JFMSE, 31(6), pp. 1649~1660, 2019.
수산해양교육연구, 제31권 제6호, 통권102호, 2019.
www.ksfme.or.kr
https://doi.org/10.13000/JFMSE.2019.12.31.6.1649

Spermatogenesis and Taxonomic Value of Sperm Ultrastructure in *Coecella chinensis* (Mesodesmatidae), and Sperm Ultrastructural Differences between Mesodesmatidae and Mactridae (Bivalvia, Heterodonta)

Jin Hee KIM† · Ki-Young LEE

†Marine Eco-Technology Institute(researcher) · Kunsan National University(professor)

퇴조개 *Coecella chinensis*의 정자 미세구조에 기초한 정자형성과정과 분류학적위치 및 개량조개과(Bivalvia, Heterodonta)와의 정자 미세구조 차이

김진희† · 이기영

†해양생태기술연구소(연구원) · 군산대학교(교수)

Abstract

Spermatogenesis and taxonomic value of sperm ultrastructure in *Coecella chinensis* (Mesodesmatidae) were investigated by transmission and scanning electron microscopy, and sperm ultrastructural differences were compared between Mesodesmatidae and Mactridae species(Bivalvia, Heterodonta). Ultrastructure of the acrosomal vesicles and nuclei of Mesodesmatidae and Mactridae species are very similar. In *C. chinensis*, the basal rings of the acrosomal vesicle of spermatozoa are composed of an electron-dense opaque part at the lower portion and an electron-dense lucent part at the upper portion. In Mactridae species, acrosomal vesicles of spermatozoa are similar. However, two different heights of acrosomal vesicle basal rings were observed in spermatozoa between the two families in subclass Heterodonta. In the case of Mesodesmatidae (*C. chinensis*), basal ring height is high toward the upper direction, whereas those of Mactridae species is low toward outer directions. Thus, height basal rings of the acrosomal vesicles of spermatozoa can be used as a key characteristic for identification of Mesodesmatidae and Mactridae species. There are four or five mitochondria in the sperm midpiece of the observed Mesodesmatidae species, whereas there are only four in previously analyzed Mactridae species. Consequently, number of mitochondria may be useful for taxonomic analysis at the family or superfamily levels.

Key words : *Coecella chinensis*, Spermatogenesis, Sperm ultrastructure, Mesodesmatidae, Mactridae

Ⅰ. Introduction

Aside from their morphological and ecological significance, Mesodesmatidae and Mactridae (subclass Heterodonta) are taxonomically important groups of bivalve molluscs. It is well known that bivalve spermatozoa ultrastructure might be related to bivalve systematics(Popham, 1974, 1979); sperm ultrastructure has long been viewed as a tool for assessing phylogenetic relationships in metazoans through spermiocladistic analysis(Jamieson, 1991). To date, comprehensive studies on bivalve

† Corresponding author : +82 (51) 611-5396, jhkim@marine-eco.co.kr

spermatogenesis in Korea have been restricted to several species in Mactridae(Longo and Anderson, 1969; Chung and Ryou, 2000; Lee et al., 2008), Veneridae (Kim, 2001; Chung et al., 2001; Kim et al., 2010; Kim and Kim, 2011), Hiatellidae (Kim, 2001), Solenidae(Chung and Park, 1998; Kim, 2001), and Myidae(Kim, 2001), Corbiculidae (Jun et al., 2009) in subclass Heterodonta, and Mytilidae (Longo and Dornfeld, 1967; Kim, 2001; Kim et al., 2010b), Pectinidae(Kim, 2001), and Ostreidae (Kim et al., 2010a; Son et al., 2014) in subclass Pteriomorphia.

Sperm morphology has been successfully used in phylogenetic examination, and knowledge of bivalve spermatozoa ultrastructure could be useful for taxonomic purposes(Franzen, 1977, 1983; Popham, 1979). Although there have been some studies regarding reproduction cycle(Kim et al., 2013), there have been no studies on the ultrastructure of spermiogenesis and mature sperm morphology of *Coecella chinensis* (Mesodesmatidae). In particular, taxonomic value of mature sperm morphology of *C. chinensis* has not been reported.

Sperm acrosome ultrastructure revealed morphological diversity in bivalves; thus, acrosomes may be useful for assessing phylogenetic relationships(Franzén, 1956). Special substructures in sperm acrosomal vesicles were used to organize the families into subclasses(Popham, 1979), and Healy (1989) reported that different bivalve subclasses have unique acrosomal morphologies. Therefore, sperm acrosomal morphology in *C. chinensis* can be compared with that of previously analyzed Mactridae species, which are closely related to Mesodesmatidae and also belong to subclass Heterodonta. In addition, the number of mitochondria in the sperm midpiece tends to be stable within families or superfamilies(Healy, 1989, 1995). Therefore, the number of mitochondria in the sperm midpiece of this species may be phylogenetically informative and should therefore be compared with those of family Mactridae.

