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Ultrastructure of Germ Cell during Spermatogenesis and the Reproductive Cycle of the Hanging Cultured Male Scallop *Patinopecten yessoensis* (Pelecypoda: Pectinidae) on the East Coast of Korea

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ABSTRACT

Ultrastructure of germ cell differentiation during spermatogenesis and the reproductive cycle in male *Patinopecten yessoensis* was studied by histological and cytological observations. The gonadosomatic index (GSI) in males rapidly increased and reached a maximum in April when seawater temperature gradually increased. Then the GSI gradually decreased from May through July when spawning occurred. Accordingly, monthly changes in the GSI in males coincided with testicular maturation and spawning periods. The sperm morphology of *P. yessoensis* belongs to the primitive type and showed general characteristics of external fertilization species. The head of the spermatozoon is approximately 3.50 μm in length: the sperm nucleus and acrosome are approximately 2.90 μm and 0.60 μm in length, respectively. The nuclear type of the spermatozoon is vase in shape, and the acrosome is cone type. The axoneme of the tail flagellum consists of nine pairs of microtubules at the periphery and a pair of central microtubules in the center. The satellite body (which is formed by the centriole) and four mitochondria appear in the middle piece of the spermatozoon. The spawning period was from April through July and the main spawning occurred from May to June when seawater temperatures gradually increased. The reproductive cycle of this species can be classified into five successive stages; early active stage (September to November), late active stage (October to March), ripe stage (February to August), spawning stage (April to July), and spent/inactive stage (July to November).

Keywords: *Patinopecten yessoensis,* Germ cell differentiation, Reproductive cycle.

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INTRODUCTION

The scallop, *Patinopecten yessoensis,* (Pelecypoda: Pectinidae) is distributed along the coasts of Korea, China and Japan (Yoo, 1976; Kwon *et al.,* 1993). Especially, in the East Sea of Korea, this species is mainly found in fine sand in the subtidal zone of Jumunjin, Gangwon-do, Korea (Park, 1998; Chung *et al.,* 2005c). Owing to the recent sharp reduction in the standing stock, which has been declining as a consequence of reckless overharvesting for commercial purpose (Park, 1998), it has been denoted as a target organism and fisheries resource that should be managed using a more reasonable fishing regimen by hanging culture that can maintain an optimal population size. For the study of reproductive mechanism and management of a living natural resource, it is important to understand its population characteristics with regard spermatogenesis and

testicular development.

Previously, there have been many studies on aspects of reproductive ecology including reproductive cycle (Maru, 1976; Mori *et al.,* 1977; Kawamata *et al.,* 1981; Kawamata, 1983; Chang *et al.,* 1985, 1997; Park, 1998), germ cell differentiation and sexual maturation (Chung *et al.,* 2005a, b) and larval distribution and growth (Yoo and Park, 1979; Yoo *et al.,* 1981; Ito *et al.,* 1988), on aspects of aquaculture including environmental condition of aquaculture (Pyen and Rho 1978; Kang et al., 1982; Park *et al.,* 2000) and aquaculture (Lee and Chang, 1977; Ventilla, 1982; Tomita *et al.,* 1982; Wildish *et al.,* 1987; Wildish and Saulnier, 1992; Kang *et al.,* 1996), on aspects of physiology including the digestive diverticula (Chang *et al.,* 1989), on ecology (Maru, 1985; Bower and Meyer, 1990), and on morphology (Bourne *et al.,* 1989). However, there are still gaps in our knowledge regarding reproductive biology. Especially, little information is available on ultrastructural study of germ cell differentiation during spermatogenesis of *Patinopecten yessoensis*. Understanding the spawning period of this species will provide necessary information for the recruitment period and age determination for aquaculture of this population. Therefore, the main aim of the present study is to describe the male germ cell differentiation during spermatogenesis, the testicular developmental cycle and spawning period of the male scallop.

