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Ultrastrucure of Germ Cells during Spermatogenesis and Some Characteristics of Sperm Morphology in Male *Mytilus coruscus* (Bivalvia: Mytilidae) on the West Coast of Korea

Jin Hee Kim[1], Ee-Yung Chung[2], Ki-Ho Choi[3], Kwan Ha Park[4], and Sung-Woo Park[4]

[1]Korea Inter-University Unstitute of Ocean Science, Pukyong National University, Busan 608-737, Korea
[2]Korea Marine Environment & Ecosystem Institute, Dive Korea, Bucheon 420-857, Korea
[3]West Sea Fisheries Research Institute, National Fisheries Research and Development Institute, Incheon 400-420, Korea
[4]Department of Aquatic Life Medicine, Kunsan National University, Kunsan 573-701, Korea

ABSTRACT

The ultrastructure of germ cells during spermatogenesis and some characteristics of sperm morphology in male *Mytilus coruscus*, which was collected on the coastal waters of Gyeokpo in western Korea, were investigated by transmission electron microscope observations. The morphology of the spermatozoon has a primitive type and is similar to those of other bivalves in that it contains a short midpiece with five mitochondria surrounding the centrioles. The morphologies of the sperm nucleus type and the acrosome shape of this species have an oval and modified cone shape, respectively. In particular, the axial rod is observed between the nucleus and acrosome of the sperm. The spermatozoon is approximately 45-50 μm in length including a sperm nucleus (about 1.46 μm in length), an acrosome (about 3.94 μm in length) and tail flagellum (approximately 40-45 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. Some special charateristics of sperm morphology of this species in the genus *Mytilus* are (1) acrosomal morphology, (2) the number of mitochondria in the midpiece of the sperm, and (3) the existence of a satellite. The axial rod appears in the acrosome and sperm nucleus as one of the characteristics seen in several species of the subclass Pteriomorphia, unlikely the subclass Heterodonta containing axial filament instead of the axial rod. The number of mitochondria in the midpiece of the sperm of this species in the family Mytilidae are five, as one of common characteristics appeared in most species in the family Mytilidae. Most of *Mytilus* species contain a satellite body which is attached to the proximal centriole in the middle piece of the sperm, as one of common characteristics of sperm morphology in the family Mytilidae.

Key words: *Mytilus coruscus*, spermatogenesis, germ cell, sperm morphology

Introduction

Recently, sperm morphology during spermatogenesis has been investigated in many species of bivalve molluscs using light and electron microscopy (Gaulejac *et al*., 1995; Chung and Ryou, 2000; Chung *et al*., 1991, 1999, 2005, 2006, 2007). Variations in the morphology of spermatozoa appear to be well correlated with the evolution of a number of bivalve group and the phylogenetic relatioship (Bacetti, 1970; Popham, 1979). And it is well-known that the ultrastructure of the spermatozoon in the bivalves might be related to the systematics of bivalves (Popham *et al*., 1974). For that reason, sperm ultrastructure has been viewed a valuable tool in assessing taxonomic and phylogenetic problems within the bivalvia (Franzén, 1970, 1983; Daniels *et al*., 1971; Popham, 1979; Healy, 1983, 1988, 1995, 1996;

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Corresponding author: Chung, Ee-Yung
Tel: +82 (32) 328-5145 e-mail: eychung@kunsan.ac.kr
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Koike, 1985; Hodgson and Bernard, 1986; Eckelbarger *et al*., 1990; Eckelbarger and Davis, 1996), and phylogenetic relationship in metazoa through the use of spermiocladistic analysis has been studied (Jamieson, 1987, 1991). Therefore, it is very important to study that sperm ultrastructures of bivalves are now widely used in taxonomic analyses (Healy, 1995).

Previoyusly, there have been some studies on Mytilidae species, especially, *Mytilus edulis* on aspects of spermatogenesis, including the fine structure of spermatid differentiation (Longo and Dornfield, 1967), spermatozoon morphology and bivalve phylogeny (Popham, 1979), ultrastructure of sperm and spermatogenesis (Hodgson and Bernard, 1986) and gametogenesis (Pipe, 1987). However, no ultrastructural study of spermatogenesis has been reported on *M. coruscus*. Little information is available on ultrastructure of germ cell development during spermatogenesis and sperm morphology associated with bivalve phylogeny of this species.