In addition to ultrastructure of germ cells during spermatogenesis, mature sperm morphology should be studied to elucidate ultrastructural characteristics. To date, this is the first study to describe taxonomic value of mature sperm morphology and compare sperm ultrastructural differences of Mesodesmatidae and Mactridae species. In particular, sperm ultrastructure revealed some special features of the acrosomal vesicles of mature sperm ultrastructure and the number of mitochondria in the sperm midpiece for phylogenetic and taxonomic analyses of Mesodesmatidae and Mactridae species. Therefore, the primary purpose of the present study was to describe some ultrastructural characteristics of the acrosomal vesicle and the other parts of spermatozoa during spermatogenesis, and to confirm the possibility of the use of acrosomal vesicle ultrastructural characteristics and the number of mitochondria in the sperm midpiece for phylogenetical classification of *C. chinensis* (Mesodesmatidae) in subclass Heterodonta. In addition, the secondary purpose of this study was to compare and clarify differences in sperm ultrastructure between Mesodesmatidae and Mactridae species (Heterodonta) for could yield taxonomic value.

Ⅱ. Materials and methods

1. Sampling and transmission electron micrographs

30 samples of *C. chinensis* (Mesodesmatidae) were collected monthly in the intertidal zone of

Changsondo, Namhae-gun, Gyeongsangnam-do, Korea, from January to December 2010. 50 male *C. chinensis* individuals of a total collected specimens from April to June were used for transmission electron microscope(TEM) and scanning electron microscope(SEM) observations.

For Mactridae species (*Mactra veneriformis* and *M. chinensis*), data from a previously published paper(Kim and Yoo, 2002) were used for phylogenetical classification analyses of sperm ultrastructure.

2. TEM observations

For TEM observations, ultrathin sections of Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 microtome and LKB ultramicrotome at a thickness of approximately 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.

3. SEM observations

A drop of sperm suspension was placed on a cover glass, prefixed with 2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.5) at 4°C for 15 min, postfixed with 1% OsO4 for 10 min, and then rinsed with cacodylate buffer. The specimens were dehydrated in a graded ethanol series, critical point dried, coated with gold, and observed under an SEM (ISI-SS4D).

Ⅲ. Results

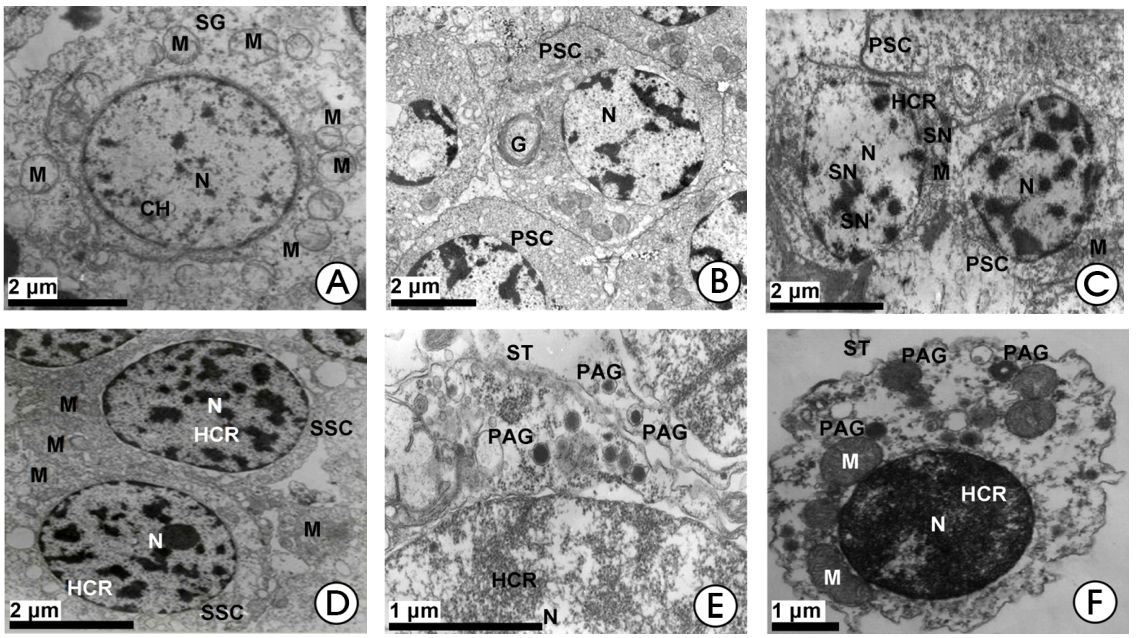
1. Germ cell differentiation during spermatogenesis

Spermatogenesis in this species occurs in numerous acini of the testis, similar to those of other bivalve species(Kim, 2001). permatogenesis in the acini of the testis can be divided into four stages as follows: (1) spermatogonia, (2) spermatocytes, (3) spermatids, and (4) spermatozoa.

Spermatogonia: As observed in other bivalves, spermatogonia are located along the internal wall of the acini. The sizes of spermatogonia are 6.4–7.4 μm in diameter, each with a spherical nucleus that is approximately 3.6 μm in diameter. The nucleus contains small clumps of electron-dense chromatin, and several small mitochondria and rough endoplasmic reticulum are present in the cytoplasm. The spermatogonia have a large nucleus with a more granular electron-dense nucleoplasm, whereas their cytoplasm is largely devoid of organelles except for scattered mitochondria[Fig. 1A].

