Germ Cell Development During Spermatogenesis and Taxonomic Values in Mature sperm Morphology in Male *Argopecten irradians irradians* (Pteriomorphia: Pectinidae) in Southern Korea

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ABSTRACT

Ultrastructural studies of germ cell development during spermatogenesis and taxonomic values in mature sperm morphology of *Argopecten irradians irradians* were investigated by transmission electron microscopic observations. In the early stage of spermatid during spermiogenesis, a few granules and proacrosomal granules are formed by the Golgi complex. In the late stage of spermatid during spermiogenesis, a proacrosomal vesicle becomes an acrosomal vesicle in the acrosome through spermiogenesis. The sperm is approximately 45-48 μm in length including a jar-shaped sperm nucleus (about 1.45 μm long), an acrosome (about 0.34 μm long) and tail flagellum. The axoneme of the sperm tail shows a 9+2 structure. As one of common characteristics of mature sperm morphologies in Pectinidae species in subclass Pteriomorphia, mature spermatozoon consists of the cone-shaped acrosomal vesicle and subacrosomal material on the invaginated jar-shaped nucleus. The acrosomal vesicle of this species is composed of electron high dense opaque part (material) from the base to the tip, as have seen in the species in the subclass Pteriomorphia. Exceptionally, five mitochondria are found in the sperm midpiece of this species, unlike four in most species of Pectinidae in subclass Pteriomorphia. However, the acrosomal vesicle of spermatozoa of *A. irradians irradians* resemble to those of other investigated Pectinidae species in subclass Pteriomorphia. Therefore, we can use sperm morphology as a tool in the resolution of taxonomic relationships within the Pectinidae species. These morphological charateristics of acrosomal vesicle belong to the family Pectinidae in the subclass Pteriomorphia.

Keywords: *Argopecten irradians irradians*, spermiogenesis, germ cell, sperm ultrastructure

INTRODUCTION

Recently, the utrastructure of the testis, spermatogenesis and mature sperm morphology have been decribed in Ostreidae species of bivalve molluscs using both light and electron microscopy (Longo and Dornfield, 1967; Longo and Anderson, 1969; Franzen, 1970, 1983; Baccetti and Afzelius, 1996; Baccetti, 1979; Popham, 1974, 1979; Healy, 1989, 1995; Sousa and Oliveria, 1994; Eckelbarger *et al*., 1990; Eckelbarger and Davis, 1996; Kim, 2001; Kim *et al*., 2010a,b). To date, sperm ultrastructure has been used as a tool in assessing taxonomic problems and phylogenetic relationships in the Metazoa through the use of spermiocladistic analysis (Jamiesen, 1991). In general, it is well-known that bivalve molluscs possess a sperm that is primitive in form (Frazen, 1956), a characteristic of many Metazoa, which discharge sperm directly into the water (Franzen, 1970, 1983). In the sperm ultrastructure and morphology in the

Received: March 22, 2012 ; Accepted: March 29, 2012
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1225-3480/24426

bivalves, there is little variation in the fine structures of the tail and the midpiece but great variation in the form of the nucleus and particularly the acrosome (Popham, 1979). Sperm morphology has been used successfully as an aid in the examination of the phylogeny of bivalvia (Popham, 1979; Bernard and Hodgson, 1985, Healy, 1989, 1995). Kim (2001) suggested that comparative ultrastructure of scallop spermatozoa might also prove taxonomically useful, provided that morphological differences between taxa could be found. However, although there are some information relating to the ultrastructure of scallop sperm (Popham, 1979; Bernard and Hodgson, 1985).

