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Anatomical Ultrastructure of Spermiogenesis and Spermatozoa of *Pseudobagrus fulvidraco* (Siluriformes: Bagridae) from Korea

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ABSTRACT The spermiogenesis and mature spermatozoa of *Pseudobagrus fulvidraco* were described by means of scanning and transmission electron microscopy. Spermiogenesis is characterized by lateral development of the flagellum, nuclear rotation, deep nuclear fossa formation and compaction into thick granules. The spermatozoa exhibit a round head containing a nucleus that lacks an acrosome, and having a midpiece and a flagellum. The midpiece is small and has a short cytoplasm including several mitochondria separated from the tail by the cytoplasmic canal. The flagellum contains the 9+2 classical axoneme structure and has two axonemal fins. The presence of axonemal fins in the flagellum is a common character in Bagridae. The interrelationships among the Bagridae as well as other teleosts are herein discussed.

Key words : Spermiogenesis, spermatozoa, Bagridae, *Pseudobagrus fulvidraco*

INTRODUCTION

Fine structure of spermiogenesis and spermatozoa reveals wide species specific differences in fish (Mattei, 1991). Organization of spermatozoa in freshwater teleostei are known only from brief characterisations or completely unknown (Jamieson, 1991). Therefore the present study describes the fine structure of spermatozoa and spermiogenesis of the river bagrid catfish, *Pseudobagrus fulvidraco* (Richardson, 1846). Although the study by Lee (1998) provided data on spermatozoon structure but the spermiogenesis and the wide considerations with other species were not enough. In light of the limited available information on either spermiogenesis or spermatozoal structure in freshwater bagridae, the present study is going to describes this characters in the *P. fulvidraco* and compares with the other data available. We also discuss spermiogenesis and the spermatozoal ultrastructure in the Siluriformes.

MATERIAL AND METHODS

Mature Korean bulhead, 3 males of *Pseudobagrus fulvidraco* were collected by stake net (5×5 mm) and scoop net (5×5 mm) during the breeding season in 2006 from Wyi stream, river of Kunwi gun, Gyeongsangbuk-Do, Korea and kept in a controlled alive environment by oxygen generator until sacrifice.

Gonad fragments from newly sacrified fish were fixed overnight 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 ultramicrotome (MTXL, RMC, USA) stained in 4% aqueous uranyl acetate, poststained with lead citrate, and examined with a Hitachi H-7500 electron microscope.

For scanning electron microscopy (SEM), testes were fixed and dehydrated using the same procedures as for TEM. They were followed by isoamylacetate and subjected to critical point drier. They were coated with gold by ion-sputter and examined with scanning electron microscope (S-4100).

RESULTS

Spermiogenesis begins at the end of meiosis and corresponds to the differentition of spermatids into spermatozoa. In *Pseudobagrus fulvidraco* spermiogenesis starts

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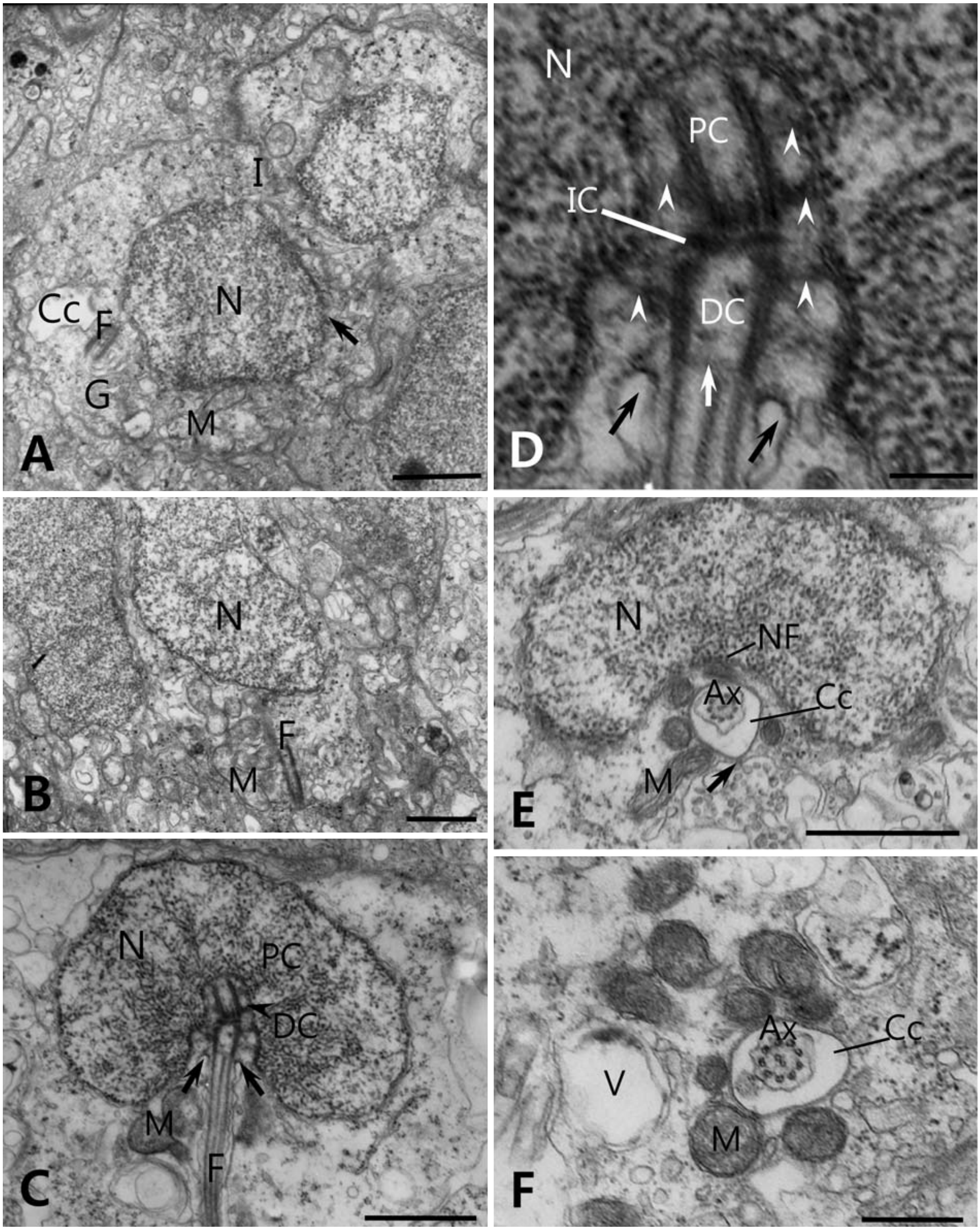