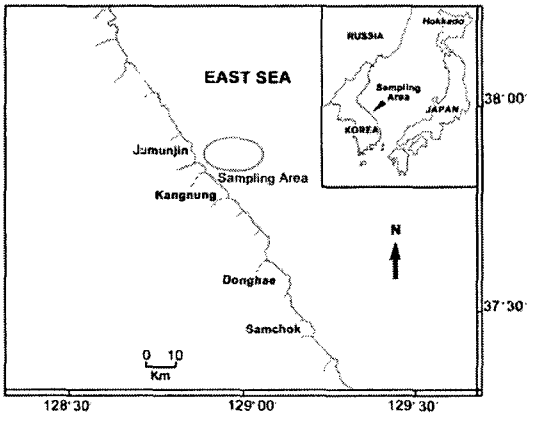


Fig. 1. Map showing the sampling area.

MATERIALS AND METHODS

1. Sampling

Specimens of the male scallop *Patinopecten yessoensis* were collected monthly from hanging culture at the subtidal zone (shellfish farm) of Jumunjin on the East Sea of Korea, for two years from January 1997 to December 1998 (Fig. 1). The scallops ranging from 91.6 to 118.5 mm in shell height were used for the present study. After the male scallops were transported alive to the laboratory, shell heights, testes weights and meat weights were immediately measured.

2. Gonadosomatic index (GSI)

A total of 226 male individuals were used to calculate the gonadosomatic index (GSI) from January 1997 to December 1998. Monthly changes in the mean GSI were calculated by the following equation: GSI = Gonad weight (g) x 100 / Meat weight (g).

3. Ultrastructure of germ cell by electron microscopic observation

For electron microscopical observations, excised pieces of the testis were cut into small pieces and immediately fixed in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hours at 4℃. After prefixation, the specimens were washed several times with the same buffer and then postfixed in 1 % osmium tetroxide dissolved in 0.2 M phosphate buffer solution (pH 7.4) for 1 hour at 4℃ Specimens were then dehydrated in a series of increasing concentrations of ethanol, cleared in propylene oxide and embedded in Epon-Araldite mixture. Ultrathin sections of Epon-embedded specimens were cut with glass knives with a Sorvall MT-2 microtome and an LKB ultramicrotome at a thickness of about 800-1000 Å. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrate, and examined with a JEM 100 CX-2 (80 kV) electron microscope.

4. Histological analysis

For light microscopic examination of histological

preparations, a total of 216 female gonad tissues were removed from shells and preserved in Bouin's fixative for 24 h and then washed with running tap water for 24 h. Tissues were then dehydrated in alcohol and embedded in paraffin molds. Embedded tissues were sectioned at 5-7 μm thickness using a rotary microtome. Sections were mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin, Mallory's triple stain and PAS stain, and examined using a light microscope.

RESULTS

1. Position and morphology of the testis

The scallop, *Patinopecten yessoensis,* is a dioecious. The gonad is separated from digestive diverticula and adductor muscle. The testis is located downward of the adductor muscle. With the testicular maturation, the testis encircle the adductor muscle near digestive diverticula (Fig. 2). The testis is a number of the acini. As maturation progresses , the external features of the sex of scallops could be easily distinguishable, because the testis shows yellowish white in color (the ovary pink). Therefore, their sexes of the scallop could be distinguishable by visual observation and dissection. After spawning the gonad degenerated, and it became difficult to distinguish thei sexes by external color or dissection.

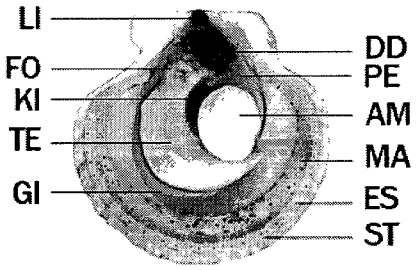


Fig. 2. Anatomy of male *Patinopectem yessoemsis.*
Abbreviations: AM, adductor muscle; DD, digestive diverticula; ES, eye spot; FO, foot; GI, gill; KI, kidney; LI, ligament; MA, mantle; PE, pericardium; ST, sensory tentacle; TE, testis.

