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Spermatid Differentiations During Spermiogenesis and Mature Sperm Ultrastructure in Male *Crassostrea nipponica* (Seki, 1934, Pteroirmorphia: Ostreidae)

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ABSTRACT

Spermatid differentiations during spermiogenesis and mature sperm ultrastructure in male *Crassostrea nipponica* were investigated by transmission electron microscope observations. The morphology of the spermatozoon of this species has a primitive type and is similar to those of other bivalves. Mature spermatozoa consist of broad, cap-shaped acrosomal vesicle and an axial rod in subacrosomal materials on an oval nucleus showing deeply invaginated anteriorly, two triplet substructure centrioles surrounded by four spherical mitochondria, and satelite fibres, which appear near the distal centriole. The acrosomal vesicle of spermatozoa of *C. nipponica* resemble to those of other investigated ostreids. Especially, two transverse bands (stripes) appear at the anterior region of the acrosomal vesicle, unlikely 2-3 transverse bands (stripes) in *C. gigas*. It is assumed that differences in this acrosomal substructure are associated with the inability of fertilization between the genus *Crassostrea* and other genus species in Ostreidae. Therefore, we can use sperm morphology in the resolution of taxonomic relationships within the Ostreidea. The sperm is approximately 48-50 μm in length including an oval sperm nucleus (about 1.0 μm in length and 1.41 μm in width), an acrosome (about 0.48 μm in length and 0.30 in width) and tail flagellum (46-48 μm). The axoneme of the sperm tail flagellum consists of nine pairs of microtubules at the periphery and a pair at the center. The axoneme of the sperm tail shows a 9 + 2 structure. These morphological charateristics of acrosomal vesicle belong to the family Ostreidae in the subclass Pteriomorphia.

Key words: *Crassostrea nipponica*, spermiogenesis, germ cell, sperm ultrastructure

INTRODUCTION

In Korea, there are ten oyster species in Ostreidae in the subclass Pteriomorphia. In general, oysters in Ostreidae comprise one of the more taxonomically perplexing groups of bivalve molluscs, in particular, at the species level (Healy and Lester, 1991). Recently, the utrastructure of the testis, spermatogenesis and mature sperm morphology have been decribed in Ostreidae species of bivalve molluscs using both light and electron microscopy (Galtsoff and Philpott, 1960; Daniels *et al*., 1971; Popham, 1974, 1979; Healy and Lester, 1991; Sousa and Oliveria, 1994; Eckelbarger and Davis, 1996; Kim, 2001; Kim *et al*., 2010). To date, sperm ultrastructure has been used as a tool in assessing taxonomic problems and phylogenetic relationships in the Metazoa through the use of spermiocladistic analysis (Jamiesen, 1987, 1991). In general, it is well-known that bivalve molluscs possess a sperm that is primitive in form (Frazen, 1956), a characteristic of many Metazoa, which discharge sperm directly into the water (Franzen, 1977). Within

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the bivalves there is little variation in the fine structures of the tail and the midpiece but great variation in the form of the nucleus and particularly the acrosome (Popham, 1979). Sperm morphology has been used successfully as an aid in the examination of the phylogeny of bivalvia (Popham, 1979; Bernard and Hodgson, 1985, Healy, 1985; 1989a, b; Healy and Lester, 1991). Daniels *et al.* (1971) suggested that comparative ultrastructure of oyster spermatozoa might also prove taxonomically useful, provided that morphological differences between taxa could be found. However, although there are some information relating to the ultrastructure of oyster sperm (Daniels *et al.*, 1971; Popham, 1974, 1979; Popham *et al.*, 1974; Bernard and Hodgson, 1985). No ultrastructural study of spermatogenesis has been reported on the Korean oyster, *C. nipponica*. And also little information is available on the ultrastructure of germ cells during spermiogenesis and morphological characteristics of mature spermatozoa associated with taxonomic problems and phylogenetic relationships of this species.

