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Ultrastructural Study of Germ Cell Development and Reproductive Cycle of the Hen Clam, *Mactra chinensis* on the West Coast of Korea

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한국 서해산 개량조개, *Mactra chinensis*의 생식세포발달의 미세구조적 연구 및 생식주기

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요 약

1992년 1월부터 12월까지 1년간에 걸쳐 전북 군산, 선연리 조하대에서 채집된 개량조개, *Mactra chinensis* Philippi를 대상으로 생식세포 발달과 생식소 발달양상을 조사하기 위해 투과형 전자현미경으로 미세구조 변화를 관찰하였고, 정확한 산란기를 규명하기 위해 조직학적으로 생식주기를 조사하였다.

개량조개는 자웅이체이다. 난황형성과정은 난모세포의 발달정도에 따라 다르게 나타나고 있다. 전난황형성기 난모세포질 내에서는 핵주변 구역에 골지장치와 수많은 공포들 및 미토콘드리아들이 출현하고 있는데 이들은 차후, 지방적 형성에 관여한다. 난황형성전기 난모세포에서는 지방적 및 지질과립들이 핵막 근처에서 출현하여 피질층쪽으로 분산되는 반면, 같은 발달단계의 난모세포질의 피질구역에서는 피질과립들 (단백질성 난황과립)이 처음으로 생성되어 난황막 근처의 피질층에서 핵주변 구역쪽으로 분산·분포된다. 난황형성후기 난모세포에서는 세포질 내의 골지장치, 공포, 미토콘드리아, 그리고 조면소포체들이 자율합성에 의해 난황과립 형성에 관여하고 있다. 반면, 외인성 물질들인 지질형태의 과립들, 단백질성 물질 및 다량의 글리코겐 입자들이 생식상피 내에서 출현하고 있는데, 이들 물질이 생식상피에서 난황막 구조물인 미세융모를 통해 난황형성 후기 난모세포의 난질 내로 통과해 들어가는 현상이 관찰되었다. 이와 같은 현상은 난황형성이 일어날 때에 heterosynthesis가 일어나고 있음을 시사한다. 완숙난모세포의 난경은 약 50~60㎛이고, 완숙정자 두부의 길이는 대략 3㎛이며, 미부의 길이는 약 30㎛ 정도이다. 정자 미부편모의 axoneme은 중앙의 2개의 미세소관 (microtubule)과 주변에 위치한 9개의 2중 미세소관 (microtubule)으로 구성되어 있다.

본 종의 산란기는 5월에서 9월 중순에 걸쳐 일어나는데, 주산란시기는 해수수온이 22℃ 이상으로 상승하는 6, 7월이다. 따라서 1년에 산란 (번식)시기가 한번 일어나고 있음을 알 수 있다.

생식주기는 초기활성기 (1~2월), 후기활성기 (2~4월), 완숙기 (4~9월), 산란기 (5~9월) 그리고 퇴화 및 비활성기 (6~12월)의 연속적인 5단계로 구분할 수 있었다. 재생산에 가담할 수 있는 암, 수개체들의 군성숙도 (%)를 조직학적으로 조사한 결과, 각장 3.5~3.9cm 범위의 개체는 55.5%이었고, 5cm 이상인 개체들은 재생산에 100% 참여하였다. 본 종의 암, 수개체들은 만 1년부터 재생산에 가담하는 것으로 추정된다.

Key words: *Mactra chinensis* Philippi, Germ cell development, Reproductive cycle, First sexual maturity.

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INTRODUCTION

The hen clam, *Mactra chinensis* Philippi (Pelecypoda : Mactridae) is distributed along the coasts of Korea, Japan and China. This clam is found especially in silty sand in the subtidal zone of the south and west coast of Korea, and is one of the important commercial shellfish (Yoo, 1976; Kwon *et al.*, 1993). However, because of the recent decline in its standing stock by reclamation work, reckless overcatching and marine pollution, it has been noted as a target organism that should be managed by a reasonable fishing regime. So far, several studies have been conducted with respect to morphometry and growth rate (Hanaoka and Shimadzu, 1949), reproduction including development of eggs (Miyasaki, 1939; Lee and Son, 1978), spawning and growth (Kim *et al.*, 1985; Hanaoka and Shimadzu, 1949), propagation (Sakai, 1976), and sexual maturation (Chung *et al.*, 1987) of the hen clam. However, any report associated with reproductive mechanism and fine structure of germ cells of this species could not be found until now. Some more informations regarding reproductive biology are desirable on this valuable food resources.

The main purpose of the present study is to understand germ cell development, the reproductive cycle with the gonad development and first sexual maturity, using histological approach. These are considered to be the mot important ecological data for resource reproductive biology.

MATERIALS AND METHODS

The clams, *Mactra chinensis* Philippi, were col-

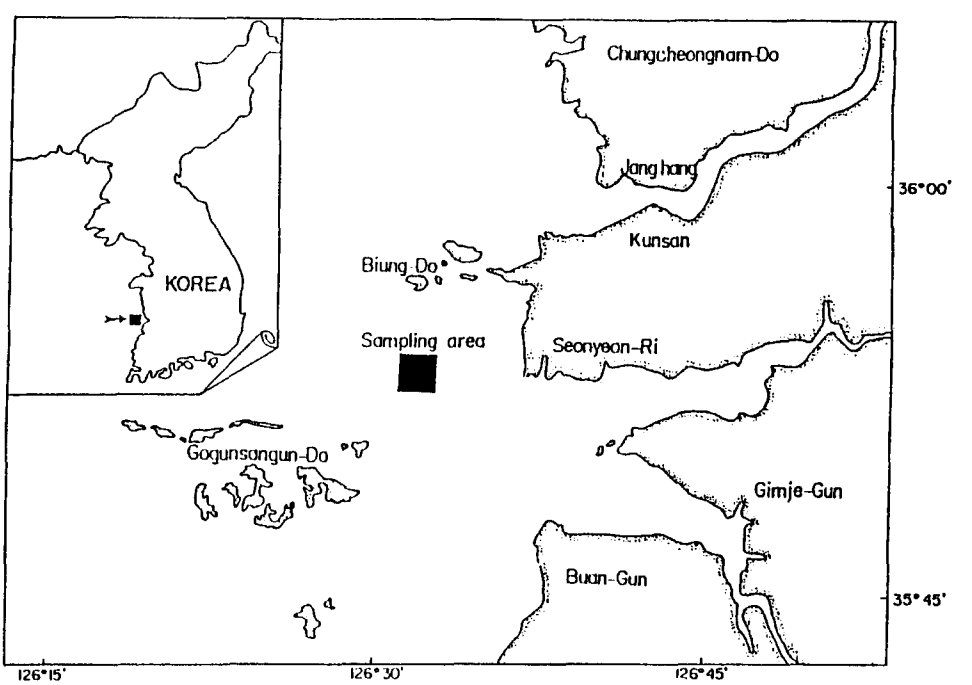

Fig. 1. Map showing the sampling area.