Spermatocytes: The spermatogonium develops into primary spermatocytes by mitotic division. At this stage, primary and secondary spermatocytes are present in the acinus wall. Primary spermatocytes are slightly smaller cells (approximately 6.0–6.2 μm in diameter) that are distinguished by nuclei (approximately 3.4 μm in diameter) with more abundant and slightly dark-staining heterochromatin. The nucleus of the primary spermatocyte is similar in shape and size to that of the spermatogonium; but the nucleolus is no longer prominent, and the chromatin is distributed in the nucleus at this stage, and their cytoplasm is largely devoid of organelles except for the Golgi complex and several mitochondria[Fig. 1B].

In the first meiotic, primary spermatocytes prophase are distinguished within the germinal layer of the testis, and zygotene/pachytene spermatocytes contain nuclei with higher condensed chromatin and synaptonemal complexes.



[Fig. 1] Transmission electron micrographs of spermatogenesis in male *Coecella chinensis* (Mesodesmatidae). **A**. A spermatogonium, with chromatin in the nucleus and mitochondria, and endoplasmic reticulum and vacuoles in the cytoplasm. **B**. Primary spermatocytes, with a large nucleus, Golgi complex, and several mitochondria in the cytoplasm. **C**. Primary spermatocytes in the first meiotic prophase stage (zygotene/pachytene stage), with several synaptonemal complexes in the nucleus and several mitochondria in the cytoplasm. **D**. Secondary spermatocytes, with several heterochromatin materials in the nucleus and several mitochondria in the cytoplasm; **E, F**. Spermatids after secondary meiotic division, with heterochromatin material in the nucleus and several proacrosomal granules in the cytoplasm. Abbreviations: G, Golgi complex; HCR, heterochromatin; M, mitochondrion; N, nucleus; PAG, proacrosomal granule; PSC, primary spermatocyte; SG, spermatogonium; SN, synaptonemal complex; ST, spermatid.

The synaptonemal complexes in the nucleus appear in the prophase during the first maturation division. Cellular outlines are oval shape, and several chromatin materials appear in the nucleus [Fig. 1C]. Primary spermatocytes differentiate into secondary spermatocytes by meiotic division of primary spermatocytes. Secondary spermatocytes are hardly observed because of the rapid first meiotic division of primary spermatocytes at this stage. Secondary spermatocytes are irregular in shape and range from approximately 4.4–5.5 μm in diameter. Spherical nuclei (approximately 3.2 μm in diameter) possess scattered chromatin that form a network. Secondary spermatocytes were frequently observed undergoing meiotic division, and the sizes of secondary spermatocytes become smaller than those of the primary spermatocytes. At this time, electron-dense heterochromatin appears in the nuclei of secondary spermatocytes[Fig. 1D].

Spermatids: During testicular development, secondary spermatocytes develop into spermatids by the secondary meiotic division. For convenience, spermiogenesis can be divided into early and late stages. In the early spermatid stage, spermatids

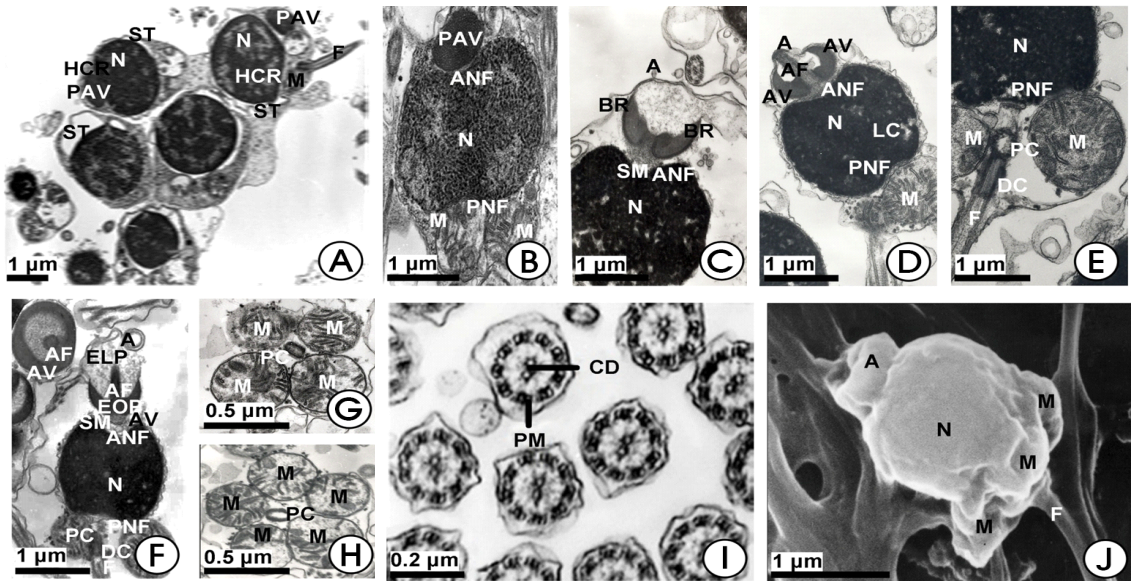
(approximately 3.5–4.0 μm in diameter) are oval in shape, and the nucleus is typically spherical or oval in shape and in the center of the cell. At this stage, spermatid nuclei (approximately 2.4–2.6 μm in diameter) contain electron-dense heterochromatin in the nucleus, and the cytoplasm contains mitochondria and several small proacrosomal granules[Fig. 1E].