2. Gonadosomatic index (GSI) in males and seawater temperature

Monthly GSI changes in males were shown in Fig. 3. In 1997, the GSI values slowly increased between February and March. Their values reached the maximum (mean 22.5) in April when seawater

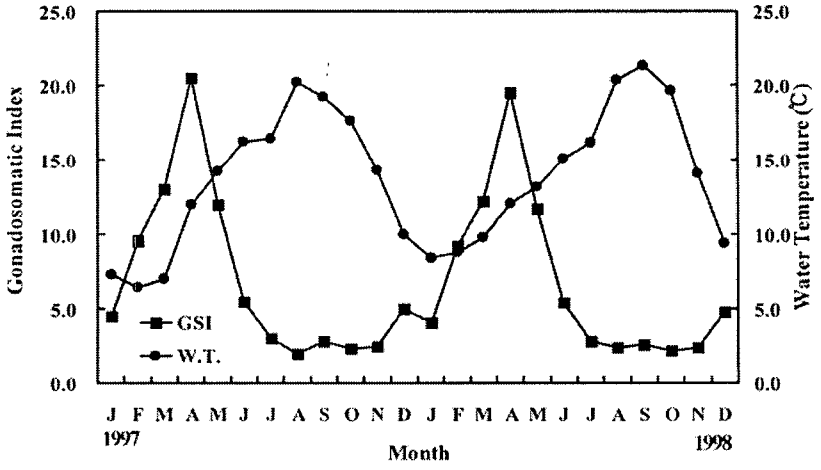
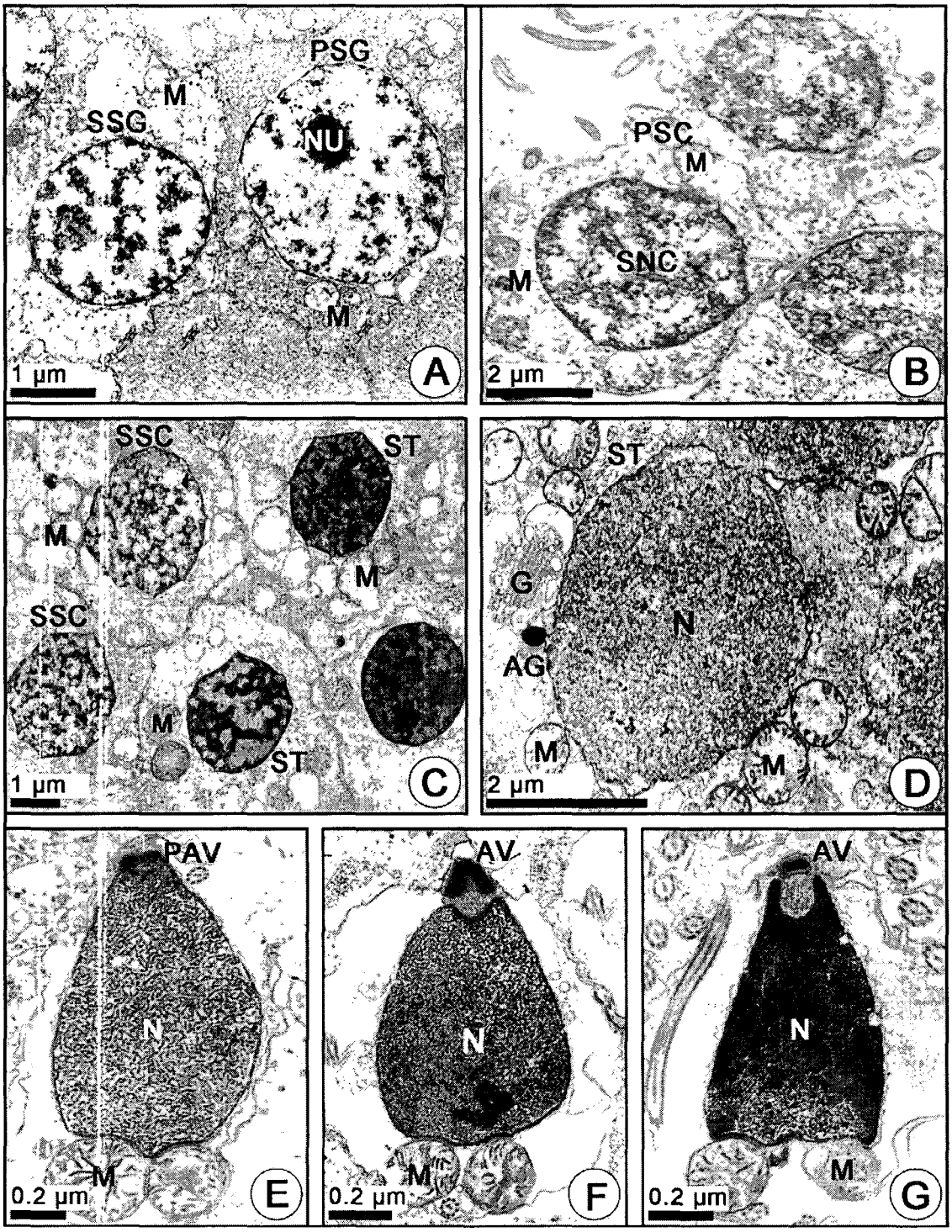


