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Ultrastructure of the Testis and Germ Cell Development During Spermatogenesis in Male *Crassostrea gigas* (Bivalvia: Ostreidae) in Western Korea

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ABSTRACT

Ultrastructural characteristics of the testis and spermatogenesis of *Crassostrea gigas* were investigated by Transmission and Scanning Electron microscope observations. The testis is a diffuse organ consisting of branching acini containing differentiating germ cells in a variety of stages. The acinus is surrounded by an intermitent layer of myoepithelial cells andis divided into subcompartments that are partially separated by pleomorphic accessory cells which remain in close contact with germ cells until late stages of development. these accessory cells contain a large quantity of glycogen particles and lipid droplets in the cytoplasm. Therefore, it is assumed that they are involved in the supplying of the nutrients for germ cell development, while any phenomena associated with phagocytosis of undischarged, residual sperms by lysosomes could be find in the cytoplasm of the accessory cells. The morphology of the spermatozoon has a primitive type and is similar to those of other bivalves. Mature spermatozoa 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 to the distal centriole and plasma membrane. Spermatozoa of *C. gigas* resemble to those of other investigated ostreids. In particular, the anterior region of the acrosomal vesicle is transversely banded. 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 spermatozoon is approximately 42-47 μm in length including an oval sperm nucleus (about 0.91 μm in length), an acrosome (about 0.42 μm in length) and tail flagellum (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. These morphological charateristics of acrosomal vesicle belong to the family Ostreidae in the subclass Pteriomorphia.

Key words: *Crassostrea gigas*, spermatogenesis, germ cell, accessory cell, sperm morphology

INTRODUCTION

Aside from their commercial significance, oysters in the family Ostreidae comprise one of the more taxonomically perplexing groups of bivalve molluscs, in particular at the species level. The utrastructure of the testis and spermatogenesis and mature sperm morphology have been decribed in many species of bivalve molluscs using both light and electron microscopy (Hodgson and Bernard, 1986; Healy, 1989; Eckelbarger *et al*, 1990; Healy and Lester, 1991; Gaulejac *et al*., 1995; Eckelbarger and Davis, 1996).

The morphology of spermatozoa appear to be well correlated with the evolution of bivalve group and the

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phylogenetic relationship (Popham *et al*., 1974; Popham, 1979; Hodgson and Bernard, 1986; Healy and Lester, 1991). Recently, it is well-known that the ultrastructure of the sperm in bivalves (Popham *et al*., 1974). For that reason, sperm ultrastructure has long been viewed as a tool in assessing taxonomic problems and phylogenetic relationships in the Metazoa through the use of spermiocladistic analysis (Jamieson, 1987, 1991). In the Mollusca, sperm morphology has been used increasingly in assessing long-standing taxonomic problems (Popham, 1979; Healy, 1983, 1988, 1995, 1996; Hodgson and Bernard, 1986).

Ultrastructural studies of mature spermatozoa have been conducted on many species in Bivalvia including those of several oyster species (Galtsoff and Philpott, 1960; Healy and Lester, 1991). To date, comprehensive studies of spermatogenesis of the Korean bivalve species have been restricted to a few commercially important species in the familes Veneridae (*Cyclina sinensis (*Chung *et al*., 1991), *Saxidomus purpratus (*Chung *et al*., 1999), *Gomphina melanaegis (*Lee *et al*., 1999), *G. veneriformis (*Park *et al*., 2003), *Meretrix petechialis (*Chung, 2005), *G. veneriformis (*Park *et al*., 2002)), Mytilidae (*Mytilus coruscus (*Kim *et al*., 2011)), Pectinidae (*Patinopecten yesoensis (*Park *et al*., 2006), *Chlamys farreri (*Chung *et al*., 2005)), and a single study within the Ostreidae (*Crassostrea nippona (*Kim, 2001)). No ultrastructural study of the testis and spermatogenesis has been reported on the Korean Pacific oyster, *C. gigas*. Although descriptions of bivalve spermatozoa are plentiful, ultrastructural studies of the bivalve testis are surprisingly rare. Therefore, little information is available on the ultrastructure of the testis, spermatogenesis 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 axial rod and satelite fiber. During spermatogenesis, sperm are closely associated with accessory cells which presumably play an important role during sperm differentiation, but the terminology used to describe these cells is confusing. We attempt to resolve this confusion and to indicate what studies are needed to assess the function of the assessory cells. The pupose of the present study is to describe and clarify the some charateristics in ultrastructures of the testis and spermatogenesis in the Pacific oyster, *C. gigas*.

