Korean Journal of Malacology, Vol. 22(2): 115-124, December 30, 2006

Ultrastructure of Germ Cells during Spermatogenesis and the Reproductive Cycle in Male *Meretrix petechialis* on the West Coast of Korea

Ee-Yung Chung

School of Marine Life Science, Kunsan National University, Gunsan 573-701, Korea

ABSTRACT

Gonad index, spermatogenesis and the reproductive cycle of *Meretrix petechialis* were investigated by cytological, histological observations. Monthly changes in the gonad index coincides the gonadal development. The morphology of the spermatozoon had a primitive type and is similar to that of other bivalves having a short mid-piece with five to six mitochondria surrounding the centrioles. The morphology of the sperm nucleus type and the acrosome shape of this species were cylindrical type and cap-like shape, respectively. The spermatozoon was approximately 40-45 μm in length including the sperm nucleus length (about 1.50 μm), acrosome length (0.60 μm) and tail flagellum. The axoneme of the tail flagellum consisted of nine pairs of microtubules at the periphery and a pair at the center. The axoneme of the sperm tail showed 9 + 2 microtubular arrangement. The spawning period was from June to September and the main spawning occurred from July to August when seawater temperatures were higher than 20°C. The reproductive cycle of this species could be categorized into five successive stages: early active stage (February to March), late active stage (February to May), ripe stage (April to July), partially spawned stage (June to September), and spent/inactive stage (September to February).

Keywords: *Meretrix petechialis*, Spermatogenesis, Reproductive cycle.

Received September 21, 2006; Accepted December 8, 2006
Corresponding author: Chung, Ee-Yung
Tel: +82 (63) 469-4592 e-mail: eychung@kunsan.ac.kr
1225-3480/22203

INTRODUCTION

Meretrix petechialis is one of the important edible bivalves in East Asian countries, including Korea and China. In Korea, this species is mainly found in silty sand in the intertidal of Simpo coastal waters of Korea (Min *et al.*, 2004). Because the recent sharp reduction in the standing stock as a consequence of reckless over harvesting of this clam, it has been denoted a target organism and fisheries resource that should be managed using a more reasonable fishing regime. For the propagation and management of a living natural resource, it is important to understand reproductive biology with regard to spermatogenesis and the reproductive cycle.

Previously there have been many studies of *Meretrix lusoria* on reproductive aspects, including artificial fertilization and development (Choi and Song, 1974), early embryonic development and growth (Choi, 1975; Hur, 1994), reproductive cycle (Lee, 1997), and on ecological aspects, including propagation (Tanaka, 1969), production (Chun *et al.*, 1981); on physiological aspects, including acute toxicity tests on some heavy metals (Ikuta, 1988a, b), effects of some hazardous substances (Lee, 1991) and trematode parasite infection (Chun and Lee, 1976). So far, there have been a few studies of *Meretrix petechialis* on classification (Kwon *et al.*, 1993), on aspect, including habitat and distribution (Min *et al.*, 2004).

Although there have been many studies on reproductive ecology of *Meretrix lusoria*. Little information is available on reproductive biology such

as germ cell differentiation during spermatogenesis and the reproductive cycle of *Meretrix petechialis*.

The results of an ultrastructural study of spermatogenesis of this species will provide important information on its reproductive mechanism. Understanding the reproductive cycle and the spawning period of this species will provide necessary information for age determination and the recruitment period of this population for the management of living natural resource.

Therefore, the purpose of the present study is to understand germ cell differentiation during spermatogenesis, the reproductive cycle with testicular developmental stages and some basic information for the propagation and management in male *Meretrix petechialis,* using cytological and histological methods.

MATERIALS AND METHODS

1. Sampling

Specimens of *Meretrix petechialis* were collected monthly from the intertidal zone at Simpo coastal waters, Gimje of Korea, from January to December, 2002. A total of 259 clams ranging from 65.0 mm to 85.0 mm in shell length was used for the study. After the clams transported alive to the laboratory, the sizes of the specimens were recorded using a Vernier caliper.

2. Gonad Index (GI)

To explore the spawning period, the mean gonad index (GI) of the hard clam was calculated using a modification of Mann's method (1979). Each histological section of gonadal tissue was also examined in detail to assess the stages of gonadal development and was scored on 0-5 scale to describe six stages of gonadal development or maturity: 0 = inactive stage (S0); 1 = spent stage (S1); 2 = early active stage (S2); 3 = late active stage (S3); 4 = partially spawned stage (S4); 5 = ripe stage (S5). The arithmetic mean of the individual scores of the whole samples was recorded as the gonad index (GI) for each month. A total of male individuals was used to calculate the gonadosomatic index (GSI). Monthly changes in the mean GI were calculated using the following equation:

GI = (N x RVS0 + N x RVS1 + N x RVS2 + N x RVS3 + N x RVS4 + N x RVS5) / Total number of clam observed by month

Where, N: number of individuals at a gonadal development stage, RVS: ranking value by stage.

