Korean J. Malacol. 26(1): 51-62, 2010

Ultrastructures of Germ Cells and the Accessory Cells During Spermatogenesis in Male *Gomphina veneriformis* (Bivalvia: Veneridae) on the East Sea of Korea

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ABSTRACT

The ultrastructures of germ cells and the accessory cells during spermatogenesis and mature sperm ultrastructure in male *Gomphina veneriformis*, which was collected on the coastal waters of Yangyang, East Sea of 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 four mitochondria surrounding the centrioles. Accessory cells are observed to be connected to adjacent germ cells, they 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 in the cytoplasm of the accessory cells after spawning was not observed in this study. The morphologies of the sperm nucleus type and the acrosome shape of this species have a cylindrical and modified long cone shape, respectively. In particular, the axial filaments in the lumen of the acrosome, and subacrosomal granular materials are observed in the subacrosomal space between the anterior nuclear fossa and the beginning part of axial filaments in the acrosome. The spermatozoon is approximately 50-55 μm in length including a long sperm nucleus (about 7.80 μm in length), an acrosome (about 1.13 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. Some charateristics of sperm morphology of this species in the family Veneridae are (1) acrosomal morphology, (2) the number of mitochondria in the midpiece of the sperm,. The axial filament appears in the acrosome as one of characteristics seen in several species of the family Veneridae in the subclass heterodonta, unlikely the subclass pteriomorphia containing axial rod instead of the axial filament. As some characteristics of the acrosome structures, the peripheral parts of two basal rings show electron opaque part (region), while the apex part of the acrosome shows electron lucent part (region). These charateristics belong to the family Veneridae in the subclass heterodonta, unlikely a characteristic of the subclass pteriomorphia showing all part of the acrosome being composed of electron opaque part (region). Therefore, it is easy to distinguish the families or the subclasses by the acrosome structures. The number of mitochondria in the midpiece of the sperm of this species are four, as one of common characteristics appeared in most species in the family Veneridae.

Key words: *Gomphina veneriformis*, spermatogenesis, germ cell, accessory cell

Introduction

Spermatogenesis and mature sperm morphology has been documented to varying degrees in many species of bivalve molluscs using both light and electron microscopy (Nizima and Dan, 1965; Chung and Ryou,

Received December 7, 2009; Revised February 1, 2010; Accepted February 17, 2010
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1225-3480/24336

2000; Chung *et al*., 1991, 2005, 2006, 2007).

In the mollusca, sperm ultrastructure is considered a valuable tool in assessing taxonomic and phylogenetic problems within the bivalvia (Franzén, 1970, 1983; Daniels *et al*., 1971; Popham, 1979; Healy, 1989, 1995; Koike, 1985; Hodgson and Bernard, 1986; Eckelbarger *et al*., 1990; Eckelbarger and Davis, 1996) and is especially useful when comparing closely related species (Popham *et al*., 1974; Popham, 1979). Therefore, sperm ultrastructures of bivalves are now widely used in taxonomic analyses (Healy, 1995).

To the date, there are several ultrastructural studies on spermatogenesis of the family Veneridae in Korea: *Ruditapes philippinarum* (Chung *et al*., 1998), *Gomphina veneriformis* (Park *et al*., 2002), *Cyclina sinensis* (Chung *et al*., 1991), *Saxidomus pupuratus* (Chung *et al*., 1999), *Meretrix lusoria* (Chung *et al*., 2006). Regarding the genus *Gomphinia*, there have been some studies on reproduction including gonadal development and reproductive cycle of *Gomphina melanaegis* (Lee *et al*., 1999), gonadal development and gametogenic cycle of *G. veneriformis* (Park *et al*., 2003). and on spermatogenesis and sperm ultrastructure (Park *et al*., 2002).

Although the reproductive ecology and sperm ultrastructure during spermatogenesis of this species has been investigated by some authors, little information is available on the ultrastructural changes of germ cell differentiations and the functions of the accessory cells associated with spermatogenesis by electron microscopic observation.

Commonly, various germ cells during spermatogenesis are present near the accessory cells in an acinus. the accessory cells (somatic cells) contain a large quantity of glycogen particles and lipid droplets in the cytoplasm (Gaulejac *et al*., 1995; Eckelbarger and Davis, 1996; Chung *et al*., 2007). Regarding the function of the accessory cell, Eckelbarger and Davis (1996) reported that accessory cells (somatic cells) are associated with germ cell development during spermatogenesis, as a source of nutrient supply. However, the functions and structures of the accessory cells with the germ cell developmental stages during spermatogenesis have not yet been clarified in detail. Above all, it is important to investigate fine structures and functions of the accessory cells associated with germ cell development in the lumen of the acinus.

