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Oogenesis and Oocyte Degeneration in *Coecella chinensis* (Bivalvia: Mesodesmatidae)

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ABSTRACT

Ultrastructural studies of oogenesis in oocytes, oocyte degeneration associated with the follicle cells in female *Coecella chinensis* were investigated for clams collected from Namhae, Geongsangnam-do, Korea. In this study, vitellogenesis during oogenesis in the oocytes occured by way of endogenous autosynthesis and exogenous heterosynthesis. Of two processes of vitellogenesis during oogenesis, the process of endogenous autosynthesis involved the combined activity of the Golgi complex, mitochondria and rough endoplasmic reticulum. whereas the process of exogenous heterosynthesis involved endocytotic incorporation of extraovarian precursors at the basal region of the oolema of the early vitellogenic oocytes prior to the formation of the vitelline coat. It is assumed that the follicle cells were involved in the development of previtellogenic and early vitellogenic oocytes and appear to play an integral role in vitellogenesis in the early and late vitellogenic oocytes by endocytosis of yolk precursors, and also they were involved in oocyte degeneration by assimilating products originating from the degenerated oocytes, thus allowed the transfer of york precursors needed for vitellogenesis (through phagocytosis by phagolysosomes after spawning). Follicle cells presumably have a lysosomal system for breakdown products of oocyte degeneration. and for reabsorption of various phagosomes (phagolysosomes) in the cytoplasm for nutrient storage during the period of oocyte degeneration.

Key words: oogenesis, vitellogenesis, oocyte degeneration, follicle cell

INTRODUCTION

The intertidal clam, *Coecella chinensis* (Mesodesmatidae) is an environmentally important edible bivalves in East Asian countries, including Korea, China, and Japan. In Korea, this species is found mainly in silty sand at the intertidal zone in the coastal waters of Namhae, Jollanam-do, Korea (Kwon *et al*., 1993; Min *et al*., 2004). Due to past reclamation project in the south coast of Korea, it has been identified as an environmental resource that should be managed for envirenmental resource regimen For the propagagation and reproduction of a living natural resource, above all, it is important to understand the reproductive biology such as reproductive physiology in association with yolk formation (vitellogenesis) during oogenesis and the functions of the follicle cells associated with the induction of oocyte degeneration of this species. Previously there have been several studies on aspect of reproduction in *Mactra* species as a closely allied species of *C. chinensis*. In particular, regarding *C. chinensis*, a few studies have been conducted on aspect of reproduction, including gametogenesis and sexual maturation (Chung *et al*., 1987; Chung and Ryou, 2000; Kim *et al*., 2013), distribution (Kwon *et al*., 1993).

To date, regarding *C. chinensis*, several studies have been conducted on aspects of reproductive ecology, including artificial discharge of reproductive substance (Iwata, 1948), propagation (Kim *et al*., 2013), reproductive cycle (Kim *et al*., 2013).

Despite this, there are still gaps in our knowledge

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regarding its reproductive biology of this species. Above all, studies on the process of vitellogenesis by oocyte developmental stages in oocytes during oogenesis, and the functions of the follicle cells associated with oocyte degeneration are required to understand reproductive biology of *C. chinensis*. In many bivalve species, it is well known that the ovaries contain follicle cells (or auxiliary cells), a kind of accessory cells, that play a role in the storage, and synthesis of yolk precursors during oogenesis (Chung, 2008). After spawning, in general, oocyte degeneration is a commonly observed phenomenon in degenerating oocytes in most bivalve species. Regarding oocyte degeneration which is known as atresia, several authors (Pipe, 1987; Dorange and Le Penec, 1989; Gaulejac *et al*., 1995; Chung *et al*., 2005; Chung, 2008) reported that after spawning, the products of lysis material created by the follicle cells (or auxiliary cells) act as sources of metabolites that can be rapidly moboilized by the organism. Previously, regarding relationships between the follicle cells and degenerating atretic oocytes, Chung (2008) clarified the functions of the auxiliary cells during oocyte degeneration of *Chlamys* (*Azumapecten*) *farreri farreri*. Accordingly, in this study, the functions of the follicle cells, which play an important role in the resorption of the lysis products of artretic oocytes of this species, should be investigated in further detail. Therefore, the purpose of present study is to describe oocyte development, vitellogenesis in the oocyte, and the functions of follicle cells during oogenesis and oocyte degeneration. In addition, the aim of this study is to clarify the reproductive mechanism on vitellogenesis of *C. chinensis* using cytological methods. The results of ultrastructural studies of the various cells of this species will provide important information on its reproductive mechanisms of yolk formation.