3. Ultrastructure of germ cells by electron microscopic observation

For electron microscope observations, the excised pieces of the testis were cut into small pieces and fixed immediately in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hrs at 4℃ After prefixation, the specimens were washed several times with the same buffer (pH 7.4) for 1 hr at 4℃ and then fixed in 1% osmium tetroxide dissolved in 0.2 M phosphate buffer solution (pH 7.4) for one hr at 4℃ The tissues were then dehydrated in a series of increasing concentrations of ethanol, cleared in propylene oxide and embedded in Epon-Araldite mixture. Ultrathin sections of the Epon-embedded specimens were cut with glass knives using a microtome (Sorvall MT-2) and LKB ultramicrotome (LKB), at thickness of 80-100 nm. The tissue sections were mounted on collodion-coated copper grids, double stained with uranyl acetate and lead citrate, and examined under the JEM 100 CX-2 (80 kv) electron microscope.

4. Histological analysis

A total of 249 males was used for histological preparation of the testes for light microscopic examination, from January to December, 2002. Testicular tissues removed from the shells were preserved in Bouin's fixative for 24 hrs, and then washed with running tap water for 24 hrs. The tissues were then dehydrated in alcohol series and embedded in paraffin mold. The embedded tissues were sectioned at 5-7 μm thickness with a rotary microtome. The sections were mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin and Mallory's triple stain, and examined under a light microscope.

RESULTS

1. Position and morphology of the testis

Meretrix petechialis is a dioecious species. The testis was located between the subregion of the midintestinal glands in the visceral cavity and the reticular connective tissue of the foot. The testis was composed of a number of the acini. Although gonadal maturation progresses the external views of the ovary and testis showed the same color. Therefore, their sexes of the clams could not easily distinguishable by external features. To make certain of location of the testis, I slightly scratched the probable region of the testis with a razor blade. When the testis was cut by the scratching, testicularis fluid containing ripe sperm flowed out from the injury. After discharging spermatozoa, the testis degenerated, and then the sex became difficult to distinguish by the external features.

2. Gonad index (GI) in males

The monthly GI changing patterns in males are shown in Fig. 1. In 2002, the GI increased rapidly from March and reached a maximum (4.50) in June when the seawater temperature rapidly increased. Then, the GI gradually decreased from July to September when relatively high water temperatures were maintained, and spawning occurred continuously. Thereafter, the value temporally reached the minimum (1.0) in October when the spawning was completely finished.

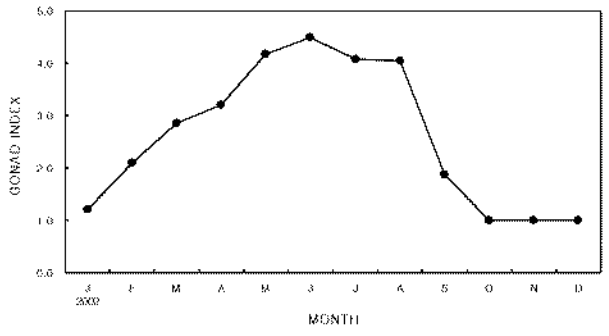

Fig. 1. Monthly changes in the gonad index of *Meretrix petechialis*.

3. Ultrastructure of germ cells during spermatogenesis

Based on the testicular development and morphological characteristics of germ cells, spermatogenesis could be classified into five phases: (1) spermatogonial, (2) primary spermatocyte, (3) secondary spermatocyte, (4) spermatid, and (5) spermatozoon phases.

1) Spermatogonial phase

The primary spermatogonia were located near the auxiliary cells. They were approximately 9-10 μm in diameter and more or less oval shaped. Each of them contained a large nucleus with chromatin. The primary spermatogonia divided mitotically to produce the secondary spermatogonia (Fig. 2A), which had smaller cells and the nuclei than the primary spermatogonia.

2) Primary spermatocyte phase

The secondary spermatogonia differentiated into the primary spermatocytes. The nucleus of the primary spermatocyte contained slightly denser chromatin. The synaptonemal complexes in the nucleus appeared in the prophase during the first maturation division. Several mitochondria appeared in the cytoplasm (Fig. 2B).

3) Secondary spermatocyte phase

The primary spermatocyte developed into the secondary spermatocyte through the first maturation division. The heterochromatin materials in the nucleus of the secondary spermatocyte showed denser and concentrated than that of the primary spermatocyte. In this phase, several mitochondria were present in the cytoplasm (Fig. 2C).

