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Oocyte Degeneration Associated with Follicle Cells in Female *Mactra chinensis* (Bivalvia: Mactridae)

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ABSTRACT : Ultrastructural studies of oocyte degeneration in the oocyte, and the functions of follicle cells during oocyte degeneration are described to clarify the reproductive mechanism on oocyte degeneration of *Mactra chinensis* using cytological methods. Commonly, the follicle cells are attached to the oocyte. Follicle cells play an important role in oocyte degeneration. In particular, the functions of follicle cells during oocyte degeneration are associated with phagocytosis and the intracellular digestion of products. In this study, morphologically similar degenerated phagosomes (various lysosomes), which were observed in the degenerated oocytes, appeared in the follicle cells. After the spawning of the oocytes, the follicle cells were involved in oocyte degeneration through phagocytosis by phagolysosomes. Therefore, it can be assumed that follicle cells reabsorb phagosomes from degenerated oocytes. In this study, the presence of lipid granules, which occurred from degenerating yolk granules, gradually increased in degenerating oocytes. The function of follicle cells can accumulate reserves of lipid granules and glycogen in the cytoplasm, which can be employed by the vitellogenic oocyte. Based on observations of follicle cells attached to degenerating oocytes after spawning, the follicle cells of this species are involved in the lysosomal induction of oocyte degeneration for the reabsorption of phagosomes (phagolysosomes) in the cytoplasm for nutrient storage, as seen in other bivalves.

Key words : *Mactra chinensis*, Oogenesis, Vitellogenesis, Oocyte degeneration, Follicle cells

INTRODUCTION

The hen clam *Mactra chinensis* is one of the most commercially important edible clams in East Asian countries, including Korea, China, and Japan. In Korea, this species is mainly found within silty sand in tidal flats and the intertidal zone in the coastal waters of Simpo, Jollabuk-do, Korea (Kwon et al., 1993; Min et al., 2004). Due to overharvesting, *M. chinensis* has been designated as a fisheries resource whose harvesting should be reasonably managed. To ensure the safe propagation and reproduction of this species, it is important to understand its reproductive physiology including the functions of follicle cells associated with the induction of oocyte degeneration. To date, several studies have been conducted on aspects of its reproductive ecology, including artificial discharge of reproductive substances (Iwata, 1948), propagation (Sakai, 1976), early development (Lee & Son, 1978), spawning and growth (Kim et al., 1985), as well as sexual maturation (Chung et al., 1987).

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In many bivalve species, the ovaries contain follicle cells (or auxiliary cells), which are a kind of accessory cell type, that play a role in the storage, and synthesis of yolk precursors during oogenesis (Wourms, 1987; Chung et al., 2005, 2007). Oocyte degeneration, which is known as atresia, is a commonly observed phenomenon after spawning in most bivalve species. Regarding oocyte degeneration, several authors (Pipe, 1987; Dorange & Le Pennec, 1989; Gaulejac et al., 1995; Chung et al., 2005, 2007; Son & Chung, 2009) have reported which the products of lysis materials created by follicle cells (or auxiliary cells) act as sources of metabolites that can be rapidly mobilized by the organism. Previously, Chung et al. (2007) clarified the functions of the follicle cells during oocyte degeneration in *Cyclina sinensis.* In this study, the functions of follicle cells, which play an important role in the resorption of the lysis products in atretic oocytes, were investigated in further detail.

Despite this, gaps in our knowledge regarding its reproductive biology still exist. Above all, further studies on the functions of follicle cells associated with oocyte degeneration are required to understand the reproductive biology of *M. chinensis*.

The purpose of the present study is to describe degeneration in the oocyte, and the functions of follicle cells during oocyte degeneration. In addition, we aim to clarify the reproductive mechanism of oocyte degeneration of *M. chinensis* using cytological methods. The results of ultrastructural studies of oocyte degeneration and the functions of the follicle cells of this species will provide important information on the reproductive mechanisms of the resorption of the lysis products in atretic oocytes for yolk formation.