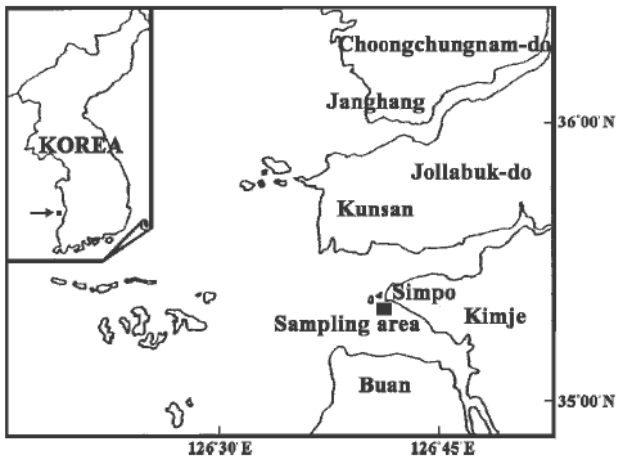

Fig. 1. Map showing the sampling area.

MATERIALS AND METHODS

1. Transmission electron microscope observation

The specimens of *C. gigas* were collected at the intertidal zone of Simpo, Jollabuk-do, Korea (Fig. 1).

A total of 52 male individuals were used for transmission electron microscope observations. 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℃. 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℃. 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 80-1,00 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.

2. Scanning electron microscope observation

A total of 22 male individuals were used for scanning electron microscope observations. A drop of sperm suspension was placed on a coverglass, prefixed with 2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.5) at 4℃ for 15 min, and post fixed with 1% OsO_4 for 10 min, before rinsing with cacodyllate buffer. The specimens were dehydrated in a graded ethanol series, critical point dried, coated with gold, and observed under a scanning electron microscope (ISI-SS4D). In addition, after dehydration, some testes were freeze-fractured in liquid nitrogen, and then submitted to the same procedure described above.

RESULTS

1. Position and ultrastructure of the mature testis

The testes are irregularly arranged from the subregions of mid-intestinal glands in the visceral cavity to the reticular connective tissues of the foot. The general morphology and the ultrastructure of the testis of *C. gigas* is similar to those of other bivalve species. The testis is a diffuse organ that consists of a series of highly branching acini lying within a matrix of support cells. Each acinus is subdivided into a number of subcompartments that partially isolate groups of developing germ cells. Within each subcompartment, germ cells are distributed in a centripetal pattern from the acinus wall to the lumen.

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 the central lumen. Developing germ cells in adjacent subcompartments are partially segregated by myoepithelial cells and pleomorphic accessory cells. Accessary cells are closely associated with all sperm stages except the mature spermatozzoon.

Each acinus is surrounded by a connective tissue compartment, the haemocoel, a fluid-filled space that contains amoeboid hemocytes. It is also partially surrounded by an intermittent, single layer of squamous myoepithelia cells which forms a partial barrier between the germinal epithelium and the hemocoel. Germ cells in adjacent subcompartments are partially segregated by myoepithelial and pleomorphic accessory cells. The myoepithelial cells (somatic cells) contain elongated nuclei, occassional electron dense granules, and dilated cisternae of rough endoplasmic reticulum. At this time, the accessory cells (somatic cells) are distributed both between and within acinal subcompartments in close association with developing germ cells. The accessory cells are amoeboid, and the cell contains an irregular nucleus, mitochondria, a few RER cisternae and scattered lipid droplets. Due to a relative scarcity of cytoplasmic ribosomes, they stain very ligthtly in contrast to the more darkly staining cytoplasm of adjacent germ cells. In this study, however, we could not find desmosomes which are observed between germ cells and accessory cells. Based on the volume of scattered condensed heterochromatin substances in the nucleus and the appearance of lysosomal-like dense granules in the cytoplasm of accessory cells (somatic cells) classified by Eckerbarger *et al*. (1990), in *C. gigas*, two types of accessory cells are observed in the inner wall of the acini during spermatogenesis. The first type of pleomorphic accessory cells (AC-I) contains a single slightly elongate nucleus containing scattered heterochromatin, and several mitochondria, abundant glycogen particles, and a few lipid droplets in the cytoplasm. However, it is difficult to find lysosome-like dense granules in the cytoplasm of AC-I. A second type of amoeboid accessory cells (AC-II) is observed in various locations throughout the acinus. This is an amoeboid-like form and contains a nucleus with patches of condensed heterochromatin, numerous cytoplasmic vesicles, small mitochondria, abundant glycogen deposits, and a few lysosomal-like dense granules, which are closely associated with developing germ cells (Fig. 2).