Previously, regarding *A. irradians irradians*, there have been several studies on reproduction aspects, including the reproductive cycle (Oh, 2000; Oh *et al*., 2002), artificial spawning, larval and spat development (Oh *et al*., 2003) and ultrastructure of spermatogenesis (Kim, 2001), comparative spermatozoon morphology and bivalve phylogeny (Popham, 1979), on aspects of aquaculture, including aquaculture (Chew and Fusui, 1993), effect of rearing density in culture cage on growth (Oh *et al*., 2000), seedling production and aquacultur (Oh, 2000) and effect of selected spat on growth (Oh *et al*., 2002a,b), on aspects of ecology, including the influence of experimental water currents (Castagna and Duggan, 1971; Kirby-Smith, 1972), suspension feeding aquaculture system (Kirby-Smith and Barber, 1974; Rhodes and Wildman, 1980), growth (Oh and Jung, 1999; Rines, 1985), growth in three rearing sites (Oh *et al*., 2003), on aspect of ecology, including distribution, habitat and classification (Kwon *et al*., 1993; Min *et al*.. 2004) of *A. irradians irradians*. Although a few studies on reproduction, ecology, aquaculture and classification of *A. irradians irradians* have been carried out already, there are still gaps in our knowledge on reproductive biology. Little information is available on ultrastructural characteristics of germ cell development during spermatogenesis and its taxonomic values of mature sperm morphology of this species. Of sperm ultrastructures, the acrosome of the sperm shows morphological diversity in the bivalve sperm, and hence it may be the most useful structure in assessing phylogenetic relations (Franzén, 1970, 1983). Therefore, the acrosomal morphology of the sperm in A. irradians iradians should be compared with the species of Pectinidae in the subclass Pteriomorphia. In case of aquaculture scallop imported from China, *A. irradians irradians*, little information is available on morphological characteristics of mature spermatozoa associated with taxonomic problems and phylogenetic relationships of this species. Therefore, it is very important to clarify some morphological differences between Pectinidae species by ultrastructures of mature spermatozoa such as the nucleus, acrosomal vesicle, the number of mitochondria, the appearance or lack of an axial rod and satellite fibres. The pupose of the present study is to describe and clarify the some ultrastructural charateristics of acrosome formation during spermiogenesis of *A. irradians irradians*. and then to discuss taxonomic values of mature sperm ultrastructures within Pectinidae and other families in Pteriormorphia.

MATERIALS AND METHODS

1. Sampling

Specimens of *Argopecten irradians iradians* were collected monthly in the scallop aquafarm in Myungsapo, Geoje island of for one year from January to December, 2007. A total of 158 male individuals were used for transmission electron microscope observations.

2. Transmission electron microscope observation

For transmission electron microscope observations, excised pieces of the gonads were cut into small pieces and fixed immediately in 2.5% paraformaldehydeglutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 2 hours at 4℃. 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 80-100 nm. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrate, and observed with a JEM 100 CX-Ⅱ (80-KV) electron microscope.

RESULTS

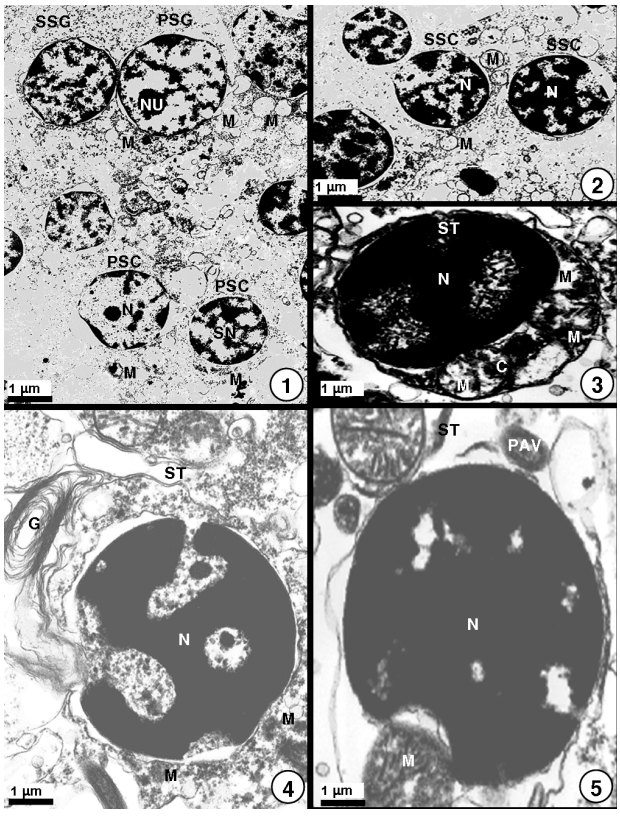
1. Ultrastructure of germ cells during spermatogenesis

Based on germ cell development during testicular development in the acini of the testis and morphologiacal characteristics of germ cell differentiations by electron microscopic observations, in general, spermatogenesis can be divided into five stages: (1) spermatogonium, (2) Primary spermatocyte, (3) secondary spermatocyte, (4) spermatid, and (5) spermatozoon stages.

