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Anatomical Ultrastructure of Spermatozoa of Korean Sharpbelly, *Hemiculter eigenmanni* (Cypriniformes, Cyprinidae)

*By Kgu-Hwan Kim**

Department of Radiologic Technology, Daegu Health College, Yeongsong-ro 15, Buk-gu, Daegu 41453, Republic of Korea

ABSTRACT **The spermatozoa of *Hemiculter eigenmanni* is similar to other cyprinid by spherical head containing a nucleus with highly condensed chromatin, a short midpiece with mitochondria and a flagellum located tangentially to the head. The fine structure of cyprinid spermatozoa described classical characteristics of Cyprinidae spermatozoon comprising the absent of acrosome, the shallow nucleal fossa, postnuclear distribution of mitochondria and the lateral insertion of flagellum. However there were some structural differences for their morphology, in the mitochondria and the orientation of centrioles. The proxomal and distal centrioles are oriented approximately 145° to each other. The mitochondria of 8 or 10 in number are arranged in two or three layers.**

Key words: Anatomical ultrastructure, seprmatozoa, Cyprinidae, *Hemiculter eigenmanni*

INTRODUCTION

Fishes have enormous spermatic diversity and the different evolutionary tendences within their group. Spermatozoal ultrastructure has been extensively investigated for taxonomic purpose using electron microscopy (Billard, 1970; Baccetti *et al*., 1984; Jamieson, 1991). Recently, spermatozoal ultrastructural features were in Gymnotiformes (Franca *et al*., 2007), Siluriformes (Quagio-Grassiotto and Oliveira, 2008), Arhythmacanthidae (Foata *et al*., 2012), Syngnathidae (Francesca *et al*., 2016) and Osmeridae (Beirão *et al*., 2016).

The family of Cyprinidae is the largest group of fishes, with over 2,000 species. Cyprinidae speces have been examined for spermatozoal ultrastructure in references to Cyprininae (Baccetti *et al*., 1984; Guan and Afzelius, 1991), Acheilognathinae (Ohta and Iwamatsu, 1983; Kim *et al*., 2008), Danioninae (Kim, 2006) and Gobioninae (Kim *et al*., 2008). Cyprinid spermatozoa have anacrosomal aquasperm, a spherical head, shallow nuclear fossa with variously angled centrioles, short mid-piece contain mithchondra and long tail with no lateral fins (Jamieson, 1991; Mattei, 1991). Although preceding search present data related to structure of spermatozoa of cyprinids, but only part of the Cyprinidae. Therefore, information on spermatozoal ultrastructure is certainly needed for this big clade of fishes.

The main aim of this study is to analyze the sperm morphology of *Hemiculter eigenmanni*. And then, the result are compared with those from other cyprinid species as a basis for phylogenetic inferences.

MATERIALS AND METHODS

Adult *Hemiculter eigenmanni* were collected during the breeding season in Andong-dam and kept in a controlled environment. For the experiment, mature spermatozoa were obtained by pressing both sides of the abdomen and kept in physiological saline in a small petri dish. For transmission electron microscopy (TEM), semen and pieces of testis were dissected and fixed in 2.5~5% glutaraldehyde in 0.1 M sodium cacodylate buffer and postfixed in 1% osmiun tetroxide in the same buffer. They were then dehydrated in a graded ethanol series and embeded in Epon 812. The samples were sectioned on a LKB ultramicrotome, stained in 4% aqueous uranyl acetate, post-stained

*Corresponding author: Kgu-Hwan Kim Tel: 82-53-320-1320, Fax: 82-53-320-1449, E-mail: guhwan@hanmail.net

with lead citrate and examined with a Hitachi H-600 electron microscope.

RESULTS

The spermatozoon of *Hemiculter eigenmanni* is a relatively simple, elongated cell composed of a spherical head, a short midpiece and a long tail region. The fllagellum is inserted tangentially in the head (Fig. 1a).

Head

The spherical nucleus of 1.83 μm in diameter is eccentrically positioned on the tail axis (Fig. 1a). The chromatin is highly electron-dense and homogeneous, and contains some vacuoles (Fig. 1a). The nuclear envelope and the plasma membrane are tightly apposed and strongly undulated (Fig. 1b, c). No acrosomal complex or vesicles are present anteriorly to the nucleus, where the undulating nuclear envelope is always in close contact with plasma membrane (Fig. 1a, c). Mediolaterally the nucleus has an invagination, the nuclear fossa, where proximal centriole and part of the distal centriole were located (Fig. 1d, e).