4) Spermatid phase

After the secondary meiotic division, the secondary spermatocyte was transformed into the spermatid with electron dense heterochromatin materials in the nucleus, and several mitochondria appeared in the cytoplasm (Fig. 2D). Spermiogenesis could expediently be divided into four phases, based on the characteristics of cell organelle differentiation; Golgi,

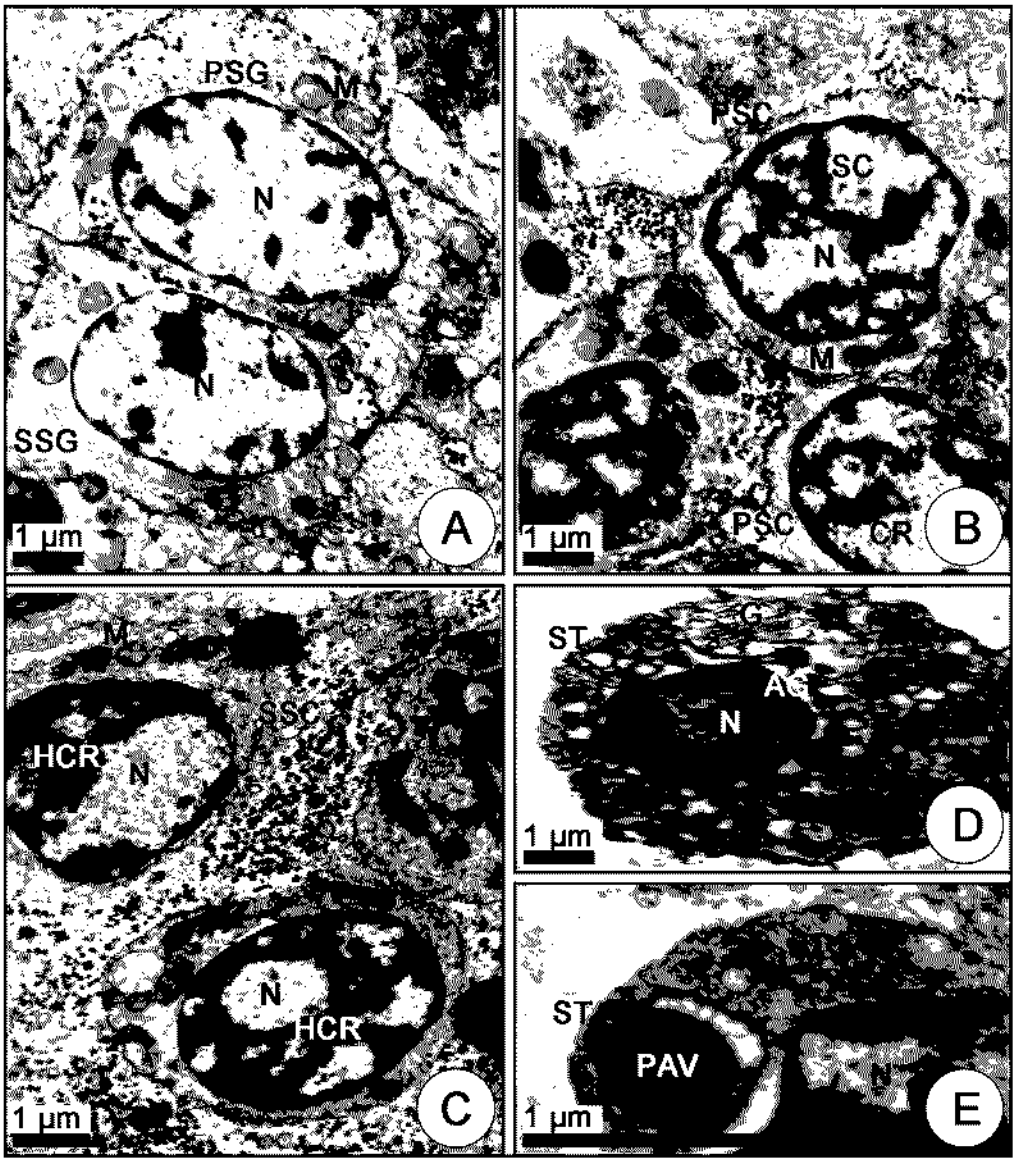

Fig. 2. Electron micrographs of spermatogenesis of *Meretrix petechialis* (A-L). **A**. Section of the primary and secondary spermatogonia, with a large nucleus with chromatin and the mitochondria in the cytoplasm; **B**, the primary spermatocytes during the meiosis, with synaptonemal complex in the nucleus and several mitochondria in the cytoplasm during the prophase of the primary maturation division; **C**, the secondary spermatocytes, with gradually condensed heterochromatin in the nucleus and several mitochondria in the cytoplasm; **D**, spermatids in the early stage of differentiation during spermiogenesis, with condensed heterochromatin in the nucleus and the Golgi complex and acrosomal granule in the cytoplasm during the Golgi phase; **E**, spermatids in the cap phase during acrosome formation during spermiogenesis, with the proacrosomal vesicle before the nucleus and several mitochondria; **F**, a spermatid in the cap phase during acrosome formation, with an acrosomal vesicles in the cytoplasm on the nucleus;

cap, acrosome, and maturation phases. The morphology of the spermatid changed gradually during the Golgi phase in the differentiation of the spermatid. At this phase the Golgi complex and small