Recently, sperm ultrastructure has been viewed in metazoa through the use of spermiocladistic analysis (Jamieson, 1987, 1991), sperm ultrastructures of bivalves are now widely used in taxonomic analyses (Healy, 1995). In particular, it is well-known that acrosomal morphology of sperms has been used to organize bivalve subclasses (Popham, 1979), the number of mitochondria in the sperm midpiece tends to be stable within any given family or superfamily. Therefore, it need to study acrosomal morphology of the sperm and the number of mitochondria in the sperm midpiece for taxonomic analyses of this species. If some characteristics obtained from sperm ultrastructure and the process of spermiogenesis are phylogenetically analyzed, the results of the ultrastructural studies on bivalve spermatozoa will provide information needed for the elucidation of relationship patterns among several bivalve subclasses (Popham *et al*., 1974, Popham, 1979; Healy, 1989, 1995). Information on sperm ultrastructure is sorely needed for this important clade of bivalves. Therefore, the main aim of the present study is to describe the ultrastructures of germ cells, as well as the ultrastructure and function of the accessory cells associated with spermatogenesis, and to confirm sperm type with sperm ultrastructure by phylogenetic analysis of *G. veneriformis*.

Materials and Methods

1. Sampling

15-20 Male specimens of *G. veneriformis* were collected monthly in the subtidal zones (3-10 m in water depth) of coastal waters of Yangyang, the East Sea of Korea, from January to December, 2006 (Fig. 1). The clams were transported to the laboratory where they were maintained in seawater at 20°C.