Therefore, it is very important to clarify some differences by Ostreidae species in ultrastructures of mature spermatozoa such as the nucleus, acrosomal vesicle, the number of mitochondria, appearance of an axial rod and satellite fibres. The pupose of the present study is to describe and clarify the some charateristics in ultrastructures of spermiogenesis in oyster, *C. nipponica*. then discuss the results on comparative sperm data and mature sperm ultrastructures within Ostreidae and other families in Pteriormorphia.

MATERIALS AND METHODS

1. Sampling

Specimens of *Crassostrea nipponica* were collected monthly in the subtidal zone of Oryukdo, Busan, Korea, for one year from January to December, 2007. A total of 158 male individuals were used for transmission electron microscope observations.

2. Transmission electron microscope observation

For transmission electron microscope observations, excised pieces of the gonads were cut into small pieces and fixed immediately in 2.5% paraformaldehydeglutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 2 hours at 4°C. After prefixation, the specimens were washed several times in the buffer solution and then postfixed in a 1% osmium tetroxide solution in 0.2 M phosphate buffer (pH 7.4) for 1hour at 4°C. Specimens then were dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in an Epon-Araldite mixture. Ultrathin sections of Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 microtome and LKB ultramicrotome at a thickness of about 80 - 100 nm. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrate, and observed with a JEM 100 CX-II (80-KV) electron microscope.

RESULTS

1. Ultrastructure of germ cells during spermiogenesis

In general, the process of spermiogenesis appear to be similar to those of other bivalve species.

Spermatids : The secondary spermatocyte is transformed into the spermatids by the secondary meiotic division. For convenience, spermiogenesis, which occur in the spermatids, has been divided arbitrarily into two stages: the early and late stages.

In the early stage of spermiogenesis, spermatids are approximately 3.5-4.0 μm diameter, the nucleus is spherical and occupies the center of the cell. Nuclei of spermatids (about 2.8-3.0 μm diameter) contain electron-dense heterochromatin materials. During spermiogenesis, the morphology of the spermatid nucleus changes gradually during the differentiation of the spermatid. After all, the morphologies of the spermatid nuclei are laterally wided, and one or a few granules which are formed by the Golgi complex in the cytoplasm of the spermatid form a proacrosomal vesicle (Fig. 1A). The nuclei of spermatids are about 2.5 μm diameter, a proacrosomal vesicle migrates to the presumptive anterior end of the spermatid, where they coalesce to form a single electron-dense acrosomal vesicle. A single acrosomal vesicle locates at

