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Ultrastructure of Spermatozoa in the Catfish, *Silurus asotus*

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메기, *Silurus asotus* 정자의 미세구조

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ABSTRACT : The spermatozoa of *Silurus asotus* are approximately 62.5 μm in length and relatively simple cells composed of a spherical head, a short midpiece and a tail as in most Siluriformes. The ultrastructure of the spermatozoa of *S. asotus* is characterized by the following features. The nucleus measuring about 1.5 μm in length is depressed with a deep nuclear fossa of about 0.6μm in length, two fifth of the nuclear diameter. The fossa contains the proximal centriole and the half of the distal centriole. Two centrioles form an angle of approximately 140° to each other. The nine satellite rays radiate from the outer surface of the distal centriole. The mitochondria surround the basal nucleus and the axoneme, and are arranged in two or three layers in the postnuclear cytoplasm. The lateral fins are lost in the sperm tail. The most significant feature is manifested in the midpiece. The midpiece comprises two parts, the mitochondria and the tubular structure unlike other teleost fishes containing only the mitochondria. The tubular structure was reported only in the spermatozoa of *Citharinus* belonging to the Characiformes of teleost fishes. Thus it is considered to be a good characteristics for the study of phylogenetic link between Siluriformes and Characiformes.

Key words : Ultrastructure, Anacrosomal spermatozoon, Midpiece, Unusual tubular structure, *Silurus asotus*.

요약 : 메기 정자는 그 길이가 약 62.5 μm 이며 구형의 핵, 짧은 중편 및 꼬리를 가진 일반적인 메기류 정자의 미세구조적 특징을 나타내었다. 정자는 대부분의 경골어류의 정자에서와 같이 첨체를 가지고 있지 않았으며 염색질은 완전히 농축되어 있었다. 핵와 (nuclear fossa) 는 약 0.6 μm 함입되어 있었고 그 속에 기부 중심립과 말단 중심립의 일부가 포함되어 있었다. 두 중심립은 140°의 각도로 배열되어 있었으며 말단 중심립에서 9개의 부수체가 원형질막을 향하여 배열되어 있었다. 미토콘드리아는 중편 세포질에서 2층 또는 3층으로 배열되어 있었으며 핵의 후반부와 꼬리의 기부를 둘러싸고 있었다. 꼬리는 축사만으로 구성되어 있었으며 lateral fins는 관찰되지 않았다. 메기 정자의 가장 큰 구조적 특징은 중편 세포질에 구성되어 있는 관구조 (tubular structure)이었다. 대부분의 경골어류의 정자는 중편 세포질에 미토콘드리아만을 포함하고 있으나, 메기 정자에서는 중편 세포질의 전반부에 미토콘드리아가 포함되어 있고, 후반부에는 소관이 모여 망상구조를 형성하는 관구조가 잘 발달되어 있었다. 이와 같은 관구조는 현재까지 Characiformes 의 정자 이외의 다른 경골어류에서는 보고된 바 없으며 이러한 구조는 Characiformes과 메기류의 계통학적 관계를 연구하는데 매우 중요한 형질로 여겨진다.

INTRODUCTION

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

Teleost spermatozoa are relatively simple cells composed of a spherical head devoid of an acrosome, a short midpiece and an elongating tail. Siluriformes have uniflagellate and biflagellate anacrosomal aquasperm. The nuclear fossa is usually deep and the middle piece is short. The length of the middle piece could be related to the type of fertilization. Internal fertilization was associated with a long middle piece and external fertilization with a short middle piece (Idelman, 1967).

Spermatozoal ultrastructure has been investigated for only a few siluroid species, *Ictalurus punctatus* (Ictaluridae) (Emel'yanova & Makeyeva, 1991a; Jaspers et al., 1976; Poirier & Nicholson, 1982; Yasuzumi, 1971), *Clarias senegalensis* (Clariidae) (Mattei, 1970), *Liocassius ussur-*

iensis (Bagridae) (Emel'yanova & Makeyeva, 1991b) and SEM only, *Rhamdia sapo* (Maggese et al., 1984). Although *Silurus asotus* is the largest catfish and the commercially important far eastern catfishes, the ultrastructure of *Silurus* spermatozoa has not been examined to date.

The present paper describes the ultrastructure of the spermatozoa in the catfish *Silrus asotus* and is compared with those of other teleost.

MATERIALS AND METHODS

Specimens of catfish, *Silurus asotus* were collected during the breeding season from Kumho river of Kyungsan City. For transmission electron microscopy, semen and pieces of testis were 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 Sorvall MT ultramicrotome, stained in 4% aqueous uranyl acetate, poststained with lead citrate and examined with a Hitachi H-600 electron microscope.