MATERIALS AND METHODS

1. Sampling

Female specimens of *C. chinensis* were collected monthly in the intertidal zone of Namhae, Jollanam-do, Korea, from January to December, 2008. The clams were then transported to the laboratory where they were maintained in seawater at 20°C.

2. Ultrastructures of germ cells and follicle cells during oogenesis and oocyte degeneration

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

RESULTS

1. Position and morphology of the ovary

The clam, *C. chinensis*, is dioecious organism. The general morphology of the ovary of *C. chinensis* is similar to those of other bivalves. The ovary is located between the digestive diverticular and the outer fibromuscular layers, which are compacted by the fibrous connective tissue and muscle fibers. As the overy matured, it extended to the lowest part of the muscular layers around the foot. The ovary is a diffused organ consisting of branching follicles containing differentiating oocytes in the various stages. Germ cells are distributed in a centripetal pattern from the follicular wall to the lumen. As ovarian maturation progresses, the external view of the mature ovary is red in color, and the testis appear lemon-yellow white, which allows the sexes of the clams to be easily distinguished by external features. At this time, if the ovary is slightly scratched with a

razor, ripe ova readily flow out.

2. Ultrastructures of germ cells and follicle cells during oogenesis and oocyte degeneration

Based on ultrastructural observations, ovarian activity and morphological characteristics of oocytes during oogenesis can be classified into four distinct phases of oogenesis were distingushed in germ cells, that is, (1) oogonia, (2) previtellogenic oocytes, (3) vitellogenic oocytes, and (4) mature oocytes (Eckelbarger and Davis, 1996). Ultrastructural characteristics in vitellogenesis by each stage of the oocytes, including oocyte degeneration, are as follow:

1) Oogonia:

The stem cells, which constitutided the boudaries of the follicles, give rise to the oogonium. An oogonium, which measure approximately 9-10 μm in diameter, is round or oval in shape. Commonly, oogonia are found individually or a cluster on the follicular walls. They possess a large ovoid nucleolus in the nucleus, in which the chromatin is reticular and marginal. Several mitochondria and vacuoles appear in the cytoplasm of oogonia. An oogonium is partially surrounded by follicle cells that maintain intimate contact with the smooth oolemma of the oocyte. Follicle cells, which measure from 4 to 5 μm in diameter, possess a dense chromatin and marginal chromatin in the nucleus of the follicle cells. In particular, several vacuoles are concentrated around the perinuclear region in the cytoplasm of oogonia. However, vitellogenic characteristics could not be found in this stage because cellular developments of cell organelles in the cytoplasm were very weak (Fig. 1A).

2) Previtellogenic oocytes:

In the first prophase of meiosis, oogonia develop into the previtellogenic oocytes. Previtellogenic oocytes are small and oval in shape, containing a nucleolus in the nucleus. In particular, these oocytes are slightly peduculated, and the diameters of the nucleus and the previtellogenic oocytes are approximately 4-5 μm and 25-30 μm in diameter, respectively. At the beginning of cytoplasmic growth of the previtellogenic oocytes, a number of mitochondria, vacuoles and the rough endoplasmic reticulum are concentrated around the nucleus, but microvilli are not yet on the oolemma. At this stage, the previtellogenic oocytes are partially surrounded by follicle cells that maintain intimate contact with the smooth oolemma of the oocyte. Follicle cells, which measure from 4 to 5 μm in diameter, possess a dense chromatin and marginal chromatin in the nucleus and contain characteristically parallel arrays of the rough endoplasmic reticulum, the Golgi complex, mitochondria, and lipid droplets in the cytoplasm. During the previtellogenic stage, with the cytoplasmic growth of the previtellogenic oocyte, the nucleus and cytoplasm of the previtellogenic oocyte increased in volume in the previtellogenic oocyte. The nucleus and oocyte diameters were 4-5 μm and about 30 μm, respectively.

However, in this stage, the activity of vitellogenic characteristics in the previtellogenic oocyte shows no evidence because yolk materials are not found by cell organelles in the cytoplasm (Fig. 1B).

3) Vitellogenic oocytes:

As the further development of previtellogenic oocytes proceeded, the oocytes developed into vitellogenic oocytes by the meiotic division. At this stage, for convenience, the vitellogenic oocyte can be divided into two vitellogenic oocytes: (1) the early vitellogenic, and (2) late vitellogenic oocytes.

(1) Early vitellogenic oocytes: At this stage, the oocytes continued to grow and differentiated. As the further development of previtellogenic oocytes proceeds, they develop into the early vitellogenic oocytes. In the early stages of oogenesis, the early vitellogenic oocytes measure 35-40 μm in diameter. The Golgi complex is present in the perinuclear region and vacuoles are scattered in the cytoplasm of the early vitellogenic oocyte. At this time, the oocytes contain a number of the mitochondria and a few lipid droplets in the cytoplasm (Fig. 1C).