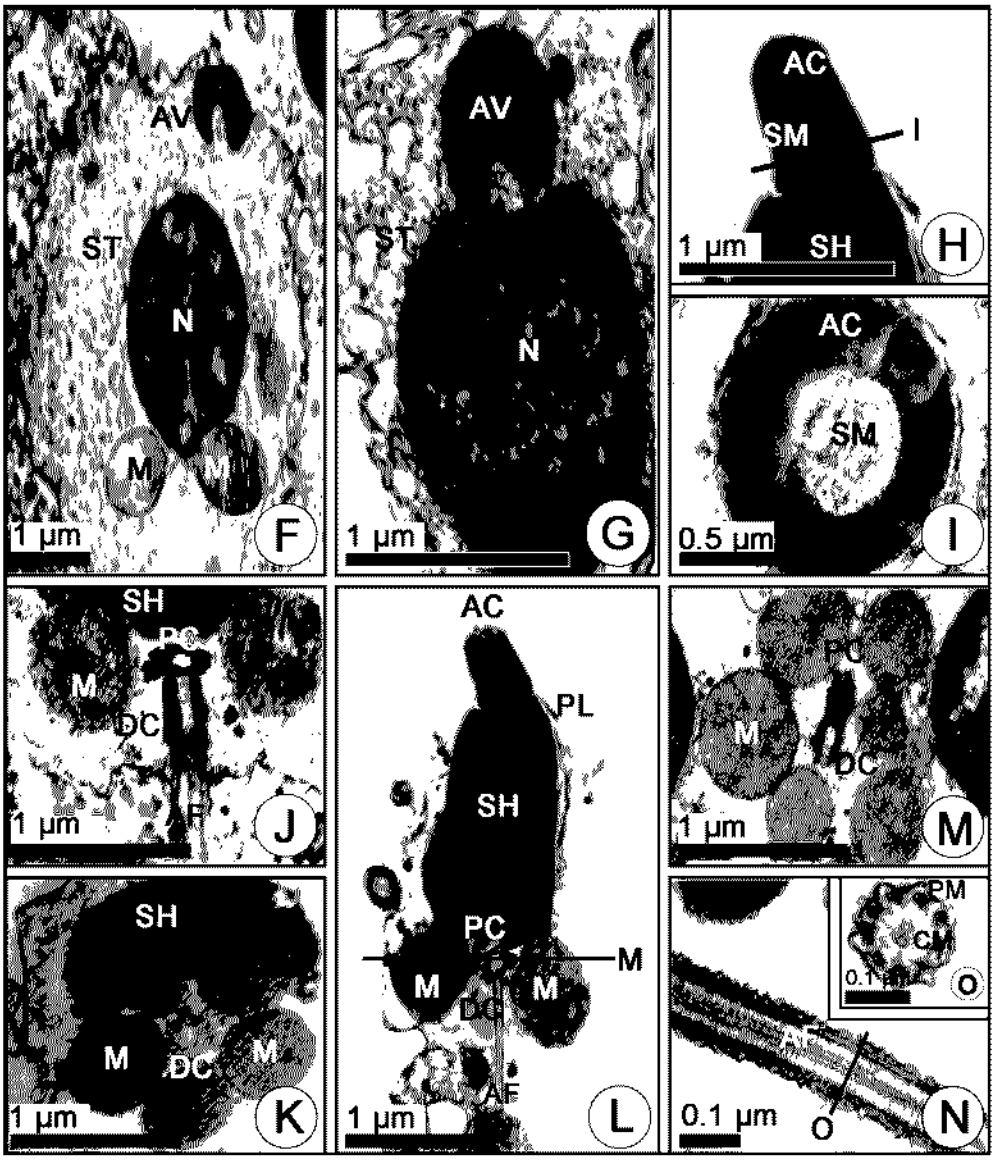

Fig. 2. (continued) **G**, a spermatid in the same stage, with an acrosomal vesicle attaching to the nucleus; **H**, .transformations of the acrosome in the late stage (acrosome phase) of spermiogenesis, with the acrosome and subacrosomal material (in the acrosome) on the nucleus; **I**, a cross sectioned acrosome in Fig 2H, with the acrosome with high electron dense part and subacrosomal material with low electron dense granules; **J**, a spermatid in the acrosome phase, with two centrioles (the proximal and distal centrioles) and a flagellum with axial filament; **K** and **M**, cross sectioned middle piece of the sperm in Fig. 2J, with the distal centriole being surrounded by five or six mitochondria; **L**, a completed spermatozoa in the maturation phase, with the acrosome, sperm head the middle piece and tail flagellum; **N**, longitudinal sectioned sperm tail, with the axial flament; **O**, cross sectioned sperm tail flagellum, with the axoneme of the sperm tail flagellum showing 9+2 structure.

Abbreviations: AC, acrosome; AF, axial filament; AG, acrosomal granule; AV, acrosomal vesicle; CM, central microtuble; DC, distal centriole; GC, the Golgi complex; HCR, heterochromatin; M, mitochondrion; N, nucleus; PAV, proacrosomal vesicle; PC, proximal centriole; PL, plasma membrane; PM, peripheral microtubule; PSC, primary spermatocyte; PSG, primary spermatogonia; SC, synaptonemal complex; SG, spermatogonium; SH, sperm head; SM, subacrosomal material; SSC, secondary spermatogonium; ST, spermatid.

acrosomal granules in the spermatid moved near to the nucleus, while the mitochondria moved to a position just behind the nucleus (Fig. 2D). During the cap phase, morphology of the nucleus was elongated, and the granule in the proacrosomal vesicle at the end of the nucleus was gradually changed and formed a slightly larger acrosomal vesicle (Fig. 2E).