MATERIALS AND METHODS

1. Sampling

Female specimens of *Mactra chinensis* Philippi, 1846 (Mactridae) were collected monthly in the intertidal zone of Simpo, Kimje, Korea, from January to December, 2009. A total of 363 clams ranging from 5.2 cm to 6.3 cm in shell length were used for the present study. After the clams were transported alive to the laboratory, they were main-tained in seawater at 20°C. And shell lengths and total weights were immediately measured.

2. Electron microscope observations

For transmission electron microscopic observations, excised pieces of the gonads from individuals in the spent and inactive stage were fixed immediately in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 2 hours at 4°C. After prefixation, the specimens were postfixed in a 1% osmium tetroxide solution in 0.2 M phosphate buffer (pH 7.4) for 1 hour at 4°C and 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 under JEM 100 CX-Ⅱ (80-kV) transmission electron microscope.

RESULTS

1. Ultrastructural characteristics of degeneration in oocytes and the functions of the follicle cells during oocyte degeneration

Based on electron microscopical observations, the ultrastructural characteristics of oocyte degeneration in the oocytes by the follicle cells were observed as follows:

2. Ultrastructure of degenerating follicle cells attached to the degenerating oocyte

After the mature oocyte (or ripe ovum) accumulated a number of mature yolk granules in the cytoplasm of mature oocyte, a number of mature yolk granules underwent degeneration by the follicle cells attached to mature oocytes. During oocyte degeneration, the follicle cells varied in shape and size according to the degree of cytoplasm repletion by engulfed material. The size of follicle cells increased during oocyte degeneration. The process of follicle cell degeneration was characterized by several phagosomes (lysosomes), a number of vacuoles, myelin-like organelles, and small number of lipid droplets in the degenerating oocyte appearing in the cytoplasm of the follicle cells, whereas glycogen particles decreased in the cytoplasm of follicle cells that were attached to the degenerated oocytes. As oocyte degeneration proceeded in the cytoplasm of follicle cells, all organelles within the follicle cells showed a good appearance (Fig. 1A). In this study, morphologically similar degenerated phagosomes (various lysosomes), which were observed in degenerated oocytes, appeared in follicle cells. After the spawning of the oocytes, the follicle cells were involved in oocyte degeneration through phagocytosis by phagolysosomes. Therefore, it can be assumed that follicle cells reabsorb phagosomes from degenerated oocytes. In this study, the presence of lipid granules, which occurred from degenerating yolk granules, gradually seems to be increased in degenerating oocytes. Particularly, in the follicle cells, myelin-like organelles, which is located near the cell membrane of the degenerating oocyte, moved into the cytoplasm of the oocyte? And at this time, several phagosomes, which appeared in the follicle cells, came from the degenerating oocyte. Therefore, morphologies of several phagosomes were similar to those in the degenerating oocyte.

3. Ultrastructure of degenerating oocytes

The nuclear content of degenerating oocytes included some electron-dense accumulation, and the nucleus and nucleolus had both deteriorated. The nuclear envelope of degenerating oocytes broke down in the end of the degeneration process. The process of oocyte degeneration was characterized by vacuolization and myelin-like organelles

