발생과 생식 제 12권 제 1호, 41~49 (2008)
Dev. Reprod. Vol. 12, No. 1, 41~49 (2008)

Ultrastructural Studies of Oogenesis and Oocyte Degeneration in Female *Ruditapes philippinarum* (Bivalvia: Veneridae) from Gomso Bay, Korea

Ki-Young Lee and Ee-Yung Chung[1,†]

School of Marine Life Science, College of Ocean Science & Technology, Kunsan National University, Gunsan 573-701, Korea

[1]Korea Marine Environment & Ecosystem Research Institute, Dive Korea, Bucheon 420-120, Korea

곰소만에 서식하는 암컷 바지락 *Ruditapes philippinarum*의 난형성과정 및 난모세포 퇴화의 미세구조적 연구

이 기 영 · 정 의 영[1,†]

군산대학교 해양과학대학 해양생명과학부, [1]다이브코리아, 한국해양환경생태연구소

ABSTRACT : Ultrastructural changes occurring during the course of development and degeneration of oocytes in female *Ruditapes philippinarum* (Adams & Reeve, 1850) are described for clams collected from Gomso Bay, Korea. During the early stages of oogenesis, desomosome-like gap junctions localized between the early vitellogenic oocyte and the follicle cells. Vitellogenesis occurs through a process of autosynthesis, involving the combined activity of the Golgi complex, mitochondria and rough endoplasmic reticulum, and heterosynthesis in which extraovarian precursors are incorporated into oocytes by endocytotic activity, involving the basal region of the early vitellogenic oocytes prior to the formation of the vitelline envelope. The follicle cells appear to play an integral role in vitellogenesis and oocyte degeneration: phagocytosis and intracellular digestion of products originating from oocyte degeneration. These functions can permit a transfer of yolk precursors necessary to vitellogenesis, and they can accumulate nutrients in the cytoplasm, as glycogen and lipids, which can be employed by the vitellogenic oocyte. During the period of oocyte degeneration, follicle cells may have lysosomal system for breakdown, and resorb various phagosomes in the cytoplasm for nutrient storage. But follicle cells probably are not the major source of yolk precursors in vitellogenesis.

Key words : *Ruditapes philippinarum*, Ultrastructure, Oogenesis, Oocyte degeneration.

요 약 : 한국 곰소만산 암컷 바지락(*Ruditapes philippinarum*)의 난모세포의 발달 및 퇴화과정 중 일어나는 미세구조적 변화에 관해 기술하였다. 난소소낭은 영양성분을 저장하는 포상결체조직세포(VCT cell)들의 기질에 의해 둘러싸여 있다. 난 형성과정 초기에 초기난황형성난모세포와 보조세포(follicle cell)들 사이에 desmosome-like gap junction들이 나타났다. 난황형성과정은 골지체, 미토톤드리아, 조면소포체가 결합되어 작용하는 자율합성과정을 통하여 일어나며, 난황막 형성에 앞서 난황형성과정의 초기와 난모세포 퇴화에 없어서는 안될 역할을 하는 것으로 나타났다: 보조세포들은 식세포작용 및 난모세포 퇴화로부터 유래되는 산물들의 세포내소화에 관여한다. 이들의 기능은 난황형성에 필요한 난황전구체들로 전환이 일어나게 한다. 또한, 보조세포들은 난황형성난모세포들이 이용하는 글리코겐과 지방적들을 세포질 내에 영양물질로 축적, 저장할 수 있다. 보조세포들은 난모세포가 퇴화되는 기간 중에는 난모세포의 붕괴를 위해 리소조옴 체제를 가지며, 영양물질 저장을 위해 세포질 내에 존재하는 여러 종류의 파고소옴(phagosome)들을 흡수하였다. 그러나 보조세포들은 난황형성과정에서 난황형성을 위한 난황전구체의 주된 공급원이 아니라고 추정된다.

INTRODUCTON

In various species of mollusks, many investigations on oogenesis were conducted at the light microscopic level

† Correspondence: Korea Marine Environment & Ecosystem Research Institute, Dive Korea, Bucheon 420-120, Korea, Tel: +82-32-328-5145, Fax: +82-32-328-5554, E-mail: eychung777@gmail.com