Fig. 1. Spermiogenesis of *Pseudobagrus fluvidraco* spermatid. A: The spermatid start to discard excess nuclear material (arrow). The centriole provide to basal portion of flagellum. bar=1 µm. B: Differentition zone showing the elongation of flagellum. bar=1 µm. C: Longitudinal section of a spermatid in a stage invagination of nuclear fossa include two centrioles. Note the elongated flagellum and cytoplasmic canal (arrows). bar=1 µm. D: Longitudinal section of spermatid showing the nuclear fossa include centriolar complex with satellite fibers (arrowheads). bar=0.2 µm. E. Transverse section of spermatid showing the axoneme and cytoplasmic canal. bar=1 µm. F: Cross section of digital part of spermatid showing the several mitochondria surround to axoneme. bar=0.5 µm. Ax: axoneme, Cc: Cytoplasmic canal, DC: digital centriole, G: Golgi's apparatus, F: flagellum, I: intercellural bridge, M: mitochondria, N: nucleus, NF: nuclear fossa, PC: proximal centriole, V: vesicle.

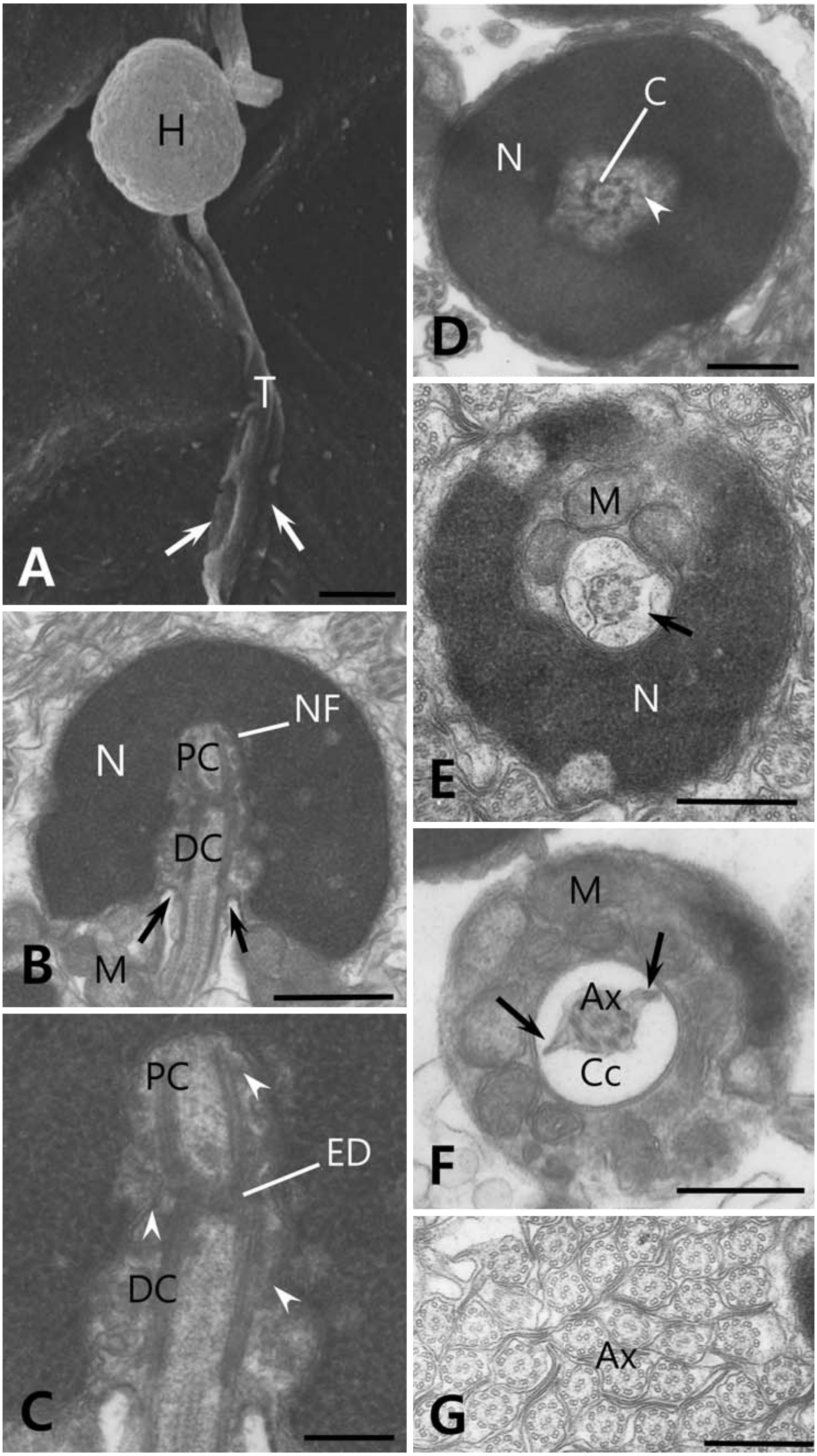

Fig. 2. Spermatozoa of *Pseudobagrus fluvidraco*. A: Scanning electrion micrograph of a spermatozoon showing a spherical head and long tail possess lateral fins (arrows). bar=1 µm. B: Longitudinal section through a head of spermatozoon showing the nucleus, two centrioles and cytoplasmic canal. bar=0.5 µm. C: Enlargement of nuclear fossa shownig deeply invaginate fossa containing two centriole. bar=0.2 µm. D: Cross-section of nucleus showing the centriole connect to nuclear membrane by satellite fibers (arrowhead). bar=0.5 µm. E: Cross-section of distal portion of nucleus showing the