show a certain structural homogeneity and provide support for the concept that ultrastructural features of spermatozoa can be useful in taxonomic studies of Pleuronectiformes. The present study also confirmed that there exist interspecific differences within the family of Pleuronectidae in the organization of the midpiece of the spermatozoa. Before the precise homology of variation in spermatozoa morphology within Pleuronectiformes can be determined, further information from additional taxa is needed to determine whether the features of *P. stellatus* are typical of Pleuronectidae or Pleuronectiformes.

**REFERENCES**


New York.
강도다리 (*Platichthys stellatus*) 정자의 미세해부학적 구조
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요 약: 강도다리 (*Platichthys stellatus*)의 정자는 대부분의 경골어류에서와 같이 첨체를 가지고 있지 않는 구형의 핵, 짧은 측면 및 꼬리를 가진 전형적인 수중형의 정자 형태를 하였다. 정자의 미세구조는 전자밀도가 높은 염색질 부위를 가지는 핵과 핵 기부에 위치한 긴 삼각형의 핵과(nuclear fossa) 그리고 그 속에 기부 중심림과 발단부 중심림이 포함되어 있었다. 두 중심림은 90도의 각도로 배열되어 있으며, 미토콘드리아는 증편 세포질에 배양되어 있으며 핵의 후반부와 꼬리의 기부를 둘러싸고 있었다. 꼬리는 축사와 2개의 lateral ribbon로 구성되어 있었다. 이러한 특징들은 가자미목의 공유형질로 나타났다. 그러나 미토콘드리아의 수와 모양, 핵과의 모양과 크기에서는 종 특이적 특징을 나타내었다. 특히 강도다리 정자의 가장 큰 구조적 특징은 basal foot, rootlet 그리고 alar sheets로 구성된 기저체이었다. 이 구조의 형태적 특징은 다른 종들 사이에서 아주 다양하게 나타내었다. 이러한 구조는 가자미목의 계통적 관계를 연구하는데 매우 중요한 형질로 여겨진다.

 찾아보기 낱말: 정자, 미세구조, 가자미목