using paraffin histology (Galtsoff, 1964; Raven, 1966; Lee, 1972; Lee & Chung, 1980; Chang & Lee, 1982; Chung et al., 1994). As a result, interpretation of the cytological features of oocyte differentiation have been limited due to the low resolution of these techniques. Many studies have examined aspects of the reproductive ecology of *R. philippinarum* in Korea, Japan, and other countries (Chung et al., 1994; Hur, 1994), including reproduction and maturation of gametes (Toba & Miyama, 1995), the spawning season (Ponurovsky & Yakovlev, 1992), the reproductive cycle (Toba et al., 1993; Toba & Miyama, 1994, 1995; Chung et al., 1994; Tsuji et al., 1994; Goshima, et al., 1996), and aspects of classification, including distribution (Yoo, 1976; Kwon et al., 1993). Neverthless, no information is available on the mechanism of vitellogenesis in *R. philippinarum*. Recently, vitellogenesis during oogenesis has received a great deal of attention in the study of the reproductive mechanism of bivalves (Jong-Brink et al., 1983). Neverthless, comprehensive ultrastructural studies of bivalve oogenesis have been reported for a relatively small number of species, mainly those of economic importance: *Mytilus edulis* (Albertini, 1985; Pipe, 1987a,b), *Brachidontes virgiliae* (Bernard et al., 1988), *Pecten maximus* (Paulet et al., 1988; Dorange & Le Pennec, 1989a,b; Paulet & Boucher, 1991; Le Pennec et al., 1991), *Cyclina sinensis* (Chung et al., 1991), *Crassostrea gigas* (Suzuki et al., 1992), *Pinna nobilis* (Gaulejac et al., 1995), *C. virginica* (Eckelbarger & Davis, 1996), *Bathymodiolus childressi* (Eckelbarger & Young, 1999), *Mactra veneriformis* (Chung & Ryou, 2000) and *Patinopecten yessoensis* (Chung et al., 2005). Regarding the functions of the acinus and vesicular connective tissue cells (VCT cells), Eckelbarger and Davis (1996) described that each acinus is bathed in hemal fluid contained within a hemocoelic space, and there is no effective cell barrier between the hemocoel and the oocytes. Therefore, the ultrastructure of the VCT cells in *R. philippinarum* requires further study in detail. In the majority of bivalve species, the ovaries commonly contain follicle cells (as a kind of the accessory cell) that play some role in the storage, mobilization and or synthesis of yolk precursors during oogenesis (Wourms, 1987). The follicle cells are intra-acinal and usually surround the previtellogenic and early vitellogenic oocytes (Eckelbarger & Davis, 1996). However, as oocyte volume increases during vitellogenesis, the follicle cells withdraw from the oocyte surface while retaining contact with the basal region of the oocyte (Pipe, 1987a). No studies have documented the ultrastructural features of developing oocyte and the follicle cells during the process of oogenesis in female *R. philippinarum*. In addition, oocyte degeneration (known as atresia) is a commonly observed phenomenon in species, as gametogenesis is under control of natural environmental conditions (Pipe, 1987a). Products of lysis by the follicle cells are a source of metabolites that can be rapidly mobilized by the organism (Herlin-Houtteville & Luet, 1975; Pipe, 1987; Dorange et al., 1989; Le Pennec et al., 1991; Gaulejac, et al., 1995). Above all, functions of the follicle cells in resorption of products of lysis from atretic oocytes of this species should be investigated in detail.

The purpose of the present study is to describe ultrastructural features of the oocyte-follicle cell relationship during oogenesis and oocyte degeneration of *R. philippinarum*, and to compare and contrast it with other bivalve species.

MATERIALS AND METHODS

1. Sampling

Specimens of *Ruditapes philippinarum* were collected monthly from the intertidal zone of Gomso Bay, on the west coast of Korea, from January to December, 2001. 12～17 female clams, ranging from 45.1 mm to 54.6 mm in shell length, were used for the cytological studies.

2. Observation of Oocyte Differentiation and Degeneration

For electron microscopy, excised pieces of ovaries were cut into smaller pieces and fixed immediately in 2.5%

paraformaldehyde, in 0.1 M phosphate buffer solution (pH 7.4) for 2 hours at 4℃. The specimens then were washed several times in the buffer solution and postfixed in 1% osmium tetroxide in a 0.2 M phosphate buffer solution (pH 7.4) at 4℃ for 1 hour. Specimens then were dehydrated in increasing concentrations of ethanol, cleared in propylene oxide, and embedded in an Epon-Araldite mixture. Ultra-thin sections of Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 microtome and a LKB ultramicrotome at a thickness of about 80~100 nm. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrate, and observed with a JEM 100CX-2 (80 kv) electron microscope.

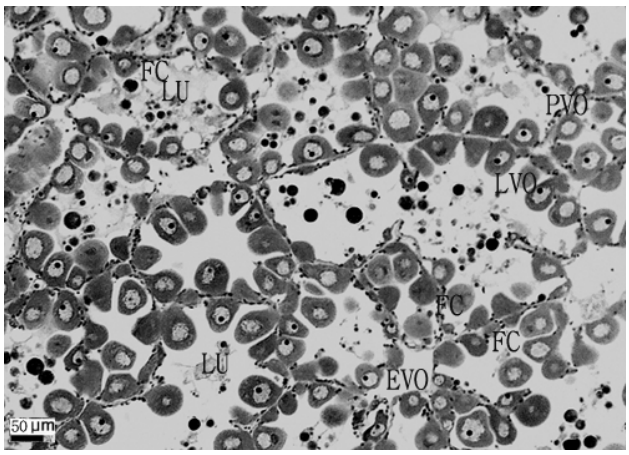

Fig. 1. A photomicrograph of the ovarian structure in female *Ruditapes philippinarum*. Abbreviations: EVO, early vitellogenic oocyte; FC, follicle cell; LU, lumen; LVO, late vitellogenic oocyte; PVO, previtellogenic oocyte.