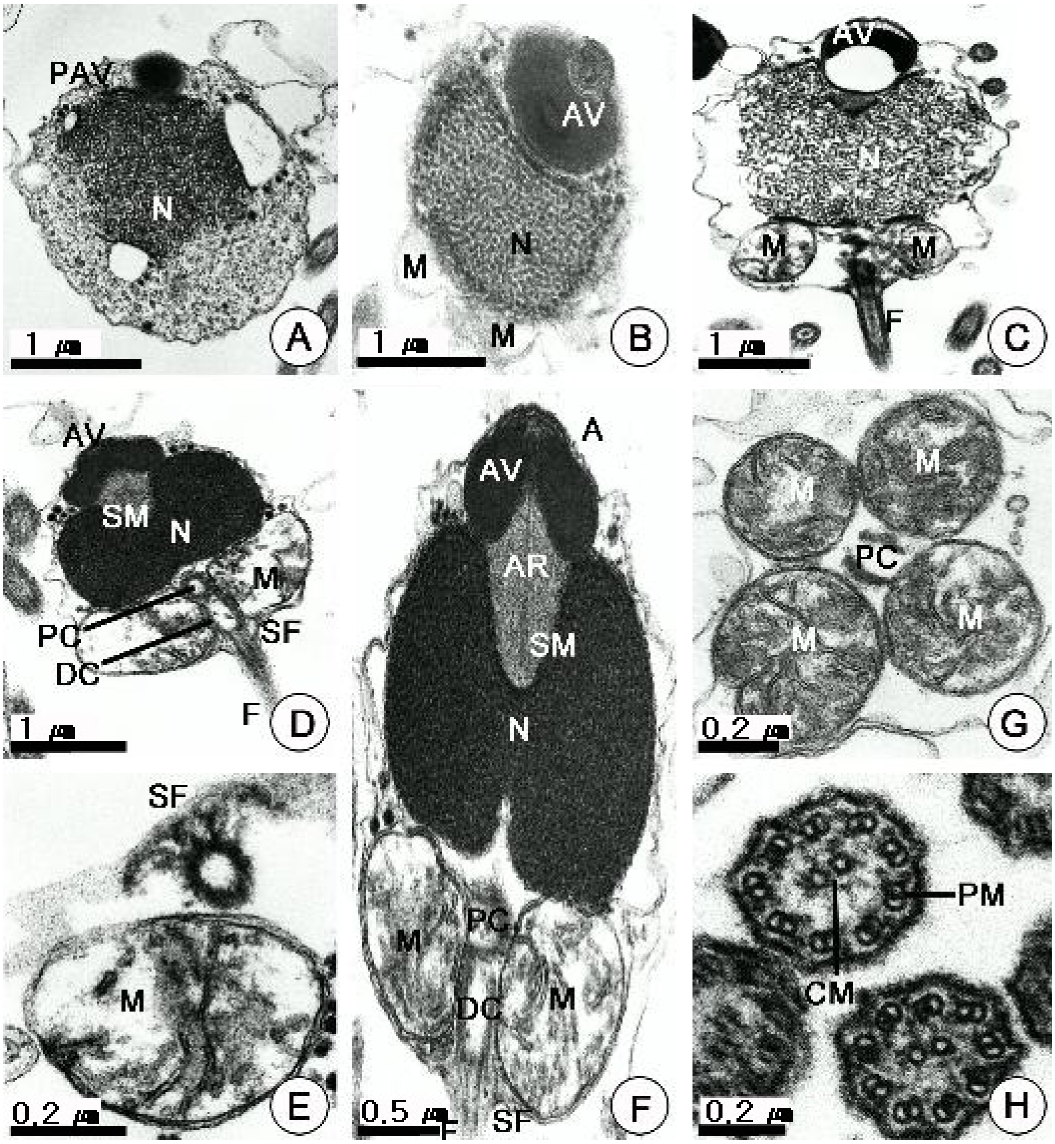

Fig. 1. Transmission electron micrographs of spermiogenesis and mature spermatozoon in male *Crassostrea nipponica*. **A**, A spermatid in the early stage of spermiogesis. Note the proacrosomal vesicle (PAV) just before the nucleus (N). **B**, **C**, The processes of the formation of the acrosomal vesicle of spermatids. Note morphological changes in acrosomal vesicles (AV) on the nucleus (N) and two spherical mitochondria (M) in the midpiece of sperm. **D**. In the late stage of spermiogenesis, formations of acrosomal vesicle (AV), the midpiece of sperm and a flagellum. Note subacrosomal materials (SM) in the acrosomal vesicle (AV) on the nucleus (N) and the proximal centriole (PC), distal centriole (DC) surrounded with spherical mitochondria (M) and a flagellum (F). **E**, A spermatid in the late stage of spermiogenesis. Note appearance of a satelite fibrer (SF) near the distal centriole and spherical mitochondria (M). **F**, A mature spermatozoon with an acrosome, which is composed of acrosomal vesicle, an axial rod (AR) in the subacrosomal materials (SM) on the nucleus (N) and the proximal centriole (PC) and distal centrioles (DC) surrounded with mitochondria (M) near the satelite fibre (SF) in the sperm midpiece and a flagellum (F). **G**, Cross sectioned sperm midpiece. Note the proximal centriole (PC) surrounded with four spherical mitochondria (M). **H**, Cross sectioned flagella of sperms. Note an axoneme showing a 9 + 2 structure containing a pair of single doublet central microtubles (CM) and nine pairs of of peripheral microtubles (PM).