The acrosomal vesicle appeared on the nucleus, and then attached to the nucleus during the acrosome phase (Fig. 2F, G). The sperm nuclear type was cylinder, and the acrosome type showed cap-like shape. There were some gaps having the distance between the nucleus and acrosome. The acrosome was composed of two parts with the density of acrosome: 1) the acrosome with electron dense part in its top of the acrosome, 2) subacrosomal materials with low electron dense granules between the nucleus and the acrosome. After the acrosome formation was completed, a prominent subacrosomal material was present at a part of cross sectioned acrosome near the sperm head (Fig. 2H, I).

During the acrosome phase, of the two centrioles lying in the middle piece of the spermatozoon, the distal centriole took up a position behind the proximal

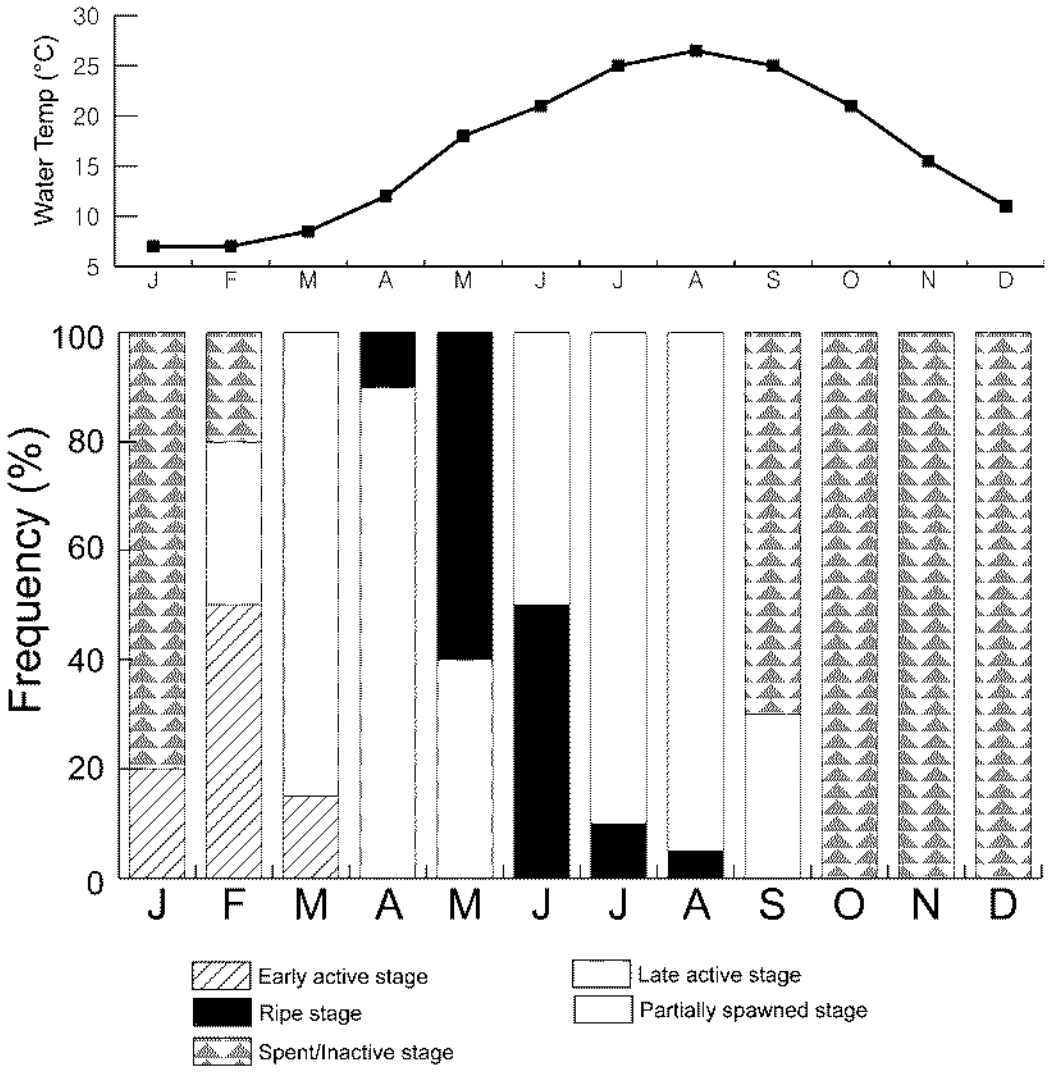

Fig. 3. Frequency of gonadal phases in male *Meretrix petechialis* and the mean seawater temperature from January to December, 2002.

and distal centrioles gave rise to the axial filament of the flagellum of the spermatozoon (Fig. 2J). At this phase, especially, a cross sectioned middle piece which were surrounded by the five or six mitochondria, appear. Five or six spherical mitochondria formed the paranucleus around the distal centriole (Fig. 2K. M)

5) Spermatozoon phase

In the maturation phase, the spermatozoon differentiation was completed and sperm morphology showed the primitive type, as found in the external fertilization species. The morphology of the sperm nucleus type and the acrosome shape of this species were cylindrical type and cap-like shape, respectively. The head of a spermatozoon was approximately 2.70 μm in length including the nucleus (1.50 μm) in length and the acrosome (about 0.60 μm in length), and its tail was approximately 40-45 μm in length (Fig. 2L). At that time, a cross sectioned tail flagellum showed that the axoneme of the tail flagellum (axial filament) of the spermatozoon consist of nine pairs of peripheral microtubules at the periphery, and one pair of central microtubules at the center (Fig. 2N, O). Thus, the axoneme of the sperm tail showed 9 + 2 microtubular arrangement.