RESULTS

1. General Morphology of the Ovary

The ovary is composed of a number of acini and is a diffuse organ consisting of highly branching acini, in which germ cells develop. Oocytes were distributed along the inner wall of each acinus in all stages of differentiation, ranging from previtellogenic oocytes to mature oocytes. The follicle cells were observed in association with oocytes at all stages (Fig. 1).

2. Oogenesis

Oogenesis occur in the ovarian follicles of the ovary. Four phases of oogenesis could be recognized: (1) oogonia, (2) previtellogenic oocytes, (3) vitellogenic oocytes, and (4) mature oocytes.

3. Oogonia

The oogonia, which measured from 9~10 μm in diameter, were round or oval in shape. They possessed a large ovoid nucleus in which the chromatin is reticular and marginal. The oogonia were characterized by high a nuclear to cytoplasmic ratio. Several mitochondria, the Golgi complex, endoplasmic reticulum and lipid droplets appeared in the cytoplasm of the oogonia. Commonly, they were present in layers of one or two cells thick along the edges of the acini (Fig. 2A).

4. Previtellogenic Oocytes

The oogonia develop into previtellogenic oocytes. The oocyte was somewhat elongated and pedunculated, and the diameters of the nuclei and oocytes were 4~5 μm and 11~15 μm in diameter, respectively. A number of the mitochondria and the endoplasmic reticulum were concentrated around the nucleus, but microvilli were not yet present on the oolemma. At this stage, the previtellogenic oocytes were partially surrounded by follicle cells that maintain intimate contact with the smooth oolemma of the oocyte. Follicle cells which measured from 6 to 8 μm in diameter appeared the periphery of the acinus wall. The follicle cells possessed a large nucleus, and contained characteristically parallel arrays of the rough endoplasmic reticulum in the cytoplasm (Fig. 2B).

5. Vitellogenic Oocytes

Early vitellogenic oocytes measured 20~25 μm. in diameter. the large nucleus and nucleolus became round or

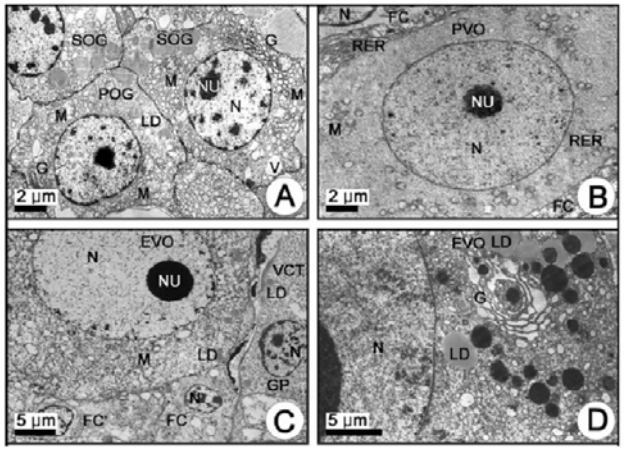

Fig. 2. Electron micrographs of oogenesis in female *Ruditapes philippinarum*. A. The primary oogonia (POG) and secondary oogonia (SOG), Note a large nucleus (N), several mitochondria (M), and Golgi complexes (G) in the cytoplasm; B. A previtellogenic oocyte (PVO) and the follicle cell (FC). Note a nucleolus (NU) in a large nucleus (N) and the mitochondria (M) and rough endoplasmic reticulum (RER) in the cytoplasm of the previtellogenic oocyte, and a follicle cell (FC) with rough endoplasmic reticulum (RER); C. The early vitellogenic oocyte (EVO), follicle cells (FC) and the vesicular connective tissue cell (VCT cell). Note a nucleolus (NU) in the nucleus (N) and the mitochondria (M) and lipid droplets (LD) in the early vitellogenic oocyte, and the VCT cell (VCT) with a large nucleus (N) and lipid droplets (LD); D. The early vitellogenic oocyte. Note a number of lipid droplets (LD) near the Golgi complex (G).

slightly oval in shape. In the early oogenesis, a number of lipid droplets and yolk precursors appeared among the rough endoplasmic reticulum, mitochondria and the Golgi complex in the cytoplasm of the oocytes.