2. Transmission electron microscope observation

For transmission electron microscope observations,

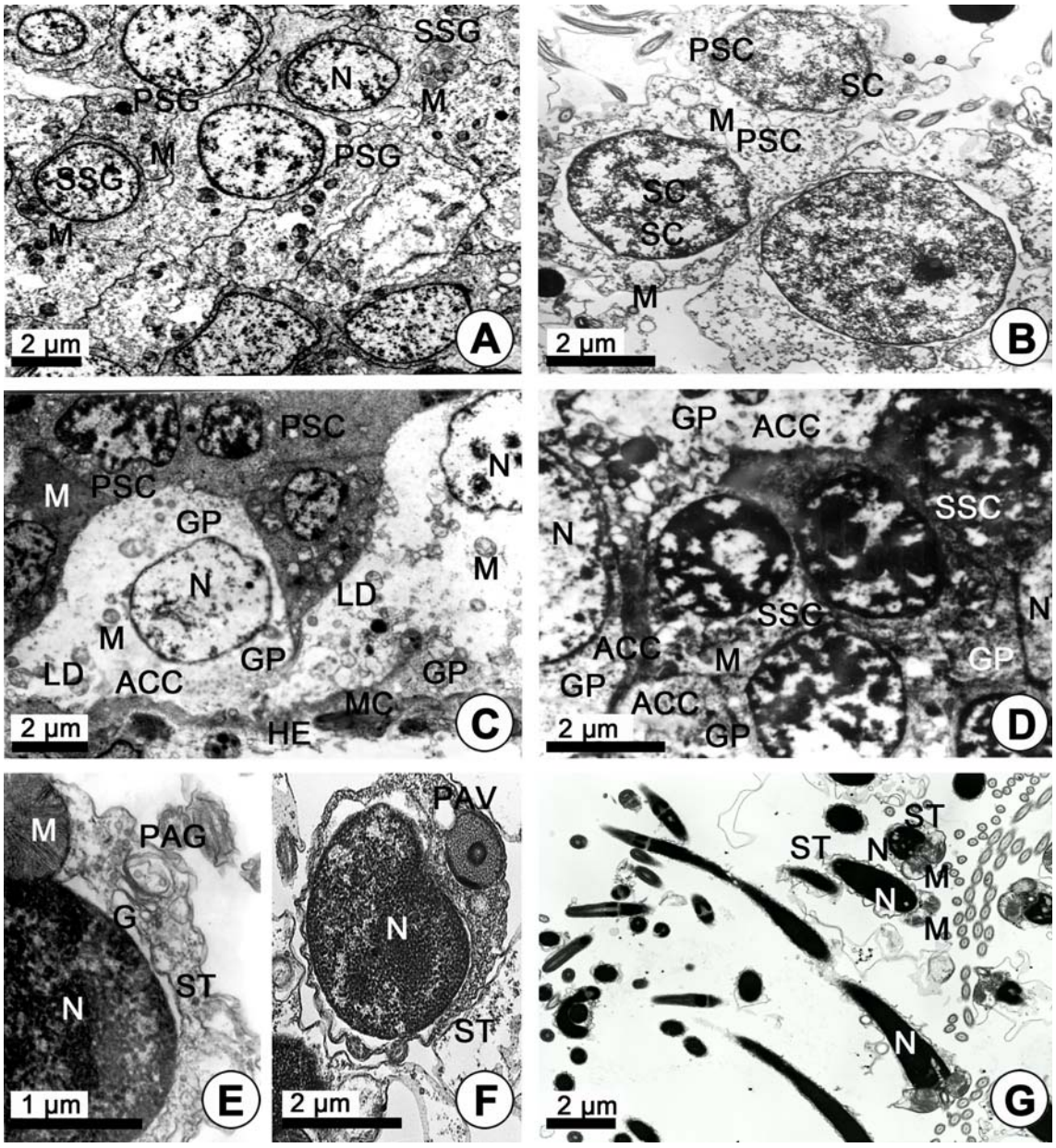

Fig. 1. Transmission electron micrographs of spermatogenesis and spermiogenesis in male *Gomphina veneriformis*. **A,** The primary spermatogonia (PSG), and the secondary spermatogonia (SSG). Note a nucleus (N) containing chromatins and several mitochondria (M) in the cytoplasm. **B.** The primary spermatocytes (PSC). Note several synaptonemal complexes (SC) in the nucleus during the prophase of the primary maturation division and mitochondria (M) in the cytoplasm. **C.** The primary spermatocytes (PSC) and the accessory cells (ACC), Note glycogen particles (GP), lipid droplets (LD), and the mitochondria (M) in the cytoplasm of the accessory cell and primary spermatocyte (PSC). **D.** Spermatids (ST) and accessory cells (ACC), Note high electron dense heterochromatin in the nucleus (N) of the spermatocytes (SSC) and glycogen particles (GP) in the accessory cells. **E.** A spermatid (ST) in the early stage of differentiation during spermiogenesis. Note the Golgi complex (G) near the proacrosomal granule (PAG) just in front of the nucleus of the spermatid (ST). **F.** A spermatid (ST) in the same stage. Note a proacrosomal vesicle just before the nucleus (N) of the spermatid (ST). G. Spermatids. Note the elongated nucleus (N) and two spherical mitochondria (M) of the spermatid (ST).

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 800-1,000 Å. 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. Structure and morphology of the testis

In general, morphology and structure of the testis of *G. veneriformis* is similar to those for the testes in bivalves. The testes are irregularly arranged from the subregions of mid-intestinal gland s in in visceral cavity to reticular connective tissues of the foot. The testis is a diffuse organ consisting of numerous branching acini containing differentiating sperm in a variety of stages. Each acinus is subdivided into a variable 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 central lumen. At the same time, accessory cells are closely associated with testicular developmental stages except the mature spermatozoon. The accessory cells are distributed within acinal subcompartments in close association with developing germ cells. However, the outer wall of the acinus is characterized by a discontinuous layer of thin squamous myoepithelial cells that forms a partial barrier between the hemocoels and developing germ cells (Figs. 1C, D).

Even though the gonads are getting mature, both sexes can not be distinguishable easily by external features (visual observation) because both mature ovary and testis are milky white in color (same color). At this time, if the testis is slightly scratched with a razor, mature sperm readily flow out after spermatozoa are discharged. Therefore, the sexes of the clams can be easily distinguished by dissection 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 occurs in the acini of the testis. Spermatogenesis follows a centripedal pattern; all developmental stages are present, including, spermatogonia, primary and secondary spermatocytes, spermatids and (4) spermatozoa.

3. Spermatogonia

The primary spermatogonia are located along the internal wall of the acini. In the first layer, spermatogonia are sometimes present near the accessory cells. Spermatogonia are approximately 6 - 7 μm in size and outlines of the cells are more or less oval-shaped. Each of the primary spermatogonia contains a large nucleus with chromatin. The nucleus is about 2 to 4 μm in size and has a patch appearance due to the arrangement of chromatin. Numerous mitochondria are distributed in the cytoplasm. The primary spermatogonia divide mitotically to produce the secondary spermatogonia, which are smaller cells with smaller nuclei compared to the primary spermatogonia. At this time mitochondria are present in the cytoplasm (Fig. 1A).