Fig. 3. Monthly changes in the mean gonadosomatic index in male *Patinopecten yessoensis* and seawater temperatures.

temperature gradually increased. Then, the GSI rapidly decreased from May to July when relatively



higher water temperatures were maintained, and spermatozoa were discharged. Thereafter, the value temporarily reached the minimum in October (mean) when the testis was degenerated and resorbed. Monthly GSI changes in 1998 showed a similar result to those in 1997.

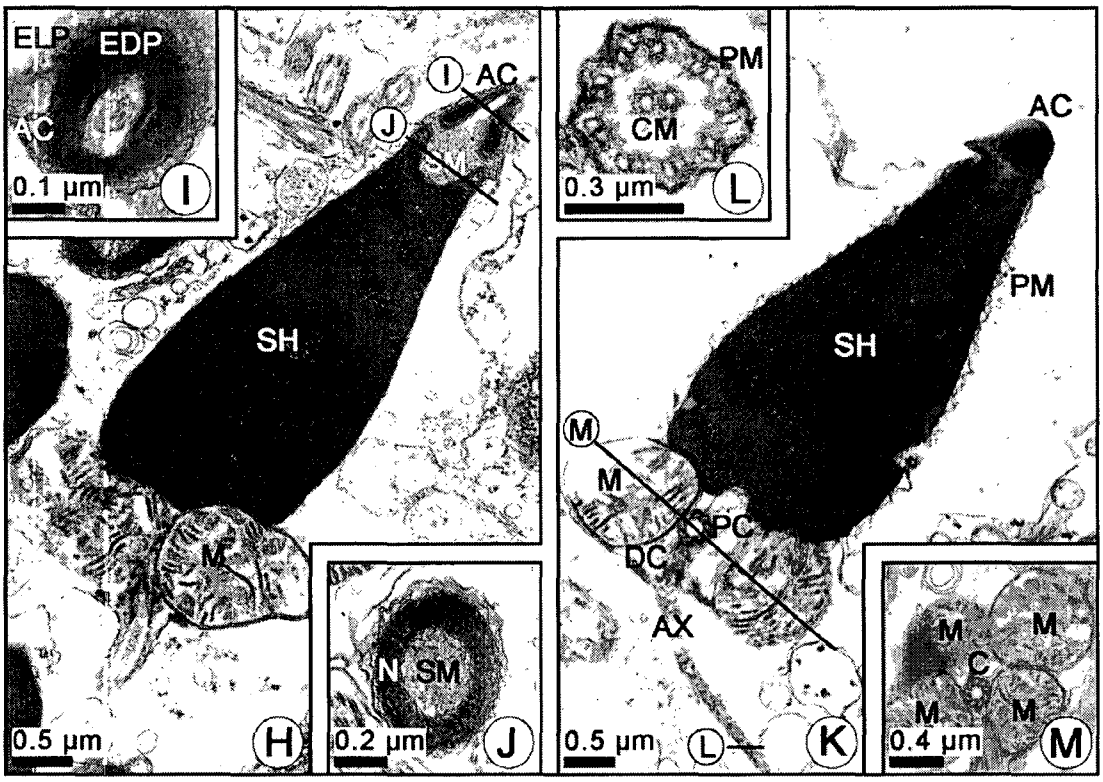


Fig. 4. Electron micrographs of spermatogenesis of *Patinopecten yessoensis* (A-M). **A.** Section of the primary and secondary spermatogonia, witha large nucleus with chromatin and several mitochondria and vacuoles in the cytoplasm **B.** the primary spermatocytes during the meiosis, with synaptonemal complex in the nucleus and several mitochondria during the prophase of the primary maturation division; **C.** the secondary spermatocytes and spermatids, with gradually condensed heterochromatin in the nucleus; **D.** spermatids in the early stage of differentiation during spermiogenesis(Golgi phase during acrosome formation), with the Golgi complex and acrosomal granule on the nucleus and several mitochondria just behind the nucleus; **E-G**, spermatids in the cap phase during acrosome formation, with the proacrosomal vesicle in the cytoplasm on the nucleus; **H.** a spermatid in the late stage (acrosome phase)of differentiation during spermiogenesis, with the completed acrosome and subacrosomal material in the sperm head **I**, Cross section of the acrosome in the maturation phase, with the acrosome (being composed of electron dense part and electron lucent part) and subacrosomal materials between the acrosome and the sperm head; **J.** cross sectioned sperm head, with subacrosomal materials in the top part of the sperm head; **K**, a completed spermatozoon, with an acrosome, sperm head, middle-piece and sperm tail flagellum; **L**, cross sectioned sperm tail flagellum, with the axoneme of the sperm tail showing 9+2 structure; **M**. cross sectioned the middle piece of the sperm, with the satellite body and five mitochondria being composed of the paranucleus.

Abbreviations: AC, acrosome; AG, acrosomal granule; AV, acrosomal vesicle; AX, axial filament; C, centriole; CM, central microtuble; DC, distal centriole; EDP, electron dense part; ELP, electron lucent part; M, mitochondrion; N, nucleus; PAV, proacrosomal vesicle; PC, proximal centriole; PL, plasma membrane; PSC, primary spermatocyte; PSG, primary spermatogonium; SH, sperm head; SM, subacrosomal membrane; SNC, synaptonemal complex; SSC, secondary spermatocyte; SSG, secondary spermatogonium; ST, spermatid.