Spermatogonial stage: The primary spermatogonia are located along the internal wall of the acini. In the first layer, spermatogonia are some times located near the accessary cells. They are approximately 6.3-7.2 μm in diameter and more or less oval-shaped. Each of spermatogonia contains a large nucleus with chromatin. 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, several mitochondria are present in the cytoplasm (Fig. 1).

Spermatocyte stage: The secondary spermatogonia differentiate into primary spermatocytes (5.3-6.4 μm in diameter). For convenience, the spermatocyte can be divided into two stages: the primary and secondary spermatocytes.

The nucleus (3.3-3.7 μm in diameter) of the primary spermatocytes contains chromatin slightly denser than that of the secondary spermatogonium. The synaptonemal complexes in the nucleus appear in the prophase during the first maturation division. Several mitochondria appear in the cytoplasm (Fig. 1). Primary spermatocytes develop into the secondary spermato cytes by the first meiotic division. At this time the heterochromatin materials in the nucleus of the secondary spermatocyte are denser and more highly concentrated than those of the primary



Figs. 1-5. Transmission electron micrographs of spermatogenesis in male *Argopecten irradians irradians*.

Fig. 1. Primary (PSG), secondary spermatogonia (SSG) and the primary spermatocyte (PSC). Note primary spermatogonia (PSG) containing nucleolus (NU) in the nucleus (N) and several mitochondria (M) and secondary spermatogonia (SSG), and several synaptonemal complexes (SN) in the nucleus of the primary spermatocytes (PSC).

Fig. 2. Secondary spermatocytes (SSC). Note the spermatocytes containing nuclei (N) and several mitochondria (M) in the cytoplasm.

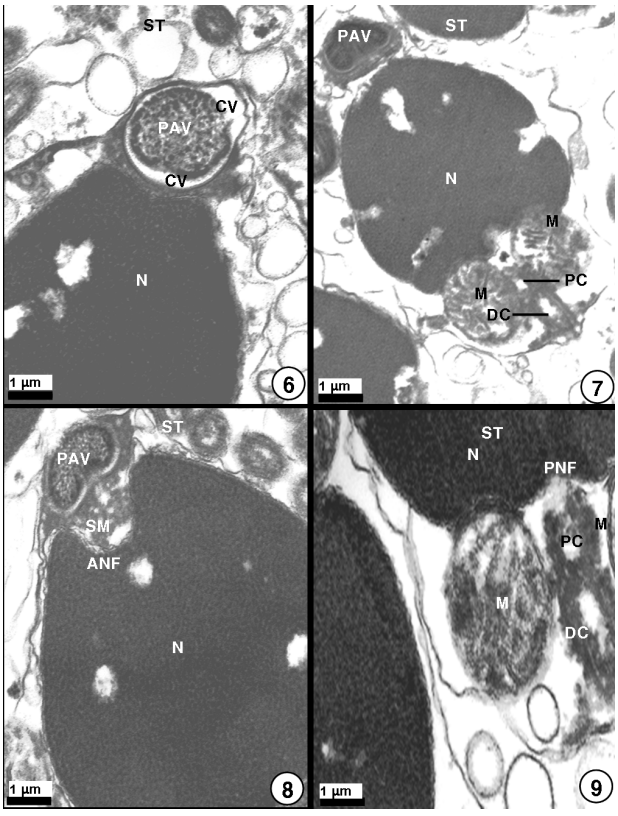
Fig. 3. A spermatid (ST) in the early stage during spermiogenesis. Note the nucleus (N) of the spermatid, centrioles (C) and several mitochondria (M) in the cytoplasm.

Fig. 4. A spermatid (ST). Note the Golgi complex (G), and granules on the nucleus (N) of the spermatid and several mitochondria (M) in the cytoplasm.