In the late spermatid stage during spermiogenesis, the spermatid nucleus morphology gradually changes during spermatid differentiation, and the spermatid nuclei become slightly narrowed (approximately 2.0 μm in diameter). At this time, small proacrosomal granules, which are formed by the Golgi complex in the cytoplasm, migrate to the anterior end of the spermatid nucleus, while mitochondria move to just behind the nucleus[Fig. 1F].

Several small proacrosomal granules mix with each other and become a larger proacrosomal vesicle, which is located on the anterior nuclear fossa of the spermatid nucleus[Fig. 2A]. Thereafter, a full-grown proacrosomal vesicle is present on the anterior nuclear fossa of the nucleus, while several mitochondria move near the posterior nuclear fossa of the spermatid nucleus[Fig. 2B].

Then, a proacrosomal vesicle forms an acrosomal vesicle. At the beginning of acrosomal formation, acrosomal vesicle formation is very complex: a single acrosomal vesicle, which is located at the presumptive anterior pole of the spermatids, is initially oval shaped. The right and left basal rings of the acrosomal vesicle appear at the lower part of the acrosomal vesicle. The acrosomal vesicle begins to invaginate on the nuclear surface, forming the characteristic hollow subacrosomal space. At this time, the acrosomal vesicle (approximately 0.85 μm long) is occupied by subacrosomal material, which is embedded in a coarsely granular matrix, and forms a membrane that is bound near the anterior nuclear fossa[Fig. 2C]. Thereafter, the right and left basal rings of the acrosomal vesicle are composed of electron-dense opaque material at the lower part. However, the upper part (about 50%) of the acrosomal vesicle is composed of electron-lucent materials. Finally, the acrosomal vesicle morphology becomes modified cap-shaped. At the same time, the axial filament, which is present at the center of the acrosomal vesicle on an anterior nuclear fossa, is easily observed. In particular, irregular electron-lucent lacunae are present in the nucleus, several small mitochondria fuse to form large spherical mitochondria under the posterior nuclear fossa, and a flagellum is located under several spherical mitochondria. The midpiece of a maturing spermatozoon contains the distal centriole and an adjacent proximal centriole under the posterior nuclear envelope at a 90° angle[Fig. 2D, 2E].

During the late stage of the spermatid, the nucleus becomes smaller (approximately 1.29 μm long), the nuclear contents continue to condense, and there is continued loss of cytoplasm by sloughing. At the same time, the number of mitochondria become reduced, but the size is increased in the midpiece of spermatid, and the larger mitochondria form a close association with the nucleus. The mitochondria come to occupy the end of the cell opposite to the acrosome, hereafter forming the sperm midpiece under further development occurs. Simultaneously, the tail appears and develops from the distal centriole; the proximal centriole appears between the nucleus and the distal centriole. However, satellite fibers, which are found in several families in subclass Pteriomorphia, are not found near the distal centriole, which is common in subclass Heterodonta[Fig. 2E].



[Fig. 2] Transmission and scanning electron micrographs showing spermatogenesis in male *Coecella chinensis* (Mesodesmatidae). **A**. Several spermatids and proacrosomal vesicle formation, with heterochromatin material in the nucleus and a proacrosomal vesicle on the nucleus. **B**. Early stage of the spermatid during acrosome formation, with a proacrosomal vesicle on the anterior nuclear fossa and several mitochondria under the posterior nuclear fossa. **C**. The right and left basal rings of the acrosomal vesicle on the anterior nuclear fossa, with subacrosomal materials (SM) in the space between the acrosomal vesicle of the acrosome and anterior nuclear fossa in the nucleus. **D**. Acrosome formation of the acrosomal vesicle and axial filament on the anterior nuclear fossa of the nucleus, with an acrosomal vesicle composed of electron-dense opaque material (the right and left basal rings of the acrosomal vesicle showed stretching toward the upper direction; however, the upper part of the acrosomal vesicle showed electron-dense lucent material). In addition, several lacunae and mitochondria appear near the posterior nuclear fossa. **E**. The midpiece of a maturing spermatozoon, with a proximal centriole and a distal centriole between two spherical mitochondria under the posterior nuclear fossa of the nucleus, and the appearance of a flagellum. **F**. A complete spermatozoon and cross-section of the axial filament in the acrosomal vesicle of the sperm, with the acrosomal vesicle composed of electron-dense opaque and electron-dense lucent parts, subacrosomal materials, a proximal centriole, and a distal centriole near the nucleus, mitochondria, and a flagellum; these traits were observed in other species of subclass Heterodonta. **G, H**. Cross-sections of midpieces of mature sperm, with four or five mitochondria surrounding centrioles in the sperm midpiece. **I**. Cross-sectioned spermatozoa flagella, with axonemes showing 9+2 structure, which means there are nine pairs of peripheral microtubules and a pair of central doublets. **J**. A scanning electron micrograph of a complete spermatozoon, with the sperm head (an acrosome and the nucleus), midpiece (mitochondria and centrioles), and tail (a flagellum). Abbreviations: AF, axial filament; ANF, anterior nuclear fossa; AV, acrosomal vesicle; BR, basal ring; CD, central doublet; DC, distal centriole; ELP, electron-dense lucent part; EOP, electron-dense opaque part; F, flagellum; HCR, heterochromatin; LC, lacunae; M, mitochondrion; N, nucleus; PAV, proacrosomal vesicle; PC, proximal centriole; PM, peripheral microtubule; PNF, posterior nuclear fossa; ST, spermatid; SM, subacrosomal material.

