

## Ultrastructure of Spermatozoa in the Bagrid Catfish, *Pseudobagrus fulvidraco* (Teleostei, Siluriformes, Bagridae)

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### 동자개 *Pseudobagrus fulvidraco* (경골어강, 메기목, 동자개과)의 정자의 미세구조

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#### 요 약

동자개 정자는 구형의 두부와 짧은 중편 및 긴 편모를 가지고 있으며 비교적 그 구조가 단순하고 그 길이는 76  $\mu\text{m}$ 이다. 대부분의 경골어류에서와 같이 첨체를 가지고 있지 않으며, 1.67  $\mu\text{m}$ 의 직경을 가진 핵에는 1.0  $\mu\text{m}$  정도로 깊이 함입된 핵와가 있고 그 속에 기부 및 말단 중심립이 들어있었다. 두 중심립은 서로 약 160°의 각도로 배열되어 있으며, 말단부 중심립의 삼중미세소관과 핵 기부에 있는 축사의 이중미세소관 각각으로부터 부수체가 핵막을 향해 접선방향으로 배열되어 있었다. 약 20개 정도의 미토콘드리아가 중편 세포질에 2층 또는 3층으로 배열되어 있으며 축사와는 세포질관에 의해 분리되어 그 주위를 둘러싸고 있었다. 축사의 이중미세소관의 A소관에는 디네인 외관이 관찰되지 않았다. 한 쌍의 lateral fins는 중심미세소관의 축에 평행하게 측면으로 돌출되어 있는데 이것은 동자개 정자 꼬리의 특징으로서 다른 메기류에서는 보고되지 않았다.

**Key words** : Ultrastructure, Spermatozoa, *Pseudobagrus fulvidraco*, Lateral fins, Nuclear fossa

#### INTRODUCTION

Siluriformes exhibit great diversity in trophic mechanisms and are far more diverse than characiforms and cypriniforms in the structure of the weberian complex, swimbladder and

caudal fin skeleton (Fuiman, 1984; Nelson, 1984; Roberts, 1973). The catfishes form a diverse group of marine to freshwater fish with about 2,400 species in 34 families with about 412 genera. However, accounts of siluroid sperm ultrastructure are scarce and restricted to *Ictalurus punctatus* (Ictaluridae) (Emel'yanova and

Makeyeva, 1991a; Jaspers *et al.*, 1976; Poirier and Nicholson, 1982; Yasuzumi, 1971), *Clarias senegalensis* (Clariidae) (Mattei, 1970), *Liocassis ussuriensis* (Bagridae) (Emel'yanova and Makeyeva, 1991b) and SEM only, *Rhamdia sapo* (Maggese *et al.*, 1984). Furthermore nothing have been reported on the fine structure of spermatozoa in Korean catfishes.

The ultrastructure of the spermatozoa has been known in about 300 species of teleost fishes (Billard, 1970; Jamieson, 1991; Mattei, 1991) and has recently served as a criterion for taxonomic and phylogenetic classification of over 200 fish species (Jamieson, 1991).

Ultrastructural studies have demonstrated the absence of an acrosome in the spermatozoa of the Teleostei (Furieri, 1962; Stanley 1965, 1966; Billard, 1970; Mattei, 1970). In Neopterygii two new characters have appeared at the spermatid level: the reduction in the size of the nucleus and the disappearance of the acrosome. This simplified structure is close to that of the gamete of aquatic invertebrates with external fertilization (Mattei, 1991).

Siluriformes have uniflagellate and biflagellate anacrosomal aquasperm (Poirier and Nicholson, 1982; Maggese *et al.*, 1984). In spermatozoa of *Ictalurus punctatus*, *Clarias senegalensis* and *Sternarchus albifrons*, the flagellar fins are absent unlike Bagridae.

Jamieson (1991) described that absence of flagellar fins in sperm of siluroids and of gymnotoids is a tenuous link between siluriforms and the Cypriniformes and Characiformes.

The present study was designed in order to examine the ultrastructure of spermatozoa of the bagrid catfish, *Pseudobagrus fulvidraco* and compared it with those of other families of siluroids.

## MATERIALS AND METHODS

Adult bagrid catfishes, *Pseudobagrus fulvidraco* were collected during the breeding season from Kumho river of Kyungsan City and kept in a controlled environment.

For the experiment, mature spermatozoa were obtained by pressing both sides of the abdomens and kept in physiological saline in a small petri dish. Part of the material was examined and photographed with phase contrast microscope. 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. They were then dehydrated in a graded ethanol series and embedded in Epon 812. The samples were sectioned with a LKB ultramicrotome, stained in 4% aqueous uranyl acetate, poststained with lead citrate, and examined with a Hitachi H-600 electron microscope.