Fig. 5. A spermatid (ST) in the early stage during spermiogenesis. Note a proacrosomal granules and a proacrosomal vesicle (PAV) on the nucleus (N) of the spermatid and mitochondria (M) in the early stage of the spermatid.

spermatocytes. At this stage, several mitochondria are present in the cytoplasm of the secondary spermatocytes (Fig. 2).

Spermatid stage: After the secondary meiotic division, the secondary spermatocytes transform into the spermatids with electron-dense heterochromatin materials in the nucleus. For convenience, spermiogenesis can be divided into two stages: the early and late stage of spermiogenesis. In the early stage of spermiogenesis, spermatids are approximately 3.5-4.0 μm diameter, the nucleus is spherical and occupies the center of the cell. Nuclei of spermatids (about 2.8-3.0 μm diameter) contain electron-dense heterochromatin materials, and several mitochondria appear in the cytoplasm of the spermatid (Fig. 3). During spermiogenesis, the morphology of the spermatid nucleus changes gradually during the differentiation of the spermatid. After all, the morphologies of the spermatid nuclei are slightly elongated, and the Golgi complex appears on the spermatid nucleus, and then a few granules or the proacrosomal granule, which are found near the Golgi complex in the cytoplasm of the spermatid, form a proacrosomal vesicle (Fig. 4). 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 proacrosomal vesicle. In the late stage of spermiogenesis, a single proacrosomal vesicle locates on the nucleus of the spermatid (Fig. 5). A single proacrosomal vesicle locates at the presumptive anterior pole of the spermatids. The proacrosomal vesicle, which is surrounded with the membrane, shows initially oval in shape. At this time an oval proacrosomal vesicle is filled with high electron dense granules surrounded by the membrane, and then large cavity is formed between granular materials in the proacrosomal vesicle and the surrounded membrane on the nucleus of the spermatid (Fig. 6). Thereafter, as an appearance of large cavity between them disappears, the triangular proacrosomal vesicle is attached to the thick membrane located on the nucleus of the spermatid in the late stage of spermiogenesis, and the proximal centriole and distal



Figs. 6-9.

Fig. 6. An oval proacrosomal vesicle (PAV) and the spermatid during spermiogenesis. Note a proacrosomal vesicle (PAV) containing large cavity (CV) and the membrane on the spermatid nucleus (N).

Fig. 7. A proacrosomal vesicle (PAV) on the nucleus (N) of a spermatid (ST) in the late stage during spermiogenesis. Note a proacrosomal vesicle (PAV) on the nucleus, proximal (PC), distal centrioles (DC) and two spherical mitochondria (M) beneath the elongated nucleus (N).

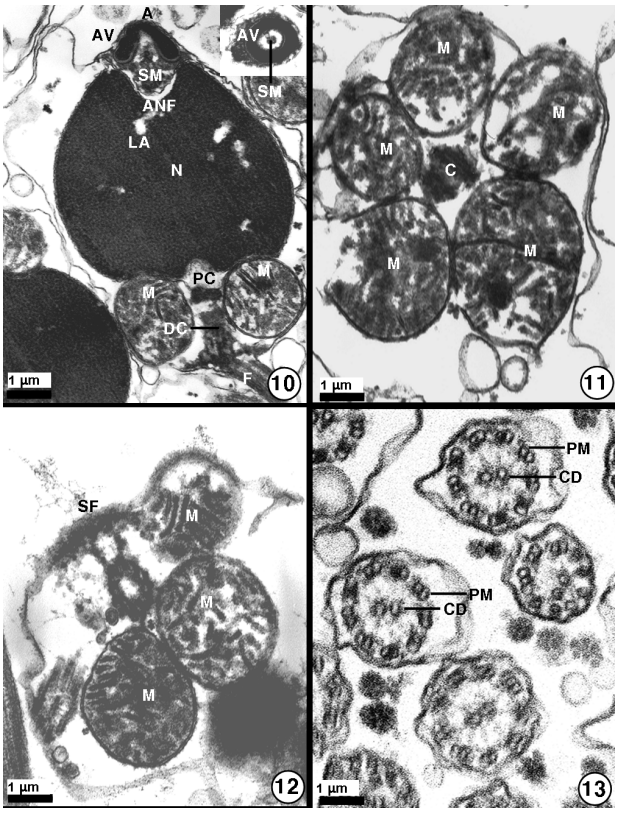
Fig. 8. A proacrosomal vesicle (PAV) on the nucleus (N) of a spermatid (ST) during spermiogenesis. Note a proacrosomal vesicle (PAV) and subacrosomal materials (SM) in the invaginated anterior nuclear fossa (ANF).