Spermatozoa: Mature spermatozoa of this species are approximately 47–50 μm long, and sperm acrosomes contain an acrosomal vesicle (approximately 0.85 μm long). The central hollow of an acrosome contains granular subacrosomal materials, which exist in a coarsely granular matrix. In addition, there is a pair of centrioles surrounded by four or five spherical mitochondria. In particular, during this stage, the acrosomal vesicle is formed by two kinds of electron-dense materials: the lower part of the right and left basal rings of the acrosomal vesicle is composed of high electron-dense opaque materials. Additionally, the right and left basal rings stretch toward the upper direction [Fig. 2D, 2E]. However, the upper part of the acrosomal vesicle contains electron-dense lucent material, which was observed in other species in subclass Heterodonta. Finally, the morphology of the acrosomal vesicle shows a modified cap-shape. The anterior part of the sperm nucleus of this species is somewhat deeply invaginated, in which differs from that of previously analyzed sperm nuclei of Mactridae species.

The shape of the nucleus (approximately 1.29 μm long) is jar-shaped. Additionally, a short, shallow basal invagination appears in the posterior nuclear fossa.

The sperm midpiece includes four or five spherical mitochondria with well-developed cristae [Fig. 2F, 2G, 2H], and the proximal and distal centrioles are found beneath the posterior nuclear fossa in the nucleus: the proximal centriole lies at 90° to the distal centriole and sperm longitudinal axis. The distal centriole is attached to the plasma membrane of the flagellum [Fig. 2E, 2F]. The flagellum is about 45–48 μm long, and exhibits a classic 9+2 microtubular substructure axoneme (that is, nine peripheral microtubules surrounding a central doublet). The flagellum, which originates from the distal centriole, is surrounded at its base by plasma membrane. After spermiogenesis, spermatozoon differentiation is complete.

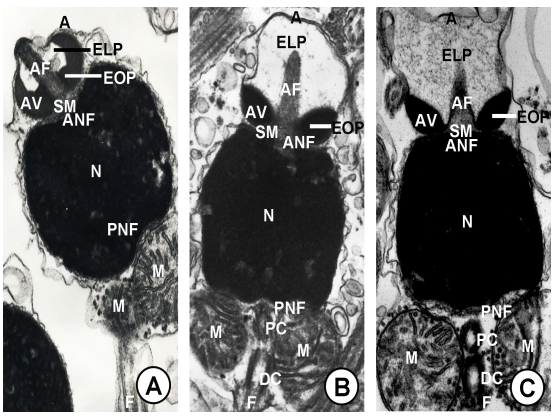
Mature sperm morphology of *C. chinensis*, which was observed by SEM, is primitive, as found in most bivalve species that undergo external fertilization. Mature sperm are approximately 48–51 μm long, and the acrosome (modified cap-shape) is positioned at the top of a jar-shaped nucleus. The size of the sperm head is approximately 2.30 μm long and includes a highly electron-dense nucleus (approximately 1.29 μm long), with a posterior nuclear fossa, and an acrosome (approximately 0.85 μm long). The morphology of the sperm nucleus and acrosome of this species are jar- and modified cap-shaped, respectively. There is also a pair of centrioles surrounded by four or five spherical mitochondria in the sperm midpiece, the cross-sectioned axoneme of spermatozoa showed 9+2 structure (nine pairs of peripheral microtubules and a pair of central doublets) [Fig. 2I], and a flagellum is present in the sperm tail[Fig. 2J].

2. Spermatozoa ultrastructural characteristic comparisons between Mesodesmatidae and Mactridae species

***Coecella chinensis* (Mesodesmatidae)**: Total sperm size of *C. chinensis* is approximately 45–50 μm long. The size of the sperm head of *C. chinensis* is smaller than those of known Mactridae species (*M. veneriformis* and *M. chinensis*). Additionally, the acrosomal vesicle of *C. chinensis* is composed of the right and left basal rings of the acrosomal vesicle. In particular, an axial filament is located in the center of the acrosomal vesicle. In case of *C. chinensis* (Mesodesmatidae), the right and left basal

rings of the acrosomal vesicle of the spermatozoa are composed of electron-dense opaque materials, whereas the upper part of the acrosomal vesicle is composed of electron-dense lucent material. In particular, in the case of the Mesodesmatidae species (*C. chinensis*), the right and left basal rings of the acrosomal vesicle of the spermatozoa stretch toward the upper direction [Fig. 3A]. However, those of Mactridae species stretch toward right and left directions [Fig. 3B, 3C]. The *C. chinensis* nucleus is jar-shaped, whereas that of known Mactridae species are modified cylinder or cylinder in shape. In particular, there are four or five mitochondria in the sperm midpiece of *C. chinensis*. No satellite fibers (located near the distal centriole) were detected, which is common of subclass Heterodonta, and there is one flagellum per spermatozoon.

***Mactra veneriformis* and *M. chinensis* (Mactridae)**: Total sperm sizes (㎛) of two species (*M. veneriformis* and *M. chinensis*) are approximately 50 μm and 50–52 μm long, respectively. In the sperm head of Mactridae species, the acrosomes are composed of the right and left basal rings that stretch toward the right and left directions[Fig. 3B, 3C]. Additionally, the axial filaments appear in the center of the acrosomes. The *M. veneriformis* nucleus is modified cylinder in shape, whereas that of M. chinensis is cylinder in shape. Additionally, in the sperm midpiece, both species have four mitochondria in the sperm midpiece, which was also seen in other Mactra species. However, there are four or five mitochondria in the sperm midpiece of *C. chinensis* (Mesodesmatidae). The satellite fibers, located near the distal centriole and mitochondria, are not detected, which is common in subclass Heterodonta. Additionally, these two Mactridae species have one flagellum per spermatozoon.