lected by dredging in the subtidal zone of Seonyeon-Ri, Kunsan, Korea, from January to December, 1992 on a monthly basis (Fig. 1). A total of 341 clams ranging from 26.8 mm to 64.8 mm in shell length were used for the study. After the clams were transported alive to the laboratory, shell lengths, heights and widths were recorded using a Vernier caliper, and total wet weights of tissues were weighed using a top-loading electrical balance.

The gonadal tissues were subjected to standard histological procedures (dehydration in alcohol series and embedding in paraffin). Embedded tissues were sectioned to 5~7 μm with 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 inspected under a light microscope.

For electron microscope observations, excised pieces of gonads were cut into small pieces and fixed immediately onto 2.5% paraformaldehyde-glutaraldehyde in 0.1M phosphate buffer soluion (pH 7.4) for 2 hr at 4℃. After prefixation, the specimens were washed several times with the buffer solution and postfixed in 1% osmium tetroxide solution in 0.2 M phosphate buffer solution (pH 7.4) for 1 hr at 4℃. The tissues were dehydrated in increasing concentrations of ethanol, rinsed in propylene oxide and embedded in an Epon-Araldite mixture. Ultra-thin sections of a Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 microtome and a LKB ultramicrotome to 800~1000 Å. Tissue sections were mounted on collodion-coated copper grids, duble stained with uranyl acetate followed by lead citrate, and observed under a JEM 100 CX-2 (80kv) electron microscope.

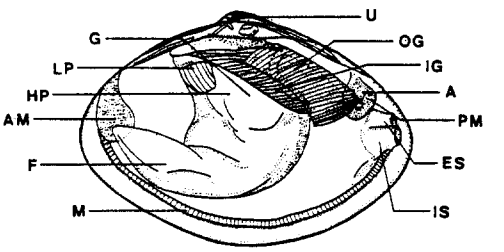

Fig. 2. Anatomy of *Mactra chinensis*.
Abbreviations; A, anus; AM, anterior adductor muscle; ES, exhalent siphon; F, foot; G, gonad; H, hepatopancreas; IG, inner gill; IS, inhalant siphon; LV, labial valve; M, mantle; OG, outer gill; PM, posterior adductor muscle; U, umbo.

The percentages of first sexual maturity were determined from the histologically prepared preparations in order to certify shell lengths of specimens that participated in reproduction before and after spawning.

RESULTS

1. Position and morphology of the gonads

Mactra chinensis are dioecious organisms composed of well defined male and female individuals. According to anatomical and histological observations, the gonads are arranged irregularly, extending from the subregions of the mid-intestinal glands in the visceral cavity to the reticular connective tissues of the foot (Fig. 2). As gonadal maturation progresses, the mature ovary appears in red, and the testis lemon-yellow in colour. At this time, if the gonads are slightly scratched, ripe eggs and milky white sperms flow out readily.

2. Electron microscope observations of the germ cell development

1) Ovary

Based on electron microscope observations, the germ cell developmental phases during oogenesis can be classified into 4 stages: 1) oogonium, 2) previtellogenic, 3) vitellogenic, and 4) mature stages.

(1) Oogonium stage

Oogonia, which multiplicated on the follicular wall (germinal epithelium), are small, oval in shape, and 9~10μm in dameter, and they are in singles or form a cluster in the oogenic follicle. Each oogonium has a large nucleus and contains several mitochondria and a endoplasmic reticulum in the cytoplasm (Fig. 3A).

(2) Previtellogenic stage

At the begining of cytoplamic growth of the previtellogenic oocyte, small, several mitochondria, Golgi apparatus and several vacuoles are concentrated around the perinuclear zone (Fig. 3 B). As development progresses, the mitochondria increase in number and usually aggregate in the cytoplasm in a region near the nucleus. The mitochondria appear as spherical or oval (Fig. 3C.) As the further development of the oocyte proceeds, in the cytoplasm of the previtellogenic oocyte, the Golgi apparatus increase in number and are scattered from the perinuclear region to the vitelline envelope of the oocyte (Fig. 3D). At that time many vacuoles formed by the Golgi apparatus appear in the cytoplasm, and lipid droplets in the vacuoles occur among the endoplasmic reticulum and a number of mitochondria in the oocyte.

(3) Vitellogenic stage

Lipid droplets and a few lipid granules appear in the perinuclear region, and microvilli on the vitelline envelope develops in the early vitellogenic oocyte (Figs. 3E, 3F). With the initiation of yolk formation, a number of cortical granules, mitochondria, small vacuoles, lipid granules and cortical granules (proteid yolk granules) occur around a well-developed cortical region of early vitellogenic oocytes. Lipid granules diffuse toward the cortical layer. The cortical granules are moderately electron dense (Figs. 3G, 3H and 3I). In the late vitellogenic oocyte, accumulation of yolk granules begins in the cortical layer and then diffusion of yolk granules gradually occurs toward the perinuclear region. Exogenous substances, viz., lipid-like granules, protein-like substances and glycogen particles appear in the germinal epithelium. Therefore, it is assumed that their substances pass into the ooplasm through the microvilli on the vitelline envelope of the late vitellogenic oocyte from the germinal epithelium (Fig. 3J).