4. Reproductive cycle with testicular developmental stage

Based on the morphological features and size of the germ cells and the tissue cells around them, the reproductive cycle with gonadal phases could be classified into five successive stages: (1) early active, (2) late active, (3) ripe, (4) partially spawned, and (5) spent/inactive stages (Fig. 3). The stages and the criteria used in defining them are as follows.

1) Early active stage

Spermatogenesis occurred in the acini of the testis. The spermatogonia and spermatocytes were 7-8 μm and 5-7 μm in diameter respectively (Fig. 4A). In comparison with the visceral mass, the volume of the testis was small. The individuals in the early active stage appeared from February to March when seawater temperatures were very low.

2) Late active stage

Spermatocytes developed into spermatids. The spermatids moved toward the center of the lumen, measuring 3-4 μm in diameter, and showed layers. As the testis developed, a number of spermatocytes, spermatids and small number of spermatozoa occupied approximately one-third to one-half of the lumina in the acini (Fig. 4B). Individuals in the late active stage were found between February to May when seawater temperatures began to increase.

3) Ripe stage

A large number of spermatids underwent transformation into differentiated spermatozoa in the center of the lumen. The ripe testis was characterized by the formation of a number of spermatozoa in the center of the lumen (Fig. 4C). Ripe testes were found from April through July when seawater temperatures were relatively high.

4) Partially spawned stage

A large number of spermatozoa in the acini were discharged into the surrounding water, and the lumen became empty. However a number of spermatozoa, as well as spermatids and spermatocytes, still remained in the lumen (Fig. 4D). The spawning period occurred once a year from June to September, and the main spawning occurred from July to August when seawater temperatures were higher than 20°C.

5) Spent / inactive stage

A small number of undischarged spermatozoa and residual spermatids were degenerated. Thereafter, a few newly formed spermatogonia on the germinal epithelium and connective tissues were rearranged between the acini at this stage (Fig. 4E, F). The individuals in this stage appeared from September through February when seawater temperatures decreased gradually.

DISCUSSION

Most of the bivalves have a primitive type of spermatozoa with a small head and cap shaped acrosome, and a short mid-piece with four to five mitochondria surrounded the centrioles (Longo and

Dornfield, 1967; Chung *et al,* 1991). In this study, I found that the morphology of the spermatozoon of *Meretrix petechialis* is similar to those of other bivalve spermatozoa in having a short mid-piece with five to six mitochondria surrounding the centrioles. However, fine structural differences in molluscan sperm structures, which are associated with the evolution of the species, are sometimes used as criteria for classification (Popham, 1979).

Franzen (1970) divided molluscan sperm morphology into two types: 1) the primitive type found in external fertilization species and 2) the modified type found in internal fertilization species. Baccetti and Afzelius (1976) 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, a biflagellate type is seen in the tripoid *Corbicula flumínea* and *C. leana* in natural populations (Komaru and Konishi, 1996; Komaru *et al.,* 1997; Choi, 2004). An aflagellate type is also found in a few crustaceans (Kim, 2001). *Meretrix petechialis* undergoes external