4. Spermatocytes

The secondary spermatogonia differentiate into primary spermatocytes by mitotic division. The nucleus of the primary spermatocyte contains slightly denser chromatin than that of the spermatogonium. The pachytene stage is characterized by the presence

of synaptonemal complexes in the nucleus The synapto- nemal complexes in the nucleus appear in the prophase during the first maturation division. Several mitochondria appear in the cytoplasm, the cytoplasm reduced, so the nucleo-cytoplasm ratio increases. Cellular outlines are oval in shape(Fig. 1B). At this time, several accessory cells are present near several secondary spermatocytes, and a large quantities of glycogen particles, several mitochondria and a few lipid droples are present in the cytoplasm of the accessory cells. In particular, the accessory cells are distributed within acinal subcompartments in close association with developing germ cells. However, the outer wall of the acinus is characterized by a discontinuous layer of thin squamous myoepithelial cells that forms a partial barrier between the hemocoels and developing germ cells (Fig. 1C).

The secondary spermatocytes are rarely observed, probably due to the rapidity of the second meiotic division of the primary spermatocytes. They are irregular in shape and range from about 3-4 μm in size. The nucleus is spherical in shape and possess scatered chromatin forming a network. The heterochromatin materials in the nucleus of the secondary spermatocyte are denser and more highly concentrated than those of the primary spermatocytes. At this time, several accessory cells are present near several secondary spermatocytes, several mitochondria and a large quantity glycogen particles are present in the cytoplasm of the accessory cells (Fig. D).

5. Spermatid

After the secondary meiotic division, the secondary spermatocyte is transformed into the spermatids. For convenience, spermiogenesis has been divided arbitrarily into two stages: the early and late stages.

Spermatids, in the early stage of spermiogenesis, are oval in shape and range from approximately 3 - 4 μm in diameter. Their round nuclei contain a scatered marginal heterochromatin and several mitochondria appear in the cytoplasm of the spermatid (Fig. 1E). Based on the characteristics of cell organelle differentiation, acrosome formation of the spermatids during spermiogenesis can be simply divided into four phases: the Golgi, cap, acrosome, and maturation. The morphology of the spermatid changes gradually during the Golgi phase in the differentiation of the spermatid. At this 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. 1E). After all the morphology of the spermatid nucleus is gradually elongated, and a few proacrosomal granules in the cytoplasm become a proacrosomal vesicle appearing just in front of the nucleus of the spermatid (Fig. 1F). However, the shape of the nucleus is modified and becomes greatly elongated. At this time the mitochondria move to a position just behind the nucleus (Fig. 1G). During the cap phase, the morphology of the spermatid nucleus is gradually elongated and a proacrosomal vesicle is modified and becomes a cap-like acrosomal vesicle on the nucleus (Fig. 2A). During the acrosome phase, a cap-like acrosomal vesicle is modified and become an acrosome (Fig. 2A). The acrosome lying on the sperm nucleus is long cone in shape. It is composed of two long electron-opaque parts (basal rings) and two long electron-lucent part. In particular, the axial filaments are present in the acrosomal lumen between two electron opaque and lucent parts. At this time, cross-sectioned the upper part and the basal ring part of the acrosomes show a headphone-like in shape and a dough nut-like in shape, respectively (Figs. 2C, 2D-a, b).

Subacrosomal granular materials are present in the subacrosomal space between the anterior invaginated part of the nucleus and the part of beginning of the axial filament in the acrosome (Fig. 2A). Cross-sectioned the upper part and the middle part of the nuclei show small and large cake-like in shapes, respectively (Figs. 2C, D-c, d). Thus, it is easy to distinguish the cross-sectioned sites by morphological differnces. In the basal part of the nucleus, mitochondria lie close to the nuclear envelope. Four spherical mitochondria surround the centrosome: they constitute the middle piece of the spermato- zoon (Figs. 2B, C, E). At this time, of the two centrioles