(4) Mature stage

In the mature oocyte, lipid granules and yolk granules are accumulated in the cytoplasm, and then their granules are mixed and become larger in size (Fig. 3K). The vitelline envelope of the mature oocyte is about 0.70~0.80μm thick. It is traversed by numerous evenly spaced microvilli. The tips of the microvilli, some of which bifurcate, extend just beyond the outer border of the vitelline envelope. The vitelline envelope of a mature oocy-

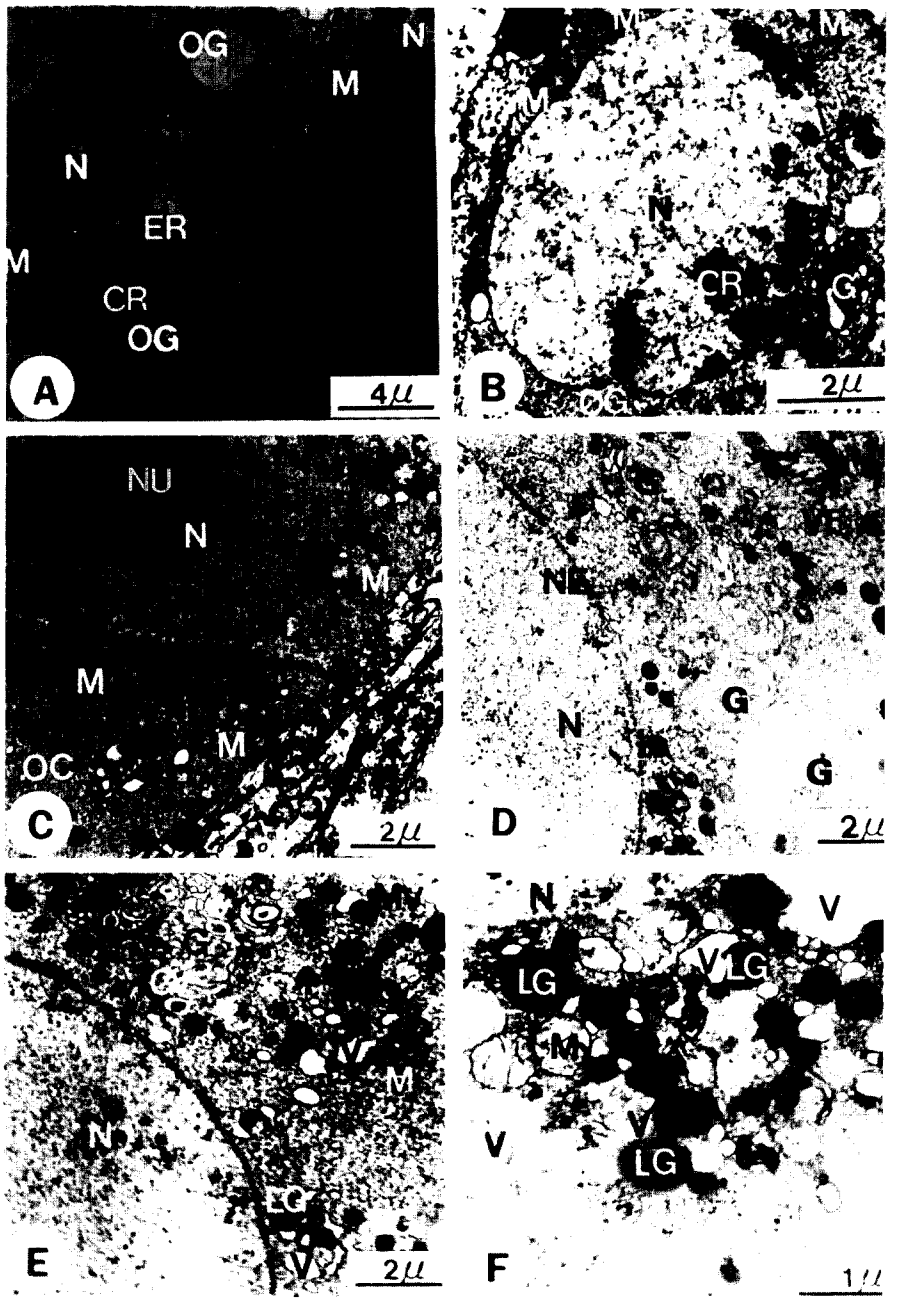

Fig. 3. Electron micrographs of oogenesis of *M. chinensis*.

A, Section of oogonia in the oogonium stage; B, an early developing oocyte in the previtellogenic stage; C, a developing oocyte; D, E, F, developing oocytes in the previtellogenic stage. Abbreviations: CR, chromatin; ER, endoplasmic reticulum; G, Golgi apparatus; LG, lipid granule; M, mitochondria; MV, microvilli; N, nucleus; NE, nuclear envelope; NU, nucleolus; OC, oocyte; OG, oogonium; V, vacuole; VE, vitelline envelope.