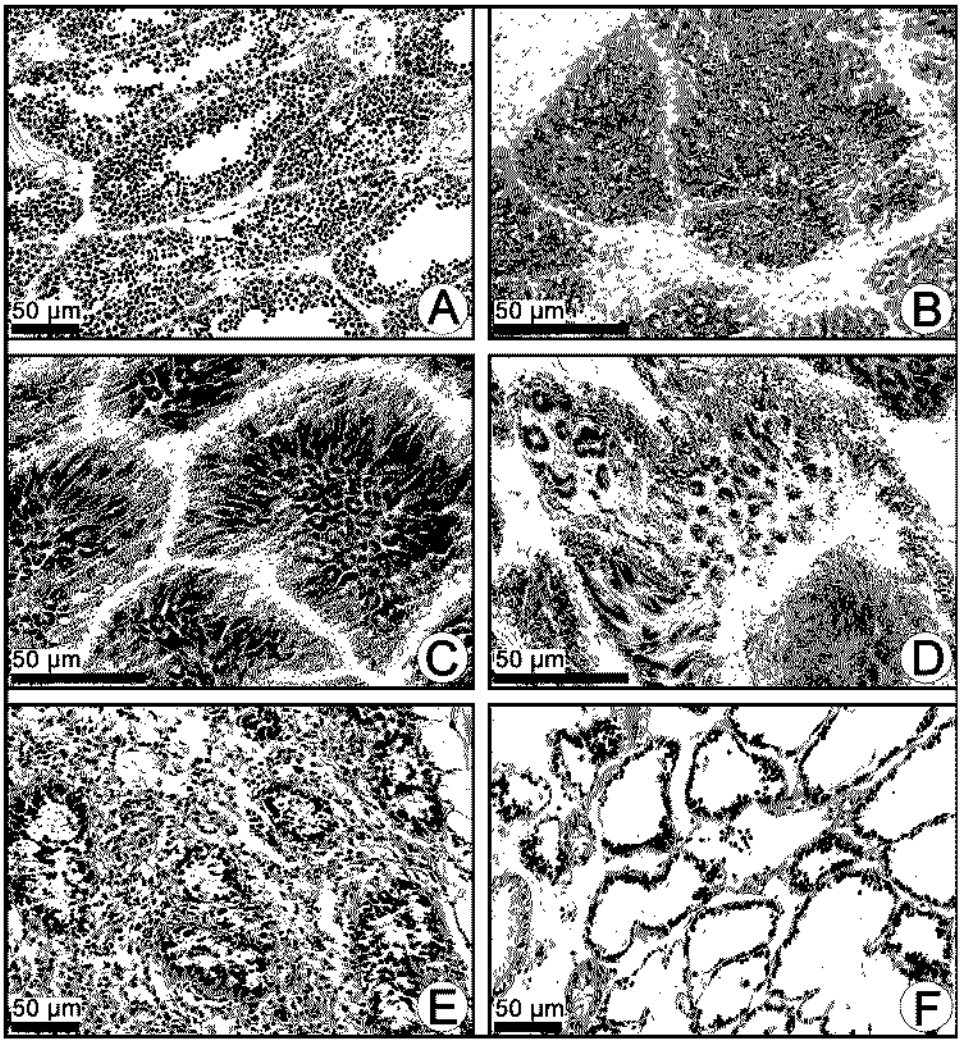

Fig. 4. Photomicrographs of gonadal phases in male *Meretrix petechialis* as seen by light microscopy. **A**, transverse section of the acini in the early active stage; **B**, section of the acini in the late active stage; **C**, section of the acini in the ripe stage; **D**, section of the acini in the partially spawned stage; **E**, and **F**, sections of the acini in the spent and inactive stage.

fertilization and possesses the primitive type of spermatozoon, unlike the modified type found in most internal fertilization gastropods.

The acrosome morphology of the sperm head differs markedly among the species (Popham, 1979). The acrosome shape can be classified into four types: cone, cap, elongate modified cone, and modified cap types. Moreover, the sperm nucleus types vary with molluscan species. Regarding the morphology of the sperm nucleus of the species, Kim (2001) reported that the sperm nuclei are cylindrical shaped in *Septifer virgatus* and some *Mactra* spp. and *Pernidia venulosa;* global in *Spisula sachalinesis* and *Tersus keenae;* oval in the Ostreidae, *Pinctata fucata martensii,* and *Atrina pinnata japonica;* jar shaped in *Solen grandis;* and arrow shaped in *Corbicula japonica*. In the present study, the morphology of the sperm nucleus type and acrosome shape of *Meretrix petechialis* are cylindrical type and cap-like shape, respectively.

Kim (2001) described that the number of the mitochondria in the mid-piece of the spermatozoon are four in the families Ostreidae, Veneridae, Mactridae, Solenidae and Corbiculidae, while five in the Arcidae, Mytilidae, Pinnidae and Veneridae. The number of the mitochondria in the mid-piece of the spermatozoon of *Patinopecten yessoensis, Chlamys farreri,* and *C. swifti* was four except for *Argopectin irradians*. *Argopectin irradians* had five mitochondria in the mid-piece of the sperm.

In the present study, the number of the mitochondria in *Meretrix petechialis* had five to six in the mid-piece of the sperm. Although it was the same species, I assume that the number of the mitochondria in the mid-piece of the sperm show slightly differences in number.

As in most other marine bivalves (Chung *et al.,* 1991; Chung and Ryou, 2000), occurrence of spermatogonia and spermatocytes appear in the early active stage, and a number of spermatids during spermiogenesis and small number of spermatozoa appear in the late active stage. Numerous fully matured spermatozoa appear in the ripe stage, and they are released in the partially spawned stage. After discharging small numbers of residual spermatozoa are degenerated and resorbed. Thereafter, newly formed spermatogonia on the germinal epithelium occur in the spent/inactive stage.

Most marine invertebrate have unique breeding patterns. According to Boolootian *et al.* (1962), breeding patterns of molluscs can be classified into three large categories, based on their spawning behavior or seasonality: 1) year-round breeders, 2) winter breeders, and 3) summer breeders. According to histological observations of its gonad, the spawning season of *Meretrix petechialis* is from June to September. Therefore, this species belongs to summer breeders.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Tae hwan Lee of the University of Michigan for helpful comments on the manuscript. Thanks are due also to Mr. Ye-Kyu Lee of the Electron Microscope Laboratory, Korea University, for his assistance with the transmission electron microscopy. This research was supported in part by funds (2005) from Coastal Research Center, Kunsan National University.