Fig. 9. The nucleus (N) of a spermatid (ST) in the late stage during spermiogenesis. Note posterior nuclear fossa (PNF) in the nucleus, the proximal (PC), distal centrioles (DC) and two spherical mitochondria (M).

cenriole appear near the spherical mitochondria in the midpiece part beneath the nucleus (Fig. 7). As further development of the proacrosomal vesicle proceeds, the morphology of the proacrosomal vesicle changes to an laterally elongated form, and the invagination at the central part of the basal region of the proacrosomal vesicle occur toward the upper part. At the same time, after the nuclear invagination occur at the the

anterior nuclear fossa of the nucleus, subacrosomal materials are filled between the proacrosomal vesicle and the nucleus (Fig. 8). At the process of the final spermatid development during spermiogenesis, the proacrosomal vesicle develops into the acrosomal vesicle. At this time two components of the acrosomal vesicle are recognized: the ultrastructure of the acrosomal vesicle and deposit of subacrosomal materials. The processes of acrosomal vesicle formation showed very complex. The acrosomal vesicle is membrane bound, consequently, become the cone in shape by way of various morphorphological changes and invaginations from initial oval shape and measures about 0.34 μm long. In Fig. 10, the acrosome is composed of the acrosomal vesicle and subacrosomal material. In particular, an acrosomal vesicle showing the cone-like in shape, being composed of high electron-dense opaque part (region) at the acrosomal membrane from the base to the tip: the apex part and the right and left lateral parts of basal rings are composed of electron-dense opaque part (region). The acrosomal vesicle is occupied by subacrosomal material which comprises embedded in a coasely granular matrix. and subacrosomal materials are filled in the subacrosomal space between the acrosomal vesicle and the nucleus. In the midpiece part of spermatid, five spherical mitochondria surrounding a pair of centrioles appear, the cristae of each mitochondrion are randomly arranged. and the proximal centriole and distal centriole also appear beneath the posterior nuclear fossa of the nucleus. In particular, the proximal centriole lies at 90° to the sperm longitudinal axis or the distal centriole near the posterior nuclear fossa of the nucleus. However, the axial rod can not find in the subacrosomal material in the acrosomal vesicle. And then a flagellum formed from the distal centriole near the satellite fibers (Fig. 9).

Spermatozoa: The morphology of the spermatozoon has a primitive type. The sperm is approximately 45-48 μm long including a jar-shaped sperm nucleus and tail flagellum (about 44-46 μm long). The sperm nucleus (about 1.45 μm in length) is the jar in shape,



Figs. 10-13.

Fig. 10. A mature spermatozoon in the mature stage. Note the acrosome (A) being composed of the acrosomal vesicle (AV) and subacrosomal materials (SM) near the anterior nuclear fossa (ANF) and lacunae (LA) in the nucleus, proximal (PC) and distal centrioles (DC), two spherical mitochondria (M) in the sperm midpiece and a flagellum (F).

Fig. 11. A cross sectioned sperm midpiece. Note a pair of centrioles (C) surrounded with five mitochondria (M).

Fig. 12. Satellite fibers (SF) in the sperm midpiece. Note satellite fibers (SF) near mitochondria (M) in the sperm midpiece.