Centriolar complex

The centriolar complex remains near the base of nucleus. The proximal centriole has orientation of 145° with respect to the distal centriole appearing tangential to the nucleus (Fig. 1e). The proximal centriole recline in the shallow nuclear fossa (Fig. 1d, e). The distal centriole is parallel to the sperm axis and posteriorly extends to the level of the anterior end of the cytoplasmic canal (Fig. 1f). A few satellite fibers project from the centriole to the nuclear membrane and plasma membrane (Fig. 1d, e). And the centrioles are connected to each other by filamentous structure (Fig. 1d, e).

Midpiece

The midpiece is short and not well defined. It is concentrated around the lateral distal region of the nucleus (Fig. 1e). The midpiece contains some vesicles (Fig. 1a, f), cytoplasmic channel and 8∼10 mitochondria arranged in two or three layers (Fig. 1a, f, g). It shapes an asymmetrical truncated cone and is invaginated sagittally by the cytoplasmic canal (Fig. 1a, f, g). The mitochondria have loose matrix, and several simple cristae of irregular layers which are sometimes difficult to distinguish (Fig. 1f, g).

Tail

The flagellum is laterally implanted toward the head (Fig. 1a). It is composed of a 9+2 microtubular doublet structure enclosed by the plasma membrane (Fig. 1h, i). The flagella membrane does not have lateral projections of fins (Fig. 1h, i). The cytoplasmic vesicles are distributed between axonemal doublet and plasma membrane and encircle the tail. They are not observed toward end part of tail (Fig. 1f-i).

DISCUSSION

The cyprinid spermatozoal fine structrue is the typical aquasperm type similar to other teleosts with external fertilization (Jamieson, 1991). The spermatozoal ultrastructure are characterized by a spherical nucleus with the shallow nuclear fossa, a midpiece containing mitochondria, a flagellum tangential to the nucleus, and no acrosome (Gwo *et al*., 1995). The ultrastructure of spermatozoa of the *Hemiculter eigenmanni* reveals some features typical of cyprinid spermatozoa. A schematic model for this species, *H. eigenmanni* is described in Figure 2.

Although the speramtozoa of *H. eigenmanni* are similar those of cyprinid. However, the ultrastructure of *H. eigenmanni* spermatozoa appear another interesting feature. The spermatozoon of *H. eigenmanni* has 8∼10 mithochondria in the postnuclear cytoplasm. In the cyprinid spermatozoa, the mitochondria number varies from one in bittering *Rhodeus* (Guan and Afzelius, 1991), *Acheilognathus* (Kim *et al*., 2007) and *Pungtungia* (Lee and Kim, 1998), two in *Barbus* and *Alburnus* to ten in *Carassius* (Bacceti *et al*., 1984). Mitochondira of Acheilognathinae (Guan and Afzelius, 1991; Kim *et al*., 2007) and *Pungtungia* (Lee and Kim, 1998) are fused single mitochondrion. According to Baccetti *et al*. (1984) and Beirão *et al*. (2015), the main interspecies difference seems to be the mitochondria shape and number. The separated mitochondria are considered as an plesiomorphic character in comparison with the fused mitochondrion (Jamieson, 1991).

In the nuclear outline, a shallow nuclear fossa containing only the proximal contriole and part of the distal centriole is found in *H. eigenmanni*. A similar case of nuclear fossa and centriolar positoning are observed in Cyprininae (Baccetti *et al*., 1984), Acheilognathinae (Ohta, 1983; Kim *et al*., 2008), Gobioninae (Kim *et al*., 2008) and Danioninae (Kim *et al*., 2006). A shallow nuclear fossa type and cetriolar positioning also exhibited by Cobitidae (Park and Kim, 1996). A shallow nuclear fossa of spermatozoa of Cyprinidae is dissimilar to Siluriformes with much deeper nuclear fossa containing the centriolar complex (Quagio-Grassiotto and Oliverira, 2008). The shallow nuclear fossa is plesiomorphic character as compared with the deep nuclear fos-