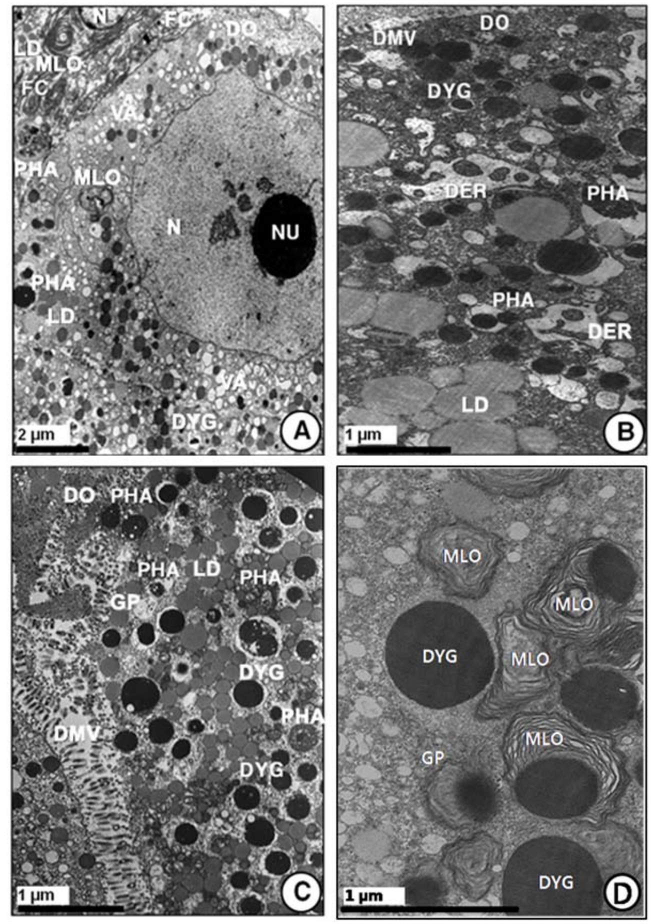

Fig. 1. Electron micrographs of oocyte degeneration and follicle cells after spawning in female *Mactra chinensis*. A, A degenerating oocyte, with a nucleolus in the irregular nucleus, a number of vacuoles, lipid droplets, myeline-like organelle, degenerating yolk granules and various phagosomes in the cytoplasm of the oocyte, and an attached follicle cell containing a nucleus, rough endoplasmic reticulum, vacuoles, a few lipid droplets, degenerating phagosomes, a number of vacuoles, and myelin-like organelles in the cytoplasm of follicle cell; B, A degenerated oocyte, with distended endoplasmic reticulum, vacuoles, degenerated yolk granules, a number of lipid granules, and phagosomes (lysosomes) near the lipid droplets, and abnormal degenerated microvillus structure on the abnormal vitelline coat; C, A degenerated oocyte, with degenerated microvillus structure on the vitelline coat, glycogen particles, a number of several lipid

droplets near the degenerated yolk granules and phagosomes (lysosomes); D, A degenerated oocyte, with myelin-like organelles attaching to degenerated yolk granules in the cytoplasm. Abbreviations: DER, distended endoplasmic reticulum; DMV, degenerated microvillus structure; DO, degenerated oocyte; DYG, degenerated yolk granule; FC, follicle cell; GP, glycogen particle; LD, lipid droplets; MLO, myelin-like organelle; N, nucleus; NU, nucleolus; PHA, phagosome; VA, vacuole.

(myelin figures) in the cytoplasm of the oocyte. In particular, myelin-like organelles and similar phagosomes (lysosomes), as seen in the cytoplasm of the follicle cells, were present in the cytoplasms of degenerating oocytes (Fig. 1A). The degenerating oocytes appeared to be slightly irregular or polyhedric near the follicle cells, and they were deformed by compression. A number of vacuoles, degenerating yolk granules, distended endoplasmic reticulum, a number of phagosomes (or phagolysosomes), and lipid droplets appeared in the cytoplasm of degenerating oocytes (Fig. 1B). At this stage, myelin-like organelles were present. Of particular interest, the Golgi complex was not observed in atretic oocytes. The endoplasmic reticulum was specifically involved during the gradual disintegration of oocytes. The smooth or rough endoplasmic reticulum became distended which led to vacuolation of the ooplasm. At this time, an abnormal microvillus structure (degenerated microvilli) appeared on the vitelline coat of the degenerated oocyte (Figs. 1B, 1C). Yolk granules disintegrated in the cytoplasm, and lysis was initiated at the cell periphery. Several vacuoles, lipid droplets, a large quantity of glycogen particles, and numerous degenerating yolk granules with similar appearances to phagosomes (lysosomes) were present in the ooplasm (Fig. 1C). In particular, many disintegrated yolk granules with myelin-like organelles (myelin figures) and phagosomes were visible at the periphery of the cytoplasm of degenerating oocyte (Fig. 1D).