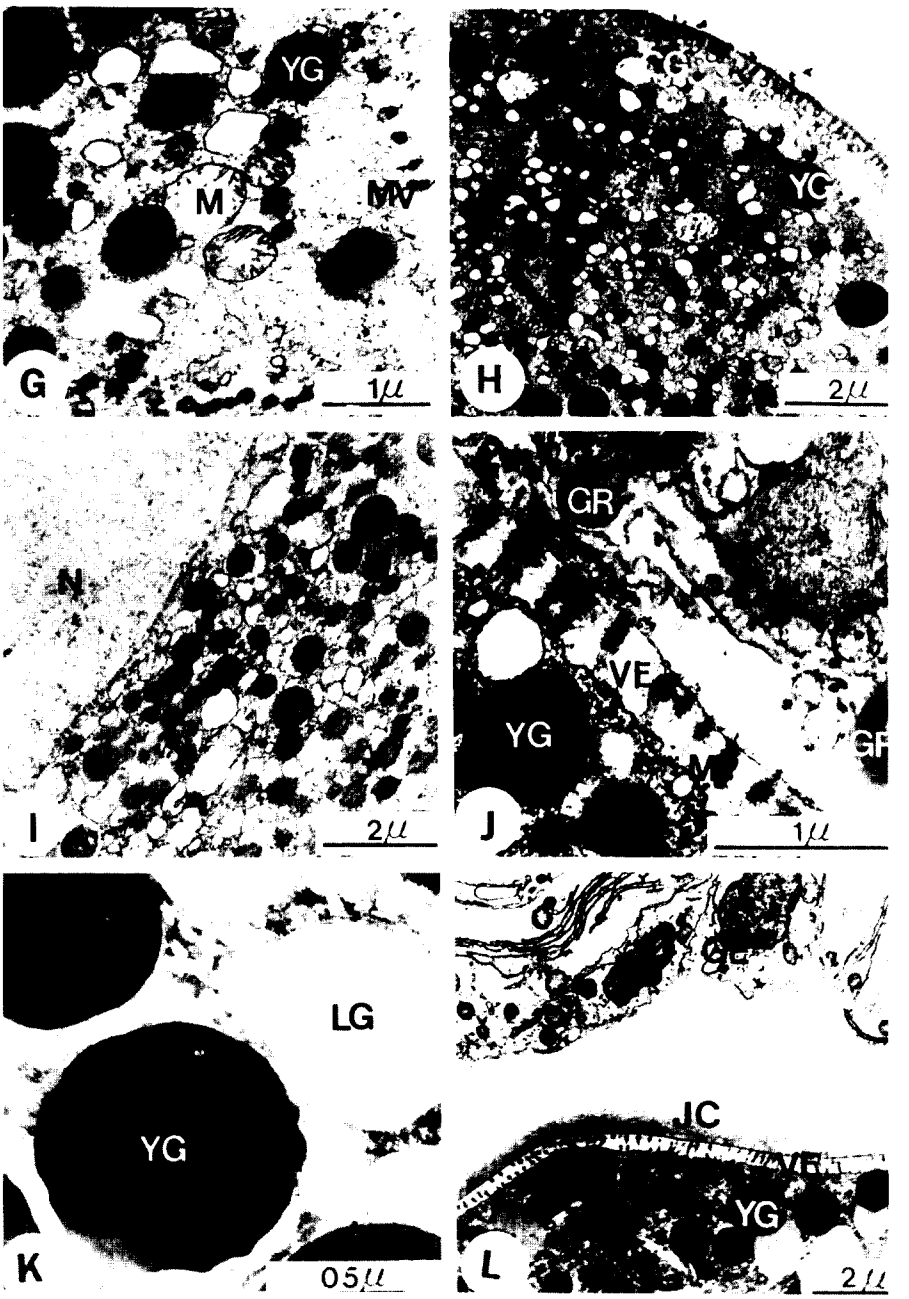

Fig. 3. continued.

G, H, I, Developments of oocytes in the vitellogenic stage; J, glycogen particles and granular substances in the germinal epithelium move to the ooplasm of developing oocyte through the vitelline envelope; K, a mature oocyte in the mature stage; L, a mature oocyte surrounded by jelly coat in the mature oocyte stage. Abbreviations: CG, cortical granule; GE, germinal epithelium; GR, granule; JC, jelly coat; LG, lipid granule; M, mitochondria; MV, microvilli; N, nucleus; VE, vitelline envelope; YG, yolk granule.

te is surrounded by a jelly coat (Fig. 3L).

2) Testis

Germ cell developmental phases during spermatogenesis can be classified into 5 stages based on electron microscope examination of germ cells : (1) spermatogonium, (2) primary spermatocyte, (3) secondary spermatocyte, (4) spermatid, and (5) spermatozoon stage.

(1) Spermatogonium stage

Spermatogenesis occurs in the spermatogenic follicle of the testis. Spermatogonia are distributed on the follicular wall (germinal epithelium) between the mesenchymal tissue and granular cells. The spermatogonia are about 9μm in diameter and are more or less oval in shape. They contain a large nucleus and a relatively small amount of cytoplasm. The nucleus contains dense and unevenly distributed heterochromatin materials. The Golgi apparatus, several mitochondria and small dense granular materials are distributed in the cytoplasm (Figs. 4A and 4B).

(2) Primary spermatocyte stage

Spermatogonia develop into the primary spermatocytes. The spermatocytes are smaller (about 7μ m in diameter) than spermatogonia. Their nuclei contain slightly more dense, heterochromatin materi-

als. The synaptonemal complexes in the nucleus appear during the prophase of meiosis. A few mitochondria are irregularly distributed in the cytoplasm (Fig. 4C).

(3) Secondary spermatocyte stage

The primary spermatocytes grow to the secondary spermatocyes through the primary maturation division. At this phase, the heterochromatin in the nucleus is more concentrated than in the previous stage. Several mitochondria are present in the cytoplasm (Fig. 4D).

(4) Spermatid stage

The secondary spermatocytes mature into spermatids. After the second maturation division, the spermatid nucleus exhibit and interphase nucleus with aggregated heterochromatin (Fig. 4E). According to the differentiation of the cell organells, the spermiogenesis could expediently be divided into Golgi, cap, acrosome and maturation phases. The morphology of the spermatid changes gradually during the early developmental stage (early Golgi phase) in the differentiation of spermatid. The Golgi apparatus and small acrosomal granule in the spermatid move to a position before the nucleus, while the mitochondria and centrosome move to a position behind the nucleus of the spermatid (Fig. 4F). The axial filament is surrounded by 4 spherical mitochondria which form the paranucleus (Fig. 4G). Small acrosomal granules merge into a larger acrosomal vesicle gradually (Fig. 4H). During the late Golgi phase, Golgi vacuole in the cytoplasm of the spermatid closely aggregates to the tip of the slightly elongating nucleus (Fig. 4I). During the mid developmental stage (cap phase) morphology of the acrosomal granule in the acrosomal vesicle is changed and form the acrosomal vesicle exhibiting two to three spines (Figs. 4J and 4K), and change to a cap-shape gradually. Acrosomal vesicle changes to an acrosome during the acrosome phase (Figs. 4L and 4M).