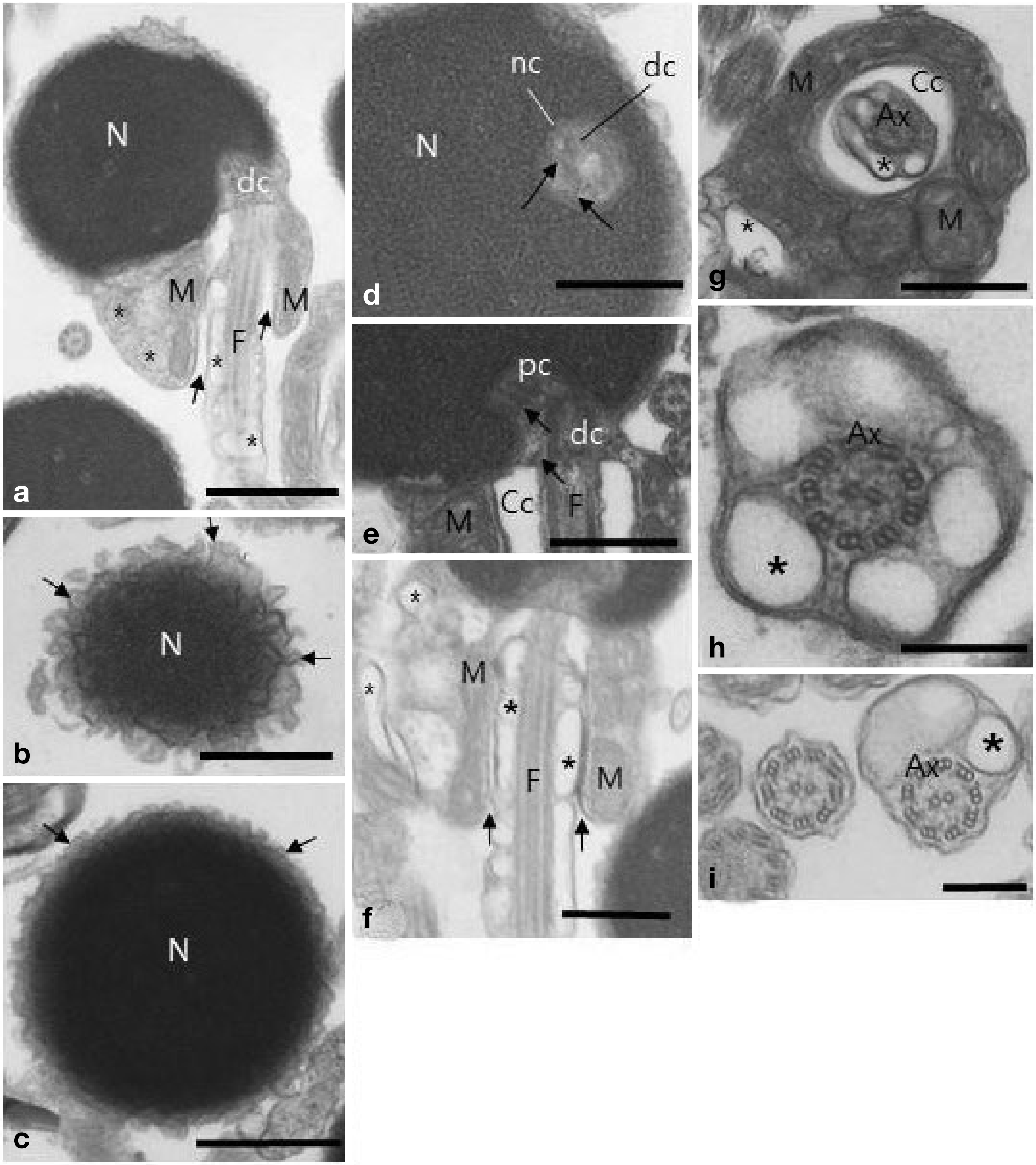

Fig. 1. The spermatozoa of *Hemiculter eigenmmanni* is peculiar in that the head is round, the nuclear fossa is moderate and containing the proximal centriole, and 8 mitochondria are arrnaged around the base of flagellum. ⓐ Longitudinal section through a head, midpiece and tail region showing the chromatin containing nucleus (N) and one or two layer arranged mithchondria (M) in the juxtanuclear pocket of the postnuclear cytoplasm. Note the lateral implantation of the flagellum (F) with respect to the nucleus (N) that the anterior portion of the distal centriole (dc) is inserted to the nuclear fossa. The flagellum containing several vesicles (*) is seperated from midpiece cytoplasm by cytoplasmic cannal (arrows). ⓑ, ⓒ Cross sections of superior and middle portion of head region, the nuclear envelope and the plasma membrane are strongly undulated (arrows). ⓓ Cross section of inferior region of head, the nuclear fossa containing anterior portion of the distal centriole. Note the satellite fibrils (arrows) attached to the nuclear envelope linging the nuclear fossa. ⓔ Longitudinal section through the nuclear fossa shallow invaginated, that contains proximal centriole and superior portion of distal centriole. The satellite fibrils (arrows) connected with nuclear envelope and plasma membrane. ⓕ Longitudinal sections of the midpiece showing the several mitochondria (M) in the postnuclear cytoplasm. Note the nuemerous vesicles (*) appear in flagellum and cytoplasm. ⓖ Transeverse section through the midpiece showing the 4 mitochondria and visecles (*) in cytoplasm and flagellum. The axoneme separated by cytoplasmic canal (Cc). ⓗ, ⓘ Cross sections of flagellum, showing the Axoneme 9+2 doublet structure (A×) and several vesicles (*). scale bar ⓐ-ⓒ = 1 μm, ⓓ-ⓖ = 0.5 μm, ⓗ, ⓘ = 0.2 μm