[Fig. 3] A schematic diagram of the acrosomes, nuclei, and midpieces of three spermatozoa in two families of subclass Heterodonta (A–C). **A.** Mesodesmatidae (*Coecella chinensis*): the right and left basal rings of the acrosomal vesicle of this family stretch toward the upper direction. **B.** Mactridae (*Mactra veneriformis*): the right and left basal rings of the acrosomal vesicle of this family stretch toward the right and left directions. **C.** Mactridae (*M. chinensis*): the right and left basal rings of the acrosomal vesicle of this family stretch toward the right and left directions. The basal rings of the acrosomal vesicles of two families are composed of electron-dense opaque parts, whereas the upper part of the acrosomes are composed of electron-dense lucent parts. Abbreviations: A, acrosome; AF, axial filament; ANF, anterior nuclear fossa; AV, acrosomal vesicle; DC, distal centriole; ELP, electron-dense lucent part; EOP, electron-dense opaque part; F, flagellum; N, nucleus; M, mitochondrion; PC, proximal centriole; PNF, posterior nuclear fossa.

Ⅳ. Discussion

1. Spermatogenesis

Spermatogenesis and associated ultrastructure of spermatozoa in *C. chinensis* were similar to those of another family in subclass Heterodonta(Hodgson and Bernard, 1986; Eckelbarger et al., 1990; Eckelbarger and Davis, 1996; Kim et al., 2010). In this study, we determined that the morphology of *C. chinensis* sperm nuclei is jar-shaped. Compared with Mactridae species, Mesodesmatidae sperm nuclei are very similar(Kim, 2001). However, sperm nucleus morphology varies by genus in Mactridae (Kim, 2001). Therefore, the sizes of sperm nuclei could not be used in taxonomic analyses, because the morphological characteristics of sperm nuclei are irregular(Healy, 1995). In this study, during spermatogenesis of germ cells, the synaptonemal complexes in the nuclei of primary spermatocytes characterized the pachytene stage of prophase during the first meiotic division. Sousa et al. (1989) suggested that the Golgi complexes may form a single acrosomal vesicle in a manner similar to that in other molluscs. As seen in spermatids of Perna perna (Bernard and Hodgson, 1985) and Pinctada martensii(Kim, 2011), the proacrosomal granule appeared near the Golgi complex in synthetic activity during the early developmental stage of spermatids. Several small electron-dense granules are also observed in the vicinity of proacrosomal granules at this time[Fig. 1E, 1F]. It is probable that the small granules, which are produced by the Golgi complex, fuse to form a single proacrosomal vesicle, as seen in externally fertilizing bivalves (Bernard and Hodgson, 1985; Hodgson and Bernard, 1986).

Numerous studies have shown that all bivalves have primitive spermatozoa(Franzén, 1983), which is typical of animals that release their gametes into the surrounding water(Gaulejac et al., 1995). Spermatogenesis in C. chinensis was observed to be similar to that in other bivalves(Eckelbarger et al., 1990; Eckelbarger and Davis, 1996; Chung and Ryou, 2000; Kim, 2001; Chung et al., 2007, 2010; Kim et al., 2010a, b). In this study, a substructure with a thick trident shape in the acrosomal vesicle of *C. chinensis*(Mesodesmatidae) and two Mactridae species was present in spermatid nuclei. In general, the acrosomal morphologies of spermatozoa in subclasses Heterodonta and Pteriomorphia can be classified into five shapes: cone, long cone, modified cone, cap, and modified cap. In this study, the acrosomal morphology of *C. chinensis* spermatozoa was modified cap-shape. However, in case of Veneridae species (Heterodonta) and Phacosoma japonicus have the cone shape, whereas Saxidomus japonicus, Meretrix lusoria, and Notochione jedoensis have the cap-shape(Kim, 2001). In the late stage of spermiogenesis, sperm ultrastructural characteristics of acrosomal vesicles were clear in *C. chinensis* (Mesodesmatidae) and Mactridae spermatozoa.

2. Taxonomic value of sperm morphology and ultrastructure

Generally, the size of bivalve sperm nuclei is not useful in taxonomic analysis, because the morphological characteristics of sperm nuclei are irregular and vary among the species within families(Healy, 1995). However, with regard to sperm ultrastructure of bivalves in particular, acrosome morphology and number of mitochondria in the midpiece of the sperm are broadly useful for taxonomic analyses(Healy, 1995; Popham, 1979).

To date, acrosome ultrastructure and morphology in many families of the two subclasses, Heterodonta and Pteriomorphia, have been investigated by several researchers. For example, Hodgson and Bernard(1986) reported that mature sperm morphology and ultrastructure can be used for classification and differentiation of genera and families based on acrosomal vesicle morphology and position of species in the subclasses Heterodonta and Pteriomorphia. To classify species based on sperm morphology, we examined some structural and morphological differences in the acrosomal vesicle to determine if this is a valuable tool or classification of *C. chinensis.*

To date, sperm ultrastructural differences of many species in the subclasses Pteriomorphia and Heterodonta have been compared. Regarding the phylogenetic classifications of the bivalve subclass Pteriomorphia, Hodgson and Bernard(1986) reported that, in general, members of this bivalve subclass have common acrosomal vesicular structural characteristics: they are cone-like in shape and are composed of electron-dense opaque material from the base to the tip.

