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Ultrastructural Study of the Process of Oocyte Degeneration and Function of the Follicle Cells in Female *Spisula sachalinensis* on the East Sea of Korea

Ee-Yung Chung[1], Ki-Young Lee[2] and Jeong-Yong Lee[3]

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

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

[3]*Marine Ranching Development & Management Center, NFRDI, Pusan 619-705, Korea*

ABSTRACT

Ultastructural studies of oocyte degeneration and follicle cells in female *Spisula sachalinensis* are described for clams collected from Jumunjin, Gangwondo, Korea. The follicle cells playan integral role in vitellogenesis and oocyte degeneration by assimilating products originating from the degenerated oocytes (thus allowed the transfer of yolk precursors needed for vitellogenesis). The functions of the follicle cells include phagocytosis and intracellular digestion of products originating from oocyte degeneration. During the period of oocyte degeneration, follicle cells of this species probably have lysosomal systems for the breakdown and reabsorption of various phagosomes(phagolysosomes) in the cytoplasm for nutrient storage; this process has been observed in other bivalves.

Key words: *Spisula sachalinensis*, Oogenesis, Ultrastructure, Oocyte Degeneration, Follicle cell

INTRODUCTION

The clam, *Spisula sachalinensis* is a commercially important bivalve in East Asian countries, including Korea, Japan, and China. On the East Sea of Korea, this species is mainly found in the sand bed in the subtidal zone at depths up to 10 m (Yoo, 1976; Kwon *et al.*, 1993; Min *et al.*, 2004). It is important that we fully understand the reproductive biology with regard to gamete degeneration. So far, there have been many utrastructural studies on oogenesis and follicle cells of *Cyclina sinensis* (Chung *et al.*, 1991), *Pinna nobilis* (Gaulejac *et al.*, 1995), *Crassostrea virginica* (Eckelbarger and Davis, 1996), *Mytilus edulis* (Pipe, 1987), *Mactra veneriformis* (Chung and Ryou, 2000), *Patinopecten yessoensis* (Chung et al., 2005), *Meretrix lusoria* (Chung, 2007), and *Mactra chinensis* (Chung and Kim, 2008).

Previously, there have been many studies on Mactridae species, especially, regarding *M. chinensis*, several studies have been conducted to

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Corresponding author: Chung, Ee-Yung
Tel: +82 (63) 469-4591 Email: eychung@kunsan.ac.kr
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investigate aspects of reproductive ecology, including propagation (Sakai, 1976), bioassay study of early development (Lee and Son, 1978), spawning and growth (Kim *et al.*, 1985), breeding season (Sakurai *et al.*, 1992), oogenesis and ovarian cycle (Chung and Kim, 2008), and spermatogenesis and sexual maturation (Kim, 2001; Chung *et al.*, 2007).

However, there have been some studies on *S. sachalinensis* on aspects of reproduction, including the reproductive cycle (Lee *et al.*, 1997; Takahashi and Takano, 1970a; Takahashi and Yamamoto, 1970b) and gametogenesis (Kim, 2001; Lee *et al.*, 2003), on aspects of ecology, including growth (Sasaki, 1981), fecundity (Sasaki, 1981), life cycle (Sasaki,1987) and mortality (Sasaki, 1988). However, there are still significant gaps in our knowledge regarding its reproductive mechanism. Studies of the oocyte degeneration and follicle cells after spawning of this species are required to understand this animal' s reproductive mechanisms for degeneration and recycling of nutrients. In the majority of bivalve species, the ovaries contain follicle cells (as a kind of accessory cell) that play a role in the storage, mobilization, and synthesis of yolk precursors during oogenesis (Wourms, 1987). However, more specifically, oocyte degeneration, which is known as atresia, is a commony observed phenomenon in most bivalve species. In bivalves, the products of lysis material created by the follicle cells as a kind of accessory cell as sources of metabolites that can be rapidly mobilized by the organism (Pipe, 1987; Dorange *et al.*, 1989; Le Pennec *et al.*, 1991; Gaulejac *et al.*, 1995). Commonly, in the most clams except for scallops, we usually use the term, "the follicle cell" as a kind of accessory cell. The functions of the follicle cells in the resorption of the lysis products of atretic oocytes of this species should be investigated in further detail. Understanding of function of follicle cells during oocyte degeneration of this species will provide information needed for the reproductive mechanism.