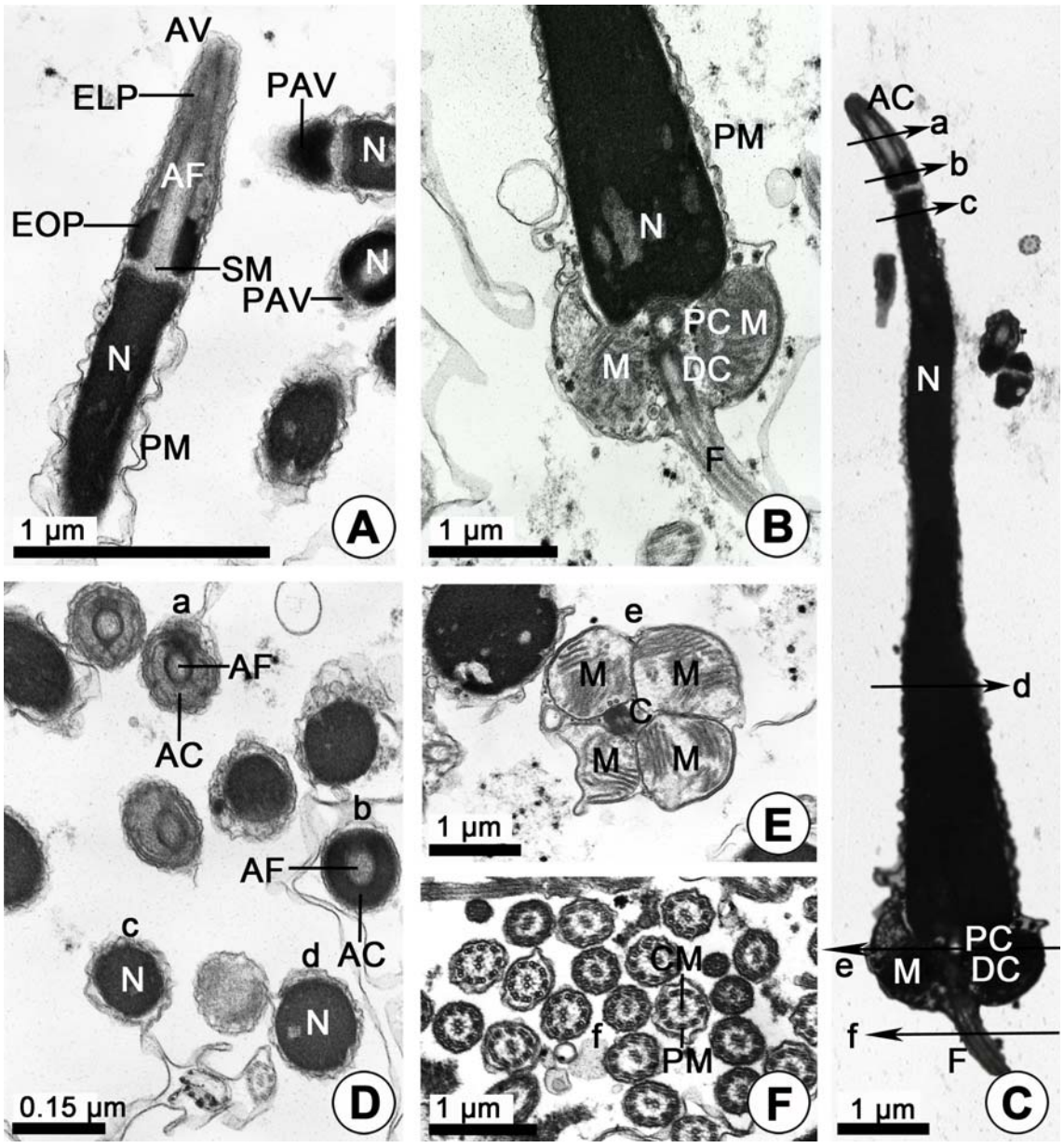

Fig. 2. Electron micrographs of spermiogenesis in male *Gomphina veneriformis*. **A.** the formation of a modified acrosome being composed of electron opaque part (EOP) and electron lucent part (ELP) of the spermatid in the late stage of differentiation. Note the axial filament (AF) in a long acrosomal vesicle (AV) modified from a proacrosomal vesicle (PAV) on the long elongated nucleus (N) in the plasma membrane (PM), and subacrosomal materials (SM) in the subacrosomal space. **B.** A spermatozoon after spermiogenesis. Note the proximal centriole (PC), distal centriole (DC), and the mitochondria (M) in the midpiece beneath the sperm nucleus (N) in the plasma membrane, and a tail flagellum (F). **C.** A completed spermatozoon. Note the sperm head part (the axial filament in the acrosome, subacrosomal materials in the subacrosomal space, two acrosomal parts and two nuclear parts cross sectioned (a, b, c, d parts), sperm midpiece part (the proximal (PC), distal centrioles (DC) and mitochondria (M)) cross sectioned (e part), sperm tail part (a flagellum) cross sectioned (f part). **D.** a, b, c, d parts (cross-sectioned) of the acrosome and sperm nucleus. Note a part (cross sectioned) showing axial filament and the acrosome, b part showing an axial filament and the acrosome, c and d parts showing the nuclei. **E.** e part (cross sectioned) of the sperm midpiece. Note four mitochondria surrounding a pair of centrioles. **F.** f part (cross sectioned) of a tail flagellum. Note d part (cross sectioned) of the axoneme showing a 9+2 structure (a pair of central microtubules and nine pair of peripheral microtubules).