2. Ultrastructure of germ cells and accessory cells during spermatogenesis

Based on the testicular development and

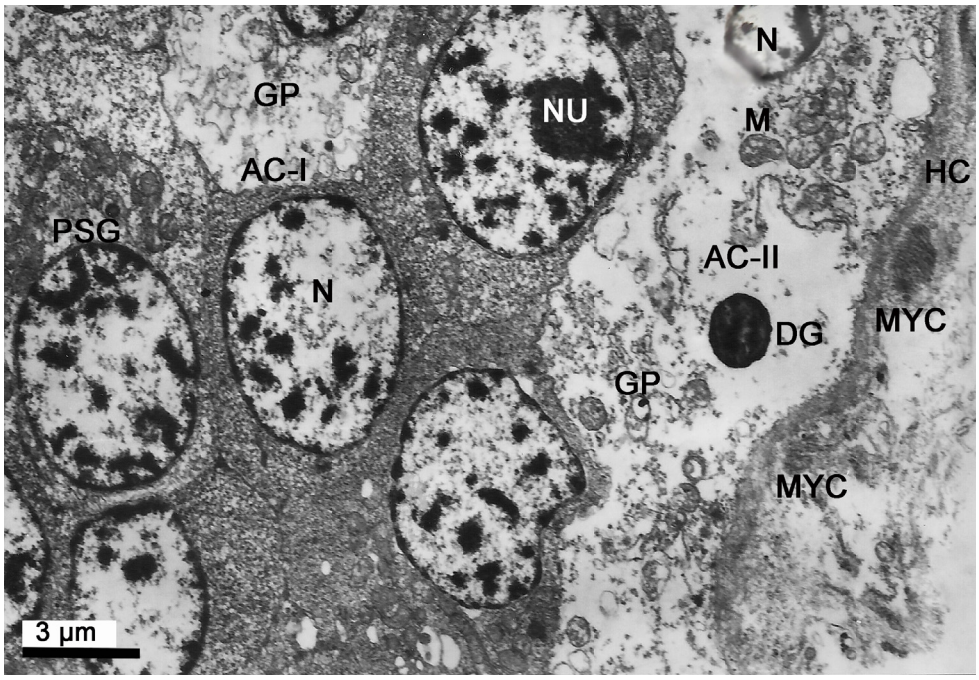

Fig. 2. A transmission electron micrograph showing the ultrastructure of the testis in male *Crassostrea gigas*. Note myoepithelial cells (MYC) and haemocoel (HC) in the outer wall and developing gern cells near the pleomorphic accessary cells (AC-I) and amoeboid accessory cells (AC-II) containing a large quantity of glycogen particle and lysosome-like dense granules in the inner wall of acini.

morphological characteristics of germ cells near the accessory cells by electron microscopic observation, spermatogenesis occurs in the acini of the testis. The mature testis normally contains most stages of spermatogenesis, and the transition from spermatogonia to spermatozoon can be observed in one section. The process of spermatogenensis appears to be similar to other bivalves. Spermatogenesis occurs in the acini of the testis and can be divided into four stages: (1) spermatogonia, (2) spermatocytes, (3) spermatids and (4) spermatozoa.

3. The process of spermatogenesis appear to be similar to those of other bivalve species.

1) Spermatoginia:

Two types of spermatogonia, designated primary and secondary, occur. The primary spermatogonia are large cells (5 to 6 μm diameter) each with a spherical nucleus (approximately 3.5 μm diameter) and a prominent elcetron-dense nucleolus. The nucleus contains small clumps of electron-dense chromatin, which are offen associated with the inner nuclear envelope. the cytoplasm contains several small mitochondria, rough endoplasmic reticulum, and vacuoles. The primary spermatogonia have an large nuleus with a more granular electron-dense nucleoplasm. Commonly, they are confined to the outer region of the acinus. Their cytoplasm is largely devoid of organelles except for scattered mitochondria (Fig. 3A).