The purpose of the present study is to describe the induction of oocyte degeneration of the follicle cells after spawning and lysis products in the degenerated oocyte of *S. sachalinensis*.

MATERIALS AND METHODS

Sampling

The specimens of *S. sachalinensis* were collected monthly by dredge in the sand bed in the subtidal zone at depths up to 10 m off Jumunjin, Gangwon-do, Korea, from January to December, 2006.

Ovarian cycle by Light Microscopical Observation

For light microscopic examination of histological preparations, female ovarian tissues were removed from animals and preserved in Bouin' s fixative for 24 hr and then washed with running tap water for 24 hr. Tissues were then dehydrated in alcohol and embedded in paraffin molds. Embedded tissues were sectioned at 5-7 μm thickness using a rotary microtome. Sections were mounted on glass slides, stained with Hansen hematoxylin-0.5% eosin, and examined using a light microscope. To identify the spawning period and describe the ovarian cycle after spawning, a total of 80 ovarian histological preparations were made from clams of 70.7-86.3 mm shell length.

Ultrastructure of degenerated oocytes and follicle cells

A total of 95 females were used for ultra-

structural study of germ cells and auxiliary cells by electron microscopy. For transmission electron microscopy, excised samples of gonads were cut into small pieces and fixed immediately in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hr at 4℃. After prefixation, the specimens were washed several times in the buffer solution and then postfixed in 1% osmium tetroxide solution in 0.2 M phosphate buffer (pH 7.4) for 1 hr at 4℃. Specimens then were dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in an Epon-Araldite mixture (Epon-812). Ultrathin sections of Epon-embedded specimens were cut to a thickness of 80-100 nm with a LKB ultramicrotome. The sections were mounted on collodion-coated copper grids, double stained with uranyl acetate followed by lead citrate, and observed under a JEM 100 CX-2 (80 kv) electron microscope.