Of sperm ultrastructures, in particular, the acrosome of the sperm shows extensive morphological diversity in the bivalve sperm, and hence it may be the most useful structure in assessing phylogenetic relations (Franzén, 1956). However, as previously noted by Bacetti (1970), the evolution of the acrosome should be considered in relation to the evolution of the egg rather than just in relation to systematics of the fauna being investigated. Recently, the acrosomal morphology of the sperm has been used to organize bivalve subclasses (Popham, 1979). In association with the acrosomal morphology, Healy (1989) reported that different subclasses of bivalves each have unique acrosomal morphologies. Therefore, the acrosomal morphology of the sperm in *M. coruscus* should be compared with other species of the subclass Pteriomorphia because *M. coruscus* belongs to the subclass Pteriomorphia.

Although the morphologies of sperm nuclei in many species of the family Mytilidae are similar, there are some differences in the morphologies of the sperm nuclei in the family Mytilidae. Therefore, the morphologies of sperm nuclei can not be a valuable tool in assessing taxonomic and phylogenetic problems within the bivalvia because the morphologies of the sperm nuclei vary with the genus names in the same family.

In addition, the number of mitochondria in the sperm midpiece tends to be stable within any given family or superfamily varying from a maximum of 14 in the mytiloid *Modiotus difficilis* (Dorozdov and Reunov, 1986) to a minimum of 4(common to many bivalve families) (Healy, 1989, 1995). Therefore, the number of mitochondria in sperm midpiece of this species should be investigated and compared with the same family Mytilidae. Beside ultrastructures of germ cells during spermatogenesis, sperm morphology should be studied to clarify ultrastructural characteristics in detail.

If some characteristics obtained from spermatogenesis and sperm ultrastructure, the results of the ultrastructural studies on bivalve spermatozoa will provide information needed for the elucidation of relationship patterns among several subclasses (Popham, 1979). Therefore, the purpose of the present study is to describe the ultrastructures of germ cells during spermatogenesis, and to confirm sperm type with sperm ultrastructure by phylogenetic analysis of *Mytilus coruscus*.

MATERIALS AND METHODS

1. Sampling

Specimens of *Mytilus coruscus* were collected monthly in the subtidal zone of Gyeokpo, Korea, for two years from January to December, 2006 (Fig. 1). A total of 127 male clams ranging from 95.6 mm to 104.7 mm in shell length were used for the studies of oogenesis by electron microscopic observations.

2. Ultrasructure of germ cells and sperm morphology

For electron microscopical observations, excised pieces of the gonads were cut into small pieces and fixed immediately in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 2 hours at 4℃. 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