When their oocytes are 30~35 μm in diameter, with the initiation of yolk formation, a number of lipid droplets, vacuoles, rough endoplasmic reticulum and

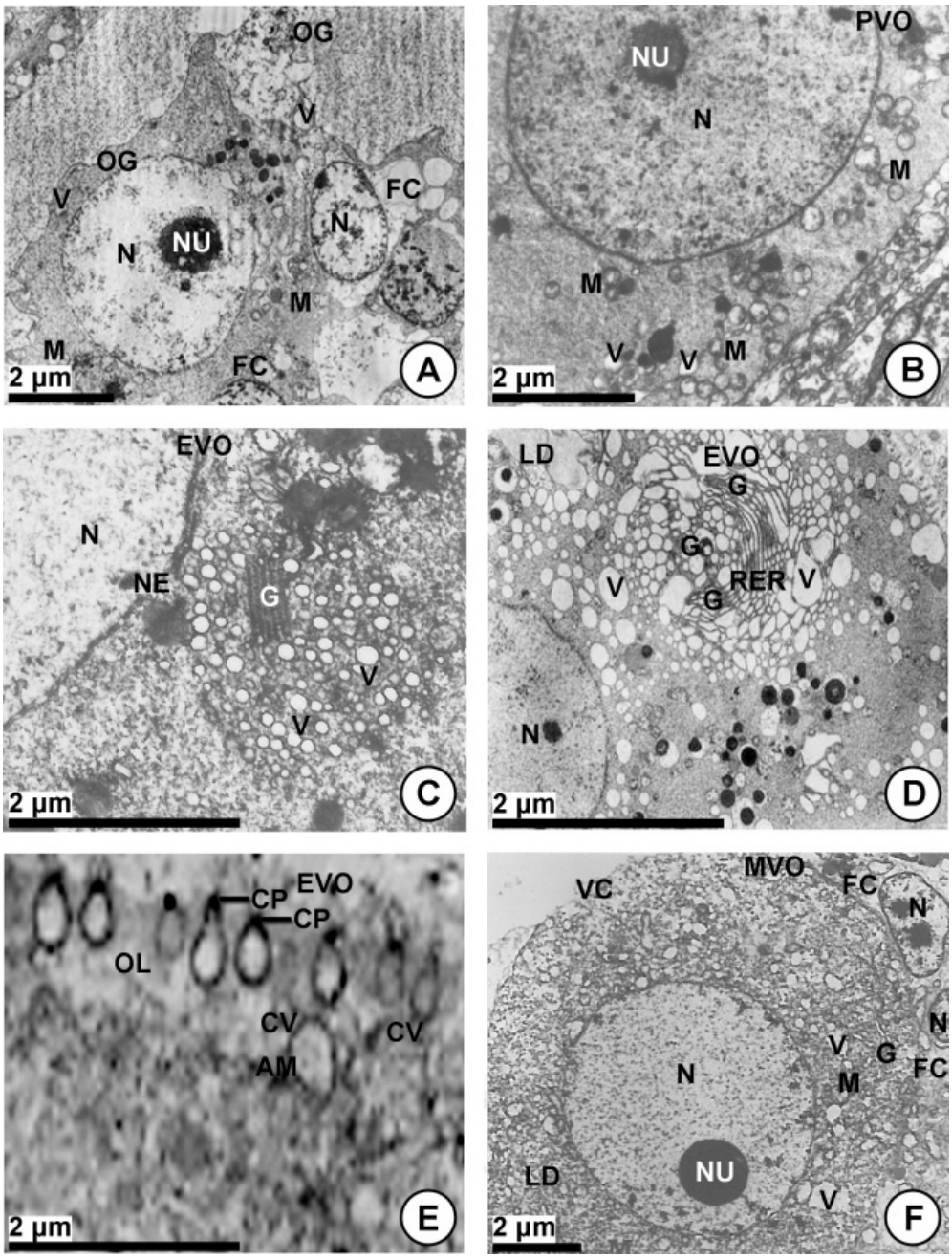

Fig. 1. Electron micrographs of oogenesis in female *Coecella chinensis* (A-F). **A**, An oogonium (OG) and the follicle cell.s. Note a large nucleus (N), several mitochondria (M) in the cytoplasm of an oogonium and the follicle cells attaching to the oogonum; **B**, A previtellogenic oocyte (PVO). Note a nucleolus (NU) in a large nucleus (N), the mitochondria (M) and a number of vacuoles (V) in the cytoplasm of a previtellogenic oocyte; **C**, An early vitellogenic oocyte (EVO). Note a nucleus (N) and the Golgi complex (G) and a number of vacuoles (V); **D**, An early vitellogenic oocyte EVO). Note the Golgi complex (G), rough endoplasmic reticulum (RER), a number of vacuoles (V) in the cytoplasm; **E**, An early vitellogenic oocyte (EVO), Note occurrences of the coated vesicles (CV) through the coated endocytotic pits (CP) formed by endocytosis at the cortical region near the oolemma (OL) and amorpous materials (AM). **F**, A middle vitellogenic oocyte (MVO) containing a round nucleolus in the nucleus and follicle cells (FC) with the elongated nucleus (N). Note the Golgi complex (G), mitochondria (M), vacuoles, lipid droplets and vitelline coat (VC) in the cytoplasm of a middle vitellogenic oocyte.

the Golgi complex appear in the cytoplasm of the early vitellogenic oocytes. At this time, a number of lipid droplets appear between the mitochondria and well-developed rough endoplasmic reticulum (Fig. 1D).

When the oocytes begin to form the microvilli on the oolemma, the initial contours of the microvilli are ovoid shape or slightly long. During the early stages of oogenesis, several coated vesicles were present due to endocytosis, and appear at the basal region of the oolemma of the oocyte. The uptake of exogenous nutritive material in the coated vesicle occurred through the formation of the coated pits on the oolemma during vitellogenesis. These exogenous substances represent many glycogen particles that were passed into the ooplasm from the outside of the oolemma. The activity of vitellogenic characteristics by endocytosis through microvilli on the oolema of the early vitellogenic oocytes was found in this stage because these exogenous yolk materials were found by cell organelles in the cytoplasm. At this time, amorphous materials that comprise the vitelline coat are deposited near the cortical region by exocytosis, and the microvillous borders then form on the oolemma (Fig. 1E).