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 (Figs. 2B, C, E). The cytoplasm is greatly reduced, and so the nucleo-cytoplasm is high.

6. 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 (Fig. 2C). At this time, a cross-sectioned tail flagellum 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. 2C, F). The morphology of the sperm nuclear type and the acrosomal shape of this species are of a cylindrical type and a long cone shape, respectively. The head of each spermatozoon is approximately 9.54 μm in length, including the nucleus (about 7.80 μm in length) and the acrosome (about 1.13 μm in length), and the tail is approximately 40 - 45 μm in length (Figs. 2C).

Discussion

In general, morphology and ultrastructure of the testis of *G. veneriformis* is similar to those of other bivalves, and spermato- genesis of this species shows similar phenomena to those of other bivalves (Park *et al*., 2002, 2003). The processes of spermatogenesis occur through the interaction between germ cells and accessory cells in the acini. During spermatogenesis, the nucleus of the primary spermatocyte contains slightly denser chromatin than that of the spermatogonium. In this study, the synaptonemal complexes in the nucleus of the primary spermatocyte appeared in the pachytene stage in the prophase during the first maturation division. It was easy to observe that in the primary spermatocyte, the pachytene stage is characterized by the presence of synaptonemal complexes in the nucleus. The main processes of spermatogenesis are morhological changes from spermatogonia to spermatozoa (including the formation of the acrosome). At this time the accessory cells, which is attached to germ cells in the acinus, provide nutrients for germ cell development.

Eckelbarger *et al*. (1990) described two types of accessory cells in an three species of *galeommatoidid* bivalves,: 1) the first, a pleomorphic "follicle cell" was confined to the outer wall of the testicular acinus and contained glycogen and lipid deposits; 2) the second, a phagocytic cell was scattered throughout the acinus in close association with developing sperm. Thus, two accessory cells have been described from the testis of a single bivalve species.

To the date, there have been some studies with respect to the presence or absence of cell junctions between accessory cells and developing germ cells (sperm) in bivalves. Accessory cells were observed to be connected to adjacent germ cells via desmosomes in the testes of *Scrabicullaria plana* (Sousa *et al*., 1989) and Crassostrea virginica (Eckelbarger and Davis, 1996), Tight junctions were reported in *Pecten maximus* (Dorange and Le Pennec, 1989), and septate juctions in *Mytilus edulis* (Pipe, 1987). These findings show that the interaction between germ cells and accessory cells. In this study, we have easily observed accessory cells near germ cells during spermatogenesis in the acini. In particular, a large quantity of glycogen particles and a few lipid droplets were easily observed in the accessory cells during spermatogenesis, therefore, it is assumed that the accessory cells may play some role in nutrition to developing germ cells. However, we could not find any desmosome (or junction) between the accessory cell and the germ cell in the acinus in *G. veneriformis*. Henceforth, it should be investigated the ultrastrucure of the accessory cells for the observations of these findings mentioned above in detail.

Gaulejac *et al*. (1995) reported that in *Pinna nobilis* the auxiliary cells with pseudopodia-like projections

between germ cells appeared to serve a resorptive function near the end of spermatogenesis. One common feature of these accessory cells is that they appear to have a phagocytic or resorptive function. In the present study, during the period of germ cell degeneration. we could not find any evidence associated with a phagocytic or resorptive function of the accessory cells reported by Gaulejac *et al*. (1995). Henceforth, we should investigate a function of the accessory cell during the period of germ cell degeneration.

Recently, some author (Sousa *et al*., 1989) suggested that the Golgi complex may form only a single acrosomal vesicle in a manner similar to other molluscs. Some authors reported that 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). Eckelbarger and Davis reported that proacrosomal vesicles were common in *C. virginica* spermatocytes. In this study, however, in *G. veneriformis*, a proacrosomal vesicle appeared spermatid stage, and this vesicle developed to an acrosomal vesicle and became an acrosome. 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.