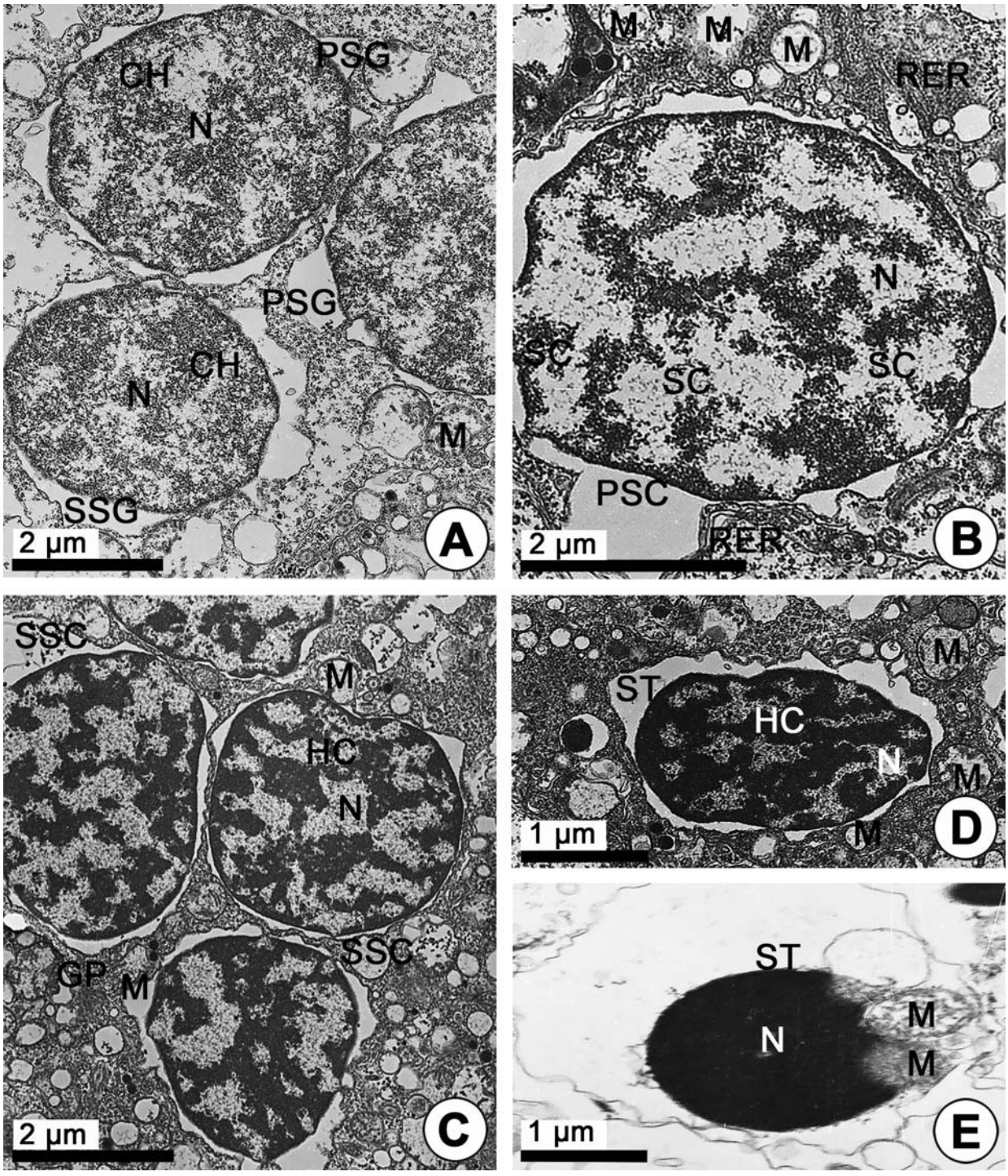

Fig. 1. Electron micrographs of spermatogenesis in male *Mytilus coruscus*. A, The primary spermatogonia (PSG), the secondary spermatogonia (SSG). Note chromatins (CH) in the nucleus (N) and the mitochondria (M) in the cytoplasm. B, The primary spermatocyte (PSC). Note several synaptonemal complexes (SC) in the nucleus during the prophase of the primary maturation division and rough endoplasmic reticula (RER). C, The secondary spermatocytes (SSC). Note glycogen particles (GP), and the mitochondria (M) in the cytoplasm. D, Spermatids (ST). Note high electron dense heterochromatin (HC) in the nucleus (N). E. A spermatid (ST) in the early stage of differentiation during spermiogenesis. Note the mitochondria (M) in the cytoplasm.

phosphate buffer (pH 7.4) for 1 hour at 4℃. 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 ace tate followed by lead citrate, and observed with a JEM 100 CX-Ⅱ(80-KV) electron microscope.

Results

1. Structure and morphology of the testis

In general, structure and morphology of the testis of the hard shelled mussel, *M. coruscus* is similar to those of other bivalves. The testis is a diffuse organ consisting of branching acini, and differentiating germ cells in a variety of stages are present in the acini. Spermatogonia are positioned nearest the inner wall of the acinus, spermatocytes and spermatids are located closer to the acinus lumen, and mature sperm are largely confined to the central lumen.

As the testis is getting mature, both sexes can be distinguishable easily by external features because mature ovary is pink in color and testis is milky white in color. Therefore, the sexes of the clams can be easily distinguished by the external colors of the gonads.

2. Ultrastructure of germ cells during spermatogenesis

Based on the testicular development and morphological characteristics of germ cells by electron microscopic observation, spermatogenesis can be classified into four stages: (1) spermatogonia, (2) spermatocytes, (3) spermatids, and (4) spermatozoa.

1) Spermatogonia

The primary spermatogonia: The primary spermatogonia appear along the internal wall of the acini. They propagate through the mitotic division in the acini. The primary spermatogonia are approximately 6-7 μm in diameter, and round or oval in shape. Each of the spermatogonia contains a large nucleus. The nuclei of primary spermatogonia are approximately 4 μm in diameter and contain irregularly scattered chromatin forming a network. At this time, several mitochondria and vacuoles are present in the cytoplasm.

The secondary spermatogonia: The primary spermatogonium divides mitotically into secondary spermatogonia. The sizes of the secondary spermatogonia are smaller than the primary spermatogonia.

The nuclei of secondary spermatogonia contain scattered chromatin forming a network. Especially, several mitochondria and vacuoles are present in the cytoplasm (Fig. 1A).

2) Spermatocytes

The primary spermatocytes: The secondary spermatogonium differentiates into the primary spermatocytes by the mitotic division.

The primary spermatocytes in early prophase have a nucleus similar in shape to that of the spermatogonia. The nucleus (approximately 3.5 μm) of the primary spermatocyte contains higher electron density heterochromatin than that of the secondary spermatogonium. In the prophase during the first maturation division, several synaptonemal complexes appear in the nucleus. In particular, the patchtene stage is characterized by the the presence of synaptonemal complexes in the nucleus. At this time, several mitochondria and several rough endoplasmic reticula appear in the cytoplasm, however, the cytoplasm is reduced, so, the nucleo-cytoplasm ratio increases (Fig. 1B).