(2) Middle vitellogenic oocytes: At the mid-vitellogenic stage, commonly, the follicle cells attached to the mid-vitellogenic oocytes. these oocytes contain a number of vacuoles and lipid droplets at the cortical region near the oolemma. In the mid-vitellogenic oocytes, as oocyte volume increases, the ooplasm is filled with the Golgi complex, lipid droplets, yolk precursors (or yolk granules), the mitochondria and vacuoles. However, at this stage, a number of microvilli of the oocyte are not found on the vitelline coat (Fig. 1F). By and by, the follicle cells gradually lose their intimate association with the oocyte surface, and the microvilli appear along the vitelline coat, where the follicle cells withdraw. In particular, the cytoplasm of the follicle cell is filled with a myelin-like organelle, the mitochondria and vacuoles (Fig. 2A).

(3) Late vitellogenic oocytes: In the late vitellogenic oocyte, yolk precursors (yolk granules), a number of cortical granules, mitochondria and lipid droplets are present in the cytoplasm, especially, a number of proteinaceous yolk granules appear among the mitochondria, lipid droplets, and yolk precursors near the vitelline coat (Fig. 2B). Thereafter, several cortical granules are found near the cortical layer, and proteinaeceous yolk granules containing several different components combined and became larger immature yolk granules (approximately 2.0 to 2.3 μm in diameter) in the cytoplasm of the oocyte (Fig. 2C)..

4) Mature oocytes:

As the further development of late vitellogenic oocytes proceeds, they develop into mature oocytes. In the mature oocytes, the thick vitelline envelope of the mature oocyte was slightly separated from follicular walls (the germinal epithelium). In the cytoplasm of the mature oocyte, small immature yolk granules continuously mixed with each other and became larger, mature yolk granules (2.7 to 3.0 μm). A mature yolk granules were composed of three components: (1) a crystalline core, (2) an electron-lucent cortex, and (3) a limiting membrane. Upon reaching maturity, mature oocytes are separated from the acinus wall (germinal epithelium) and protrude into the lumen of the acinus. At this stage, the tips of the microvilli protrude and extend just beyond the outer border of the vitelline coat. The vitelline coat of the mature oocye is covered with a jelly coat. Mature oocytes that contain numerous mature yolk granules in the cytoplasm measure approximately 60-67 μm in diameter. Finally, the mature oocyte is separated from the follicular wall (germinal epithelium) (Fig. 2D).