nucleus, the mitochondria, cytoplasmic canal and axoneme. bar=0.5 µm. F: Transverse section of midpiece showing the several mitochondria, cytoplasmic canal and axoneme possess lateral fins. bar=0.5 µm. G. Cross-section of flagellum showing the 9+2 doublet structure and lateral fins. bar=0.5 µm. Ax: axoneme, C: centriole, DC: digital centriole, ED: electron-dense material, H: head, M: mitochondria, N: nucleus, NF: nuclear fossa, PC: proximal centriole, T: tail.

with the nucleus condensation and the flagellum formation from distal centriole (Fig. 1A). The nucleus shows diffuse choromatin and has a circular outline (Fig. 1A). The cytoplasm connected by intercelluar bridge and contains some vesicles (Fig. 1A, 1B). The mitochondria are rounded to elongate, with strict membrane and an electron dense matrix (Fig. 1B, 1C). The centriolar complex lies lateral to the nucleus forming the the flagellum (Fig. 1B). The proximal centriole slightly oblique to the distal centriole (Fig. 1C, 1D). When the nucleus starts to rotate, a depression is formed in the nuclear fossa (Fig. 1C-1E). The distal centriole, that is anchored by radial fibrils and differentiated into the basal body, remains associated with the plasma membrane and nuclear enveloper (Fig. 1D). Two centriole themselve were fastened to the nuclear envelope by microfibrils and interconnected each other (Fig. 1D). The centriolar complex penetrates into the nuclear fossa. The nuclear fossa is relatively deep and counterly positioned (Fig. 1C). The cytoplasmic mass moves around the tail and gives rise the midpiece of the future spermatozoon (Fig. 1C, 1E).