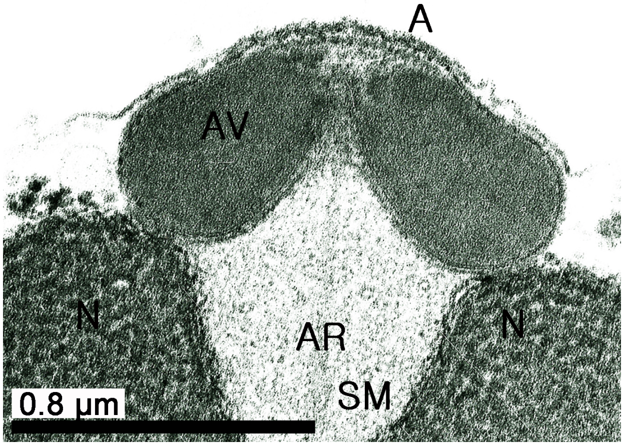

Fig. 2. A transmission electron micrograph of the acrosomal vesicle of a spermatozoon in male *Crassostrea nipponica*. Note an acrosomal vesicle ultrasructure of the spermatozoon showing two transverse bands (stripes) on the anterior region between two basal rings, and an axial rod (AR) in subacrosomal materials (SM) in the anterior nuclear invagination on the sperm nucleus (N).

the presumptive anterior pole of the spermatids. In the late stage of spermiogenesis, the acrosomal vesicle is initially oval in shape (Fig. 1B) but gradually assumes a cap-like form with a slightly pointed anterior prominence and a sharply invaginated posterior face. Two components of the acrosomal vesicle can be recognized (Fig. 1C): the acrosomal vesicle and an extensive deposit of subacrosomal (extravesicular) materials. The processes of acrosomal vesicle formation are very complex. The acrosomal vesicle is membrane bound, consequently, become cap-shaped form by way of various morphorphological changes (Fig. 1D). The satellite fibres appear near the distal centriole and spherical mitochondria (Fig. 1E).

Spermatozoa : The morphology of the spermatozoon has a primitive type. The sperm is approximately 48-50 μm long including an oval sperm nucleus and tail flagellum (about 46-48 μm long). The sperm nucleus is oval in shape, and the acrosome is modified cap in shape. An acrosome (0.48 μm long and 0.30 μm width) on the nucleus is composed of the acrosomal vesicle (being composed of the cap-shaped basal rings) and subacrosomal materials which comprises an axial rod embedded in a coasely granular matrix. An acrosomal vesicle shows high electron dense opaque at the acrosomal membrane, In particular, two transverse bands (stripes) are present in the anterior region of acrosomal vesicle (Fig. 2), as seen in *C. gigas* of the subclass Pteriomorphia.

Anteriorly oval nucleus (1.00 μm long and 1.41 μm width) is deeply invaginated, and then the space is occupied by subacrosomal materials. Posteriorly, the nucleus shows only a weak indentation into which the centrioles appear. The nuclear contents are highly electron dense and granular in texture. In the cross sectioned acrosomal vesicle, an axial rod is present in the subacrosomal granular materials. Posterior to the nucleus is the midpiece. This region consists of four spherical mitochondria surrounding a pair of triplet substructure centrioles (Fig. 1G). The cristae of each mitochondrion are randomly arranged, and the proximal centriole lies at 90° to the sperm longitudinal axis or the distal centriole near the basal invagination of the nucleus. The distal centrile lies parallel to the sperm longitudinal axis and forms the point of origin of flagellar axoneme. The flagellum is composed of a 9 + 2 substructure axoneme enclosed by the plasma membrane and measures approximately 46-48 μm long: that is, nine peripheral doublets (microtubules) surrounding a central pair of sigle microtubles (Fig. 1H).