Sperm ultrastructure of bivalves is considered a valuable tool in assessing taxonomic and phylogenetic problems within the bivalvia (Franzén, 1970, 1983; Popham, 1979; Eckelbarger *et al*., 1990), and it is now widely used in taxonomic analyses (Healy, 1995): for example, 1) acrosomal morphology, and 2) the number of mitochondria in the sperm midpiece. Regarding molluscan sperm morphology, Franzén (1970) divided molluscan sperm morphology into two types: 1) the primitive type found in species that perform external fertilization, and 2) the modified type found in internal fertilization species. Also, Verdonk *et al*. (1983) divided sperm morphology into four types: 1) primitive, 2) modified, 3) biflagellate, and 4) aflagellate types. In addition to the primitive type and partially modified type of molluscan sperm, the biflagellate type is seen in the triploid *Corbicula fluminea* and *C. leana* in natural populations (Komaru and Konishi, 1996; Komaru *et al*., 1997). The aflagellate type was also found in a few crustaceans (Kim, 2001). In this study, *G. veneriformis* undergoes external fertilization and possesses the primitive spermatozoon type, unlike the modified type found in most gastropods that perform internal fertilization.

Compared with the morphologies of the sperm nuclei in in Veneridae species (Table 1), the types of the sperm nuclei are of the long cylinder type in *G. veneriformis*, the cylinder type in *M. lusoria*, *C. sinensis* and *Notochione jedoensis*, the bend cylinder type in *R. philippinarum*, *Saxidomus purpuratus*, *Dosinorbis japonicus* and *Mercenaria stinpsoni* (Kim, 2001). The angles of the sperm nucleus in the family Veneridae ranged from 0° (*Notochione jedoensis*) to 80° (*Mercenaria stinpsoni*), and that of *G. veneriformis* was slightly bend (5°). On the whole, the morphologies of the sperm nuclei in Veneridae species varied with the species. The sizes of sperm nuclei in Veneridae species ranged from 2.13 μm (*C. sinensis*) to 7.80 μm in diameter (*G. veneriformis*). Therefore, of 8 species, in particular, the size of sperm nucleus of *G. veneriformis* was the longest in length (Table 1). Thus, although several species belong to the same Veneridae, the morphologies of the sperm nucleus can not be used in taxonomic analyses because of irregular morphological characteristics of the nuclei (Healy, 1995).

Of sperm ultrastructures of bivalves, acrosomal morphology are now widely used in taxonomic analyses (Healy, 1995) because its morphological characteristics has been used to organize bivalve subclasses (Popham, 1979), Recently, Healy (1989) showed that different subclasses of bivalves each have unique acrosomal morphologies.

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. Hodgson and Bernard

(1986) reported that the acrosomes can be distinguisable by acrosomal morphologies.

To the date, we have investigated the morphologies of the acrosomes in many families in two subclasses (Pteriormorphia and Heterodonta). According to the results investigated, the acrosomes can be distinguisable those of the genuses and families by the morphologies and positions of the acrosomes. The family Veneridae belongs to the subclass Heterodonta. In this study, all species in the subclass Heterodonta in the bivalves have a common structural characteristics of the acrosomal vesicles showing the cone-like in shape, being composed of electron high density (opaque) materials (from the basal ring parts to the lateral part) and electron lucent materials (the apex part) (Hodgson and Bernard, 1986). However, the subclass Pteriomorphia in the bivalves have a common structural characteristics of the acrosomal vesicles showing the cone-like in shape, being composed of electron high density (opaque) materials in all parts (the basal parts, the lateral part, the apex part) (Hodgson and Bernard, 1986).

In this study, *G. veneriformis* belongs to the family Veneridae in the subclass Heterodonta has the acrosomal vesicles showing the long cone in shape, being composed of electron high density (opaque) materials (from the basal ring part to the lateral parts) and electron lucent materials (the apex part) as reported by Hodgson and Bernard (1986). Therefore, our results are coincide with opinions of Hodgson and Bernard (1986).