3. Ultrastructure of germ cells during spermatogenesis

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

1) Spermatogonial phase

The spermatogonia are located near the auxiliary cells. They are approximately 9-10 μm in diameter and more or less oval shaped. The primary spermatogonia divided mitotically to produce secondary spermatogonia, which have smaller cells and nuclei than the primary spermatogonia. Each of them contained a large nucleus with electron dense chromatin, and several mitochondria and vacuoles appeared in the cytoplasm (Fig. 4A).

2) Primary spermatocyte phase

Secondary spermatogonia differentiate into primary spermatocytes. The primary spermatocytes have a large nucleus with slightly denser chromatin. Synaptonemal complexes in the nucleus appear in prophase during the first maturation division, and several mitochondria appear in the cytoplasm of the primary spermatocyte (Fig. 4B).

3) Secondary spermatocyte phase

The primary spermatocyte develops into the secondary spermatocyte through the first meiotic division. At this time, dense heterogeneous chromatins in the nucleus of the secondary spermatocyte show more denser, and concentrate than that of the primary spermatocyte. In this phase, several mitochondria are present in the cytoplasm (Fig. 4C).

4) Spermatid phase

The secondary spermatocytes develop into the spermatids as a result of the second meiotic division. At this time, the spermatid nucleus had the typical structure of the nucleus with aggregated chromatin, and several mitochondria appeared in the cytoplasm (Fig. 4C). Spermiogenesis can expediently be divided into four phases based on the characteristics of cell organelle differentiations; Golgi, cap, acrosome and maturation phases. The morphology of the spermatid changes gradually during the Golgi phase in the early differentiation of the spermatid. At this phase the Golgi complex and acrosomal granules in the spermatid moved to a position just before the nucleus, while the mitochondria moved to a position just behind the nucleus (Fig. 4D). 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. At this phase, which are surrounded two or more mitochondria appears in the middle piece (Fig. 4E-G). Four spherical mitochondria form the paranucleus (Fig. 4M).