2) Spermatocytes:

Two stages of spermatocyte development, presumed to be primary and secondary, can be observed in the acinus walls of some specimens. Primary spermatocytes are slightly smaller cells (4.0 to 5.0 μm diameter) that are distinguished by nuclei (aprroximately 3.0 μm diameter) with more abundant and more darkly staining heterochromatin. The nucleus of the primary spermatocyte is similar in size and shape to that of the secondary spermatogonium, however, the nucleolus is no longer prominent, and the chromatin is in the form of a patch work.

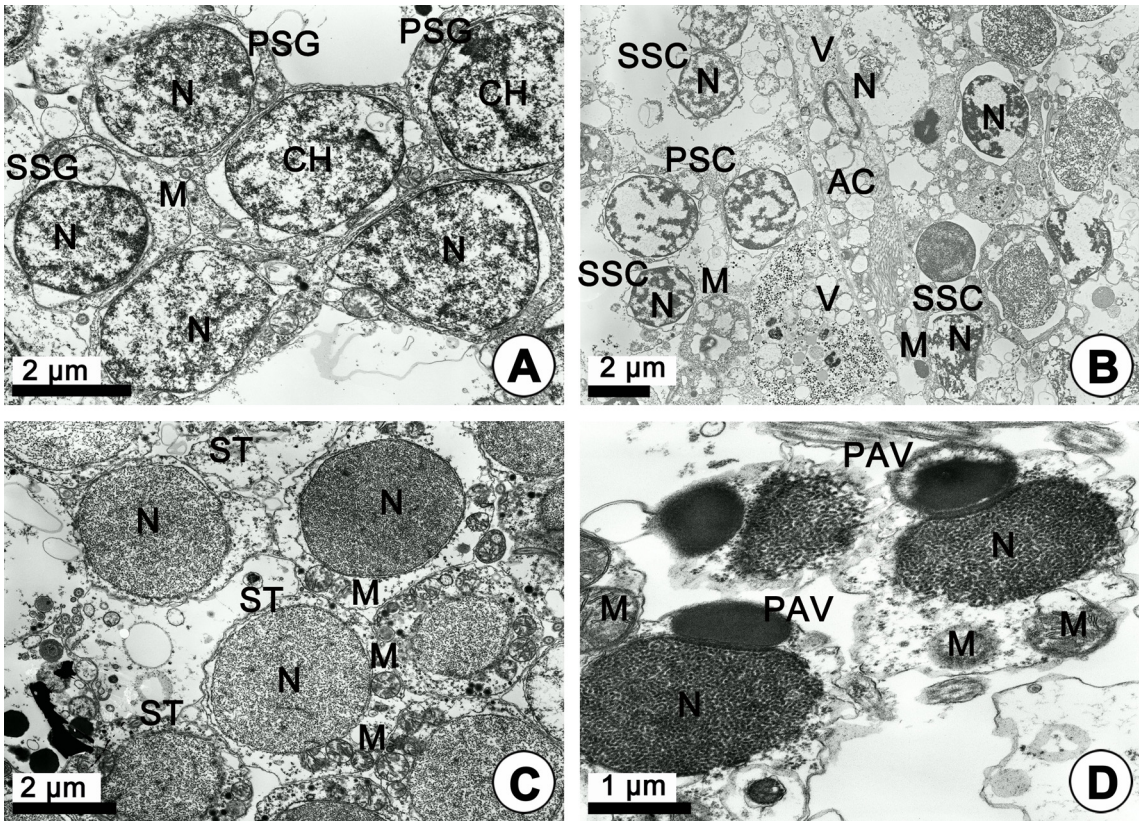

Fig. 3. Transmission electron micrographs of spermatogenesis in male *Crassostrea gigas*. **A**, Primary spermatogonia (PSG) and secondary spermatogonia (SSG). Note chromatins (CH) in the nucleus (N) and mitochondria (M) in the cytoplasm. **B**, The primary spermatocyte (PSC), secondary spermatocytes (SSC) and accessory cells (AC). Note several synaptonemal complexes in the nucleus (N) of primary spermatocytes and chromatin materials in the nucleus and mitochondria (M) in the cytoplasm of secondary spermatocytes near the accessary cell containing glycogen particles and vacuoles (V) in the cytoplasm. **C**, Spermatids (ST). Note heterochromatin materials in the nucleus (N), mitochondria (M) and a few granules formed by the Golgi complex in the cytoplasm.. **D**, A spermatids. in the early stage of spermiogenesis. Note a proacrosomal vesicle (PAV). on the nucleus.(N) and mitochondria in the cytoplasm.