The acrosomal morphology of the sperm head differs markedly among the species (Popham, 1979). The acrosome can be classified into four shapes: cone, long cone, modified cone, cap, modified cap shapes. The acrosomal morphologies of the sperms in the 8

Table 1. Comparisons of the morphologies and structures of eight species in Veneridae (Kim, 2001)

species	Size of a sperm (㎛) except for a flagellum	Head part							Middle piece	Axial filament	satellite body
		Morphology of the sperm nucleus	nucleus length (㎛)	nucleus width[2)] (㎛)	angle of nuclear	acrosome			no. of mitochondria		
						shape	length (㎛)	width (㎛)			
Ruditapes philippinarium	6.23	bend cylinder	3.36	0.94 (0.28)	26°	modified cone	2.36	0.05	4	filament	absent
Saxidomus prupruatus	4.59	bend cylinder	3.74	0.67 (0.35)	15°	cap	0.39	0.13	5	absent	absent
Meretrix lusoria	2.69	cylinder	1.49	0.99 (0.69)	10°	cap	0.58	0.24	5	absent	absent
Gomphina veneriformis melanaegis	9.54	long cylinder	7.80	1.02 (0.4)	5°	long cone	1.13	0.15	4	filament	absent
Cyclina sinensis	3.42	cylinder	2.13	0.98 (0.71)	9°	cone	0.69	0.19	5	absent	absent
Dosinorbis japonicus	5.21	bend cylinder	3.69	0.95 (0.40)	44°	cone	0.53	0.15	4	absent	absent
Mercenaria stinpsoni	5.15	deep bend cylinder	4.18	1.05 (0.47)	80°	cap	0.52	0.19	4	absent	absent
Notochione jedoensis	3.49	cylinder	2.43	0.96 (0.52)	-	cap	0.44	0.16	4	absent	absent

[1)] ( ) means the minimum width of the nucleus.

Korean species in Veneridae were the cone shapes in *C. sinensis* and *D. japonicus*, and a modified cone shape in *R. philippinarum*, and the cap shapes in *Saxidomus japonicus*, *M. lusoria*, *Mercenaria stinpdoni*, *Notochione jedoensis*.

In the present study, the acrosomal morphology of *G. veneriformis* has a long cone shape as seen in the family Veneridae. Compared with the morphology of the acrosomal vesicles in species of other families, the morphological or phylogenetical characteristics of *G. veneriformis* is the presence of long cone acrosomal vesicles during spermatogenesis. Therefore, we assume that the presence of a special acrosomal vesicle during spermatogenesis could be used as a key characteristic for identification of species of the genus *Gomphina*, as seen in the family Veneridae: *Ruditapes philippinarum*, *Cyclina sinensis*, *Dosinorbis japonicus*.

In addition, of sperm ultrastructures of bivalves, the number of mitochondria in the sperm midpiece are now widely used in taxonomic analyses (Healy, 1995). That is the reason that 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).

Recently, some authors (Chung and Ryou, 2000; Kim, 2001; Chung *et al*., 2006) described that the number of mitochondria in the midpiece of the spermatozoon is four in families Ostreidae, Veneridae, Solenidae, and Corbiculidae, while this number is five in the Arcidae, Mytilidae, Pinnidae, and Veneridae. Kim (2001) reported that the number of mitochondria in the midpiece of the sperms of the species in the family Veneridae are four in *R. philippinarum*, *D. japonicus*, *M. stinppsoni* and *N. jedoensis*, and five in *S. purpuratus*, *M. lusoria* and *C. sinensis* (Table 1).

In the present study, we found that there are four mitochondria in the midpiece of the sperm in *G. veneriformis*. Sometimes, however, within one species, we assume that the number of mitochondria in the midpiece of the sperm show slight differences in number.

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).

Correlations between acrosomal morphology and features of the egg envelope have been proposed (Popham, 1974; Franzén, 1983), but in several bivalve species the evolution of elongated sperm nuclei has been more highly correlated with the evolution of large, yolky eggs (Franzén, 1983). Therefore, it is supposed that the long cylinder type of the sperm nucleus and long cone shape of the acrosme of this species is closely related with the acrosomal reaction for fertilization between the acrosomal structure of the sperm and features of the egg envelope.

In particular, the most consistent feature of the sperm investigated in this work is that they have the axial filament in the acrosome as part of the apparatus making up the acrosome, while the axial rod is not found in this species, as seen in the mid-central line hole of the nucleus and the acrosome of the sperm of *Mytilus coruscus*.

Acknowledgements

The authors are grateful to Dr. Tae Hwan Lee, the University of Michigan, for helpful comments on the manuscript. This research was supported in part by the funds from the Research Projects (2005) of the Fisheries Science Institute, Kunsan National University.

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