The acrosomal vesicle changes into an acrosome during the acrosome phase. The sperm nucleus and acrosome are 2.90 μm and 0.60 μm, respectively. The sperm nuclear type is vase in shape, and the acrosome shows cone type. The acrosome is composed of two parts with the density of the acrosome: 1) the acrosome with electron dense part and electron lucent part in its top or front of the acrosome, 2) subacrosomal materials with low electron dens granules between the nucleus and front part of the acrosome.

There are some gaps having the distance between the nucleus and acrosome. After the acrosome formation is completed, a prominent subacrosomal material is surrounded by the sperm head near the acrosome (Fig. 4H-J). At this time, of the two centrioles lying in the middle piece of the spermatozoon, the distal centriole takes up a position behind, the proximal centriole and the distal centriole give rise to the axial filament of the flagellum of the spermatozoon. According to the results of a cross sectioned sperm tail, The axoneme of the sperm tail flagellum consists of nine pairs of peripheral microtubles at the periphery, and one pair of central microtubles at the center. The satellite body (which is formed by the centriole) and four mitochondria appear in the middle piece (Fig. 4K-M).

5) Spermatozoon phase

During the maturation phase, the spematozoon differentiation was completed, The head of a spermatozoon is approximately 3.50 μm in length including the acrosome measuring about 0.60 μm in length, and its tail is approximately 30 μm (Fig. 4L).

4. Reproductive cycle and gonadal stage

Based on morphological features and sizes of the germ cells and accompanying cells, the gametogenic cycle can be classified into five successive stages as follows;

Early Active Stage: The spermatogonia and spermatocytes were 8-9 and 6-7 μm in diameter, respectively, and appeared in several layers along the acinus wall (Fig. 5A). Male individuals in the early active stage appeared from September to November when seawater temperatures gradually decreased.