Primary spermatocytes in the stages of first meiotic prophase have been distinguished within the germinal layer of the testis, Zygotene/patchtene spermatocytes contain nuclei with more highly condensed chromatin and synaptonemal complexes. The synaptonemal complexes in the nucleus appear in the prophase during the first maturation division. Only several mitochondria and various sizes of vacuoles appear in the cytoplasm, the volumes of the cytoplasms reduce, so the nucleo-cytoplasm ratio increase, cellular outlines are oval in shape.

Primary spermatocytes differentiate into secondary spermatocytes by way of the meiotic division of primary spermatocytes. In fact, the secondary spermatocytes are rarely observed, probably due to the rapidity of the first meiotic division of the primary spermatocytes. They are irregular in shape and range from about 4-5 μm in size. Spherical nucleus possess scattered chromatin forming a network. Secondary spermatocytes are frequently observed undergoing mitotic division, and the sizes of secondary spermatocytes become smaller than those of the primary spermatocytes.

At this time, several pleomorphic accessory cells are present near several primary and secondary spermatocytes, and a large quantity of glycogen particles, several mitochondria and a few lipid droplets are present in the cytoplasm of the accessory

cells (somatic cells). In particular, the accessory cells are distributed within acinal subcompartments in close association with developing germ cells (Fig. 3B).

3) Spermatids:

The secondary spermatocyte is transformed into the spermatids by the secondary meiotic division. For convenience, spermiogenesis has been divided arbitrarily into two stages: the early and late stages. In the early stage of spermatid development (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, and the cytoplasm contains a number of mitochondria and the Golgi complex (Fig. 3C). Based on the characteristics of cell organelle differentiation, the prossesses of acrosome formation of the spermatids occur during spermiogenesis as follows. In the late stage of spermiogenesis, the morphology of the spermatid nucleus changes gradually during the differentiation of the spermatid. At this time, small granules, which are formed by the Golgi complex in the cytoplasm, move to a position just in front of the nucleus, while mitochondria move to a position just behind the nucleus. 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. 3D). 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 the presumptive anterior pole of the spermatids. If the acrosomal vesicle is well developed, its length occupies approximately one third of the sperm head. The acrosomal vesicle is initially oval in shape (Fig. 4A) 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: the acrosomal vesicle and an extensive deposit of subacrosomal (extravesicular) materials. The processes of acrosomal vesicle formation are very complex as follows. The acrosomal vesicle is membrane bound, consequently, become cap-shaped form by way of various morphorphological changes and invaginations from initial oval shape and measures about 0.42 μm long (Figs. 4B-4D). Contents of the acrosomal vesicle are finely granular, moderately electron dense, and anteriorly, differentiated into a series of transverse bands (two or three bands interleaved by relatively elctron lucent bands) as some characteristics of the acrosomal vesicle of Ostreidae species (Fig. 4E and see magnification of Fig. 4E in Fig. 6).

4) Spermatozoa:

The morphology of the spermatozoon has a primitive type and is similar to those of other bivalves (Fig. 5). An acrosome on the nucleus is composed of the acrosomal vesicle (which comprises both right and left basal rings) and subacrosomal materials which comprises an axial rod embedded in a coasely granular matrix. An acrosomal vesicle (about 0.42 μm in length) shows high electron dense opaque at the acrosomal membrane, while two or three bands (alternating electron dense and electron lucent bands) are present in the anterior region of acrosomal vesicle. And both right and left lateral parts of basal rings in an acrosomal vesicle show electron lucent regions except for the both lateral parts along the acrosomal membrane (electron dense opaque), as seen in the subclass Pteriomorphia species. The nucleus (0.91 μm long and 1.36-1.38 μm wide) is oval. At this time, anterorly the nucleus is deeply invaginated, this space being 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 (Fig. 4F). Occassional electron lucent lacunae are also visible in some sections. In the cross sectioned acrosomal vesicle, an axial rod is present in the subacrosomal granular materials (Fig. 4G). Posterior to the nucleus is the midpiece. This region consists of four equal-sized, spherical mitochondria surrounding a pair of triplet substructure centrioles (Fig. 4H). The cristae of each