Fig. 13. A cross sectioned tail flagellum of mature sperm. Note the axoneme showing a 9+2 structure (a pair of central doublets (CD) and nine pair of peripheral microtubles (PM)).

and the acrosome is the cone in shape. An acrosome (0.48 μm long and 0.30 μm width) on the nucleus is composed of the acrosomal vesicle (being composed of the cone-shaped basal rings) and subacrosomal materials embedded in a coasely granular matrix. An acrosomal vesicle shows high electron dense opaque part (region) at the acrosomal membrane from the base to tip, as seen in Pectinidae of the subclass Pteriomorphia. Anteriorly the jar-shaped nucleus is

deeply invaginated, and then the space is occupied by subacrosomal material (Fig. 10). Posteriorly, the centrioles appear. Posterior to the nucleus is the midpiece. This region consists of five spherical mitochondria surrounding a pair of triplet substructure centrioles. The cristae of each mitochondrion are randomly arranged (Fig. 11), 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. However, the axial rod can not find in the subacrosomal material in the acrosomal vesicle, but the satellite fibers are present near the distal centriole (Fig. 12). And then a flagellum formed from the distal centriole near the satellite fibers. The flagellum is composed of a 9 + 2 substructure axoneme enclosed by the plasma membrane and measures approximately 44-46 μm long: that is, nine peripheral microtubules surrounding a central pair of sigle doublets (Fig. 13).

DISCUSSION

1. Spermatogenesis

In general, the process of spermatogenesis in *A. irradians irradians* showed similar phenomena to those of other bivalves and more specifically of Pteriormorphia species (Eckelbarger *et al*., 1990; Eckelbarger and Davis, 1996; Gaulejac *et al*., 1995; Chung *et al*., 2007, 2010; Kim *et al*., 2010a,b). Spermatogenesis occurred through the interaction between germ cells in the acini. To date, many studies have shown that most bivalves have primitive spermatozoa (Franzen, 1983; Chung *et al*., 2007; Kim *et al*., 2010a,b), typical bivalves which release their gametes into the surrouding water (Franzen, 1983; Gaulejac *et al*., 1995; Eckelbarger *et al*., 1990; Kim, 2001; Chung *et al*., 2007; Kim *et al*., 2010a,b). In this study, during the process of spermatogenesis of germ cells, the synaptonemal complexes in the nucleus of the primary spermatocyte appeared in the pachytene stage in the prophase during the first maturation division. Commonly, it was easy to observe that the pachytene stage in the primary spermatocyte was characterized by the presence of synaptonemal complexes in the nucleus. Recently, Sousa *et al*., (1989) suggested that the Golgi complex may form only a single acrosomal vesicle in a manner similar to other molluscs. As seen in the spermatid stage in *Perna perna* (Bernard and Hodgson, 1985) and *Pecten maximus* (Dorange and Le Pennec, 1989), in this study, a proacrosomal vesicle appeared in the spermatid stage, and this vesicle developed to an acrosomal vesicle and became an mature acrosome. In this study, morphologies and sizes of the sperm acrosomes in Pectinidae species showed similar morphological and ultrastructural characteristics, as seen in other family species. In general, the acrosome could be classified into five shapes: cone, long cone, modified cone, cap, modified cap shapes. In this study, of Pectinidae species, the acrosomal morphologies of the sperms of *A. irradians irradians* were a cone shape, however, of the species in Veneridae, *C. sinensis* and *Phacosoma japonicus* were the cone shape, and *Saxidomus japonicus, Meretrix lusoria*, *Notochione jedoensis* were the cap shape (Kim, 2001).

To date, many researchers (Longo and Dornfeld, 1967; Longo and Anderson, 1969; Baccetti and Afzelius, 1976; Bacetti, 1979; Bernard and Hodgson, 1985; Healy, 1989, 1988, 1995) described on the modes of acrosmal developments and formations in bivalves and gastropods associated with external or internal fertilizations. Healy (1989) reported that acrosomal development in the Mollusca can be classified into three modes. The first mode of the acrosomeal development could be viewed that numerous electron-dense proacrosomal vesicles, which are at first formed by the Golgi complex, become later the definitive acrosomal vesicle by the fusion of several Golgi-derived vesicles. This pattern belongs to the first mode of the acrosomeal development. the first mode of the acrosomeal developmen is commony observed in numerous other externally-fertilizing bivalves and other invertebrate. The second mode of acrosomal development could be viewed that the initial definitive acrosomal vesicle is formed by the Golgi complex of a large receptacle vesicle, and then the growth of which is achieved through fusion of