Additionally, Hodgson and Bernard(1986) reported that, in general, members of Heterodonta have right and left parts of basal rings that are composed of electron-dense opaque materials, whereas the upper part of the acrosome is composed of electron-dense lucent materials.

Regarding the acrosomal vesicle composition, the differing electron opacity of materials probably reflect the differing functions of the acrosome during fertilization (Hodgson and Bernard, 1986). In addition, it is assumed that the axial filament is closely related to the acrosomal reaction during fertilization between the acrosomal structure of the sperm and the egg envelope.

Sperm ultrastructural differences comparisons between Mesodesmatidae and Mactridae species (Heterodonta) were studied. Ultrastructure of the acrosomal vesicles and nuclei of Mesodesmatidae and Mactridae species are very similar. In *C. chinensis*, the right and left basal rings of the acrosomal vesicle of the spermatozoon are composed of electron-dense opaque materials, whereas the upper part of the acrosomal vesicle is composed of electron-dense lucent materials. For the known Mactridae species, the same results were observed in the acrosomal vesicles. However, two different stretching directions of the acrosomal vesicles were found in Mesodesmatidae and Mactridae species: in the Mesodesmatidae species (*C. chinensis*), the right and left basal rings of an acrosomal vesicle of the spermatozoa stretch toward the upper direction, whereas those of the Mactridae species stretch toward the right and left directions. Thus, stretching direction of the right and left basal rings of the acrosomal vesicle of the spermatozoa can be used as a key characteristic for identification of Mesodesmatidae and Mactridae species.

In general, an axial filament, which is present and unique in the center of the acrosome, is located in acrosomes associated with fertilization. In particular, an axial filament is detected in the acrosome of Mesodesmatidae(*C. chinensis*) and Mactridae species (*M. veneriformis* and *M. chinensis*). Therefore, it is possible that the axial filament is closely related to the acrosomal reaction that occurs during fertilization between the acrosomal structure of the sperm and the egg envelope.

In this study, the acrosomal vesicle in *C. chinensis* (Mesodesmatidae) showed a modified cap-shape during spermatogenesis. Consequently, we

inferred that the presence of a special acrosomal vesicle during spermatogenesis can be used as a key characteristic for species identification. In particular, if some species have similar acrosomal vesicle characteristics, they may belong to subclass Heterodonta.

3. Number of mitochondria in the sperm midpiece

Healy(1995) reported that, in bivalve sperm ultrastructure characteristics, such as number of mitochondria in the sperm midpiece, could be widely used in taxonomic analyses. That is the reason that the number of mitochondria in the sperm midpiece tends to be stable within any given family or superfamily(Healy, 1989, 1995).

Recently, some researchers(Chung and Ryou, 2000; Kim, 2001; Chung et al., 2007; 2010) described that, in subclass Pteriomorphia, there were five mitochondria in the spermatozoon midpiece in Pinnidae, Arcidae, Mytilidae, and Pteriidae and four mitochondria in Ostreidae, and, in subclass Heterodonta, five mitochondria were observed in most species of Veneridae, Solenidae, and Corbiculidae(Kim, 2001; Chung et al., 2007, 2010). However, the number of mitochondria in the sperm midpiece may differ by species within families. Additionally, within *C. chinensis*, the number of mitochondria in the sperm midpiece showed slight differences in number. In this study, we found that there are four or five mitochondria in the sperm midpiece of *C. chinensis* (Mesodesmatidae, Heterodonta). Therefore, the number of mitochondria in the sperm midpiece was not found to be associated with subclasses, but it may potentially be associated with family or superfamily (Healy, 1995). Therefore, our results on the number of mitochondria are consistent with the findings of Healy(1995).

References

Bernard RTF and Hodgson AN(1985). The fine structure of the sperm and spermatid differentiation in the brown mussel *Perna perna*. South Africa J Zool. 20, 5~9. https://doi.org/10.1080/02541858.1985.11447904

Chung EY and Ryou DK(2000). Gametogenesis and sexual maturation of the surf clam, *Mactra veneriformis* on the west coast of Korea. Malacologia. 42, 149~163.

Chung EY, Kim EJ and Park GM(2007). Spermatogenesis and sexual maturation in male *Mactra chinensis* (Bivalvia: Mactridae) of Korea. Integra. Biosci 11, 227~234. https://doi.org/10.1080/17386357.2007.9647339

Chung EY, Chung CH, Kim JH, Park SW amd Park KH(2010). Ultrastructures of germ cells and the accessory cells during spermatogenesis in male *Gomphina veneriformis* (Bivalvia: Veneridae) on the east coast of Korea. Kor J Malacol. 26, 51~62.