Secondary spermatocytes: The primary spermatocytes develop into the secondary spermatocytes by the first maturation division. The secondary spermatocytes are irregular in shape and rage from about 4-5 μm. The secondary spermatocytes contain denser and more highly concentrated heterochromatin materials in the nuclei (approximately 3 μm) than those of the primary spermatocytes. At this time, several mitochondria, rough endoplasmic reticula and a large quantity of glycogen particles are present in the cytoplasm of the

secondary spermatocytes (Fig. 1C).

3) Spermatids

The secondary spermatocytes develope into the spermatids by the secondary meiotic division. For convenience, the differentiation of the spermatids during spermiogenesis has been divided arbitrarily into two stages: the early and late stages of the differentiation of the spermatids.

Before the differentiation of the spermatid, spermatids are slightly oval in shape and range from approximately 2.5 μm in diameter. Their nuclei (approximately 2 μm) contain a scattered electron-dense heterochromatin. And many mitochondria, rough endoplasmic reticula, and glycogen particles appear in the perinuclear region in the cytoplasm of the spermatid (Fig. 1D).

In the early stage of differentiation of the spermatid during spermiogenesis, the cytoplasm is reduced, several mitochondria in the cytoplasm move to the posterior part of the nucleus of the spermatid (Fig. 1E). Acrosome formation of the spermatids during spermiogenesis can be simply divided into three phases based on the characteristics of cell organelle differentiation: the Golgi, acrosomal vesicle, acrosome phases. The morphology of the spermatid changes gradually during the Golgi phase in the differentiation of the spermatid. At the Golgi phase, the Golgi complex and small proacrosomal granules in the cytoplasm move to a position just in front of the nucleus, while the mitochondria move to a position just behind the nucleus (Fig. 2A). A finding of the fact that "the occurrence of the proacrosomal granule is originated from the Golgi complex", is easily confirmed. Thereafter, a proacrosomal granule develops into a proacrosomal vesicle, and then a proacrosomal vesicle is attached to the anterior part of the nucleus (Fig. 2B). At the acrosomal vesicle phase, a proacrosomal vesicle develops into an acrosomal vesicle on the nucleus. At the initial phase of the acrosomal vesicle formation, several large and small vacuoles at the left and right sides of the anterior part of the proacrosomal vesicle are formed in the proacrosomal vesicle and the mid-central line hole is formed in the nuceus (Fig. 2C), Consequently, after several vacuoles are fused, two large vacuoles are formed in an acrosomal vesicle. At the same time, the axial rod in the mid-central line hole is formed in the nucleus, and the centrioles with two spherical mitochondria in the midpiece appear at the posterior part of the nuclear fossa (Fig. 2D).

During the acrosomal phase, first, the basal rings at the left and right side are formed by two vacuoles at the left and right side part of the acrosomal vesicle. At this time, the central part of the acrosomal vesicle is invaginated, and the axial rod is formed between two parts (at the left and right side part) of the acrosomal vesicle. The borders of the nucleus and the acrosomal vesicle become attached. The narrow subacrosomal space between the peripheral parts of the two basal rings of the acrosomal vesicle and the anterior part of the nucleus is filled with subacrosomal materials (Fig. 2E).

In the late stage of differentiation of spermatid (spermiogenesis), especially, the nucleus changes morphologically and a centrosome, which is surrounded with the mitochondria, appear near the posterior nuclear fossa. A mid-central line hole and a few vacuoles are present in the nucleus, In particular, an axial rod is present from the posterior nuclear fossa part to the anterior nuclear fossa parts. In the acrosomal vesicle, an axial rod is present at the upper-central parts of the acrosomal vesicle. In the maturation phase, the acrosomal vesicle elongate forward and become a modified cone-like acrosome. The subacrosomal space between the anterior nuclear fossa and the part of two basal rings is filled with subacrosomal materials (Fig. 3A). At this time, cross-sectioned a, b, c parts of the acrosomes are as follows: 1) a part shows an axial rod (electron lucent part) and the acrosome (electron opaque part); 2) b part shows an axial rod (electron lucent part) and the acrosome (electron opaque part); 3) c part shows an axial rod (electron lucent part), subacrosomal materials (electron lucent part) and the acrosome (electron opaque part) (Figs. 3A, 3B-a, b, c). Subacrosomal granular materials are present in the subacrosomal space between the anterior invaginated