DISCUSSION

1. Ultrastructural comparisons of Ostreidae species

Early investigations of bivalve sperm ultrastructure demonstrated the taxonomic value of comparative studies (Franzen, 1956, 1977; Galtsoff and Phillpott, 1960; Popham, 1979), and such studies are now widely used in taxonomic anlyses (Hodgson and Bernard, 1983, 1986; Healy, 1988, 1995, 1996). The primitive sperm show sufficient structural variability that they are useful in taxonomic studies.

In this study, as seen in the morphology of spermatozoon of *C. gigas* (Kim *et al.*, 2010), mature spermatozoa of *C. nipponica* consist of broad, cap-shaped acrosomal vesicle, subacrosomal material (containing axial rod embedded in a granular matrix), a oval nucleus showing deeply invaginated anteriorly, two triplet substructure centrioles surrounded by four spherical mitochondria, and satelite fibres appear near the distal centriole and plasma membrane.

Results of the present study support the sugestion of Daniels *et al.* (1971) that sperm ultrastructure may be of taxonomic use in the Ostreidae. In general, morphology of ostreid sperm shows broad, cap-shaped acrosome, extensive subacrosomal deposit (an axial rod), round nucleus, four pericentriolar mitochondria (Healy and Lester, 1991; Kim *et al.*, 2010). Therefore, compared with most structures and morphologies, spermatozoa of *C. nipponica* closely resemble those of other investigated ostreids (Galtsoff and Philpott, 1960; Daniels *et al.*, 1971; Osanai and Kyozuka, 1982; Healy and Lester, 1991).

In this study, the acrosomal contents have a relatively uniform electron density. In case of Ostreidae species unlikely other family of bivales, the nucleus of the spermatid become wider than long, and a finely granular subacrosomal materials are present in the anterior invagination region of the nucleus. It is one of special characteristics of spermatozoa of Ostreidae species.

Regarding a central filamentous axial rod, in this study, the subacrosomal matterial contains a central filamentous axial rod composed of anterio-posterior-oriented filaments as seen in the family Ostreidae species of the subclass Pteriomorphia.

Hodgson and Bernard (1986) and Healy (1989) stated that different subclasses of bivalves each have unique acrosomal morphologies, and the number of mitochondria in the sperm midpiece tends to be stale within any family or superfamily varying from a maximum of 14 in the mytilord *Modiolus difficilis* (Drozdov and Reunov, 1986) to a minimum of 4 (common to many bivalve families, Healy, 1989, 1995).

In this study, the number of mitochondria at the midpiece of the spermatozoon are four, and satellite fibres are found, as seen in *C. gigas*. Judging from the resuts on the ultrastructure of mature spermatozoon, it is supposed that this species belongs to family Ostreidae in the subclass Pteriomorphia because the spermatozoon of this specis has special structural charateristics of the acrosomal vesicle containing an axial rod and satellite fibres.

The taxonomic value of sperm morpholgy at the level of species or genus in the Ostreidae will be determined only through a broad, comparative study. In this study, two transverse banding (stripes) appeared in *C. nipponica* (Fig. 2), and two to three transverse banding appeared at the anterior region of the acrosomal vesicle in *C. gigas* (Kim *et al.*, 2010). However, Healy and Lester (1991) reported that three to four horizontal bandings appeared at the anterior region of the acrosomal vesicle in S. commecials may perhaps be limited to this species-which would in itself, be a significant finding-or prove to be a diagnostic feature of the genus Saccostrea.

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REFERENCES

Bernard, R.T.F. and Hodgson, A.N. (1985) Fine structure of the sperm and spermatid differentiation in the brown mussel *Perna perna*. *South African Journal of Zoology,* **20**: 5-9.

Daniels, E.W., Longwell, A.C., McNiff, J.M. and Wolfgang, R.W. (1971) A re-investigation of the ultrastructure of the spermatozoa fromthe american oyster *Crassostrea virginica*. *Transection American Microscope Society*, **90**: 275-282.

Drozdov, T.A. and Reunov, A.A. (1986) Spermatogenesis and the sperm ultrastructure in the mussel *Modiolus difficillis*. *Tsitologiia*, **28**: 1069-1074.