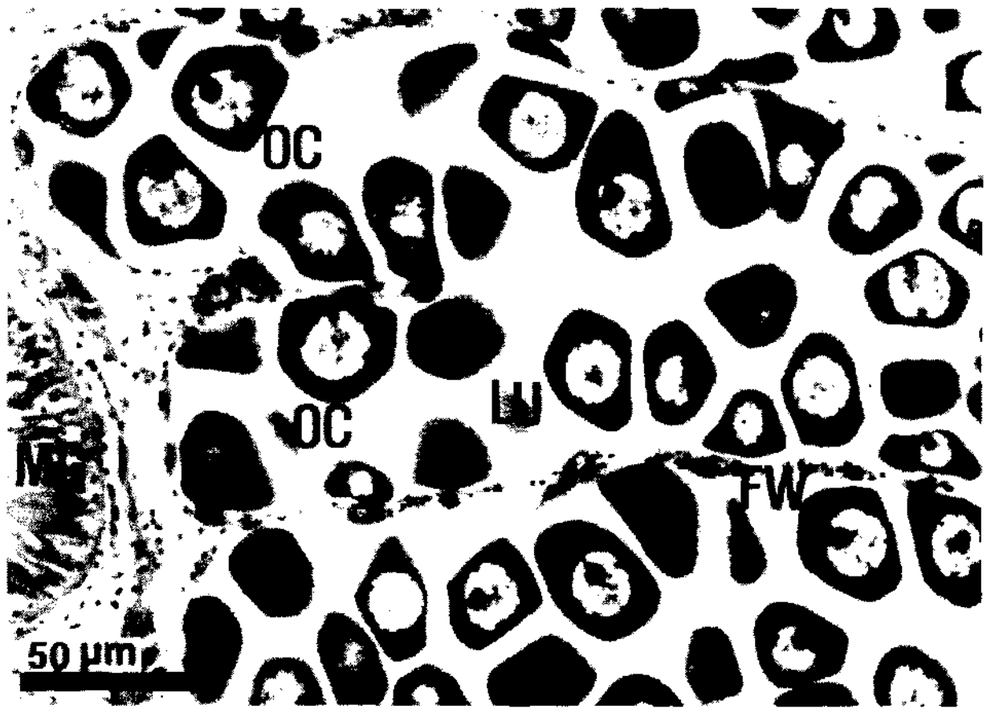

Fig. 1. Photomicrograph of the structure of the ovary. Abbreviations: FW, follicular wall; LU, lumen; MG, mid-intestinal gland; OC, oocyte.

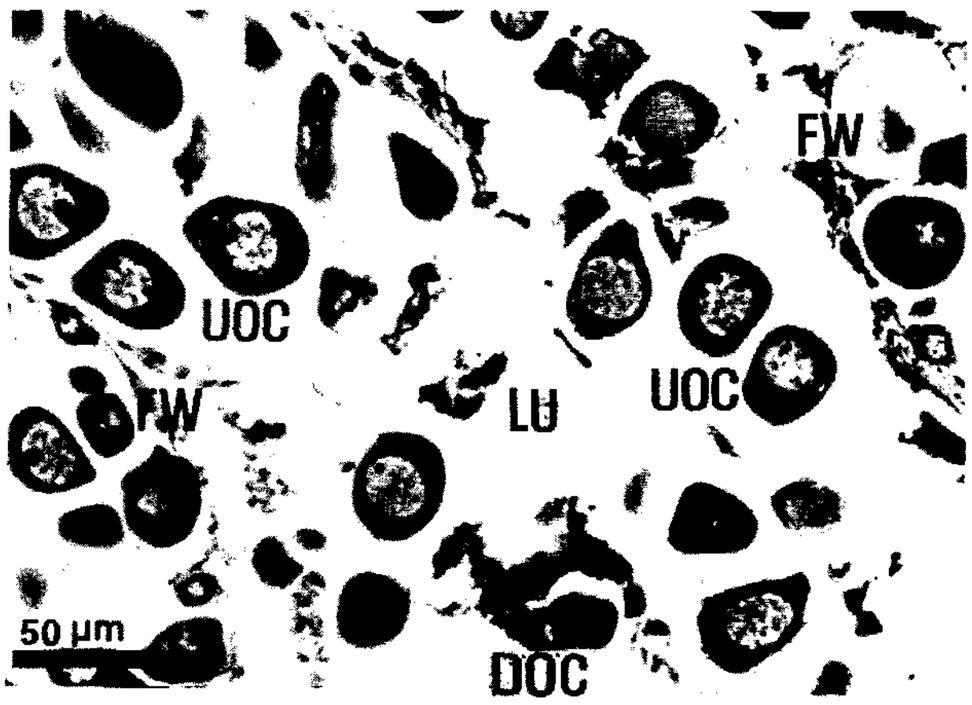

Fig. 2. Photomicrographs of the gonadal stage in female *Spisula sachalinensis*. Section of oogenic follicles in the spent stage. Abbreviations: DOC, degenerating oocyte; FW, follicular wall; LU, lumen; UOC, undischarged oocyte.

RESULTS

Position and Morphology of the Ovary

The general morphology of the ovary of *S. sachalinensis* is similar to that of other bivalves. The ovary is distributed from the subregion of visceral mass (the mid-gut gland, digestive diverticular) to the reticular connective tissue of the foot. The ovary is a diffuse organ that is composed of highly branching follicles (acini), in which germ cells develop (Fig. 1).