5) Ultrastructure of degenerating oocytes:

The degenerating oocytes appear slightly irregular or polyhedric near the follicle cells, and are deformed by compression of the oocyte. In the degenerating oocytes, several degenerating yolk granules, lipid droplets, and a number of vacuoles are found in the cytoplasm. a number of vacuoles and lipid droplets are found in the cytoplasm of degenerating oocyte. As characteristics of oocyte degeneration, the mitochondria and yolk

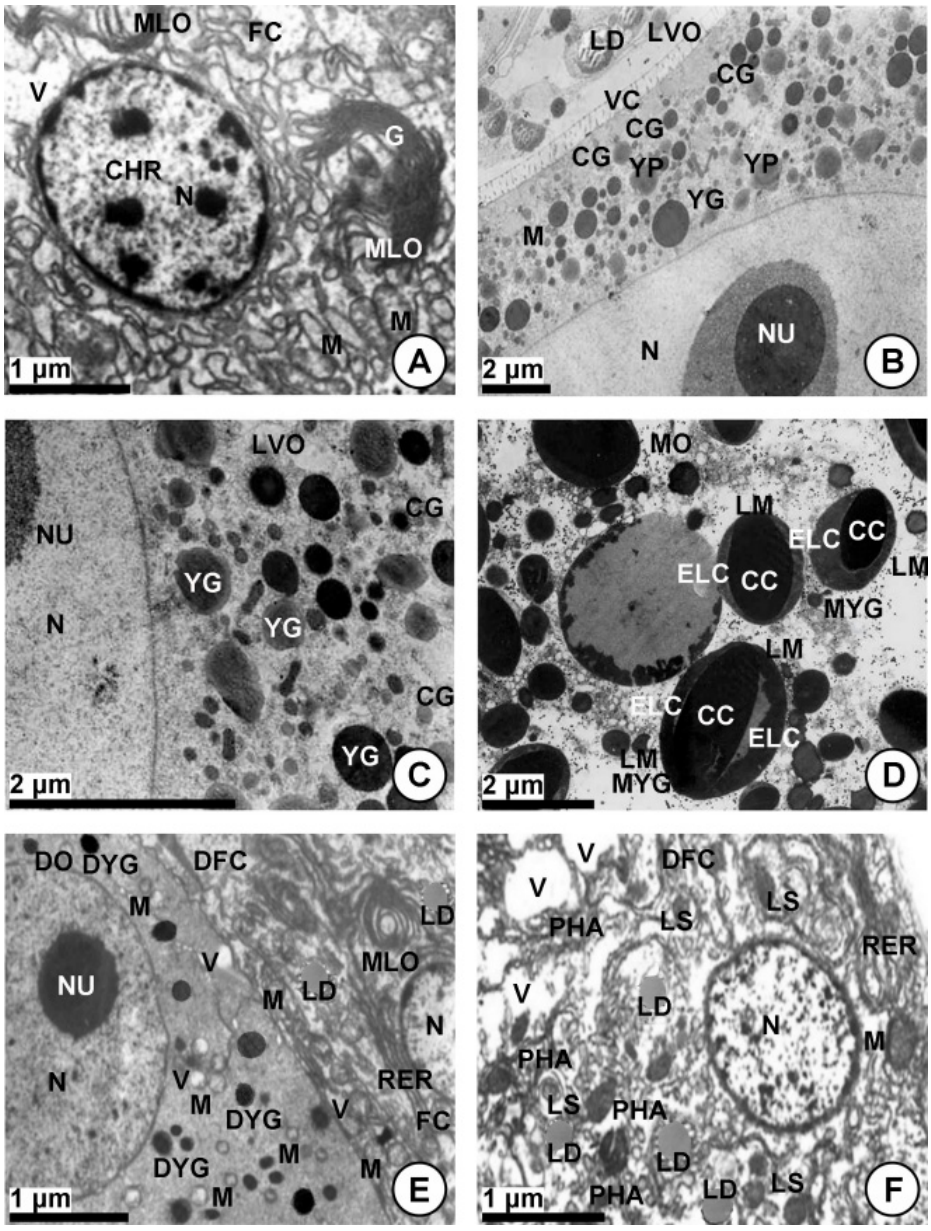

Fig. 2. Electron micrographs of vitellogeneis during oogenesis and oocyte degeneration in female *Coecella chinensis* (A-F). **A**, A follicle cell attaching to the middle vitellogenic oocyte. Note chromatin materials in a round nucleus and the mitochondria (M), the Golgi complex (G) and myelin-like organelles (MLO) in the cytoplasm; **B**, A late vitellogenic oocyte (LVO), with a large nucleolus in the nucleus. Note a number of cortical granules (CG), mitochondria (M), yolk precursors (YP), lipid droplets and a number of yolk granules (YG) in the cytoplasm, microvilli on the vitelline coat (VC); **C**, A latevitellogenic oocyte with a large nucleolus (NU) in the nucleus. Note a number of yolk granules and cortical granules at the cortical layer of cytoplasm; **D**, A mature oocyte (MO) with a number of mature yolk granule (MYG) being composed of three parts: (1) crystalline core (CC), (2) electron lucent cortex (ELC), and (3) a limiting membrane (LM); **E**, A degenerating oocyte (DO) with a nucleolus in the nucleus and a degenerating follicle cell (DFC) containing an oval nucleus (N), rough endoplasmic reticulum (RER), vacuoles (V), lipid droplets, a number of phagosome (PHA) or lysosomes (LS) and myelin-like organelles. Note a number of mitochondria, vacuoles and degenerating yolk granules (DYG), lipid droplets (LD), various phagosomes (PHA) or lysosome in the cytoplasm of a degenerating oocyte; **F**, A degenerating follicle cell (DFC) with an oval nucleus (N). Note the rough endoplasmic reticulum (RER), number of lipid droplets (LD), vacuoles (V), various phagosomes (PHA) or lysosome (LS) in the cytoplasm of a degenerating follicle cell (DFC).

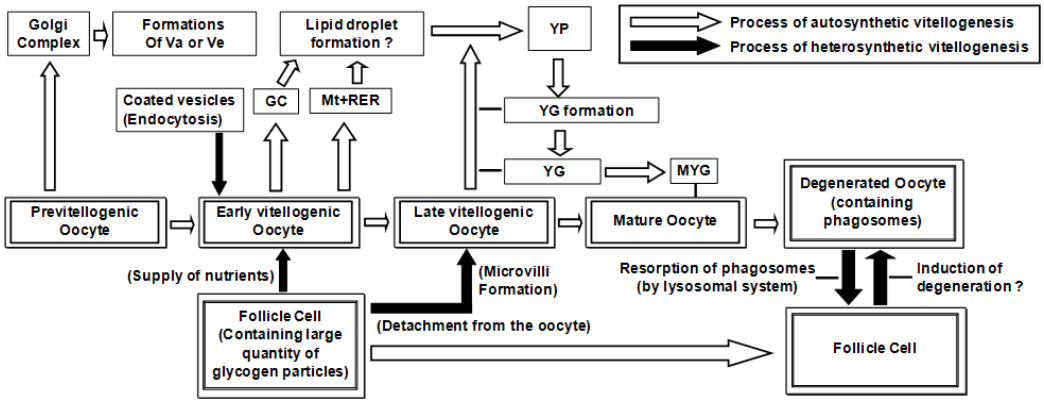

Fig. 3. Schematic diagrams of the process of vitellogenesis during oogenesis and oocyte degeneration in female *Coecella chinensis*. Abbreviations: GC, Golgi complex; M, mitochondrion; MYG, mature yolk granule; Va, vacuole; Ve, vesicle; YG, yolk granule; YP, yolk precursor.

granules disintegrate in the cytoplasm, and lysis is initiated at the periphery of the oocyte. At this time, many disintegrated granules are visible at the periphery of the oocyte.