For scanning electron microscopy (SEM), testes were fixed and dehydrated using the same procedures as for transmission. They were followed by isoamylacetate and subjected to critical point dryer. They were coated with gold by ion-sputter and examined with scanning electron microscope.

## RESULTS

The spermatozoon of *Pseudobagrus fulvidraco* is approximately 76  $\mu\text{m}$  in length, and a relatively simple, elongated cell composed of a round head, a short midpiece, and a tail with two lateral fins (Fig. 1). There is thus no acrosome.

### 1. Head

The nucleus is spherical, measuring about

1.67  $\mu\text{m}$  in diameter and length, and covered by a nuclear envelope. The nuclear envelope and the plasma membrane are undulated through the length of the spermatozoa (Fig. 2). The chromatin is highly electron-dense and granular in appearance. Inconsistencies in the dense granularity appear internally and frequently at the periphery of the nucleus (Fig. 3).

The nuclear fossa, the length of which is approximately 1.0  $\mu\text{m}$ , about three-fifths of the nuclear diameter, contains the centriolar complex and cytoplasm, and intrudes into the posterior part of the nucleus (Fig. 2). The nuclear fossa is bell shaped in longitudinal section. A series of transverse sections reveal it to be circular (Figs. 4, 5).

## 2. Centriolar complex

The proximal centriole is at approximately  $160^\circ$  to the distal and oriented at approximately  $20^\circ$  to the longitudinal axis of the spermatozoon (Figs. 2, 6). Both centrioles have a conventional 9+0 microtubular triplet construction (Fig. 4). Both of the centrioles are located in a basal fossa of the nucleus. The anterior region of the nuclear fossa is occupied by the proximal centriole, and the distal centriole is located in the posterior region of the fossa. The distal centriole lies on the longitudinal axis of the cell and extends to the level of the anterior end of the cytoplasmic canal (Figs. 2, 6).

An osmiophilic filamentous plate abuts the proximal and the distal centrioles, which implies that they connect the pair of centrioles (Figs. 2, 6). Electron dense filamentous material is in the nuclear fossa (Figs. 2, 4, 5), and in transverse sections gives rise to satellite appendages arranged tangentially from the triplets (Fig. 4) and the doublets of the anterior end of the axoneme (Fig. 5) toward nuclear envelope lining the nu-

clear fossa.

## 3. Midpiece

The mitochondria are not fused and appear circular to oblong in section. They are situated in a cytoplasmic collar which adjoins the base of the head, averages 0.8  $\mu\text{m}$  in length and has a width of 1.25  $\mu\text{m}$ .

Numerous mitochondria associated with a little cytoplasm, are arranged in two or three layers and surround the cytoplasmic canal. The mitochondria are not in physical contact with the flagellum and are separated from the axoneme by the cytoplasmic canal (Fig. 7). Their number is 20 or more. The mitochondrial matrix is rather loose and the internal membranes or cristae are sometimes difficult to distinguish.

The distribution of the mitochondria is shown in levels of the basal nucleus and the midpiece (Figs. 8–11). A few mitochondria are distributed at the level of the basal nucleus (Figs. 8, 9). At the middle and posterior levels of the cytoplasmic collar, many mitochondria are arranged in two rings around the axoneme in transverse sections (Figs. 10, 11).

The cytoplasmic canal is formed during spermiogenesis and is about 0.95  $\mu\text{m}$  long in the spermatozoa. Structural relationship of the middle piece to the head and flagellum is shown in figures 2 and 7.

## 4. Axoneme

The flagellum is about 74  $\mu\text{m}$  long, and the axoneme has the typical 9+2 microtubular pattern. Each of the nine doublets consists of subtubules A and B. The inner dynein arm arises from subtubule A of each doublet and extends toward the next tubule, however the outer dynein arms are not visible (Fig. 12). Radial spokes can be present in the axoneme. The

cytoplasmic sheath which contains the axoneme is initially cylindrical in outline. Most distally this sheath becomes flattened into an undulating membrane which, from the point on, envelops its individual flagellum. The two lateral fins of the cytoplasmic sheath are roughly in the plane of the two central microtubules, the doublets 3 and 8 (Fig. 12). They are rarely observed along the proximal portion of the tail and not at the end piece.