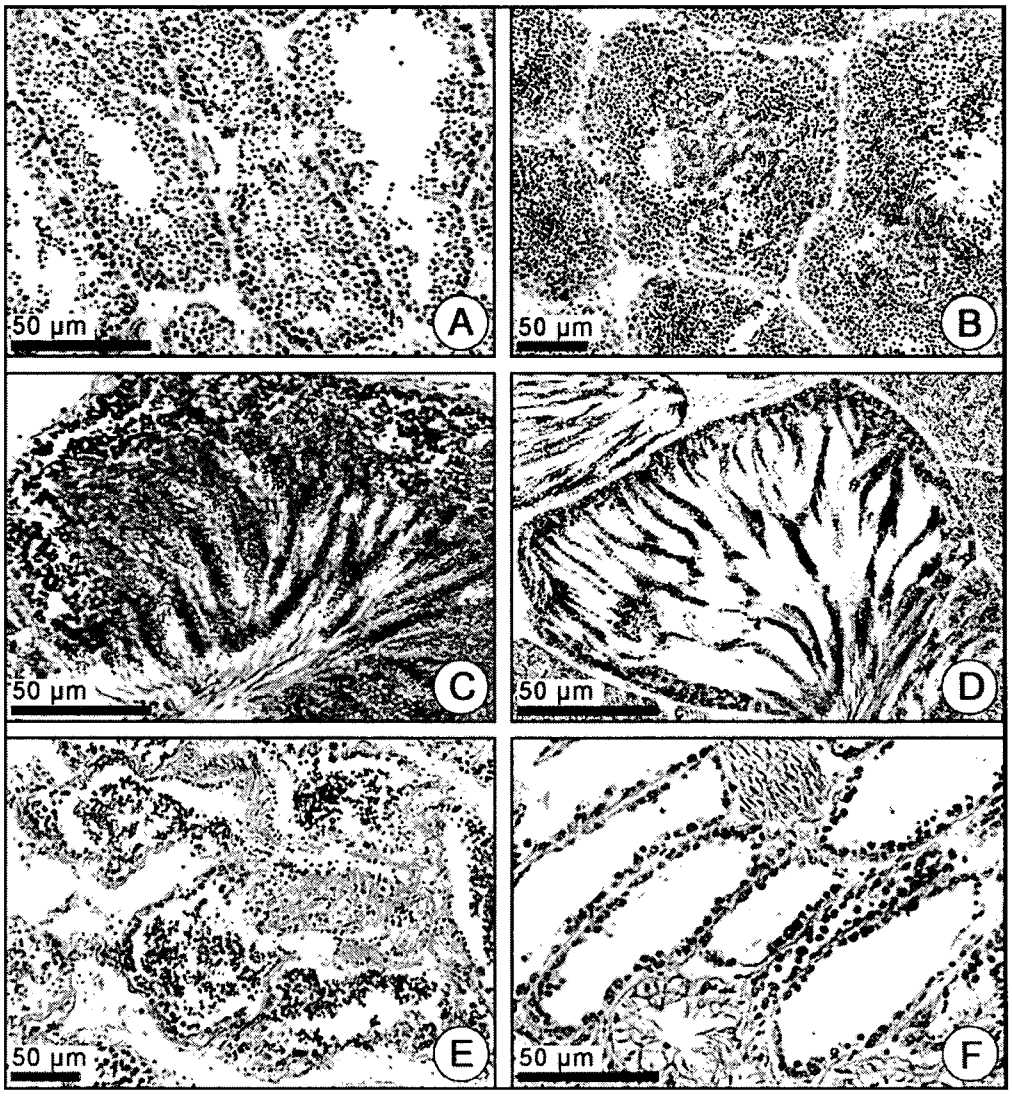


Fig. 5. Photomicrograph of gonadal phases in male *Pectinopecten yessoensis* as seen by light microscopy. **A**, transverse section of the acini in the early active stage; **B**, section of the acini in the late active stage; **C**, section of the acini in the ripe stage; **D**, section of the acini in the spawning stage; **F**, section of the acini in the spent /inactive stage.

Late Active Stage: A number of spermatocytes and spermatids were measuring 6-7 μm and 3-4 μm in diameter, respectively and appeared in the acinus. As the spermatogenesis progresses, a number of spermatocytes, spermatids and a small number of spermatozoa occupied approximately one-third to one-half of the lumina in the acini (Fig. 5B). The individuals in the late active stage were found from October to March when seawater temperatures gradually decreased.

Ripe Stage: A number of spermatids began to transform into differentiated spermatozoa in the center of the lumen. The ripe testis was characterized by the formation of a number of spermatozoa (Fig. 5C). Male individuals in the ripe stage were found from February to August when seawater temperatures gradually increased.

Spawning Stage: A large number of spermatozoa in the acini were discharged into the surrounding water, and although the lumen empties, a number of undischarged spermatozoa as well as spermatids and spermatocytes remained in the lumen (Fig. 5D). The spawning period in males occurred once a year from April to July, with the main spawning occurring between May and June when seawater temperatures rapidly increased.

Spent/resting stage: After discharging of spermatozoa, a small number of undischarged spermatozoa and spermatids in the acini underwent cytolysis (Fig. 5E). The products of gamete atresia were resorbed. Thereafter, the rearrangement of a few newly formed spermatogonia and connective tissues occurred in the acini in this stage (Fig. 5F). The individuals in this stage were found from July to November when seawater temperatures were relatively high.

DISCUSSION

Germ cell differentiation during spermatogenesis in *Patinopecten yesoensis* is very similar to other bivalves that undergo external fertilization (Kim, 2001). 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 is found in external fertilization species and 2) the modified type is found in internal fertilization species (Chung and Kim, 1997; Chung *et al.,* 2005a). Verdonk *et al.* (1983) divided sperm morphology into four types; 1) primitive, 2) modified, 3) biflagellate, and 4) aflagellate types. In addition to the primitive type and partially modified type of molluscan sperm, a biflagellate type is seen in the tripoid *Corbicula fluminea* and *C. leana* in natural populations (Komaru and Konishi, 1996; Komaru *et al.,* 1997: Choi, 2005). An aflagellate type is also found in a few crustacean (Kim, 2001). *Patinopecten yessoensis* undergoes external fertilization and possesses the primitive type of spermatozoon, unlike the modified type found in most internal fertilization gastropods (Chung and Kim, 1997; Chung *et al.,* 2005c).