Even though gonad maturation progressed, the external features of the ovary and testis showed the same color (light milky white or light milky yellow) without any change. Therefore, sex could not be easily determined from external features. However, when the ovary was slightly scratched using the razor, mature oocytes readily emerged. At this time their sexes could be easily determined by disection. If germ cells in the ovary were degenerated after spawning, the sexes became difficult to distinguish by disection.

Ovarian Developmental Stage by histological observation

Spent / inactive stage: After spawning, each fol

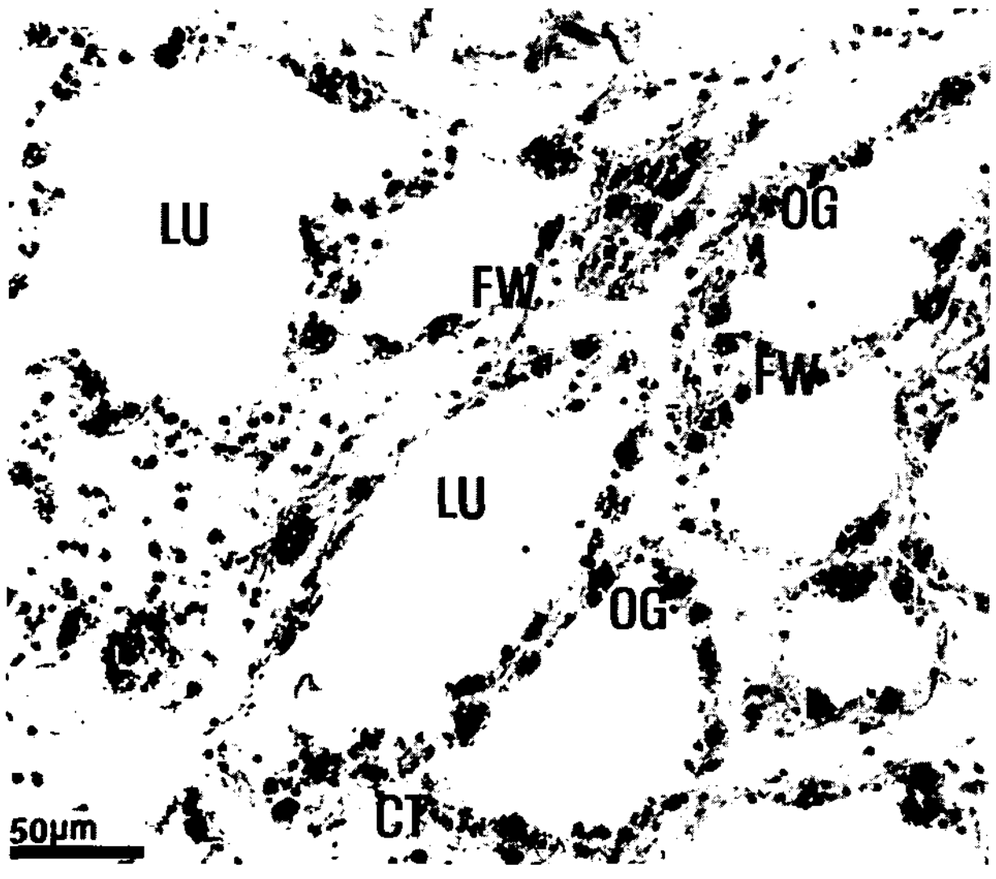

Fig. 3. Photomicrographs of the gonadal stage in female *Spisula sachalinensis*. Section of oogenic follicles in the inactive stage. Abbreviations: CT, connective tissue; FW, follicular wall; LU, lumen; OG, oogonia.

licle contracted and degenerated, and the undischarged oocytes in the lumen of the follicle underwent cytolysis. The products of gamete atresia were resorbed (Fig. 2). Thereafter, a rearrangement of the connective tissues was observed. Occasionally a few oogonia were present on the follicular walls in the inactive stage (Fig. 3)