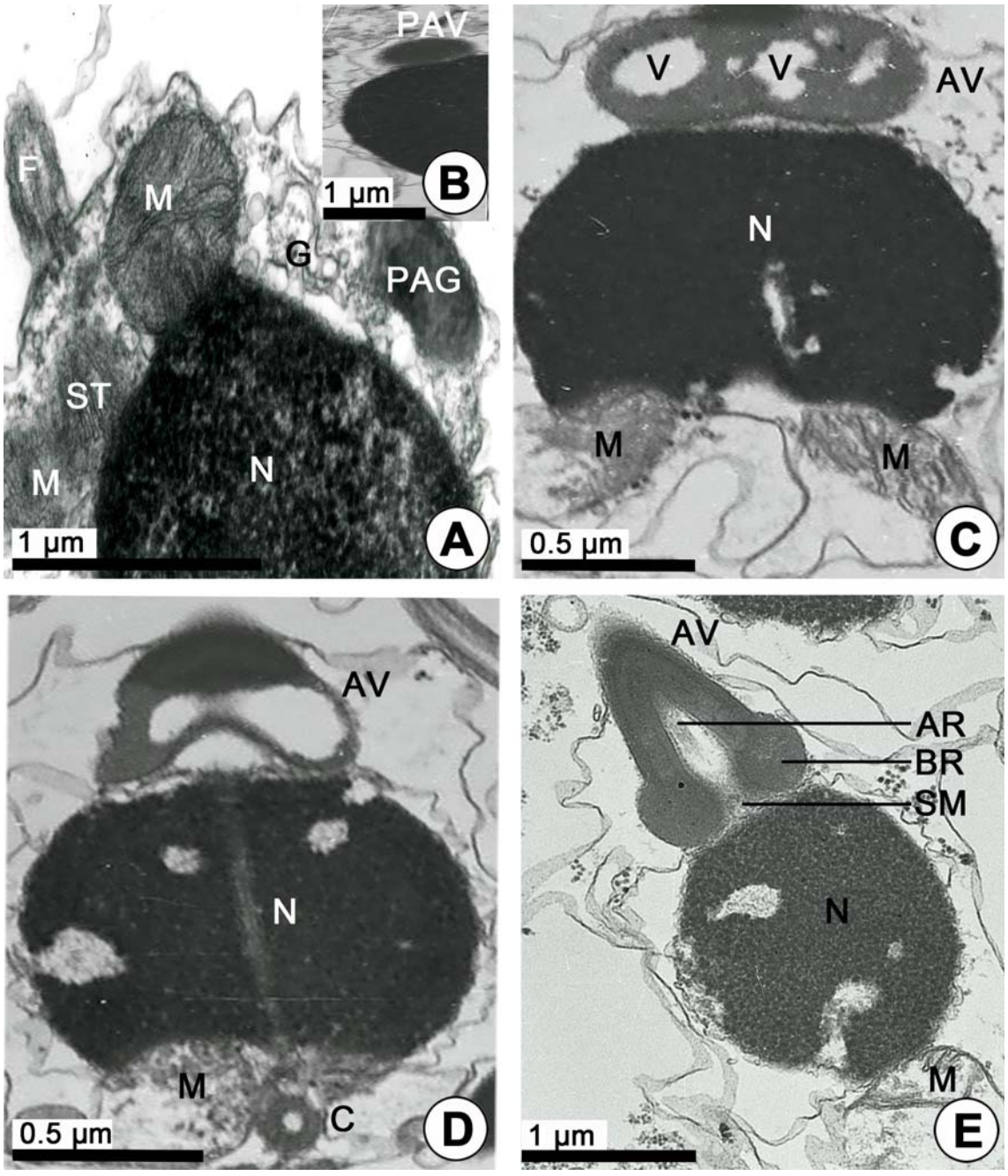

Fig. 2. Electron micrographs of spermiogenesis in male *Mytilus coruscus*. A, A spermatid in the early stage of differentiation. Note the Golgi complex (G) and a proacrosomal granule (PAG) just before the nuceus and spherical mitochondrion (M) beneath the nucleus. B, A spermatid during differentiation. Note a proacrosomal vesicle (PAV) on the nucleus (N). C, A spermatid in the middle stage of differentiation. Note several vacuoles in a proacrosome on the nucleus (N) and two large mitochondria (M) beneath the nucleus. D, A spermatid. Note two large vacuoles at the right and left side of an acrosomal vesicle, the mid-central line hole in the nucleus and the centrosome (C) surrouding with mitochondria (M). E, A spermatid in the late stage of differentiation. Note two basal rings at the right and left sides, the axial rod in the center of an acrosomal vesicle, and subacrosomal materials in the acrosomal space between two basal rings and the anterior part of the nuclear fossa.