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 type vary with molluscan species. In the present study, the morphologies of the sperm nucleus type and acrosome shape of *Patinopecten yessoensis* and other Pectinidae scallops except for *Argopectin irradians* (jar type and cone type) are vase type and cone type, respectively (Kim, 2001). In addition, Kim (2001) reportedthat the sperm nuclei are cylindrical in *Septifer virgatus* and some *Mactra* spp. and *Pernidia venulosa* global in *Spisula sachalinesis* and *Tersus keenae* ovoid in the Ostreidae, *Pinctata fucata martensii,* and *Atrina pinnata japonica* vase shaped in *Solen grandis* and arrow shaped in *Corbicula japonica.*

Kim (2001) described that the number of the mitochondria in the middle piece of the spermatozoon are four in the families Ostreidae, Veneridae, Mactridae, Pectinidae, Solenidae and Corbiculidae, while five in the Arcidae, Mytilidae, Pinnidae and Veneridae (Ji, 2002; Moon, 2002; Kwak, 2005; Choi, 2005). In the present study, the number of the mitochondria in the middle piece of the spermatozoon of *Patinopecten yessoensis* are four as seen in

Table 1. A comparison of spawning periods of *Patinopecten yessoensis* with locality.

Locality (latitude)	Culture method	Spawning periods	References
Jumunjin, Kangwon-do, Korea (37° 50' N)	Hanging	April-July (Male)	Present study
Jumunjin, Kangwon-do, Korea (37° 50' N)	Hanging	April-June (Female)	Chung *et al.*, 2005
Gosung-gun, Kangwon-do, Korea (38° 20' N)	Hanging	April-June (Female)	Chang *et al.*, 1997
Töni Bay, Iwate Prefecture, Japan (39° N)	Hanging	April-May (Female)	Mori *et al.*, 1977
Funka Bay, Hokkaido, Japan (42° N)	Sowing	April-June (Female)	Kawamata *et al.*, 1981; Kawamata., 1983
Saroma lake, Hokkaido, Japan (44° N)	Hanging	May-June (Female)	Maru, 1978
Abashiri waters, Hokkaido, Japan (44° N)	Sowing	May-July (Female)	Chang *et al.*, 1985

Chlamys swifti. However, *Argopectin irradians* and *C. farreri* have has five mitochondria in the middle piece of the sperm (Kim, 2001; Chung *et al.*, 2005b). Especially, the Pectinidae scallops such as *Patinopecten yessoensis, Chlamys farreri, C. swifti,* and *Argopectin irradians* have the satellite body in the middle piece of the spermatozoa.

From the present study by histological observations, in case of Korea, spawning in male *Patinopecten yessoensis* in Jumunjin, the East Sea of Korea occurs from April to June. And that in the scallop from Goseong-gun, the East Sea is also from April to July (Chang *et al.*, 1997). Therefore, our result about the spawning period shows a similar pattern to Goseong-gun, the East Sea of Korea. As shown in Table 2, the Japanese *P. yessoensis* has been reported to spawn once a year from April through May-June in Töni Bay and Funka Bay, Japan. And spawning occur from May through June-July in Lake Saroma and Abashiri waters, Hokkaido, Japan. Therefore, our results coincide with the result of Mori *et al.* (1977) and Kawamata (1983). However, the spawning period of the scallop in Jumunjin, Korea is 20-30 days faster than that of Lake Saroma in Japan. Slight discrepancy in the spawning period between these several studies might be related to geographic differences in environmental conditions such as water temperature and food availability as reported in *Mactra veneriformis* (Chung and Ryou, 2000).

As shown in Table 1, the spawning season in female *Patinopecten yessoensis* in Korea was from April to June (Chung *et al.*, 2005c), and in case of male individuals, spawning occurred from April through July. Therefore, this species may be classified as a summer breeder, based on the criteria for classification of the breeding season of marine molluscs by Boolootian *et al.* (1962).

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