## DISCUSSION

The ultrastructure of the bagrid catfish spermatozoon, *Pseudobagrus fulvidraco* agrees with that of the most lower teleosts. It thus shows the characteristic features: a round nucleus and a short midpiece, two lateral fins of the sperm tail, and no acrosome. The spermatozoa of another catfishes, *Ictalurus punctatus* (Poirier and Nicholson, 1982) and *Rhamdia sapo* (Maggese *et al.*, 1984), have been examined and shown to lack an acrosome and to be biflagellate and roundish nucleus which is composed of dense and homogeneous chromatin. The sperm of the African catfish *Clarias senegalensis* is uniflagellate unlike *Ictalurus* and *Rhamdia*, but there is a moderate basal nuclear fossa and there are numerous mitochondria in the midpiece as in *Ictalurus*.

The proximal centriole is at approximately 160° to the distal in *P. fulvidraco*. The arrangement of two centrioles is variable in siluroids.

The two centrioles are arranged mutually perpendicular in *Silurus glanis* (Siluridae) with the proximal centriole located above the distal centriole at an obtuse angle as in *Liocassis ussuriensis* (Bagridae) (Gwo *et al.*, 1995), or absent with two distal centrioles as found in *Amiurus nebulosus* (Ictaluridae) (Emel'yanova

and Makeyeva, 1991b).

The midpiece of *P. fulvidraco* is short and contains about 20 or more mitochondria as in most catfishes. The gametes with a short midpiece are found in fishes that employ external fertilization. Mattei (1991) has demonstrated a reduction in the length of the midpiece in Dipnoi and Actinopterygii and that this character must be linked to the biology of fertilization in these fishes.

Siluridae have 2 to 20 mitochondria located in the midpiece (Emel'yanova and Makeyeva, 1991b). Baccetti *et al.* (1984) suggested in cyprinid species that the mitochondria never fuse to form a mitochondrial derivative and their number varies from 2 to 10, with highly frequency of 3 to 4 and that the number of mitochondria of all the spermatozoal characteristics is the only character closely linked with phylogeny.

The flagellar doublets have inner but no outer dynein arms in *P. fulvidraco*. While in Cyprinodontidae, they show only the external arm, except for two species that completely lack them (Thiaw *et al.*, 1986). Such armless flagella have been described for pycnogonid Nymphon sperm (El-Hawawi and King, 1978). Yet, these types of flagella are motile. In *Ictalurus punctatus*, the axoneme is of the 9+2 type but the outer dynein arms are absent (Poirier and Nicholson, 1982), and the flagellum is devoid of a lateral ridge, while in *Liocassis ussuriensis*, a lateral ridge is present (Emel'yanova and Makeyeva, 1991b). The sperm flagella of a number of animal species lack the dynein arms or lateral ridge while retaining motility. Afzelius (1982) reported that just as there is a great diversity in sperm flagellar ultrastructure there is great diversity in the motility patterns.

The tail of the bagrid catfish spermatozoon has two lateral fins or sidefins. Such a peri-

pheral morphology has been described in spermatozoa of various fishes, Crossopterigians (Tuzet and Millot, 1959), Dipneustes (Boisson *et al.*, 1967; Jespersen, 1971), Chondrosteans (Ginsburg, 1977), Holosteans (Afzelius, 1978), and Teleosteans (Billard, 1970; Nicander, 1970). There are generally two lateral fins, except in *Esox lucius* (Billard, 1970; Nicander, 1970), where the flagellum shows only one lateral fin. In trouts, Billard (1970) states that there are some flagella with three lateral fins besides the classical flagella with two fins. In the Cyprinodontidae family, the number of lateral fins associated with the spermatic flagellum varies. However the lateral fins are apparently lacking in channel catfish (Poirier and Nicholson, 1982).

The lateral fins are not reported in other siluroids except in Bagridae. However they occur throughout the spermatozoa of various fishes and are plesiomorphic characters. *P. fulvidraco* is primitive species among siluroid. Jamieson (1991) reported that in view of the widespread occurrence of flagellar fins throughout the Osteichthyes, absence in cyprinids as in all the investigated Ostariophysi is an apomorphic loss and an ostariophysian synapomorphy.

Numerous authors have suggested that the sidefins might increase the efficiency of flagellar movement. However, Afzelius (1978) reported that the lateral fins are unlikely to improve the efficiency of the ciliary beat, in spite of the fact that they increase the surface of the flagellum in the direction perpendicular to the beat direction. He described that the existence of lateral fins on the sperm tail is not only for many teleosts but also for echinoderm species and for four species of the polychaet family *Nereis* and suggested parallel evolution of lateral fins on the sperm tail in one polychaet genus, an echinoderm genus, and in the fishes.