With the beggining of degeneraing oocytes, follicle cells that are attached to mature oocytes lost their intimate association with the surface of the degenerating oocyte, and microvilli appeared along the vitelline coat where the follicle cells had withdrawn. At this time, a few lysosomes, a number of vacuoles, myelin-like organelles (myelin figures) appear in the cytoplasmic deformation of the follicle cells which attached to the degerating oocyte (Fig. 2E).

In the degenerating follicle cells, these cells function in the resorption of phagosomes from the degenerated oocyte because morphologically similar degenerating phagosomes (various lysosomes) and a number of vacuoles. which are observed in the degenerated oocytes, appear in the follicle cells: lipid droplets (or granules) gradually increase, whereas glycogen contents decreased in the follicle cell. The degenerating follicle cells, which are attached to degenerated oocytes, induce oocyte degeneration and the resorb a number of degenerating phagosomes (lysosomes) by the various lysomes (Fig. 2F).

DISCUSSION

1. Vitellogenesis in oocytes

The ultrastructural characteristics of oocyte development and vitellogeneis in oocytes during oogenesis of *C. chinensis* have first been investigated in this study. In particular, vitellogenesis in oocytes during oogenesis of this species showed similar phenomenona to other bivalve species such as *Mytilus edulis* (Pipe, 1987), *Pecten maximus* (Dorange and Le Pennec, 1989), *Pinna nobilis* (Gaulejac *et al*., 1995), *C. verginica* (Eckelbarger and Davis, 1996), *Patinopecten yessoensis* (Chung *et al*., 2005) and *Chlamys farreri farreri* (Chung, 2008). The process of vitellogenesis in the oocytes during oogenesis of *C. chinensis* was summarized in Fig.3. In this study, in the early vitellogenic stage, several coated vesicles were present due to endocytosis, that appeared at the basal region of the oolemma of early vitellogenic oocytes, and prior to the formation of the vitelline envelop. The uptake of nutritive material, that is, extraovarian precursors in coated vesicles occurred through the formation of coated pits on the oolemma during vitellogenesis (Eckelbarger and Davis, 1996; Chung *et al*., 2005; Chung, 2008). As shown in Fig. , exogenous heterosynthetic vitellogenesis occurred through the incorporation of extraovarian precursors into oocytes by endocytosis before the formation of the vitelline envelope. From these findings, it is assumed that vitellogenesis in *M. chinensis* occur through the processes of endogenous autosynthetic and exogenous heterosynthetic vitellogensis. Thus, the processes of