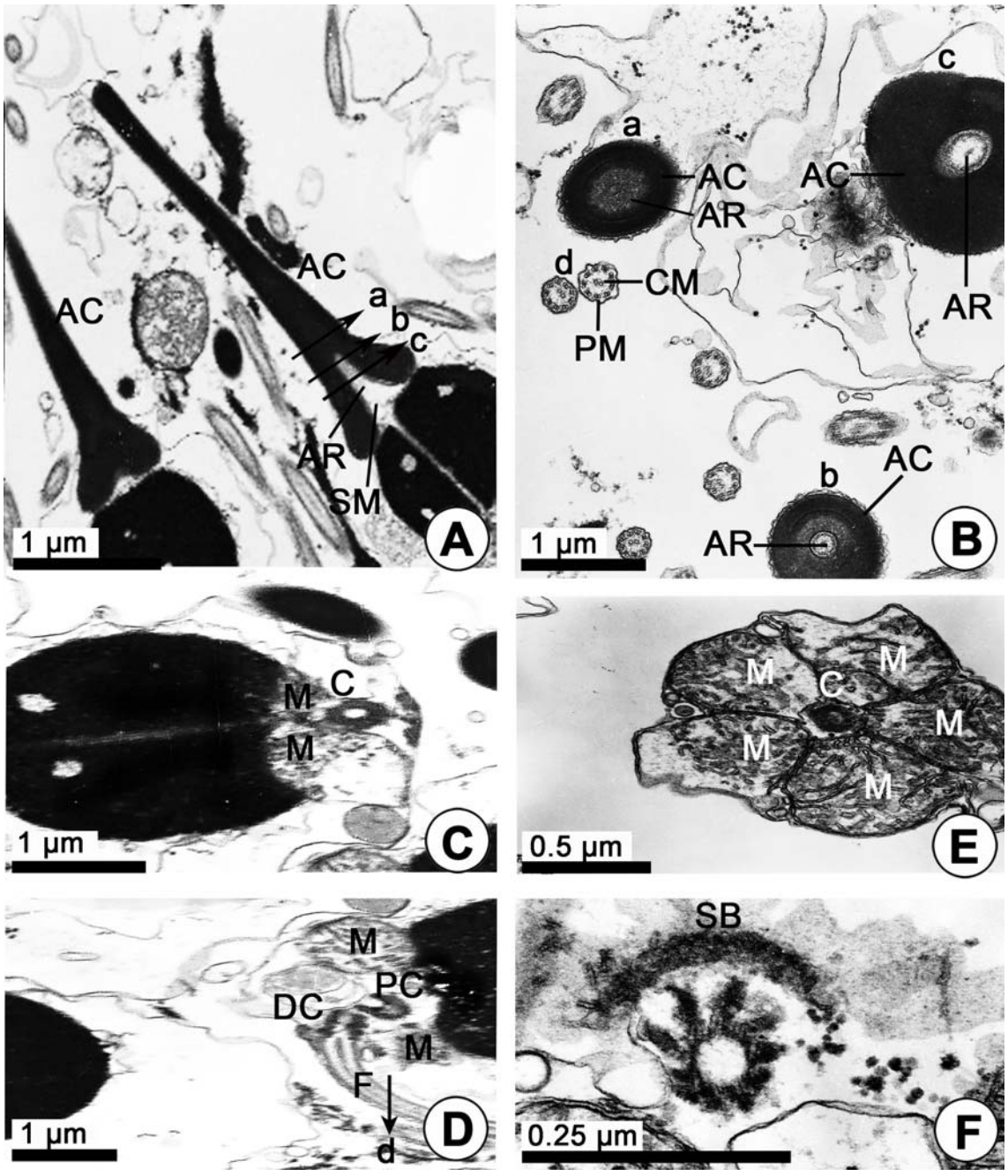

Fig. 3. Electron micrographs of sperm morphology in male *Mytilus coruscus*. A, Formation of a modified long cone-like acrosome. Note the axial rod in the acrosome, subacrosomal materials in the subacrosomal space, and three acrosomal part cross sectioned (a, b, c parts). B, cross-sectioned a, b, c parts of the acrosomes. Note a part showing axial rod and the acrosome, b part showing an axial rod and the acrosome and c part showing an axial rod and an acrosome. C, Sperm nucleus and the mid-piece of a spermatozoon. Note a few vacuoles (V), an axial rod in the mid-central line of the sperm nucleus and a pair of centrioles (C) and mitochondria (M). D, Sperm nucleus, the proximal centriole (PC), distal centriole (DC), mitochondria (M), and cross sectioned d part (axoneme) showing a 9+2 structure (nine pairs of microtubules at the periphery (PM) and a pair at the center (CM)). (Fig. B-d). E. A proximal centriole. Note 5 mitochondria surrounding a proximal centriole. F. a satellite body. Note a satellite body which is attached to the proximal centriole in the middle piece of a sperm (Figs. 3E, F).