DISCUSSION

1. Functions of follicle cell-oocytes

In general, accessory cells termed follicle cells appear in invertebrate gonads and are often suspected of playing a role in oocyte nutrition during oogenesis (Wourms 1987; Eckelbarger & Davis, 1996). Follicle cells at the periphery of oogenic follicles (or acinus) initially appear close to previtellogenic oocyte in the early stages of oogenesis, and thereafter, progressively surround a part of the oocyte. At this stage, a small number of vacuoles are visible in the cytoplasm of the follicle cells near the adherence zone. The attached follicle cells also showed cytological modifycations as their cytoplasmic volume increased in *C. virginica* (Eckelbarger & Davis, 1996) and *M. edulis* (Pipe, 1987), after which they gradually detach from vitellogenic oocytes. Therefore, it can be assumed that follicle cells function as nutritive cells in the early development of the oocytes (Chung et al., 2005, 2007, 2008; Chung, 2007, 2008).

According to the number and arrangement of follicle cells attached to oocytes, Jong-Brink et al. (1983) reported that oocyte-follicle cell relationships can be divided into three categories (types). In the first type, oocytes are completely surrounded by an increasing number of follicle cells; in the second type, oocytes are surrounded by a small, distinct number of follicle cells; in the third type, a small number of follicle cells surround oocytes only during the early stages of oogenesis. In this study, oocytes of *M. chinensis*is were surrounded by a small number of follicle cells during the early and late stages of oogenesis. As a result, this species can be classified into the third type of oocyte - follicle cell relationships.

At the adherence zone of the follicle cell-vitellogenic oocytes, lipid droplets were surrounded by myelin-like organelles (myelin figures) in the cytoplasm of the oocytes. At this time, a number of vacuoles and myelin-like organelles appeared in the cytoplasm of the follicle cells, which suggests membrane breakdown, as seen in *Mytilus edulis*

(Pipe, 1987). Pipes (1987) reported that endocytotic figures appeared between vitellogenic oocytes and follicle cells, indicating a transfer of nutrients in *M. edulis*. In this study, similar ultrastructural changes were observed in the follicle cells of this species. Follicle cells, which were attached to previtellogenic oocytes, were in the early developmental stage. In particular, glycogen particles, several vacuoles, and a few lipid droplets were visible in the cytoplasm of follicle cells attached to previtellogenic oocytes. However, at the adherence zone of the follicle cells and vitellogenic oocytes, several lipid droplets, myelin-like organelles (or myelin figures) and several vacuoles appeared in the cytoplasm of late vitellogenic oocytes.

In the sexually ripe stage, it is hard to identify follicle cells since some disappear from mature oocytes. Therefore, it can be assumed that follicle cells function as nutritive cells in the formation and development of germ cells during heterosynthetic vitellogenesis. After spawning, cellular organelles in the cytoplasm of the follicle cells showed unprecedented phenomenona associated with the cytoplasm of degenerating oocytes.