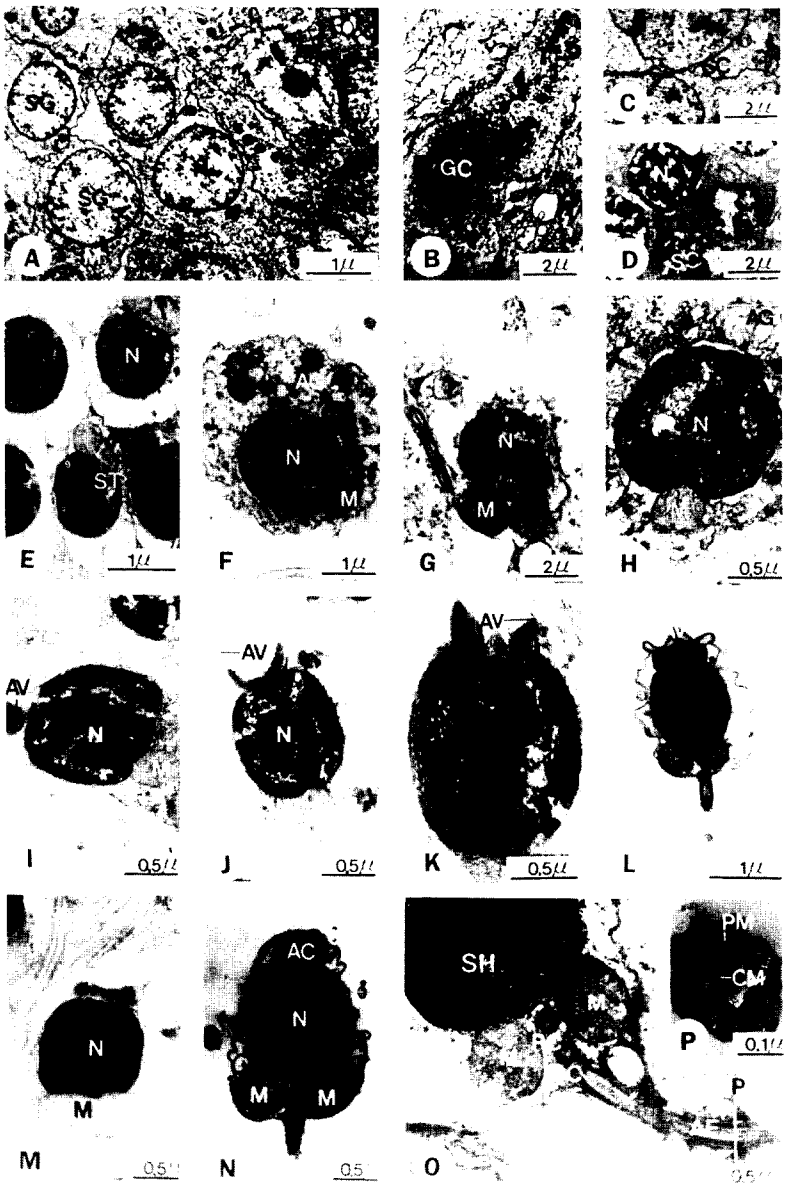

Fig. 4. Electron micrographs of spermatogenesis of *M. chinensis*.

A, Section of the spermatogonia around the mesenchymal tissues; B, a granular cell with granules; C, the primary spermatocytes during the first maturation division; D, the secondary spermatocytes during the second maturation division; E, the spermatid with concentrated heterochromatin; F, the spermatid in the early stage of the differentiation; G, cross sectioned middle region of the spermatid during spermiogeneis; H-N, acrosome formation in the late stage of the differentiation; O, sperm head, the paranucleus, the middle region and the tail of the completed spermatozoon; P, cross sectioned axial filament of sperm tail. Abbreviations: AC, acrosome; AF, axial filament; AG, acrosomal granule; AV, acrosomal vesicle; CR, chromatin; CM, central microtubules; DC, distal centriole; GC, granular cell; GR, granule; M, mitochondria; N, nucleus; PC, proximal centriole; PM, peripheral microtubles; SG, spermatogonium; SC, spermatocyte; SH, sperm head; ST, spermatid; SZN, nucleus of spermatozoon.

(5) Spermatozoon stage

The mass of mitochondria around the mid region of spermatozoon forms the paranucleus around the centrioles. The paranucleus is formed by 4 mitochondria. Of the two centrioles, the proximal centriole gives rise to the axial filament of the flagellum, the distal centriole gives rise to the axial filament of the flagellum. When spermiogenesis is completed, the head of a ripe spermatozoon is approximately 3 μm in length, and its tail is about 30 μm (Figs. 4N and 4O). The axoneme of the tail flagellum consists of nine pairs of microtubules at the periphery and one pair at the center (Fig. 4P).

3. Gonadal development and reproductive cycle

The gonadal development were investigated to confirm the timing of gamete maturation and the spawning period of the gonads. Based on histological observation of the germ cells and the somatic tissue, the gonadal development can be classified into five successive stages (Fig. 5). Gonadal phases show a periodicity. The five reproductive stages are as follows:

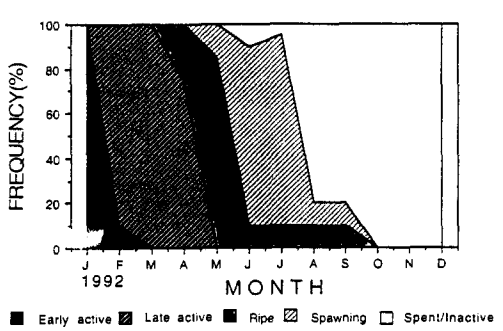

Fig. 5. Frequency of gonadal phases of *Mactra chinensis* from January to December, 1992.

(1) Early active stage

In females, oogenesis occurs in the oogenic follicles of the ovary. A large number of oogonia and a few small oocytes appear along the follicular wall. The oogonia are about 10 μm in diameter, and the early oocytes about 16~20 μm in diameter. The lumina of the oogenic follicles are empty during the early active stage (Fig. 6A).

In males, spermatogenesis occurs in the spermatogenic follicles of the testis. A number of spermatogonia, spermatocytes and mesenchymal tissue appear along the follicular wall. The lumina of the follicles are empty. The spermatogonia are about 8~9 μm, and the spermatocytes 6~7 μm in diameter (Fig. 7A). The individuals in the early active stage appear from January to February.