part of the nucleus and the peripheral part of the basal rings the acrosome (Fig. 3A, B). In the posterior part of the nucleus, mitochondria lie close to the nuclear envelope (Figs. 3C, D). A pair of centrioles is surrounded with five spherical mitochondria: they constitute the middle piece of the spermatozoon (Fig. 3E). At this time, of the two centrioles lying in the midpiece of the spermatozoon, the two centrioles, at right angles, show the classic nine triplets of microtubles. The proximal one lies in the posterior fossa of the nucleus and is perpendicular to the axoneme. It appears unconnected to the nuclear envelope. The distal centriole occupying the basal portion of the flagellum constitutes the basal body of the flagellum, and it gives rise to the axial filament of the flagellum of the spermatozoon (Figs. 3B, 3D). The cytoplasm is greatly reduced, and so the nucleo-cytoplasm shows high ratio.

4) Spermatozoa

In the maturation phase, the differentiation of spermatozoon is completed and sperm morphology shows the primitive type, as found in species that perform external fertilization. At this time, a cross-sectioned tail flagellum (d part) shows that the axoneme of the tail flagellum of the spermatozoon consist of nine pairs of peripheral microtubules at the periphery and one pair of central microtubules at the center. The axoneme of the sperm tail shows a 9+2 structure (Figs. 3B-d). In particular, at the site of a proximal centriole, a satellite body, which is attached to the proximal centriole, is found in the middle piece of spermatozoa (Figs. 3E, F). The morphology of the sperm nuclear type and the acrosomal shape of this species are of a cylindrical type and a modified cone shape, respectively. The head of each spermatozoon is approximately 5.40 μm in length, including the nucleus (about 1.46 μm in length) and the acrosome (about 3.94 μm in length), the mid-piece is about 0.54 μm in length, and the tail is approximately 40 - 45 μm in length.

Discussion

The morphology of the spermatozoa appears to be a feature necessary for assessing phylogenetic relationships. In general, the ultrastructures of the bivalve sperm has a primitive or basic form (Franzén, 1970). That is, the sperm usually consists of a elipsoid or conical nucleus, an acrosome of variable complexity, a middle piece conssisting of an aggregation of usually 4-5 mitochondria surrounding a pair of centrioles, and a flagellum or sperm tail (Franzén, 1970).

In this study, morphology and ultrastructure of the sperm of *M. coruscus* is similar to those in the sperm in bivalves.

To the date, The sperm of more than 70 species have been described from 10 orders and nearly 30 families (Eckelbarger and Davis, 1996). Eckelbarger and Davis (1996) reported that of ultrastructures of the spermatozoa, in particular, the main variable structures appear to be acrosome, centrioles and mitochondria and the tail, In this study, the main variable structures appear to be acrosome, centrioles and mitochondria as reported by Eckelbarger and Davis (1996).

Popham (1979) described that ultrastructures of the spermatozoa in 5 subclasses of the bivalves have some differences in the morphologies and positions of the acrosomes of the sperms. And he stated that in particular, the subclasses, Pteriomorphia and Heterodonta have very similar morphologies of the acrosomes, while Hodgson and Bernard (1986) reported that the acrosomes can be distinguisable by the morphologies of the acrosomes. To the date, in this study, we have investigated the morphologies of the acrosomes in many families in two subclasses, Pteriormorphia and Heterodonta. From these obtained results, the acrosomes can be distinguisable those of the genuses and families by the morphologies and positions of the acrosomes. In this study, all species in the subclass Pteriormorphia in the bivalves have the acrosomal vesicles showing the cone-like in shape, with electron high density (opaque) materials from the basal parts to the apex parts, while all species in the subclass Heterodonta in the bivalves have the acrosomal vesicles showing the long cone or cap in shape, with electron high density (opaque)

materials from the basal parts (basal ring) to the lateral parts (Hodgson and Bernard, 1986). In this study, *M. coruscus* belongs to the family Mytilidae in the subclass Pteriomorphia, and this species have a acrosomal vesicles showing the modified cone-like in shape, and containing electron high density (opaque) materials from the basal part to the apex part as reported by Hodgson and Bernard (1986). Therefore, our results are coincide with opinions of Hodgson and Bernard (1986).