RESULTS

The spermatozoon of *Silurus asotus* is approximately 62.5 μm in length and a relatively simple and elongated cell composed of a head devoid of an acrosome, a short midpiece and an elongated tail (Fig. 1). The nucleus measuring about 1.5 μm in length is spherical and contains electron dense chromatin. The nuclear envelope is undulated and is in close contact with plasma membrane at the anterior region of the nucleus (Fig. 1). The base of the nucleus is indented by a nuclear fossa, which is approximately 0.6 μm long and two fifths of the length of the nucleus. The nuclear fossa is circular in transverse section (Fig. 2A). There is no acrosome in the sperm head.

The proximal and distal centrioles are located at an angle of approximately 140° to each other. The distal centriole is parallel to the main axis of the sperm and extends from the nuclear fossa to the level of the anterior end of the cytoplasmic canal (Fig. 1). The midpiece is approximately 1.0 μ m long and consists of two parts. The anterior part contains the distal centriole and mitochondria near the base of the nucleus (Figs. 1, 2B and 2C). The posterior part has a globular structure which is made up of tubules (Figs. 1, 2E). The tubular structure contains the axoneme which is centrally located and surrounded by the cytoplasmic canal (Fig. 2E). This tubular structure shows similar aspects in the longitudinal (Fig. 1) and transverse (Figs. 2D and 2E) sections. The tubules are approximately 60 nm in diameter and are linked one to another. They are smooth and as a whole form a globular net-work measuring about 1.3 μm in outer diameter (Figs. 2D and 2E).

The mitochondria are arranged in two or three layers,

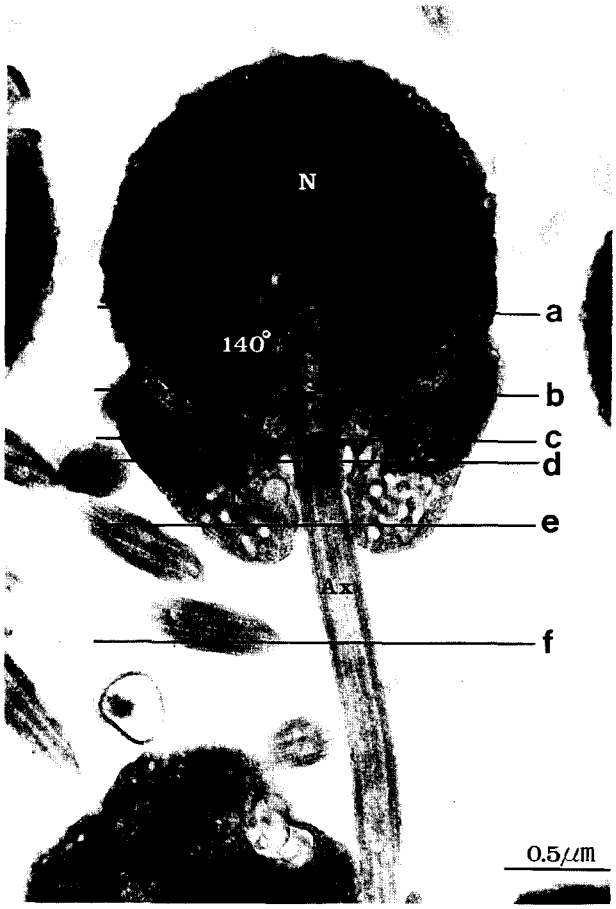

Fig. 1. Longitudinal section through a spermatozoon showing the spherical nucleus (N) containing the proximal centriole and the half of the distal centriole in a nuclear fossa, the midpiece comprising two parts, the mitochondria (M) and the tubular structure (TS), and the axoneme (Ax).

Note that two centrioles form an angle of approximately 140° to each other. a-f denote the level of transverse section in Fig. 2.

and surround the basal nucleus and the basal flagellum. They are completely separated from the axoneme by a cytoplasmic canal (Fig. 2D, arrow). Nine satellite rays radiate from triplets of the distal centriole (Fig. 2C).

The sperm tail is approximately 61 μm long and contains an axoneme of the typical 9+2 pattern. The lateral fins are not observed in the flagellum (Figs. 1 and 2F).

DISCUSSION

The ultrastructure of the spermatozoa of *Silurus asotus* is similar to those of the other teleosts with external fertilization. The similarity is especially manifested in the spherical head devoid of an acrosome and the elongated tail. However, there are some differences in the depth of the nuclear fossa, the number and arrangement of the mitochondria, the orientation of two centrioles and the midpiece.