2. Oocyte degeneration and resorption by the follicle cells

Oocyte degeneration is a continuous phenomenon observed in *M. chinensi*. During oocyte degeneration, follicle cells varied in shape and size according to the degree of cytoplasm repletion by engulfed materials. The size of follicle cells increased during oocyte degeneration. This process of oocyte degeneration was characterized by vacuolezation of the oocyte cytoplasm. During degeneration of oocytes, a number of degenerating yolk granules showing hydrolytic enzyme activity as well as a few myelin-like organelles appeared in the cytoplasm of degenerated oocytes in *M. chinensis*. At this time, several phagosomes (or lysosomes) and lipid droplets increased in number in the cytoplasm of the follicle cells, which were attached to degenerated oocytes. However, glycogen particles decreased in the cytoplasm of follicle cells, as seen in *Mytilus edulis* (Pipe, 1987) and *Pecten maximus* (Dorange & Le Pennec, 1989). Therefore, it can be assumed that this function can permit a transfer of yolk precursors necessary for vitellogenesis, and allows for the accumulation of reserves in the cytoplasm, such as glycogen and lipids, which can be used by vitellogenic oocyte (Gaulejac et al., 1995; Chung et al., 2005). Several lipid droplets and the formation of myelin-like organelles by lysosomal enzyme activity appeared in the cytoplasm of follicle cells, and a number of degenerating yolk granules with myelin-like organelles exhibiting lysosomal enzyme activity appeared in the cytoplasm of degenerated oocytes in *M. chinensis*. At this point, degenerating mitochondria, distended endoplasmic reticulum, phagosomes (or lysosomes), and lipid droplets all increased in number in the cytoplasm of degenerated oocytes (Chung et al., 2007). Morphologically, a similar number of degenerated yolk granules and phagosomes (lysosomes), which were easily observed in the cytoplasm of degenerated oocytes, was detected in the cytoplasm of follicle cells, as was reported for *Patinopecten yessoensis* (Chung et al., 2005), *Sinonovacula constricta* (Chung et al., 2008), and *Cyclina sinensis* (Chung et al., 2007). When the oocytes were degenerated, the presence of glycogen particles decreased in the cytoplasm of the follicle cells attaching to degenerated oocytes, whereas a number of lipid droplets rapidly increased in the cytoplasm of the follicle cells, as reported when follicle cells attached to the degenerated oocytes of *Pinna nobilis* (Gaulejac et al., 1995) and *P. yessoensis* (Chung et al., 2005). However, these findings investigated by ultrastructural studies were not clear. Therefore, to clarify the contents of glycogen particles and lipid droplets, we should investigate their contents in detail by chemical analysis. Accordingly, it is assumed that the function of follicle cells play an integral role in vitellogenesis and oocyte degeneration. Regarding the functions of follicle cells during oocyte degeneration, Gaulejac et al. (1995) reported that, in *P. nobilis*, the functions of follicle cells are phagocytosis and the intracellular digestion of products originating from degenerated oocytes, which was also reported for *C. sinensis* (Chung et al., 2007). The function of follicle cells appeared to be associated with the induction of oocyte degeneration as seen in *C. sinensis*. In particular, follicle cells were involved in the development of previtellogenic and early vitellogenic oocytes by supplying nutrients, as well as vitellogenesis in early and late vitellogenic oocytes by the endocytosis of yolk precursors. After the spawning of the oocytes, the follicle cells were involved in oocyte degeneration through phagocytosis by phagolysosomes. Therefore, it can be assumed that follicle cells reabsorb phagosomes from degenerated oocytes, since morphologically similar degenerated phagosomes (various lysosomes), which were observed in degenerated oocytes, appeared in follicle cells. In this study, the presence of lipid granules gradually increased in degenerating oocytes, whereas glycogen contents decreased in follicle cells with gametogenesis. As previously reported by Gaulejac et al. (1995), “this function of follicle cells can permit a transfer of yolk precursors necessary for vitellogenesis and accumulate reserves of glycogen and lipids in the cytoplasm, which can be employed by the vitellogenic oocyte.” Follicle cells attached to degenerating oocytes are shown to be involved in the induction of oocyte degeneration and resorption of degenerated phagosomes (lysosomes) and degenerating yolk granules by lysosomes, as seen in *P. yessoensis* (Chung et al., 2005), *S. constricta* (Chung et al., 2008) and *C. sinensis* (Chung et al., 2007). In addition, it can be assumed that follicle cells attached to degenerated oocytes have a lysosomal system for the breakdown of ingested materials. Further, they might be involved in the induction of oocyte degeneration and also resorb various degenerating phagosomes (lysosomes) in the cytoplasm for nutrient storage (such as lipid droplets) during oocyte degeneration, as seen in *M. chinensis.*

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