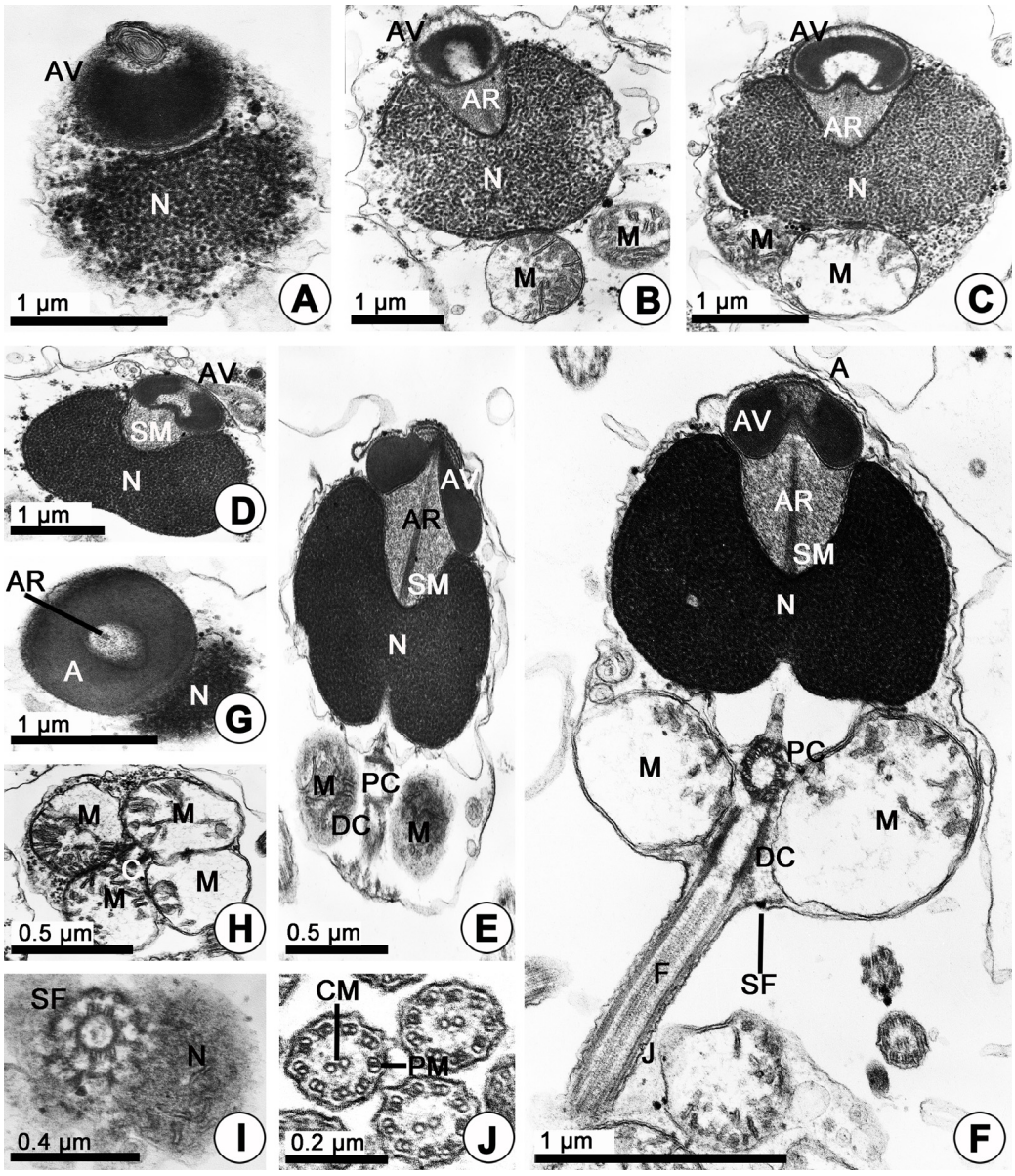

Fig. 4. Transmission electron micrographs of spermiogenesis in male *Crassostrea gigas*. **A**, A spermatid in the early stage of spermiogesis. Note the acrosomal vesicle (AV) just before the nucleus. **B**, **C**, **D**, Spermatids during differentiation of the acrosomal vesicle. Note an axial rod (AR), subacrosomal granular materials in the acrosomal vesicle (AV) on the nucleus (N) and spherical mitochondria (M). **E**, A Spermatid in the late stage of spermiogenesis. Note two or three transversed band at the anterior part of two basal rings and the axial rod (AR) in the acrosomal vesicle (AV) on the nucleus (N), large mitochondria (M) surrounding proximal and distal centrioles beneath the nucleus (N). **F**, A completed spermatozoon. Note basal rings, axial rod (AR), subacrosomal granular materials (SM) in the acrosomal vesicle (AV), proximal centriole (PC), distal centriole (DC), satelite fibers (SF) and a flagellum of a spermatozoon. **G**, Cross sectioned acrosome (A). Note the axial rod (AR) in the acrosome on the nucleus (N). **H**, Cross sectioned the sperm midpiece. Note centrioles surrounded with four mitochondria (M). **I**, Satelite fibers (SF) near mitochondria in the sperm middle piece (Figs. 3E, F).. **J**, Cross sectioned the flagellum of the sperm. Note an axoneme showing a 9 + 2 structure (a pair of single doublet central microtubles (CM) and nine pairs of peripheral microtubules (PM)).

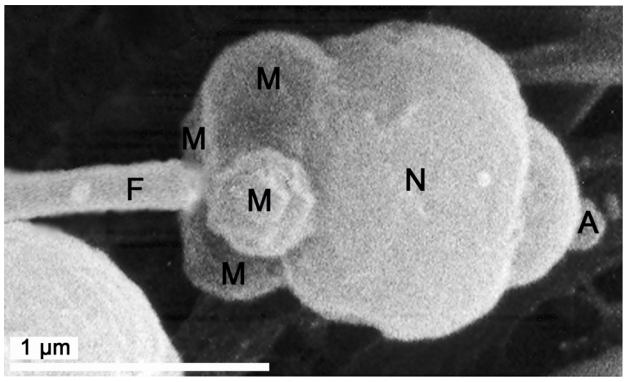

Fig. 5. A scanning electron micrograph of sperm morphology in male *Crassostrea gigas*. Note a complete spermatozoon showing an acrosome (A) on the nucleus (N), four spherical mitochondria (N) in the midpiece and a long flagellum.

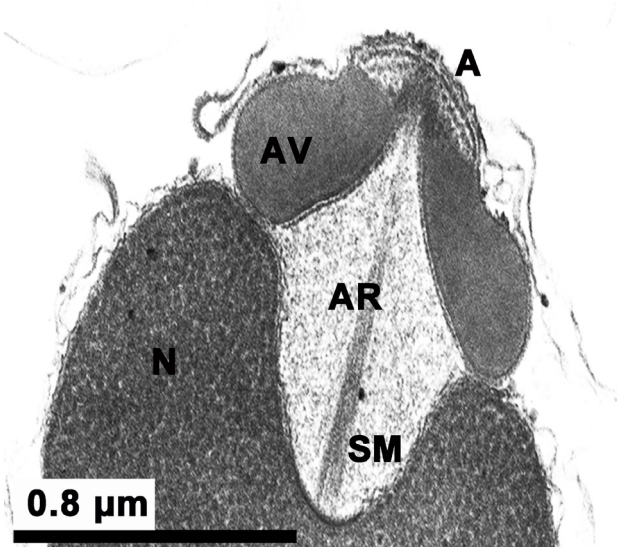

Fig. 6. A transmission electron micrograph of the acrosomal vesicle of a spermatozoon in male *Crassostrea gigas*. Note an acrosomal vesicle ultrastructure of the spermatozoon showing two or three transversed bands on the anterior region between two basal riings, axial rod (AR) and subacrosomal granular materials (SM) in the anterior nuclear invagination on the sperm. nucleus (N).