The nucleus of *S. asotus* has a deep nuclear fossa at its base. The deep nuclear fossa is also found in other teleost species (Jasper et al., 1976; Poirier & Nicolson, 1982; Emel'yanova & Makeyeva, 1991a, b; Gwo & Gwo, 1993; Gwo et al., 1994a, b, 1996). But in Cypriniformes (Baccetti et al., 1984) the nuclear fossa is much smaller. The deep nuclear fossa is considered to be apomorphic as compared with the shallow nuclear fossa (Jamieson, 1991).

Two centrioles vary considerably in their relative position in teleost spermatozoa. Siluriformes show three types of orientation of the centrioles. In the first type they are perpendicular to each other. The second type is characterized by the centrioles forming an obtuse angle. This type is seen in *Liocassius ussuriensis* and *Clarias senegalensis* (Mattei, 1970). In the third type their angle is absent because the proximal and distal centrioles are parallel and each forms a flagellum. This type has been observed in *Amiurus nebulosus* (Emel'yanova & Makeyeva, 1991b).

The two centrioles of *S. asotus* are located at an angle of approximately 140° each other and the distal centriole is parallel to the main axis. Baccetti et al. (1984) reported in seven cyprinid species that the variations in centriolar geometry are correlated with the position of the nucleus with respect to the axis of the tail, where the spermatozoa have the lateral implantation of the flagellum. However the correlation of the nuclear position and the tail axis is not observed in this species. Jamieson (1991) reported that the proximal centriole perpendicular to distal centriole is a plesiomorphic feature in fish spermatozoa.

The satellite rays of the *S. asotus* have been also observed in the *Leuciscus souffia* of Cyprinidae, and Perciformes and Atheriniformes of some advanced Neopterygii (Mattei & Mattei, 1976; Jamieson, 1989). But the structure has been rarely reported in teleost fish spermatozoa with exception of the above species. According to Afzelius (1979), nine satellite appendages are very common in lower metazoan sperm but are gradually lost toward the higher phyla. These structures attach the distal centriole to the plasma membrane by the tips of their rays and are also responsible for the stabilization of the spatial relationship between the nucleus and the centriolar complex which is important during sperm tail movement (Gwo et al., 1996).

The midpiece of teleost fish spermatozoa is less developed than those in other vertebrate groups (Billard, 1970a) and generally contains only mitochondria disposed around the axoneme. Mattei et al. (1989) mentioned the presence of numerous vesicles at the base of the midpiece in *Ophidion*. Vesicles were described around the axoneme in the portion which extend beyond the midpiece in cyprinids (Billard, 1970a; Baccetti et al., 1984). Tubular structure was described for the first time in the spermatozoa of *Citharinus* belonging to the Characiformes of teleost fishes (Mattei et al., 1995). A similar structure was reported only in the protist, *Leptomonas collosoma* (Linder & Staehelin, 1980).

The tubular structure of *Citharinus* is also observed in the midpiece of *S. asotus* spermatozoa. It apparently exhibits phylogenetic link between Characiformes and Siluriformes. Fink & Fink (1981) suggested that the Siluriformes are the apomorph sister-group of the Characiformes, emphasizing shared specializations of the otophysic connection. The unusual midpiece structure is not observed in the other siluroid species.

The mitochondria are various in their numbers and distribution in teleost sperm (Jamieson, 1991; Gwo et al., 1992, 1994b, 1995; Afzelius & Mims, 1995; Gwo, 1995). Baccetti et al. (1984) suggested that the specific chara-