small vesicles budded from the Golgi cisternae, or from materials channeled directly from the cisternae. The second mode is commony observed in internally fertilizing molluscs (higher prosobranch gastropods, opisthobranch and pulmonate gastropods) and in many other internally fertilizing animal groups. The third mode of acrosomal development could be viewed as a variation on the first mode of acrosomal development. In case of the freshwater clam *Neotrigonia* (Unionoidea), acrosome formation is formed through production of multiple proacrosomal vesicles which do not fuse into a single acrosomal vesicle. Therefore, this pattern could be viewed as a variation on the first mode of acrosomal development. In this study, in the early stage of the spermatid during spermiogenesis, a few electron-dense granules, which are at first formed by the Golgi complex, became later the definitive acrosomal vesicle by the fusion of small proacrosomal granules. Therefore, of three modes of acrosomal development and formation, the processes of *Argopecten irradians iradians* belongs to the first mode of acrosomal development and formation.

2. Taxonomic value of sperm morphology and ultrastructure

Ultrastructures of the spermatozoa in 5 subclasses of the bivalves have some differences in the morphologies and positions of the acrosomes of the sperms (Popham, 1979). Recently, sperm ultrastructures of bivalves and acrosomal morphology and the number of mitochondria at the midpiece of the sperm are widely used in taxonomic analyses (Healy, 1995; Popham, 1979). In general, the sizes of sperm nuclei could not be used in taxonomic analyses because morphological characteristics of sperm nuclei were irregular and varied with the species in the family (Healy, 1995). As shown in Figs. 6-8, in the formation of the proacrosomal vesicle in *A. irradians irradians* (Pectinidae), a large oval proacrosomal vesicle, which are filled with a number of rough and coarsed granules (high electron dense material), is covered with the membrane surrounded with the edge of the round cavity, which is formed in the oval proacrosomal vesicle. In this study, the ultrastructures of spermatozoa of *A. irradians irradians* and *P. yessoensis* showed some similarities in proacrosomal vesicle formation and nuclear morphological changes between these two species in Pectinidae in subclass Pteriormorphia (Kim, 2001), unlike the processes of proacrosomal vesicle formation during spermiogenesis of *Chlamys* spp. (Kim, 2001). In this study, in case of *A. irradians irradians*, the large cavity in the proacrosomal vesicle which is filled with relatively coarsed granules, and granular materials of an oval proacrosomal vesicle is uplifted from the center part of the basal region into the forward direction, as have reported in the proacrosomal vesicle of *P. yessoensis* (Kim, 2001). However, on the course of proacrosomal vesicle formation of Chlamys species, a similar phenomenon such as the formation of large cavity in the proacrosomal vesicle were not found (Kim, 2001). Therefore, in case of Pectinidae species such as *A. irradians irradians*, *P. yessoensis*, and *Chlamys* species, from the processes of the proacrosomal vesicle formations until the acrosomal vesicle formation, each species showed slightly differences in the species or the genus. Exceptionally, the invagination processes of the nuclei during spermiogenesis in all species in Pectinidae were very similar, as have reported in *P. yessoensis* and *C. farreri farreri* (Kim, 2001).

According to the results investigated on the processes of proacrosomal vesicle formation and nuclear morphological changes, In particular, of Pectinidae species, *A. irradians irradians* and *P. yessoensis* showed very similar between two genera (Argopecten and Patinopecten) in Pectinidae (subclass Pteriomorphia), unlike the genus *Chlamys*. However, Kim (2001) reported that the processes of the proacrosomal vesicle formation in *C. farreri farreri* and *C. swiftii* showed some similar patterns.

To date, we have investigated the morphologies of the acrosomes in many families in two subclasses (Pteriormorphia and Heterodonta) by electron microscopic observations. Therefore, we can confirm that the Pteriomorphia and Heterodonta can be separated according to acrosome morphology and position. Regarding subclasses Pteriormorphia and

Heterodonta, Hodgson and Bernard (1986) described the morphological and characteristics of the acrosomal vesicles of the Pteriomorphia and the Heterodonta as follows: the Pteriomorphia all have acrosomes that are in the shape of a cone, albeit of varing dimensions, and that contain electrone-dense opaque material from the base to the tip.