Eckelbarger KJ and Davis CV(1996). Ultrastructure of the gona and gametogenesis in the eastern oyster, Crassostrea virginica. II. Testis and spermatogenesis. Mar Biol. 127, 89~96. https://doi.org/10.1007/BF00993648

Eckelbarger KJ, Bieler R and Mikkelsen PM(1990). Ultrastructure of sperm development and mature sperm morphology in three species of commensal bivalves (Mollusca: Galeommatoidea). J Morph. 205, 63~75. https://doi.org/10.1002/jmor.1052050107

Franzén Å(1956). On spermatogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrate. Zool Bidr Upsala 31, 355~482.

Franzén Å(1970). Phylogenetic aspects of the mophology spermatozoa and spermiogenesis in Baccetti B (ed): "Comparative spermatology". Accad. Natl Dei Lincei, Rome. 573pp.

Franzén Å(1983). Ultrastructural studies of spermatozoa in three bivalve species with notes on evolution of elongated sperm nucleus in primitive spermatozoa. Gamete Res. 7, 199~214.

https://doi.org/10.1002/mrd.1120070302
Gaulejac de J, Jenry M and Vicente N(1995). An ultrastructural study of gametogenesis of the marine bivalve *Pinna nobilis* (Linnaeus, 1758). II. Spermatogenesis. J Mollusc Stu. 61, 393~403. https://doi.org/10.1093/mollus/61.3.393
Healy JM(1989). Spermiogenesis and spermatozoa in the relict bivalve genus Neotrigonia: relevance to trigonioid relationships, particularly Unionoidea. Mar Biol. 103, 75~85. https://doi.org/10.1007/BF00391066
Healy JM(1995). Sperm ultrastructure in in the marine bivalve families Carditidae and Crassatellidae and its bearing on unification of the Crasssatelloidea with the Carditoidea. Zool Sci. 24, 21~28. https://doi.org/10.1111/j.1463-6409.1995.tb00472.x
Hodgson AN and Bernard RTF(1986). Ultrastructure of the sperm and spermatogenesis of three species of Mytilidae (Mollusca, Bivalvia). Gamete Res. 15, 123~135. https://doi.org/10.1002/mrd.1120150204|
Jamieson BGM(1991). Fish evolution and systematics: evidence from spermatozoa. Cambridge Univ. Press, Cambridge. pp. 181~194.
Jun JC, Kim BS, Chung EY, Kim JH, Park GM and Park SW(2009). Spermatogenesis and ultrastructural characteristics of spermatozoon of brackish water diploid clam, *Corbicula japonica* (Bivalvia: Corbiculidae). Dev Reprod. 13, 115~122.
Kim JH, Chung EY, Choi KH, Lee KY and Choi MS(2010a). Ultrastructure of the testis and germ cell development during spermatogenesis in male *Crassostrea gigas* (Bivalvia: Ostreidae) in western Korea. Kor J Malacol. 26, 235~244.
Kim JH, Chung EY, Choi KH, Park KH and Park SW(2010b). Ultrastructure of germcells during spermatogenesis and some characteristics of sperm morphology in male *Mytilus coruscus* (Bivalvia: Mitilidae) on the west coast of Korea. Kor J Malacol. 26, 33~43.
Kim JH(2001). Spermatogenesis and comparative ultrastructure of spermatozoa in several species of Korean economic bivalves (13 families, 34 species). Ph.D. thesis, Pukyung National University 161pp.
Kim JH and MS Yoo(2002). Spermatozoan ultrastructure of 4 species in Mactridae. J. Korean Fish. Soc. 35(5), 504~511.
Kim JH, Sung HK and Lee KY(2011). Germ Cell Differentiations During Spermatogenensis and Taxonomic Values of Mature Sperm Morphology of Pinctada martensii (Bivalvia, Pteriomorphia, Pteriidae). Kor. J. Malacol. 27(3), 273~282.
Kim JH, Son MH, Kang HW and Lee KY(2013). Reproductive cycle of sulf clam, Coecella chinensis (MesodesmatidaeL Bivalvia). Kor. J. Malacol. 29(2), 113~120. https://doi.org/10.9710/kjm.2013.29.2.113
Lee KY, Chung EY and Lee JY(2008). Spermatogenesis and reproductive cycle in male Spisula sachalinensis (Bivalvia: Mactridae) of Korea. Korean. J Malacol. 24, 1~10.
Longo FJ and Dornfeld EJ(1967) The fine structure of spermatid differentiation in the mussel *Mytilus edulis*. J Ultrastruct Res. 20, 462~480. https://doi.org/10.1016/S0022-5320(67)80113-8
Longo FJ and Anderson E(1969). Spermatogenesis in the surf clam *Spisular solidissima* with special reference to the formation of the acrosomal vesicle. J Ultrastruct Res. 27, 435~443.
Popham JD(1974). Comparative morphometrics of the acrosomes of the sperms of externally and internally fertilizing sperms of the sperms of the shipworms (Teredinidae, Bivalvia, Mollusca). Cell Tissue Res. 150, 291~297. https://doi.org/10.1007/BF00220138
Popham JD(1979). Comparative spermatozoon morphology and bivalve phylogeny. Malacol Rev. 12, 1~20.
Sousa M, Corral L and Azevedo C(1989). Ultrastructural and cytochemical study of spermatogenesis in *Scrobicula riaplana* (Mollusca, Bivalvia). Gamete Res. 24, 393~401. https://doi.org/10.1002/mrd.1120240406

• Received : 05 November, 2019
• Revised : 02 December, 2019
• Accepted : 05 December, 2019