yolk formation by endogenous autosynthesis and exogenous heterosynthesis in *C. chinensis* are similar to those of *C. virginica* (Eckelbarger and Davis 1996), *M. edulis* (Pipe, 1987), *P. yessoensis* (Chung *et al*., 2005), *C.* (*A.*) *farreri farreri* (Chung 2008).

2. Functions of follicle cells attached to the oocyte

Commonly, the follicle cells at the periphery of the oogenic follicle (or acinus) initially appears close to the oogonia or previtellogenic oocyte, and thereafter, progressively surrounds a part of the oocyte. At this stage, a small number of vacuoles were visible in the cytoplasm of the follicle cells near the adherence zone. The attached follicle cells also showed cytological modifications as their cytoplasmic volume increased in *C. virginica* (Eckelbarger and Davis, 1996) and *M. edulis* (Pipe, 1987). Because the follicle cells attach to the oocyte in the early stages of oogenesis and gradually detach from the vitellogenic oocyte, it is assumed that follicle cells function as nutritive cells in the early development of the oocytes (Chung *et al*., 2005; Chung, 2007). During oocyte degeneration, follicle cells varied in shape and size according to the degree of cytoplasm replention by engulfed material. The size of follicle cells increased during oocyte degeneration. The process of oocyte degeneration was characterized by the vacuolization of the oocyte cytoplasm at the beginning. The degenerating oocytes appeared slightly irregular or polyhedric near the follicle cells, and were deformed by compression in the follicle. A number of vacuoles, degenerating yolk granules, distended endoplasmic reticulum, and a few phagosomes (phagolysosomes) and lipid droplets appeared in the ooplasm of the degenerating oocytes. At this stage, degenerating mitochondria and myelin-like organelles were present. In particular, no Golgi complex was observed in atretic oocytes.

In the present study, the characteristics of a functional role of lysosomes and a number of lipid droplets or degenerating yolk granules containing a few myelin-like organelle figure appeared in the ooplasm of the degenerated oocytes in *C. chinensis*. At the same time, several phagosomes (or lysosomes) and lipid droplets, in particular, increased in the cytoplasm of the follicle cells, which are attached to the degenerated oocytes. However, the number of glycogen particles decreased in the cytoplasm of the follicle cells, as reported in *M. edulis* (Pipe, 1987) and *Pecten maximus* (Dorange and Le Pennec, 1989). In this study, morphologically similar phagosomes (lysosomes), which were easily observed in the cytoplasm of degenerated oocytes, also appeared in the follicle cells. Thus, the follicle cells appear to play an integral role in vitellogenesis and oocyte degeneration. During the period of oocyte degeneration, the follicle cells function in phagocytosis and intracellular digestion of products originating from oocyte degeneration; these cells might also have a function associated with the induction of oocyte degeneration, and it is assumed that they are also active in the resorption of phagosomes (lysosomes) from the degenerated oocyte because lipid droplets and degenerating phagosomes appeared in the follicle cells. In this study, the number of lipid granules gradually increased in follicle cells during gametogenesis; this function can permit a transfer of yolk precursors necessary for vitellogenesis and allow for the accumulation of reserves in the cytoplasm as glycogen particles and lipids, which can be employed by vitellogenic oocytes (Gaulejac *et al*., 1995). However, it is assumed that the follicle cells, which are attached to degenerated oocytes, presumably have a lysosomal system for breakdown of ingested material, and they might be involved in the induction of oocyte degeneration, and might also resorb various degenerating phagosomes (lysosomes) in the cytoplasm for nutrient storage (such as lipid droplets) during oocyte degeneration, as seen in *M. lusoria* (Chung, 2007).