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. A system of nine terminally forked satellite fibers link the distal centriole and initial portion of the flagellum to the plasma membrane (Fig. 4I). Transverse sections reveal that the triplets of the distal centriole posteriorly transform into doublet are continuos with the nine doublets of the flagellar axoneme. The flagellum is composed of a 9 +2 substructure axoneme (that is, nine peripheral doublets (microtubules) surrounding a central pair of sigle microtubles, Fig. 4J) enclosed by the plasma membrane and measures approximately 40-45 μm.

DISCUSSION

Regarding germ cell development and the functions of the accessory cells, many authors have described bivalve accessory cells using a variety of terms including "follicle cells" (Eckelbarger *et al*., 1990), "auxiliary cells" (Dorange and Le Pennec, 1989; Gaulejac *et al*., 1995), "Sertoli cells" (Pipe, 1987).

However, few studies have documented that the ultrastructural features of testicular accessory cells in bivalves, and most descriptions are too cursory to be useful in assessing homology. However, we believe that some attempt should be made to reassess and standarize the terminology used to describe these cells.

Accessory cells were observed to be connected to adjacent germ cells via desmosomes in the testes of *Scobicularia plana* (Sousa *et al*., 1994), and *C. virginica* (Eckerbarger and Davis, 1996).

These observations show that the interaction between germ cells and accessory cells varies significantly in different species suggesting that they may play different physiological roles.

The remainining ultrastructural descriptions of testicular accessory cells are so limited, it is impossible to determine if they are homologous. Accessory cells also appear to vary ultrastructurally during spermatogenesis, so different authors may have described a single cell type during different stages of ontogeny. Until more information on their function is available, testicular somatic cells should probably be assigned the neutral term "accessory cells".

In general, the morphology of the spermatozoon of bivalve species has a primitive type and is similar to

those of other bivalves. In this study, mature spermatozoa of *C. gigas* consists 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 to the distal centriole and plasma membrane. The morphology of the spermatozoon has a primitive type and is similar to those of other bivalves. In most characteristics, such as shape of the acrosomal vesicle and nucleus, number of mitochondria, and position of centrioles, spermatozoa of *C. gigas* closely resemble those of other investigated ostreids (*C. virginica*-Galtsoff and Philpott, 1960; Daniels *et al*., 1971). On the whole, *C. gigas* shows almost same patterns from *Crassostrea* spp.

In this study, the anterior region of the acrosomal vesicle consisted of alternating electron dense and electron lucent bands. In paricular, the acrosomal membrane in the acrosomal vesicle exhibited high electron dense opaque region, while two or three bands are present in the anterior region of the acrosomal vesicle which was exhibited electron-lucent whorls (Fig. 6). A similar whorled substructure might also occur in acrosomes of *C. virginica*, though the micrograph presented by Daniels *et al*., (1971) were not detailed enough to confirm this. Healy and Lester (1991) reported that a similar phenomenon mentioned above appeared in *Saccostrea commercialis*: they found three or four bands showing electron-lucents whorls at the anterior region of the acrosomal vesicle. Compared our result with that reported by Healy and Lester (1991), we could find some differences in the anterior region of the acrosomal substructure: two or three bands showing whorled substructures in *C. gigas*, while three or four bands showing electron-lucents whorls at the anterior region of the acrosomal vesicle. in *Saccostrea commercialis*.

For that reason, they reported that *S. commercialis* could not be fertilized with *Crassostrea* spp. and could not produce some oyster larvae because of differences (between two species) in the number of alternating transversely bands showing a reticulum of electron-lucents whorls as a acrosomal substructure. In case of subsructure of the acrosomal vesicle, both right and left lateral parts of basal rings in an acrosomal vesicle show to be electron lucent regions except for the both lateral parts along the acrosomal membrane (electron dense opaque), as seen in the subclass Pteriomorphia species.

Early investigations of bivalve sperm ultrastructure demonstrated the taxonomic value of comparative studies (Popham, 1979), and such studies are now widely used in taxonomic anlyses (Healy, 1995). The primitive sperm show sufficient structural variability that they are useful in taxonomic studies.

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. The subacrosomal matterial contains a central filamentous axial rod composed of anterio-posterior-oriented filaments.

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 satelite fibers are found in family Ostreidae. Judging from the resuts on the ultrastructure of mature spermatozoon, it is supposed that this species belongs to family Ostreidae and subclass Pteriomorphia because the spermatozoon of this specis has special structural charateristics such as substructure of the acrosomal vesicle containing an axial rod and satelite fibers.

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