Spermatozoa of *P. fulvidraco* have a head, a midpiece and a flagellum. The head exhibits no acrosomal vesicle (Fig. 2A). It contains a spherical nucleus, compactly dense homogenerous chromatin material, 1.7 μm in diameter, surrounded by a marrow strip of cytoplasm with no organelles in anterior part of nucleus (Fig. 2B). Posterior part of nucleus, deeply invaginated nuclear fossa is approximately 1.2 μm, contains the two centrioles toward nuclear enveloper connecting the nuclear foss (Fig. 2C, 2D). On the transverse section of midpiece, the mitochondria not fused and appear round shape. Numerous mitochondria are placed in an nuclear fossa and a cytoplasm of midpiece (Fig. 2E, 2F). The midpiece cytoplasm is separated from the fagellum by the cytoplasmic canal (Fig. 2F). The flagellum has a typical 9+2 microtubular structure. All length of falgellum is appeared the side fins from the proximal part in nuclear fossa to distal part of end piece (Fig. 2E, 2F)

DISCUSSION

External fertilization teleostei, the flagellum generally laterally develops to the nucleus in the spermatid. According to Mattei (1970), the flagellar axis may be either parallel or perpendicular to the nucleus depending on weather or not rotation occur of nucleus. The rotation of nucleus during spermiogenesis has been observed in *Pseudobagrus fulvidraco*, and apparently occurs in almost other Bagridae (Kim and Lee, 2003) and Siluridae (Kwon and Kim, 2004). When the rotation of the nucleus is incomplete, the nuclear fossa is eccentric and so is the flagellum, which is perpendicular tod the nucleus. The flagellum of *P. fulvidraco* develops lateral side of nucleus. This initial position may change during spermiogenesis depending on nuclear rotation. A spermatozoon developing in this way is termed by Mattei (1970) a Type I sperm. In the nucleus of the spermatozoa of *P. fulvidraco*, the chromatin forms highly condensed granules. The highly condensed chroamtin may also be seen in almost numbers of the family Bagridae (Kim and Lee, 2003) and Cyprinidae (Baccetti *et al.*, 1984; Kim *et al.*, 2008). The previously scattered mitochondria aggregate around the base of the falgellum. The mitochondria migration to the centriolar area to settled at the end of the nucleus. These process were similar to most of the Curimatidae (Quagio-Gassiotto *et al.*, 2003), Blenniidae (Silveira *et al.*, 1990), Cyprinidae (Kim *et al.*, 2008).

In Bagridae, the mature ultrastructure of spermatozoa was similar to that of the other teleostei. The organization of teleostei spermatozoa there exist wide divergences (Mattei, 1991). In general spermatozoa of external fertilizers can be characterised by a varying shape of the head region from spherical to elongated, a small midpiece and a long flagellum (Jamieson, 1991). The divergences in sperm structure are considered to be mainly pylogenetic not to depend on the type of development (Jamieson, 1991). The organization of of spermatozoa of *P. fulvidraco* were similar to typical for external fertilizing teleostei like in Bagridae (Kim and Lee, 2000, 2003; Kim *et al.*, 2007). They have no acrosome, and the small midpiece include several mitochondria and a long flagellum separated from midpiece by cytoplasmic canal. The short midpiece contains numerous mitochondria as in most Sillufiromes (Lee and Kim, 1999; Kim and Lee, 2003; Kim *et al.*, 2007). The presence of dinein arms and radial spokes with no particular morphological changes also suggest normal flagellar movement. The lateral fins of flagellum are appear in Bagridae (Kim and Lee, 2003; Kim *et al.*, 2007) and Amblycipitidae (Lee and Kim, 1999) except Siluridae (Lee and Kim, 2001) and Pimelodidae (Santos *et al.*, 2001) among the Siluriformes. It is common in the teleosts, absent in Siuridae (Lee and Kim, 2001) and Cyprinidae (Baccetti *et al.*, 1984; Kim *et al.*, 2008) are an apomorphic loss (Jamieson, 1991). Afzelius (1982) reported that sperm flagellar ultrastructure is great diversity according species which has many way in the motility patterns.

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한국산 동자개(*Pseudobagrus fulvidraco*)의 정세포변형과정과 성숙한 정자의 미세해부학적 구조(Siluriformes: Bagridae)

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요 약 : 한국산 동자개 *Pseudobagrus fulvidraco*의 정세포변형과 성숙한 정자의 미세구조를 주사 및 투과전자현미경으로 관찰하였다. 정세포변형은 측면에서 발생되는 편모, 핵의 회전, 염색물질로 채워진 핵의 함입과 핵와의 형성이 특징적이었다. 성숙한 정자의 구조는 첨체가 없는 둥근두부와 짧은중편 그리고 긴 편모로 구성되어 있었다. 짧은 중편에는 다수의 미토콘드리아가 있으며 cytoplasmic canal에 의하여 편모와 분리되어 있었다. 편모는 전형적인 9+2 구조이며 axonemal fins를 가지고 있었다. Axonemal fins는 동자개과에서 공통적으로 나타나는 형질로 여겨지며 그 외 다른 특징을 경골어류에서 비교하였다.

찾아보기 낱말 : 정자, 정세포변형, 동자개과, 동자개