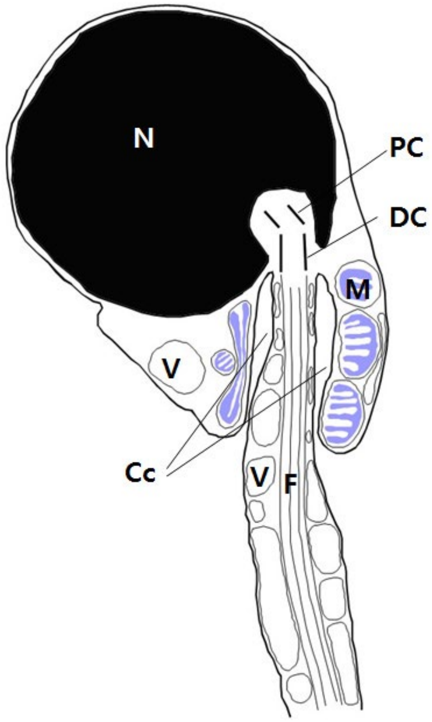

Fig. 2. Schematic diagram of *Hemiculter eigenmanni* spermatozoon. Cc: cytoplasmic canal; DC: distal centriole; F: flagellum; N: nucleus; PC: proximal centriole; M: mithochondria; V: vesicles.

sa (Jamieson, 1991).

Two centrioles vary considerably in their relative position in teleost spermatozoa (Mattei, 1991). Baccetti *et al.* (1984) reported that the orientation of two centrioles in seven cyprinid species is variable from 40° to 140° and that the position of the nucleus with respect to the axis of the tail is correlated to the arrangement of centrioles. In silurid, two centrioles form an obtuse angle (Mattei, 1970; Quagio-Grassiotto and Oliverira, 2008) as in most cyprinids but the nucleus is parallel to the sperm tail unlike cyprinids having the sperm tail positioned tangentially to the nucleus. The correlation of the nuclear position and the centriolar arrangement in cyprinids is not shown in silurid (Kwon *et al.*, 1998). The proximal centriole perpendicular to distal centriole is a plesiomorphic feature in fish spermatozoa (Jamieson, 1991).

Billard (1970) reported, the midpiece is less developed in teleost spermatozoa than in other vertebrae group. The midpiece of *H. eigenmanni* spermatozoon is rather short, which also has been observed on teleosts utilizing external fertilization. According to Mattei (1991), the elongated of the midpiece is found in the teleosteans that retain internal fertilization, but this is not general. In the midpiece, the cytoplasmic vesicles are also observed in many cyprinid species but their locations are differing each other. The vesicles of most of cyprinid species are located in the anterior region of the axoneme (Lee and Kim, 1998; Kim, 2006), while in *H. eigenmanni* and *Rutilus* (Baccetti *et al.*, 1984) are located along almost whole length of flagellum, and in *Alburnus* (Baccetti *et al.*, 1984) appear in the cytoplasmic cannal portion. The cytoplasmic vesicles of cyprinid are composed of tubular SER (endoplasmic reticulum) and some their membranes even fuse with flagellar plasmalemma and communicate with external environment (Kudo, 1980).

The flagellum of *H. eigenmanni* is laterally inserted to the nucleus and as a result, the spermatozoa appears an asymetrical structure. This asymmetrical structure have been observed in Cyprininae (Baccetti *et al.*, 1984), Acheilognathinae (Ohta, 1983; Kim *et al.*, 2008), Gobioninae (Kim *et al.*, 2008) and Danioninae (Kim *et al.*, 2006). In spermatozoa, flagellum axis may be either perpendicular or parallel to the nucleus, depending on whether nuclear rotation occur during spermiogenesis (type I) or not (type II) (Mattei, 1970).

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치리 *Hemiculter eigenmanni* 정자의 미세해부학적 구조 (잉어목, 잉어과)

김구환

대구보건대학교 방사선과

요 약 : 치리 *Hemiculter eigenmanni* 정자의 미세구조를 전자현미경으로 관찰하였다. 치리 정자의 미세구조는 잉어류 정자의 일반적인 구조와 유사하게 첨체가 없는 둥근두부, 미토콘드리아를 포함하는 짧은 중편 그리고 긴 단편모로 구성되어 있었다. 핵물질은 매우 농축되어 있고 핵와는 얕게 함입되어 있었다. 중편부에는 8∼10개의 미토콘드리아가 2∼3층으로 배열되어 있었다. 중편부의 세포질과 소기관은 비대칭적으로 분포하고 있으며, 두 중심립 사이의 각도는 145°로 나타났다. 핵과 편모는 두 중심립의 각도만큼 기울어져 있었다.

찾아보기 낱말 : 미세해부학, 정자, 잉어목, 치리