The early vitellogenic oocyte was still attached to the acinus wall, and its apex bulges into the lumen of the acinus. At this time, follicle cells contained the rough endoplasmic reticulum, glycogen particles and a few lipid droplets in the cytoplasm. The follicle cells were connected to an early vitellogenic oocyte near the acinus wall. At this stage, the vesicular connective tissue cells (VCT cells) were attached to the outer wall of the acinus. The acini were encircled by a connective tissue compartment and hemocoel that were separated them from the matrix of surrounding VCT cells. The outer wall of the acinus was defined by a discontinuous layer of thin squamous myoepithelial cells that forms a partial barrier between vitellogenic oocytes and the hemocoel. The ultrastructure of VCT cells in this species was bag-like in shape, with an irregularly shaped nucleus containing a single nucleolus. And the cytoplasm was filled with large quantity of glycogen particles, a few mitochondria and a small number of lipid droplets during vitellogenesis (Fig. 2C).

In the same stage oocytes, lipid droplets appeared near the Golgi product in the Golgi complex (Fig. 2D). A number of lipid droplets also appeared between the mitochondria and well-developed rough endoplasmic reticulum (Fig. 3A). When oocytes began to form microvilli on the oolemma, the initial contours of the microvilli were ovoid in shape. During early oogenesis, several coated vesicles, at the basal region of the oocyte, formed membrane-bound vesicles by endocytosis. Uptake of nutritive material in the coated vesicle formed by receptor-mediated endocytosis was through the coated pits on the oolemma during vitellogenesis. The microvillous borders are formed on the oolemma (Fig. 3B). In late vitellogenesis, as oocyte volume increases, the ooplasm of the stalked region was filled with a number of lipid droplets, numerous yolk granules, mitochondria, and rough endoplasmic reticulum. Follicle cells gradually were lost their intimate association with the entire oocyte surface, and microvilli appeared along the vitelline envelope where the follicle cells withdrew. The cytoplasm of the follicle cell was filled with myelin-like organelles, mitochondria and vacuoles (Fig. 3C). In this stage, yolk precursors, well-developed endoplasmic reticulum, mitochondria and lipid droplets appeared in the cytoplasm (Fig. 3D). Proteinaceous yolk granules also appeared between the mitochondria and cortical granule near the vitelline envelope (Fig. 4A). Immature yolk granules containing several different components were intermingled and formed larger yolk granules (approximately 2.0 to 2.5 μm in diameter) in the cytoplasm of the oocyte (Fig. 4B).

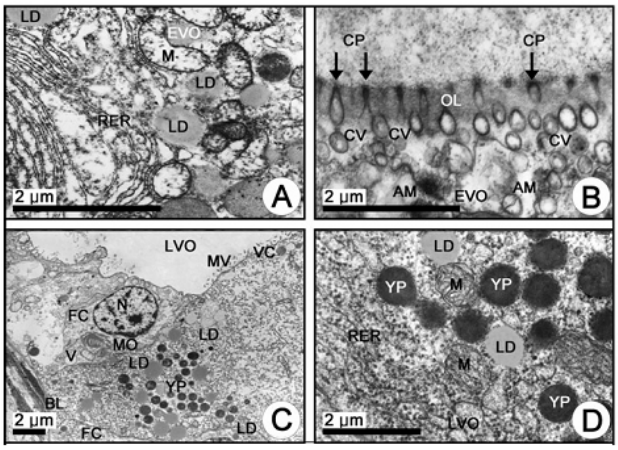

Fig. 3. Electron micrographs of oogenesis in female *Ruditapes philippinarum*. A. The early vitellogenic oocyte (EVO). Note a number of lipid droplets (LD) between well-developed rough endoplasmic reticulum (RER) and the mitochondria (M); B. The oolemma and basal region (cortical region) of the early vitellogenic oocyte (EVO). Note occurrences of the coated vesicles (CV) through the coated pits by endocytosis and amorphous materials (AM) formed by exocytosis at the basal region of the oolemma of the early vitellogenic oocyte; C. Follicle cells which are attached to the stalk region of the late vitellogenic oocyte (LVO). Note an oval nucleus (N), vacuoles (V) and myeline-like organelles (MO) in the follicle cell (FC), the microvilli (MV) on the vitelline coat (VC) and the cytoplasm of the stalk region containing a number of yolk precursors (YP) and lipid droplets (LD); D. A late vitellogenic oocyte (LVO) during yolk formation. Note a number of yolk precursors (YP) among the well-developed endoplasmic reticulum (RER), the mitochondria (M) and lipid droplets (LD).