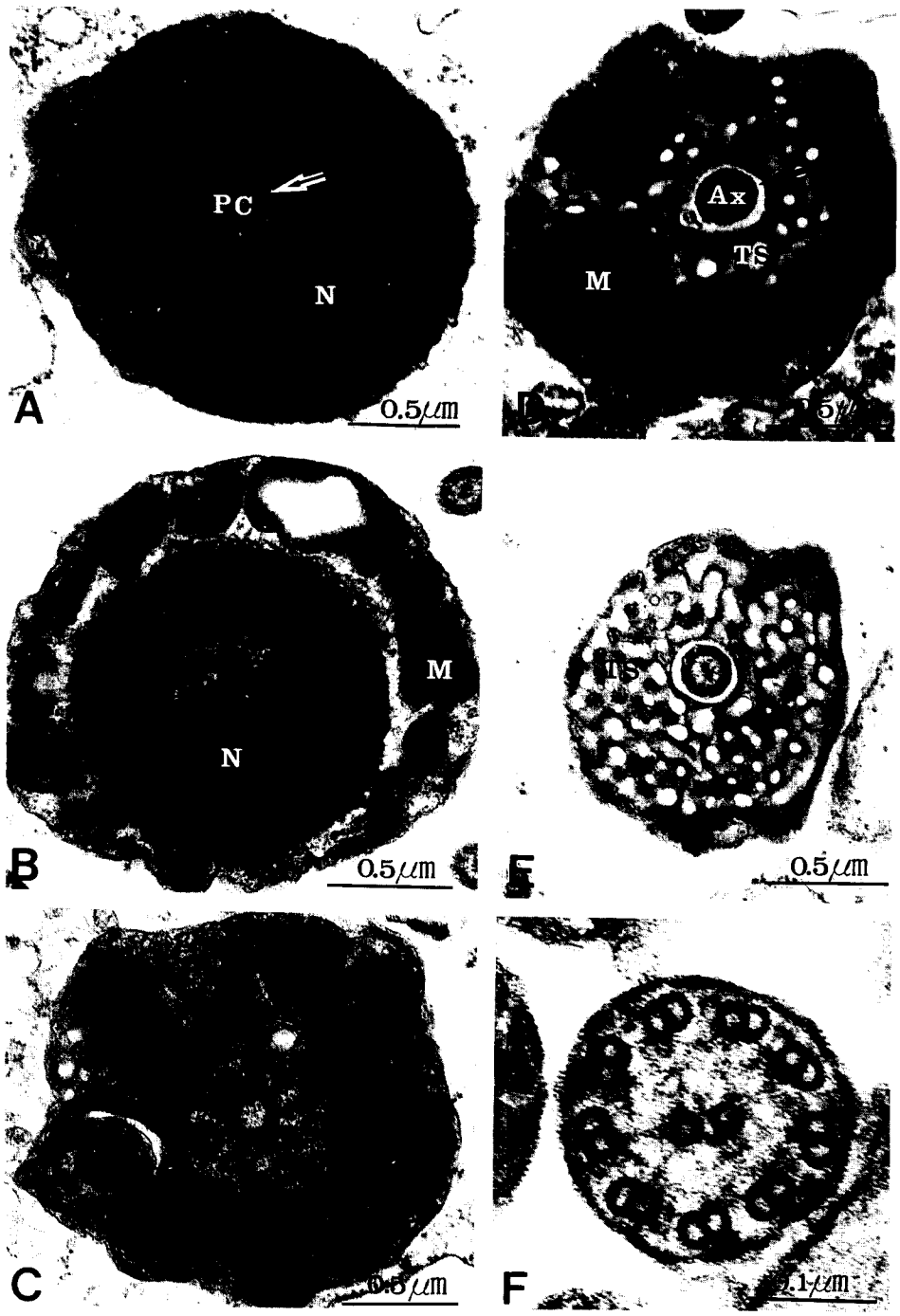

Fig. 2. Transverse section of catfish spermatozoa.

A. Transverse section of the nucleus (N) at the level of the proximal centriole (PC) in the nuclear fossa (arrow).

B. Transverse section through the basal nucleus containing the distal centriole in nuclear fossa. Note the mitochondria (M) surrounding the nucleus (N).

C. Transverse section through the anterior midpiece showing the distal centriole and the surrounding mitochondria. Note nine satellite rays (arrowheads) radiating from the outer surface of centriole.

D. Transverse section through the midpiece containing mitochondria (M) and tubular structure (TS). The mitochondria are separated from the axoneme (Ax) by the cytoplasmic canal (arrow). Note the tubular structure inside the midpiece.

E. Transverse section of the posterior level of the midpiece showing the tubular structure (TS) surrounding the axoneme and the cytoplasmic canal.

F. Transverse section through the sperm tail showing the axoneme of 9+2 microtubular pattern. Note the absence of lateral fins.

cteristics of the cyprinid spermatozoa is the number of mitochondria, which is a good characteristics from a phylogenetic point of view.

The flagellar apparatus of spermatozoa exhibits a great diversity among species (Afzelius, 1982). Lateral fins have been observed in many species of fish (Billard, 1970a, b; Nicander, 1970; Ginsburg, 1977; Afzelius, 1978; Gardiner, 1978; Stein, 1981). In Siluriformes, they are lacking with the exception of one species, *Liocassius ussuriensis* (Emel'yanova & Makeyeva, 1991b). In view of the widespread occurrence of lateral fins throughout Osteichthyes the absence of lateral fins in Siluriformes appears to be an apomorphic loss and an ostariophysian synapomorphy (Jamieson, 1991). Lateral fins are also reported in a few invertebrates (Afzelius, 1978) but not found in the gametes of other vertebrates.

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