In general, the nucleus shows greater morphological diversity than the mitochondria since it can be ovoid (Mytilus spp., Nijima and Dan, 1965), short and conical (*Lyrodus pedicellatus*; Popham, 1974), nearly spherical (*Crassostrea virginica*; Galtsoff and Phipott, 1960), barrel-shaped (*Spisula solicidissima*; Longo and Anderson, 1969), pencil-shaped (*Corbicula japonica*; Jun *et al*., 2009) or long, tapering and curved (*Ruditapes philippinarum*; Chung *et al*., 1998).

In this study, the morphology of the sperm nucleus of *M. coruscus* is cylinder type.

Proacrosomal vesicles were first observed in the spermatogonial stage in *Crassostrea angulata* and *Ostrea edulis* (Sousa and Oliveira, 1994), but not until the spermatid stage in Perna perna (Bernard and Hodgson, 1985), *Pecten maximus* (Dorange and Le Pennec, 1989) and *Brachiodontes variabilis* (Al-Haij, 1990). While we did not confirm their presence at the spermatogonial stage in the present study, proacrosomal vesicles were common in *C. virginica* spermatocytes. The above studies collectively show that the mechanism of acrosomal vesicle formation in mollusc sperm are diverse and that no single mechanism characterizes bivalve sperm.

This study showed that the fine structure of the marine bivalves is more variable than hitherto realized. This variability can be largely ascribed to variations in the morphology, composition and location of the acrosome within the spermatozoon, although variations in the morphology of the nucleus and morphology and number of mitochondria appear to occur within rather narrow limits. For example, as shown in this work, the nucleus can be spherical, ovoid or cylindrical, as previously reported for other bivalve species.

Bivalve sperm generally show the greatest morphological variation in the nucleus and acrosome (Franzén, 1983). The primary function of the acrosome is to penetrate barrers to the egg during fertilization, acrosomal variation between species is believed to reflect differences in functional demands at sperm penetration (Anderson and Personne, 1975; Franzén, 1983).

The variability in size composition, morphology and position of the acrosome in the sperm of the present bivalves is astounding. The essential functions of the acrosome are to dissolve egg coats and permit fussion of the sperm plasma with egg plasma membrane (Franklin, 1970; Longo, 1973). Hence, it is highly probable that some of the variation in the characteristics of the acrosome may reflect functional differences associated with egg coat structure (Bacetti, 1970) rather than random mutations which have been maintained in the species. For example, the large acrosome of the sperm of *Mytilus edulis* may be due to the fact that the eggs of this species are covered with a thick coat of jelly (Humphreys, 1962). In this study, a similar finding was also found in a large acrosome showing long cone-like of the spermatozoa of M. edulis (Popham *et al*., 1974) and *M. galloprovincialis* by Kim (2001).

The presence of an anterior nuclear fossa of the sperm nucleus can be correlated with the presence of an axial rod in the acrosome. Therefore, the most important feature of the sperms investigated in this work is that they have an axial rod as part of the apparatus making up the acrosome. Popham *et al*. (1974) reported that *M. edulis* and *C. virginica* have sperms showing special morphological characteristics associated with the placement of the axial rod which appear in the sperm nucleus and the acrosome. In the process of the formation of the axial rod, the periacrosomal material may partly consist of an axial rod, a structure usually found occupying much of the volume of the lumen created by the invagination of the acrosomal vesicle. In some species having an axial rod, it may long and penetrate deeply into an anterior

nuclear fossa in *Mytilus* spp. (Nijimma and Dan, 1965) and *C. virginica* (Galtsoff and Philipott, 1960; Daniels *et al*., 1971; Popham, 1979). In this study, a long axial rod appeared to penetrate deeply into an anterior nuclear fossa of sperm nucleus from a posterior nuclear fossa, and its appearance continued to the apex of the acrosome as reported in *Mytilus* spp (Nijimma and Dan, 1965). Probably, it is assumed that the axial rod as part of the apparatus is involved in the making up the acrosome associated with the acrosomal reaction for fertilization. The mitochondria have been found to be intimately asociated with the nuclear envelope, spherical to ovoid in shape and 4-5 in number in *C. virginica* (Galtsoff and Philpott, 1960; Eckelbarger and Davis, 1996), *M. edulis* (Longo & Dornfield, 1967), *S. solidissima* (Longo & In general, it is easy to see a satellite body attached to the proximal centriole. In many bivalve species, the mitochondria surround a pair of centrioles oriented to 90° to each other. The proximal centriole has a satelite body which is inserted into the posterior nuclear fossa. In this study, five mitochondria surround a pair of centrioles, and a satellite body, which is attached to the proximal centriole, appeared remarkably near the posterior nuclear fossa. It is assumed that a satellite body as part of the apparatus is involved in the formation of a flagellum in *M. coruscus*.

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