(2) Late active stage

In females, when the oocytes grow to 30~40 μm in diameter, each of them forms an egg-stalk connected to the follicular wall (germinal epithelium) (Fig. 6B). At this phase, each egg stalk appears to be a positive reaction to PAS stain.

In males, as gonadal development in the testis proceeds, each of the spermatogenic follicles contains spermatogonia, spermatocytes, spermatids and spermatozoa (Fig. 7B). Late active stage are observed from the clams collected during February and mid-April.

(3) Ripe stage

In females, maturing and ripe oocytes increase in size to 30~50 μm in diameter, become round or oval, and are located in the center of the lumen. The follicular wall becomes very thin (Fig. 6C).

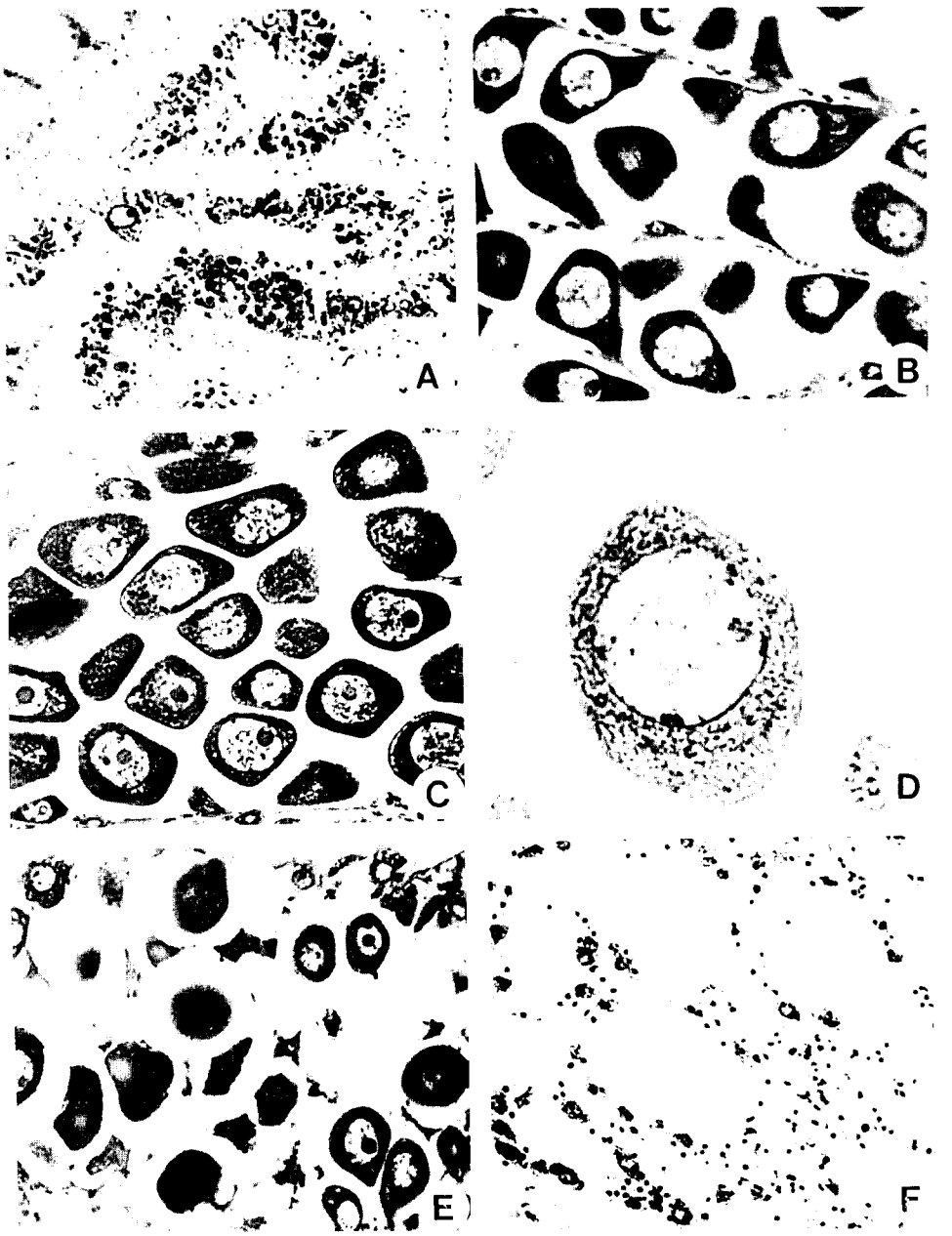

Fig. 6. Gonadal phases of female *M. chinensis* observed under light microscope.

A, Transverse section of oogenic follicles in the early active stage; B, secton of the follicles in the late active stage; C, section of a ripe ovary in the ripe stage; D, fully ripe oocytes magnified in the same stage; E, secion of the follicles in the spawning stage; F, secion of the oogenic follicles in the spent/inactive stage. A, B, C, E, F × 200; D × 600.