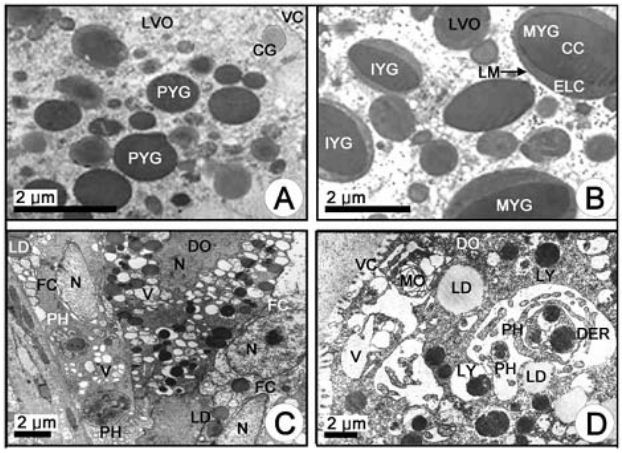

Fig. 4. Electron micrographs of oogenesis and oocyte degeneration in female *Ruditapes philippinarum*. A. A late vitellogenic oocyte. Note with a number of proteinaceous yolk granules (PYG) and several cortical granules (CG) near the vitelline envelope (VE); B. A mature oocyte. Note immature yolk granules (IYG) and mature yolk granules (MYG) being composed of three parts: 1) crystalline core (CC), 2) electron lucient cortex (ELC), and 3) a limiting membrane (LM); C. A degenerated oocyte and the follicle cells. Note a degenerated oocyte containing irregular nucleus (N) and degenerating yolk granules and phagosomes by lysosome, and the follicle cell containing a elongated nucleus, lipid droplets (LD) and various phagosomes and a number of vacuoles; D. Vitelline coat (VC) and phagosomes by lysosomes in the degenerated oocyte (DO). Note distended endoplasmic reticulum (DER), a number of vacuoles, lipid droplets, degenerated yolk granules, myeline- like organelles (MO), various phagosomes (PH) and lysosomes (LY) in the degenerated oocyte (DO).

6. Mature Oocytes

In the mature oocyte, small immature yolk granules were intermingled and formed larger mature yolk granules (2.5 to 3.0 μm). A mature yolk granule in the cytoplasm of a mature oocyte is composed of three parts: (1) a crystalline core, (2) an electron lucient cortex, and (3) a limiting membrane. Mature oocytes containing numerous yolk granules in the cytoplasm were approximately 55~65 μm in diameter. Finally, the mature oocyte was separated from the acinus wall (germinal epithelium) (Fig. 4B).

7. Oocyte Degeneration

The degenerating oocytes appeared slightly irregular or polyhedric near the follicle cells and are deformed by compression in the acinus. A number of vacuoles, degenerating yolk granules and lipid droplets appeared in the ooplasm. A few phagosomes (lysosomes), a number of vacuoles and a small number of lipid droplets appeared in the cytoplasm of the follicle cells, while glycogen particles decrease in the cytoplasm of follicle cells attached to the degenerating oocyte (Fig. 4C).

As oocytes disintegrate, the endoplasmic reticulum, the

first oocytic organelle, was involved in the degenerative process. The rough endoplasmic reticulum became distended, leading to vacuolation of ooplasm. The mitochondria and yolk granules were disintegrated in the ooplasm, and lysis began at the periphery of the oocyte; numerous heterogenous, dense granules similar to phagosomes (lysosomes) and several vacuoles were also present in the ooplasm. Many disintegrated granules with myelin-like organelles were visible at the periphery of the oocyte (Fig. 4D).

DISCUSSION

1. Functions of the Oocyte and Follicle Cell during Oogenesis

Commonly, lipid droplets appeared in *R. philippinarum* at the beginning of the vitellogenic stage. Gaulejac et al. (1995) described that the lipid may be produced outside the oocyte by auxiliary cells. So far, no clear morphological evidence was available for the processes involved in lipid droplet formation.

From the ultrastructural study, vitellogenesis can be classified into two processes: autosynthetic and heterosynthetic yolk formation (Eckelbarger & Eckelbarger, 1989). It is assumed that vitellogenesis occurs through a process of autosynthesis, involving the combined activity of the Golgi complex and rough endoplasmic reticulum (Fig. 5). On the other hand, Pipe (1987a) reported endocytotic activity in the oocytes of *M. edulis*, and Eckelbarger and Davis (1996) presented evidence for heterosynthetic yolk formation in the oocytes of *C. virginica*. In the present study, extra-ovarian precursors were incorporated into oocytes by endocytosis at the basal region of the early vitellogenic oocytes evidence for heterosythetic yolk formation. Beside heterosynthetic yolk formation by endocytosis and exocytosis, for nutrient supply to vitellogenic oocytes, follicle cells may be involved in the process of heterosythetic yolk formation.

Pipe (1987a) reported that in *M. edulis*, once the follicle cells withdraw, the microvilli appear along the oolemma of the oocyte. In the present study, we observed the same phenomenon.