REFERENCES

Baccetti, B. and Afzelius, B.A. (1976) The biology of the sperm cell. Monographs in Developmental Biology, Volume 10. 254 p. Karger, Basel and New York.

Boolootian, R.A., Farmanfarmain, A. and Giese, A.C. (1962) On the reproductive cycle and breeding habits of two western species of *Haliotis*. *Biological Bulletin,* **122**: 183-192.

Choi, K.H. (2004) Karyotype analysis and reproductive characteristics of the diploid brackish water clam, *C. fluminea,* 51 p. Master Thesis, Kunsan National University.

Choi, S.S. and Song, Y.K. (1974) Studies on the artificial fertilization and development of *Meretrix lusoria*. *Bulletin of the Korean Fisheries Society,* **7**: 1-6.

Choi, S.S. (1975) Comparative studies on the early embryonic development and growth of *Meretrix lusoria* and *Cyclina sinensis*. *Bulletin of the Korean Fisheries Society*, **8**: 185-195.

Chun, S.K. and Lee, J.B. (1976) Studies on the trematode lervae infected in the hard clam, *Meretrix lusoria*. *Bulletin of the Korean Fisheries Society*, **9**: 35-42.

Chun, S.K., Chang, D.S., Park, C.K., Kim, Y.G. and Rho.

Y.G. (1981) Basic studies for the production of the hard clam *Meretrix lusoria* (RÖDING) in Jeonbug farming area. *Bulletin of Fisheries Research and Development Agency*, **26**: 7-36.

Chung, E.Y., Lee, T.Y. and An, C.M. (1991) Sexual maturation of the venus slam, *Cyclina sinensis,* on the west coast of Korea. *Journal of Medical and Applied Malacology*, **3**: 125-136.

Chung, E.Y. and Ryou, D.K. (2000) Gametogenesis and sexual maturation of the surf clam *Mactra veneriformis* on the west coast of Korea. *Malacologia,* **42**: 149-163.

Franzen, A. (1970) Phylogenetic aspects of the morphology spermatozoa and spermiogenesis. *In:* Comparative Spermatology. (ed. by Baccetti B.) pp. 29-46. Academic Press, New York.

Hur, Y.B. (1994) Comparative studies on the embryonic development and the growth of larvae of eight bivalve species. 82 p. Master Degree, National Fisheries University of Pusan. [in Korean]

Ikuta, K. (1988a) Heavy metal concentrations and year-class structure of a venus clam *Meretrix lusoria*. *Nippon Suisan Gakkaishi,* **54**: 709-715.

Ikuta, K. (1988b) Seasonal variations of some heavy metal concentrations in a venus clam *Meretrix lusoria*. *Nippon Suisan Gakkaishi,* **54**: 817-822.

Kim, J.H. (2001) Spermatogenesis and comparative ultrastructure of spermatozoa in several species of Korean economic bivalves (13 families, 34 species). Ph. D. thesis, Pukyung National University, 161 pp.

Komaru, A. and Konishi, K. (1996) Ultrastructure of biflagellate spermatozoa in the freshwater clam, *Corbicula leana* (Prime). *Invertebrate Reproduction and Development,* **29**: 193-197.

Komaru, A., Konishi, K., Nakayama, I., Kobayashi, T., Sakai, H. and Kawamaru, K. (1997) Hermaphroditic freshwater clams in the genus *Corbicula* produce non-reductional spermatozoa with somatic DNA content. *Biological Bulletin,* **193**: 320-323.

Kwon, O.K., Park, G.M. and Lee, J.S. (1993) Coloured Shells of Korea. Academy Publishing Company, Seoul 285 pp. [in Korean]

Lee, J.H. (1997) Histological studies on the gametogenesis and reproductive cycle of the hard clam, *Meretrix lusoria*. *The Korean Journal of Malacology,* **13**: 131-141.

Lee, J.Y. (1991) Effects of some hazardous substances on the physiological function for hard clam, *Meretrix lusoria*. *Bulletin of Kunsan Fisheries Junior College*, **25**: 29-33.

Longo, F.J. and Dornfield, E.J. (1967) The fine structure of spermatid differentiation in the mussel, *Mytilus edulis*. *Journal of Ultrastructural Research*, **20**: 462-480.

Mann, R., (1979) Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. *Journal of the Marine Biological Association U.K.*, **59**: 95-110.

Min, D.K., Lee, J.S., Koh, D.B. and Je, J.G. (2004) Mollusks in Korea (revised supplementary edition). 566 p. Min Molluscan Research Institute, Seoul. [in Korean]

Popham, J.D. (1979) Comparative spermatozoon morphology and bivalve phylogeny. *Malacological Review*, **12**: 1-20.

Tanaka, Y. (1969) Studies on propagation of a hard clam, *Meretrix lamarckii*- I. Artificial breeding. *Bulletin of Tokai Region Fisheries Research Laboratory,* **58**: 163-168.