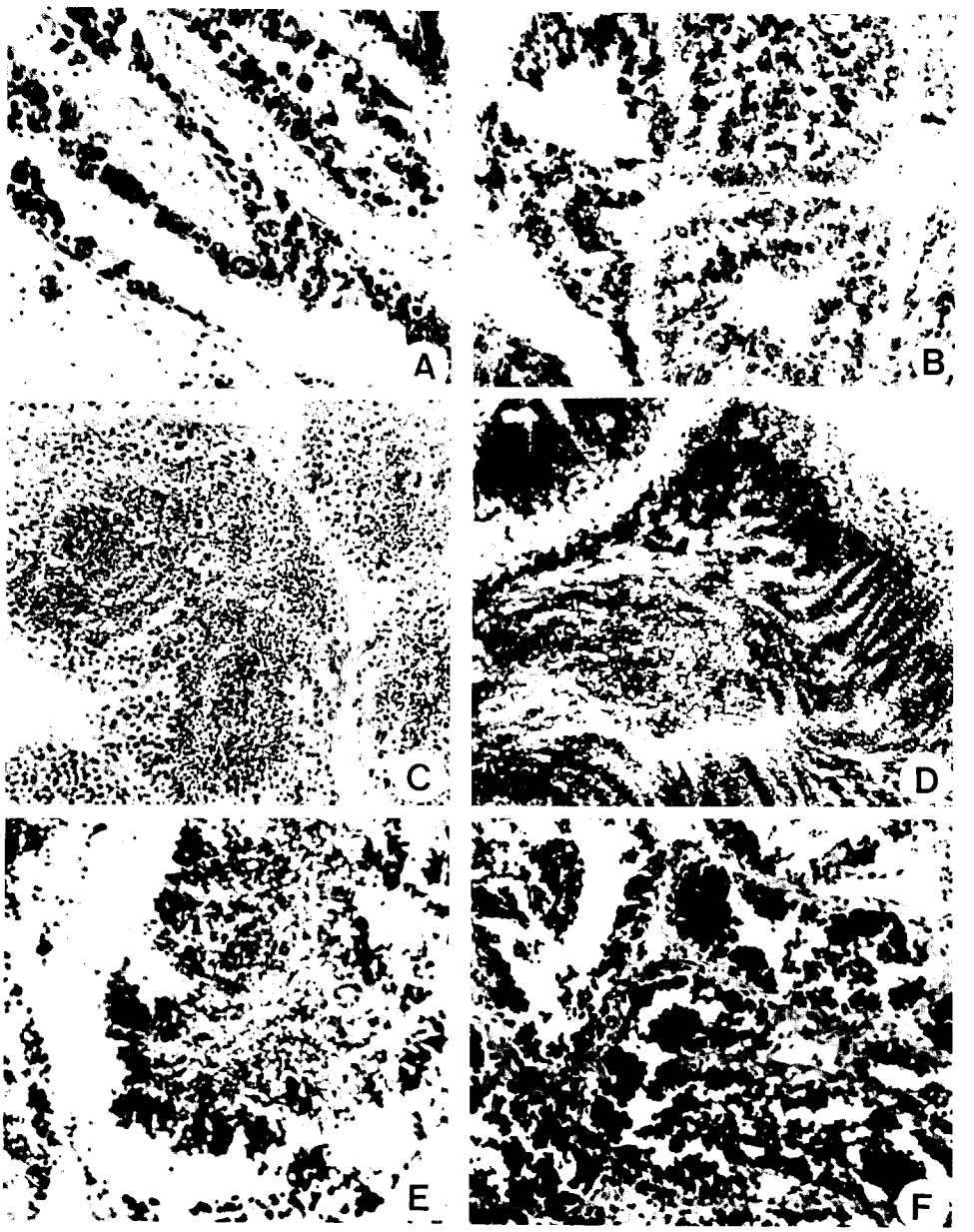

Fig. 7. Gonadal phases of male *M. chinensis* as observed under light microscope.

A, Transverse section of the spermatogenic follicles in the early active stage; B, Setion of the spermatogenic follicles in the late active stage; C, Section of the testis in the ripe stage. Note numerous spermatozoa in the lumen of the follicles; D, Section of a fully ripe testis magnified in the same stage; E, Section of the follicles in the spawning stage; F, Section of the follicles in the spent /inactive stage. A, D, E × 300; B, C, F × 200.

Each ripe oocyte, 50~60 μm in diameter, is surrounded by gelatinous membrane and its cytoplasm exhibits a large number of yolk and a few lipid granules. Germinal vesicle and a basophilic nucleolus are also observed in ripe oocyte (Fig. 6D).

In males, the spermatocytes grow into spermatids, and the spermatids undergo transformation into differentiated spermatozoa. Numerous spermatozoa occur in the center of the lumina of the follicles (Fig. 7C). A fully ripe testis is characterized by the formation of stream of numerous spermatozoa in their follicles (Fig. 7D).

Individuals in the ripe stage appear from the begining of April to September.

(4) Spawning stage

In females, the ripe oocytes in the oogenic follicles are discharged and a few ripe oocytes undischarged are remained as well as developing oocytes (Fig. 6E).

In males, spermatozoa in the lumen are discharged and the lumina of the spermetogenic follicles becomes empty. However, a few undischarged spermatozoa, as well as spermatids and spermatocytes, remain in the follicle (Fig. 7E).

The spawning period appear once a year from the end of May to mid-September, with the spawning peaks between June and July when seawater temperatures reaches over 22.0℃.

(5) Spent/ Inactive stage

In females, each follicle of the ovary is contracted after spawning and degenerated (Fig. 6F). Thereafter, the rearrangement of the connective tissues are observed, occassionally few or no sex cells present in this stage.

In males, after spawning, a few remaining spermatozoa and spermatocytes are scattered in the follicles, however, they begin to degenerate (Fig. 7F).

Individuals in these stages are found from June to December.

4. First sexual maturity

The first sexual maturity of a total of 341 individuals of *M. chinensis*, ranging from 2.6 to 6.5 cm in shell length, was investigated histologically in order to certify the shell lengths that participated in reproduction from May to September as shown in Table 1. Percentages of the first sexual maturity of female and male individuals ranging from 3.5~3.9 cm were 54.5% and 58.3%, respectively, and from those over 5.0 cm in shell length, it was 100%.

Table 1. Shell length of first sexual maturity of *Mactra chinensis* during the spawning period from May to September.

Shell	Female		Male	
length(cm)	Number	Maturity(%)	Number	Maturity(%)
2.6~2.9	23	0	25	0
3.0~3.4	24	20.8	21	28.6
3.5~3.9	22	54.5	24	58.3
4.0~4.4	20	80.0	26	80.8
4.5~4.9	21	90.5	20	95.0
5.0~5.4	17	100.0	23	100.0
5.5~5.9	18	100.0	16	100.0
6.0~6.3	23	100.0	18	100.0
Total	168		173	

DISCUSSION

1. Gonad development

It is well-known that gonad development of bivalves are closely related to water temperature (Sastry, 1966, 1968; Chang and Lee, 1981; Chung *et al.*, 1991, Chung *et al.*, 1994), food availability (Sastry, 1966, 1968; Griffiths, 1977; Chung *et al.*, 1991) and day length (Simpson, 1982). In the present study, rapid gonadal development and maturation of *M. chinensis* occurs spring-summer seasons when the seawater temperature rises with abundant food organisms. Accordingly, the period of food availability and gonadal development coincides at the study area. Inactive stage observed during fall to under season also coincides with low food availability and low seawater temperature as reported by several studies.