In bivalve ovaries, including *C. virginica*, several follicle cells are associated with each oocyte during early stages of oogenesis (Eckelbarger & Davis, 1996). However, in the scallop *Pecten maximus*, only a single "auxiliary cell" is associated with each oocyte (Dorange & Le Pennec 1989b). In contrast, in the pectinid, *Patinopecten yessoensis*, and the mussel *Crenomytilus grayana*, "auxiliary cells"

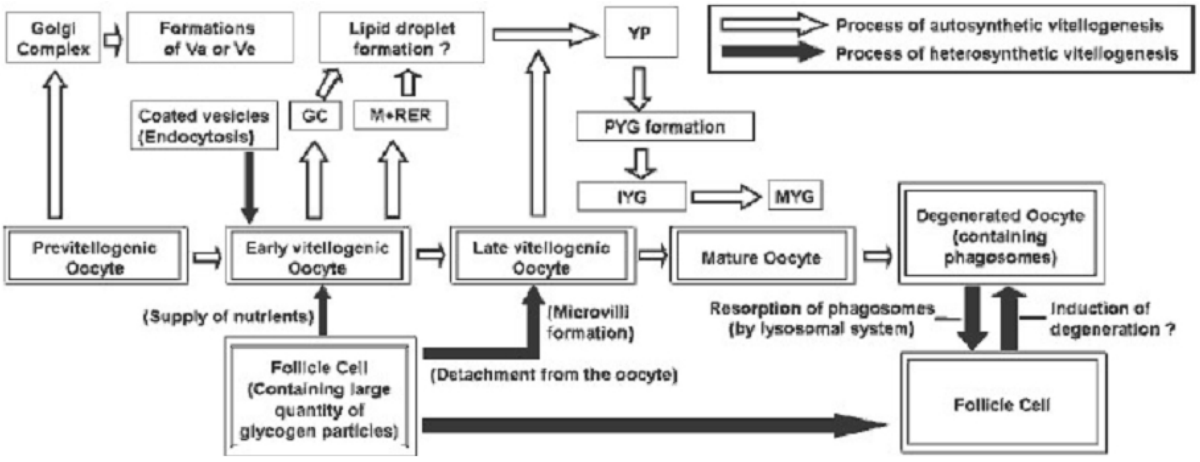

Fig. 5. Schematic diagrams of the process of vitellogenesis during oogenesis and oocyte degeneration in female *Ruditapes philippinarum*. Abbreviations: GC, Golgi complex; IYG, immature yolk granule; M, mitochondria; MYG, mature yolk granule; PYG, proteinaceous yolk granule; Va, vacuole; Ve, vesicle; YP, yolk precursor.

completely encompass oocytes that are free in the acinus lumen, and they are alleged to transfer metabolites to the oocytes by pinocytosis (Mortavkine & Varaksine 1983). In the present study, a few follicle cells of *R. philippinarum* are associated with each oocyte during early stages of oogenesis, to *C. virginica* (Eckelbarger & Davis, 1996).

Jong-Brink et al. (1983) distinguished three categories of oocyte-follicle cell relationships by the number and arrangement of the follicle cells; In the first, the oocyte is completely surrounded by increasing numbers of follicle cells; in the second, the oocyte is surrounded by a small, distinct number of follicle cells; and in the third, a small number of follicle cells surround the oocyte only during the early oogenesis. In the present study, an oocyte of *R. philippinarum* is surrounded with a small number of follicle cells during early oogenesis. Therefore, this species belongs to the third type of oocyte-follicle cell relationship.

Pipe (1987a) described that follicle cells, which are attached to the oocyte in *Mytilus edulis*, appear to play an integral role in oocyte development, using electron microscopic observation. However, Galtsof (1964) doubted that follicle cells played any role in oocyte nutrition in *Crassostrea virginica*, due to their small size and number.

Regarding functions of the follicle cell, Eckerbarger and Davis (1996) considered that follicle cells in *C. virginica* are unlikely to be the primary source of yolk precursors for the following reasons: (1) they are generally relatively small in volume in relation to the oocytes and are few in number; and (2) little or no endocytotic activity is observed along the follicle cell-oocyte interface. However, they also assumed that follicle cells control the microenvironment around the oocytes by regulating and mediating the flow of metabolites (small ions and molecules) to the oocytes, rather than playing a direct, proteosynthetic role. In our study, follicle cells in *R. philippinarum* were small in number and volum, therefore, we agree with Eckelbarger and Davis (1996).

Regarding physiological functions (such as supply of nutrients oocytes) of the ovarian follicle cells and the VCT cells, Eckerbarger and Davis (1996) argued that ovarian follicle cells are unlikely to be the primary source of yolk precursors in *C. virginica*. They suggested that VCT cells are more likely a major source of yolk precursors (Pipe 1987b). In the present study, large VCT cells were found at the extra-acinus near the oocyte. These cells contain large quantities of glycogen particles and several lipid droplets in the cytoplasm. Follicle cells in contrast contain a relatively small quantity of glycogen, and a few lipid droplets in the cytoplasm. Therefore, it is assumed that VCT cells are source of yolk precursors, as in by Eckelbarger & Davis (1996). The function of these cells should be investigated in detail.