3. Vitellogenesis in the oocyte and the functions of follicle cells during oogenesis

From the ultrastructural study of *C. chinensis*, vitellogenesis during oogenesis can be classified into two separated processes: autosynthetic and heterosynthetic yolk formations (Eckelbarger and Davis, 1996). Vitellogenesis occurs through a process of autosynthesis, which involves the combined activity of the Golgi complex, mitochondria and rough

endoplasmic reticulum. In contrast, Pipe (1987) reported endocytotic activity in the oocytes of *M. edulis*, and Eckelbarger and Davis (1996) suggested an evidence for heterosynthetic yolk formation in the oocytes of *C. virginica*. In the present study, extraovarian precursors were found to be incorporated into oocytes by endocytosis at the basal region of the early vitellogenic oocytes which acts as evidence for heterosythetic yolk formation. Beside the process of heterosynthetic yolk formation by endocytosis, it is assumed that the follicle cells may be involved in supplying nutrients to vitellogenic oocytes in the process of heterosynthetic yolk formation.

Regarding the relationship between the follicle cells and the formation of microvilli on the oolemma of the oocyte, Pipe (1987) reported that once the follicle cells withdraw in *M. edulis*, the microvilli appear along the oolemma of the oocyte. In this study, the same phenomenone was observed as that shown in *M. edulis* (Pipe, 1987) and *M. lusoria* (Chung, 2007). Therefore, it is suggested that the follicle cells, which are attached to the oolemma of the oocyte, play a role in the formation of the microvilli on the oolemma (Pipe, 1987).

As shown in Fig. 3, vitellogenesis showed a possibility of autosynthetic and heterosythetic yolk formation. The process of yolk formation by endogenous autosynthesis and exogenous heterosynthesis of *C. chinensis* were similar to those of *C. virginica* (Eckelbarger and Devis, 1996) and *M. edulis* (Pipe, 1987).

4. Induction of oocyte degeneration and resorption by the follicle cells

During the period of oocyte degeneration, a number of degenerating yolk granules showed characteristics of a functional role for hydrolytic enzyme activity, and lipid droplets also appeared in the ooplasm of the degenerating oocytes in *C. chinensis*. At this time, a few phagosomes (lysosomes) and lipid droplets increased in number in the cytoplasm of the follicle cells, which are attached to the degenerating oocyte, however, glycogen particles decreased in the cytoplasm of the follicle cells, as seen in *M. edulis* (Pipe 1987) and P. maximus (Dorange and Le Pennec, 1989). Therefore, it is assumed that "this function can permit a transfer of yolk precursors necessary for vitellogenesis, and allows for the accumulation of reserves in the cytoplasm, as glycogen and lipids, which can be used by vitellogenic oocyte, as reported by Gaulejac *et al*. (1995).

In this study, more specifically, morphologically similar phagosomes (lysosomes), which were easily observed also in the cytoplasm of degenerated oocytes, also appeared in the follicle cells of *C. chinensis*, as seen in *M. lusoria* (Chung, 2007). Thus, the follicle cells appeared to play an integral role in vitellogenesis and oocyte degeneration: the follicle cells function in phagocytosis and intracellular digestion of products originating from oocyte degeneration through a lysosomal system (Chung, 2007), as those seen in P. nobilis (Gaulejac *et al*., 1995) and *M. lusoria* (Chung, 2007).

Judging from the observations of the follicle cells, as shown in Fig. 3, they probably perform a function associated with the induction of oocyte degeneration, and it is assumed that they also function in resorption of phagosomes from the degenerated oocyte because morphologically similar degenerating phagosomes (various lysosomes), which were observed in the degenerated oocyte, appeared in the follicle cells. Therefore, it is assumed that the follicle cells, which are attached to degenerated oocytes, may be involved in the induction of oocyte degeneration and resorption of degenerating phagosomes (lysosomes) by the lysosomal system.

5. Fates of the gametes

The presently observed gamete resorption phenomenon in the follicles attached to the oocyte after spawning has been described previously in *P. yessoensis* (Chung *et al*., 2005) and *M. lusoria* (Chung, 2007). Regarding reproductive energy allocation to the production of gametes, Morvan and Ansell (1988) reported that "the continuous production and resorption of gametes may be regarded as an adaptation to environmental temperature and food availability". "If the reproductive energy allocated to

the production of gametes is too large, nutritive reserves cannot be provided to all eggs to reach their critical size for spawning". In this case, "the products of gamete atresia would be reabsorbed, and the energy would be reallocated to still developing oocytes or used for other metabolic functions by the bivalves" (Dorange and Le Pennec, 1989). Therefore, it is assumed that *C. chinensis* must possess a physiological mechanism on the products of gamete atresia for resorption of gamates, and use nutritive reserves originated from hopeless gametes, as seen in other mollusks (Morvan and Ansell 1988, Mortavkine and Varaksine, 1989; Dorange and Le Pennec, 1989, Chung *et al*., 1987, 2005 2006; Chung and Ryou, 2000; Chung, 2007).

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