2. Gameted development and vitellogenesis

Lipid droplets often arise in the cytoplasm of the oocytes before proteinaceous yolk synthesis begins (Humpreys, 1962). Reverberi (1971) reported that the Golgi apparatus present in the perinuclear region is involved in lipid droplet formation in *Mytilus edulis*. Chung *et al.* (1991) reported that in the vitellogenic oocytes of *Cyclina sinensis*, the Golgi apparatus and mitochondria in the perinuclear region are involved in lipid droplet and lipid granule formations. It is possible that the Golgi apparatus and mitochondria present in the perinuclear region may be involved in lipid droplet formation in the oocyte as observed in *Cyclina sinensis*. The yolk granules first originate in the cortical regions of the oocyte and then fill the entire ooplasm of the oocyte. Yolk granules vary in different regions of the egg. Therefore, various cell organelles, viz., the Golgi apparatus, mitochondria and vacuoles, are thought to be involved in endogenous formation of yolk granules in the cytoplasm, Exogeneous electron dense granular substances and glycogen particles in the germinal epithelium are passed into the ooplasm of the oocyte through the microvilli of the vitelline envelope of the vitellogenic oocyte. Vitellogenesis may be occurred by way of the endogenous synthesis in the oocyte and input of exogenous substances to ooplasm.

Most bivalves have a primitive type of spermatozoon with a small head and cap-shaped acrosome, a short mid-piece with four to five mitochondria near the centrioles (Longo and Dornfield, 1967; Lee, 1983). However, spermatozoa of *M. chinensis* in this study exhibit some differences compared to other bivalve spermatozoa, with a cap-shaped acrosome, a short mid-piece with four mitochondria.

3. Fate of the gametes

Some authors (Morvan and Ansell, 1988; Paulet, 1990) stated that continuous production and resorption of gametes depend on environmental temperature and food availability. After spawning, gamete resorptions common in the follicles of the gonads are also observed in *M. chinensis*. For resorptions of reproductive energy, a number of undischarged gametes or hopeless gametes in the follicles of the gonads should be resorbed by way of the gamete atresia. Therefore, it is supposed that *M. chinensis* have a mechanism to resorb the gametes and utilize the high nutritive reserves for

allocation of the reproductive energy for developing oocytes or to use for other metabolic purposes as observed in other bivalves (Dorange and LePennec, 1989; Motavkine and Veraksine, 1989).

4. Breeding pattern and spawning

Marine invertebrates have unique breeding patterns. Boolootian *et al.* (1962) categorizes breeding pattern of molluscs 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 and cytological observations of its gonads, *M. chinensis* belongs to summer breeders as most marine bivalves (Chung *et al.*, 1991).

Regarding the spawning season, Miyasaki (1939) reported that the optimum water temperature for spawning in the hen clam is 22~28℃. In the present study, the water temperature varies from 20.3 to 27℃ during the spawning period, and it coincides well with Miyasaki's observation.

The spawning period of the Japanese hen clam, *Mactra sulcataria* is between May and June in Tokyo Bay (Hanaoka and Shimadzu, 1949). However, the spawning peaks of the Korean hen clam, *M. chinensis*, is observed from late May to mid-September. Defferent spawning period of the two hen clam species might be related to water temperature due to the geographical distance (Belding, 1930; Chung *et al.*, 1991), time of the food availability (Chung *et al.*, 1991), and some other environmental factors.

5. First sexual maturity

Kim *et al.* (1985) reported that percentage of first sexual maturity of the hen clams was 50% among those individuals of 3.64 cm (corresponding to one year old) and from those over 4.75 cm in shell length, it was 100%.

In the present study, the percentage of first sexual maturity of clams ranging from 3.5 to 3.9 cm was over 50% and from those over 5.0 cm in shell length, it was 100%. Therefore, individuals ranging from 3.5 to 3.9 cm in shell length are considered to be one year old (Kim *et al.*, 1985). It is probable that the hen clam may sexually mature at one-year old in males and females.

ABSTRACT

Germ cell development, gonadal development, and reproductive cycle of *M. chinensis* Philippi were investigated using individuals collected from the subtidal zone of Seonyeon-Ri, Chollabuk-Do, on the west coast of Korea, on a monthly basis from January to December, 1992.

M. chinensis exhibit distinct male and female. The processes of vitellogenesis vary with developmental stages of oocytes. In the cytoplasm of the previtellogenic oocyte, the Golgi apparatus, vacuoles and mitochondria in the perinuclear region are involved in lipid droplet formation. In the vitellogenic oocyte, lipid granules occur near the nuclear envelope and are dispersed toward the cortical layer, The proteid yolk granules first originated in the cortical regions of the oocyte are dispersed from the cortical layer near the vitelline envelope to the perinuclear region. In the late vitellogenic oocytes, an extensive development of granular endoplasmic reticulum, Golgi apparatus, mitochondria, and vacuoles in the cytoplasm are

involved in the formation of yolk granules autosynthetically. Exogenous substances, viz., lipid-like granules, protein-like substances and glycogen particles appear in the germinal epithelium pass into the ooplasm through the microvilli on the vitelline envelope of the vitellogenic oocyte from the germinal epithelium. This phenomenon shows a posibility of heterosynthetic yolk formation. Ripe oocytes are about 50~60 μm in diameter, the head of a ripe spermatozoon is approximately 3 μm in length and its tail is about 30 μm. The axoneme of the tail flagellum consists of a pair at the center and nine pairs of microtubules at the periphery.

The spawning period continues from May and mid-September, with a peak between June and July when the seawater temperature reaches over 22℃. The reproductive cycle can be classified into five succesive stages: early active (January to February), late active (February to April), ripe (April to September), spawning (May to September), and spent/inactive stages (June to December).

Percentage of first sexual maturity in female and male clams ranging from 3.5 to 3.9 cm in shell length was over 55.5%, and for clams over 5.0 cm in shell length it was 100%. Therefore, individuals ranging from 3.5 to 3.9 cm in shell length are considered to be one year old. It is assumed that the hen clams are sexually mature after one year old in males and females.

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