2. Oocyte Degeneration and Resorption by the Follicle Cells

Our observations of the follicle cells and degenerated oocytes suggest a functional role for hydrolytic enzyme activity. As shown in Fig. 4, a number of degenerating yolk granules (with lysosomal enzyme activity or myeline-like organellea) and lipid droplets appeared in the ooplasm of the degenerating oocytes in *R. philippinarum*. At the same time, phagosomes (or lysosomes) and lipid droplets increased in the cytoplasm of the follicle cells attached to the degenerating oocyte. However, glycogen particles decreased in the cytoplasm of the follicle cells, as seen in *M. edulis* (Pipe, 1987a) and *P. maximus* (Dorange & Le Pennec, 1989b). Morphologically similar phagosomes, which were also observed in the cytoplasm of degenerated oocytes, appeared in the follicle cells. Thus, follicle cells appeared to play an integral role in vitellogenesis and oocyte degeneration. During oocyte degeneration, functions of the follicle cells include (1) the phagocytosis and intracellular digestion of products originating from oocyte degeneration, (2) the induction of the oocyte degeneration, and (3) the resorption of phagosomes from the degenerated oocyte, because lipid droplets and degenerating phagosomes appeared in follicle cells. In our study, lipid granules gradually increase in the follicle cell with gametogenesis, permitting

transfer of yolk precursors necessary to vitellogenesis and accumulation of reserves in the cytoplasm, as glycogen and lipids, which can be employed in the vitellogenic oocyte (Gaulejac et al., 1995). Therefore, follicle cells, which are attached to degenerated oocytes, may induce oocyte degeneration and resorption of degenerating phagosomes (lysosomes) by the lysosomal system (Fig. 5).

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Tae Hwan Lee of the University of Michigan for helpful comments on the manuscript. This research was supported by the fund (2007) from Fisheries Science Institute of Kunsan National University.

REFERENCES

Albertini L (1985) Recherdhes cytologiques et expérimentales sur I‘ ovegoènese chez la moule(*Mytilus edulis* L., mollusque bivalve). Thèse de 3ième cycle, Université de Caen, Caen.

Bernard RTF, Davies BR, Hodgson AN (1988) Reproduction in a brackish-water mytilid: gametogenesis and embryonic development. Veliger 30:278-290.

Chang YJ, Lee TY (1982) Gametogenesis and reproductive cycle of the cockle, *Fulvia mutica* (Reeve). Bulletin of the Korea Fisheries Society 15:241-253.

Chung EY, Lee TY, An CM (1991) Sexual maturation of the venus clam, *Cyclina sinensis*, on the west coast of Korea. Journal of Medical and Applied Malacology 3: 125-136.

Chung EY, Ryou DK, Lee JH (1994) Gonadal development, age and growth of the shortnecked clam, *Ruditapes philippinarum* (Pelecypoda: Veneridae), on the coast of Kimje. The Korean Journal of Malacology 19:38-54.

Chung EY, 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, Park YJ, Lee JY, Ryu DK (2005) Germ cell differentiation and sexual maturation of the hanging cultured female scallop *Patinopecten yessoensis* on the east coast of Korea. Journal of Shellfish Research 24:913-921.

Dohmen MR (1983) Gametogenesis. In: N.H. Verdonk, J.A.M. van den Biggelaar, A.S. Tompa. eds. The Mollusca, 3: Devcelopment. Academic Press. London. 88:1-48.

Dorange G, Paulet YM, Pennec ML (1989a) Etude cytologique de la partie femelle de la gonade de *Pecten maximus* recolte en baie de Saint-Brieu. 2. Ovogenese et Lyse ovocytaire. Haliotis 19:299-314.

Dorange G, Paulet YM, Pennec ML (1989b) Ultrastructural study of oogenesis and oocytic degeneration in *Pecten maximus* form the Bay of St. Brieyc. Marine Biology 103:339-348.

Eckelbarger KJ, Eckelbarger PIB (1989) Structure of the Ovotestis and evidence for heterosynthetic incorporation of yolk precursors in the oocytes of the nudibranch mollusk, *Spurilla neapolitana*. Journal of Morphology 201:105-118.

Eckelbarger KJ, Davis CV (1996) Utrastructure of the gonad and gametogenesis in the eastern oyster, *Crassostrea virginica*. 1. Ovary and oogenesis. Marine Biology 127: 79-87.

Eckelbarger KJ, Young CM (1999) Ultrastructure of gametogenesis in a chemosynthetic mytilid bivalve (*Bathymodiolus childressi*) from a bathyal, methane seep environment (northern Gulf of Mexico). Marine Biology 135:635-646.