## ABSTRACT

The spermatozoa of bagrid catfish, *Pseudobagrus fulvidraco* are approximately 76  $\mu\text{m}$  in length, and a relatively simple and elongated cell composed of a spherical head, a short middle piece and a tail. The ultrastructure of spermatozoa of *P. fulvidraco* is characterized by the following features. The acrosome is absent as in most teleost. The round nucleus measuring about 1.67  $\mu\text{m}$  in length and diameter is depressed with a deep nuclear fossa. The nuclear fossa, the length of which is about three-fifths of the nuclear diameter, contains the proximal and distal centrioles. The two centrioles are oriented approximately  $160^\circ$  to each other. The filamentous materials give rise to satellite appendages arranged tangentially from the triplets of the distal centriole and the doublets of the anterior end of the axoneme toward the nuclear envelope. The mitochondria are not fused and their number is 20 or more. They are arranged in two or three layers and two rings within the cytoplasmic collar and surround the axoneme.

They are separated from the axoneme by the cytoplasmic canal. The axoneme is of the 9+2 microtubular pattern and has inner but no outer dynein arms. The two lateral fins are in the same plane with the two central microtubules, the doublets 3 and 8, which are ultrastructural characteristics of the sperm tail unlike other siluroids lacking the lateral fins.

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**FIGURE LEGENDS**

- Fig. 1, 1a.** Scanning electron micrographs of a spermatozoon showing a spherical head (H), a short midpiece (Mi) and a tail (T). Note the two lateral fins of the sperm tail in figure 1 (white arrowheads) and figure 1a (black arrowheads). Scale bar is 1  $\mu\text{m}$ .
- Fig. 2.** Longitudinal section through the head and midpiece of the bagrid catfish spermatozoon. The deep nuclear fossa contains the proximal (PC) and the distal centrioles (DC) showing an orientation of approximately  $160^\circ$  and the filamentous plate (arrow) abuts two centrioles. Electron dense filamentous materials (white arrowheads) present between the centriole and the nuclear envelope lining the nuclear fossa. Note the undulating nuclear envelope and plasma membrane (black arrowheads) through the length of the spermatozoa. Note the absence of the acrosome. Scale bar is 0.5  $\mu\text{m}$ .
- Fig. 3.** Longitudinal section through the nucleus showing the chromatin containing nuclear vacuoles (arrowheads) internally and frequently at the periphery of the nucleus. Scale bar is 0.5  $\mu\text{m}$ .
- Figs. 4, 5.** Transverse sections at the level of the distal centriole showing nine triplets in figure 4 and the anterior end of the axoneme showing nine doublets in figure 5, each with a satellite appendage (arrowhead) arranged tangentially toward the nuclear envelope. Scale bar is 0.5  $\mu\text{m}$ .
- Fig. 6.** Longitudinal section through the nuclear fossa and two centrioles showing the arrangement of approximately  $160^\circ$ . Electron dense filamentous materials (white arrowheads) are between the centriole and the nuclear envelope lining the nuclear fossa. Note the filamentous plate between the proximal and the distal centrioles and that the distal centriole extends to the level of the anterior end of the cytoplasmic canal (black arrowheads). Scale bar is 0.5  $\mu\text{m}$ .
- Fig. 7.** Longitudinal section through the basal nucleus and the midpiece showing the cytoplasmic collar (CC) containing the mitochondria (M) arranged in two or three layers. The mitochondria are separated from the axoneme by the cytoplasmic canal (arrowheads). Scale bar is 0.5  $\mu\text{m}$ .
- Figs. 8-11.** Transverse sections through various levels of the cytoplasmic collar showing the distribution of the mitochondria, which is separated from the axoneme by the cytoplasmic canal (C).
- Fig. 8.** Transverse section at the level of the basal nucleus containing one mitochondrion (M). Note the cytoplasmic canal (arrow). Scale bar is 0.5  $\mu\text{m}$ .
- Fig. 9.** Transverse section at the level of the anterior part of the cytoplasmic collar containing three mitochondria. Note the lateral fins (arrow) of the axoneme. Scale bar is 0.5  $\mu\text{m}$ .
- Fig. 10.** Transverse section at the middle level of the cytoplasmic collar containing many mitochondria arranged in two rings. Scale bar is 0.5  $\mu\text{m}$ .
- Fig. 11.** Transverse section at the posterior level of the cytoplasmic collar containing ten or more mitochondria arranged in two rings. Scale bar is 0.5  $\mu\text{m}$ .
- Fig. 12.** Transverse section through two sperm tails. The tails have two lateral fins (arrows) which are in the same plane of the two central microtubules. Note the absence of outer dynein arm. Scale bar is 0.5  $\mu\text{m}$ .