Eckelbarger, K.J. and Davis, C.V. (1996) Ultrastructure of the gonad and gametogenesis in the eastern oyster, *Crassostrea virginica*. II. Testis and spermatogenesis. *Marine Biology*, **127**: 89-96.

Franzen, A. (1956) On spermatogenesis. Morphology of the spermatozoon, and biology of fertilization among invertebrates. *Zoology Bidrung Uppsala*, **31**: 355-482.

Galtsoff, F.S. and Phillpott, D.E. (1960) ultrastructure of the spermatozoon of the oyster, *Crassostrea virginica*. *Journal of Ultrastructural Research*, **3**: 241-253.

Healy, J.M. (1983) Ultrastructure of euspermatozoa of cerithiacean gastropods (Prosobranchia: Mesogastropoda). *Journal of Morphology*, **178**: 57-75.

Healy, J.M. (1988) Sperm morphology and its systematic importanc in the Gastropoda. ***In:*** Ponder W.F. (ed) Prosobranch phylogeny. *Malacological Review*, **4**:

251-266.

Healy, J.M. (1989) Spermiogenesis and spermatozoa in the relict bivalve genus Neotrigonia: relevance to trigonioid relationships, particularly Unionoidea. *Marine Biology*, **103**: 75-85.

Healy, J.M. and Lester, R.J.G. (1991) Spermultrastructure in the Australian oyster *Saccostrea commercialis* (Iredale & Roughey) (Bivalvia: Ostreoidea). *Journal of Molluscan Studies*, 57: 219-224.

Healy, J.M. (1995) Sperm ultrastructure in the marine bivalve families Carditidae and Crassatellidae and its bearing on unification of the Crasssatelloidea with the Carditoidea. *Zoological Science*, **24**: 21-28.

Healy, J.M. (1996) *Molluscan sperm* ultrastructure: correlation with taxonomic units within the *Gastropoda*, *Cephalopoda* and *Bivalvia*. ***In:*** Taylor J (ed) Origin and evolutionary radiation of the Mollusca. Oxford University Press, London p. 99-113.

Hodgson, A.N. and Bernard, R.T.F. (1986) Ultrastructure of the sperm and spermatogenesis of three species of *Mytilidae* (Mollusca, Bivalvia). *Gamete Research,* **15**: 123-135.

Jamieson, B.G.M. (1987) The ultrastructure and phylogeny of insect spermatozoa. Cambridge University Press, Cambridge.

Jamieson, B.G.M. (1991) Fish evolution and systematics: evidence from spermatozoa. Cambridge University Press, Cambridge. pp. 181-194.

Kim, J.H. (2001) Spermatogenesis and comparative ultrastructure of spermatozoa in several species of Korean economic bivalves (13 families, 34 species). Ph. D. thesis, Pukyung National University 161 pp.

Kim, J.H., Chung, E.Y., Choi, K.H., Lee, K.Y. and Choi, M.S. (2010) Ultrastructure of the testis and germ cell deve3lopment during spermatogenesis in male *Crassostrea gigas* (Bivalvia: Ostreidae) in western Korea. *Korean Journal of Malacology,* **26**: 235-244.

Osanai, K. and Kyozuka, K. (1982) Cross fertilization between sea urchin eggs and oysterspermatozoa. *Gamete Research*, **5**: 49-60.

Popham, J.D. (1974) Comparative morphometrics of the acrosomes of the sperms of externally and internally fertilizing sperms of the sperms of the shipworms (Teredinidae, Bivalvia, Mollusca). *Cell Tissue Research*, **150**: 291-297.

Popham, J.D. (1979) Comparative spermatozoon morphology and bivalve phylogeny. *Malacological Review*, **12**: 1-20.

Sousa, M. and Oliveria. E. (1994) An ultrastructural study of *Crassostrea angulat*a (Mollusca, Bivalvia) spermatogenesis. *Marine Biology*, **120**: 41-47.