Galtsoff PS (1964) The American oyster, *Crassostrea virginica* Gmelin. Fisheries Bulletin US 64:1-480.

Gaulejac DeB, Henry M, Vicente N (1995) An ultrastructural study of gametogenesis of the marine bivalve *Pinna nobilis* (Linnaeus 1758) 1. Oogenesis. Journal of Molluscan Study 61:375-392.

Goshima S, Ide N, Fujiyoshi Y, Noda T, Nakao S (1996) Reproductive cycle and shell growth of transplanted Manila clam *Ruditapes philippinarum* in Saroma lagoon.

Nippon Suisan Gakkaishi 62:195-200. [in Japanese]

Guraya SS (1979) Recent advances in the morphology, cytochemistry, and function of Balbiani's vitelline body in animal oocytes. International Review of Cytology 59:249-321.

Herlin-Houtteville P, Lubet P (1975) The sexuality of pelecypod mollusks. In: Intersexuality in the Animal Kingdom (R. Reinboth. Ed.). Springer-Verlag. Berlin. Heidelberg. New York. pp 179-187.

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

Jong-Brink deM, Boer HH, Joosse J (1983) Mollusca. In: Adiyodi Vol. 1. Oogenesis, Oviposition, and Oosorption. John Wiley & Sonse, New York, pp 297-355.

Le Pennec M, Beninger PG, Drange G, Paulet YM (1991) Trophic sources andpathways to the developing gametes of *P. maximus* (Bivalvia: Pectinidae). Journal of the Marine Biological Association of the U.K 71:451-463.

Kwon OK, Park GM, Lee JS (1993) Coloured Shells of Korea. Academy Publishing Corporation, Seoul. [in Korean]

Lee TY (1972) Gametogenesis and development of germ cells in *Pinctada matensii* (Dünker). Publications of The Marine Laboratory Busan Fisheries College 5:21-30.

Lee TY, Chung EY (1980) Reproductive cycle of marsh clam, *Corbicula fluminea* (V. Müller). Publications of Institute of Marine Sciences National Fisheries University of Busan 12:47-54.

Mortavkine PA, Varaksine AA (1983) Histophysiologie du systeme nerveux et regulation de la reproduction chez les mollusques bivalves. Sciences, Moscu pp 208.

Paulet YM, Lucas A, Gerard A (1988) Reproduction and larval development in two *Pecten maximus* (L.) populations form Brittany. Journal of Experimental Marine Biology and Ecology 119:145-156.

Paulet YM, Boucher J (1991) Is reproduction mainly regulated by temperature or photoperiod in *Pecten maximus.* Invertebrate Reproduction and Development 19(1):61-70.

Pipe RK (1987a) Oogenesis in the marine mussel *Mytilus edulis*: an ultrastructural study. Marine Biology 95:405-414.

Pipe RK (1987b) Ultrastructural and cytochemical study on interactions between nutrient storage cells and gametogenesis in the mussel *Mytilus edulis*. Marine Biology 96: 519-528.

Ponurovsky SK, Yakovlev YM (1992) The reproductive biology of the Japanese littleneck. *Tapes philippinarum* (A. Adams and Reeve, 1850) (Bivalvia:Veneridae). Journal of Shellfish Research 11:265-277.

Raven CP (1966) Morphogenesis: The Analysis of Molluscan Development Oxford, Pergamon Press. pp 311.

Suzuki T, Hara A, Yamaguchi K, Mori K (1992) Purification and immunolocalization of a vitellin-like protein from the Pacific oyster *Crassostrea gigas.* Marine Biology 113:239-245.

Toba M, Natsume Y, Yamakaw H (1993) Reproductive cycles of Manila clam colleted from Funabashi waters, Tokyo Bay. Nippon Suisan Gakkaishi 59:15-22. [in Japanese]

Toba M, Miyama Y (1994) Relationship of size to gonadal maturation and spawning in artificially conditioned Manila clams. Nippon Suisan Gakkaishi 60:173-178. [in Japanese]

Toba M, Miyama Y (1995) Influence of temperature on the sexual maturation in Manila clam, *Ruditapes philippinarum.* Suisanzoshoku 43:305-314.

Tsuji S, Munekiyo M, Itani M, Douke A (1994) Reproductive cycle of a Manila clam in Maizuru Bay. Bulletin of Kyoto Ocean Center pp 1-9. [in Japanese]

Wourms JP (1987) Oogenesis. In: A.C. Giese., Pearse J.S., Pearse VB, eds, Reproduction of Marine Invertebrates. Vol. IX. General aspects: seeking unity in diversity. Blackwell Scientific Publications, Palo Alto, California and Boxwood Press, Pacific Grove, California, pp 50-157.

Yoo JS (1976) Korean Shells in Colour. Ilgisa, Seoul. [in Korean]