However, he described that the acrosomes of the Heterodonta are characterized by restriction of the electron-dense opaque material (region) to the base or lateral regions, with such area joined by the acrosome membrane only.

Taxonomically, *A. irradians irradians* belongs to Pectinidae in the subclass Pteriomorphia. In this ultrastructural study for taxonomic confirmation of this species, this species in Pectinidae have a common structural characteristics of the acrosomal vesicles showing the cone-like in shape, being composed of electron-dense opaque part (region) from the base to the tip. As it were, the apex part and the right and left lateral parts of basal rings are composed of electron-dense opaque part (region) (Hodgson and Bernard, 1986). From the results of observations by the ultrastructural characteristics of the acrosomal vesicle, we can confirm that *A. irradians irradians* belongs to the Pteriomorphia containing the acrosomal vesicles showing the cone shape, as reported by Hodgson and Bernard (1986). Therefore, our results coincide with those observed by Hodgson and Bernard (1986). For the identification of *A. irradians irradians* in Pectinidae in the subclass Pteriomorphia, we assume that sperm ultrastructure of the acrosomal vesicle during spermatogenesis of bivalves can be considered a valuable tool in assessing taxonomic and phylogenetic problems.

In this study, this species have some special characteristics during the process of acrosome formation: After granules or a proacrosomal granule are formed by the Golgi complex, a proacrosomal vesicle on the nucleus of spermatid is formed by the proacrosomal granule. These similar phenomena were only found in the process of the proacrosomal vesicle formation (the acrosomal vesicle formation) of *Patinopecten yessoensis* in Pectinidae of (sublass Pteriomorphia). Even though it is the same Pectinidae species, the process of acrosomal vesicle formation of *A. irradians irradians* vary with those of *Chlamys* spp. and other Pectinidae species except for that of *P. yessoensis* (Kim, 2001). Therefore, we assume that the presence of a special acrosomal vesicle formation during spermatogenesis can be used as a key characteristic for identification of species of the genus Argopecten as have seen in the family Pectinidae.

Although *A. irradians irradians* belongs to Pectinidae in subclass Pteriomorphia, the axial rod was not found in this species, unlike Ostreidae species (*Crassostrea gigas* and *C. nipponica*) and Mytilidae species (*Mytilus coruscus*) in the subclass Pteriomorphia contained the axial rod in subacrosomal materials in the subacrosomal space (Kim, 2001; Kim *et al*., 2010a,b). All family species in the subclass Heterodonta do not have satellite fiber near the distal centriole in the sperm midpiece. However, commonly, satelite fibers were found in all species of Pectinidae, Ostreidae and Mytilidae and Arcidae in subclass Pteriomorphia. Recently, the number of mitochondria in the sperm midpiece have been now widely used in taxonomic analyses because the number of mitochondria in the sperm midpiece tends to be stable within any given family or superfamily (Healy, 1989, 1995). To date, many authors (Chung and Ryou, 2000; Kim, 2001; Chung *et al*., 2007; 2010) described that the number of mitochondria in the midpiece of the spermatozoon were four in families Ostreidae and Pectinidae in the subclass Pteriomorphia, and also Veneridae, Solenidae and Corbiculidae in the subclass Heterodonta. However, these numbers are five in Arcidae, Mytilidae, Pinnidae in the subclass Pteriomorphia, and in part of Veneridae in subclass Heterodonta.

However, even though it is the same species, sometimes, the number of mitochondria in the sperm miedpiece showed some differences containing four or five. Occasionaly, within the same family, the number of mitochondria in the sperm midpiece of most species in Pectinidae in subclass Pteriomorphia are four, however, exceptionally, five in *A. irradians irradians* in the same Pectinidae in subclass Pteriomorphia.

Thus, the number of the mitochondria in the sperm midpiece showed slight differences in number and varied with the species or with the species within the same family. Thus, the number of mitochondria in the sperm midpiece were not concerned with the subclasses, but in general, their numbers were concerned with family or superfamily (Healy, 1995). Therefore, our results on the number of mitochondria coincide with Healy's opinion (1995).

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Emeritus, Ee-Yung Chung of Kunsan National University for helpful revision and comments on the manuscript. Thanks are also to two reviewers for helpful comments.

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