Ultrastructures of Degenerated Oocyte and Follicle Cells after spawning

After spawning, the degenerating oocytes appeared irregular or polyhedral near the follicle cells and were deformed by compression in the follicle. A number of vacuoles, degenerating yolk granules, a few phagosomes (lysosomes), and lipid droplets appeared in the cytoplasm of degenerating oocyte. Especially, mitochondria and yolk granules disintegrated in the ooplasm, and lysis was initiated at the cell periphery, several vacuoles and numerous heterogenous, dense granules that appear similar to phagosomes (lysosomes) were present in the ooplasm (Fig. 4). At this stage, especially, a few phagosomes

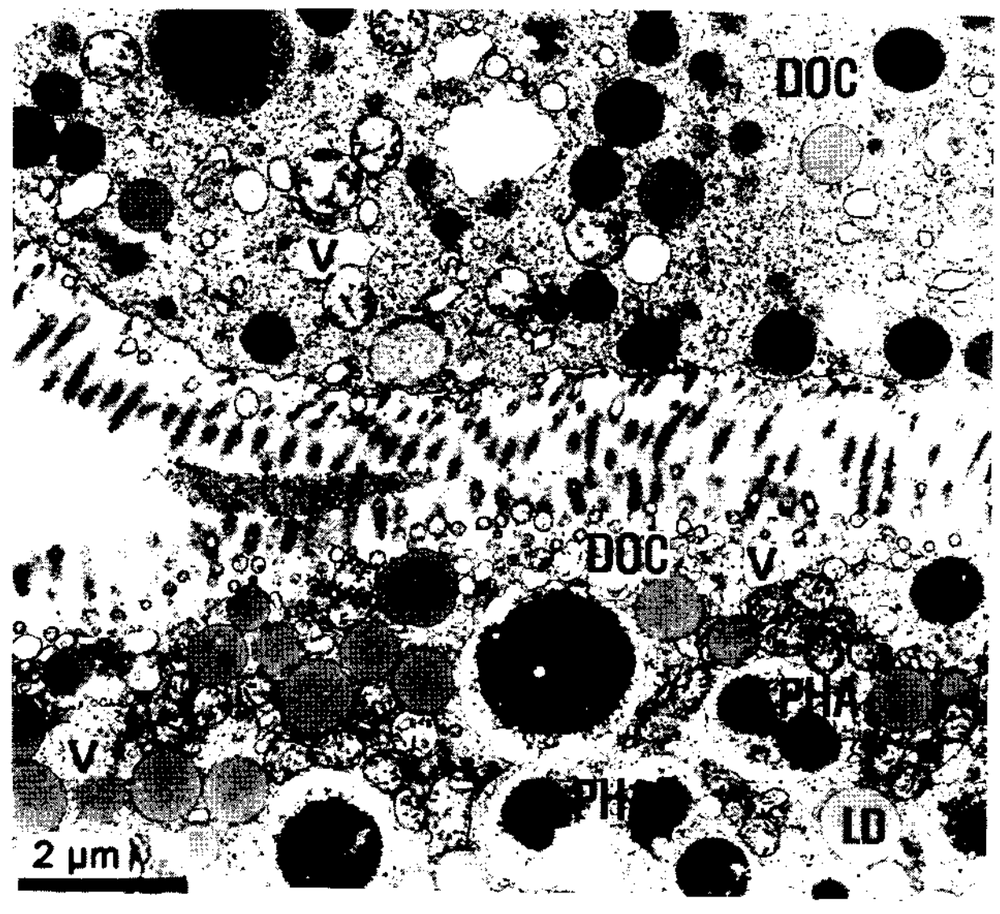

Fig. 4. Electron micrographs of oocyte degeneration in female *Spisular sachalinensis.*, with a degenerating oocyte containing degenerating yolk granules, lipid droplets and phagosomes by lysosome in the cytoplasm. Abbreviations: DOC, degenerating oocyte; LD, lipid droplet; PHA, phagosome.

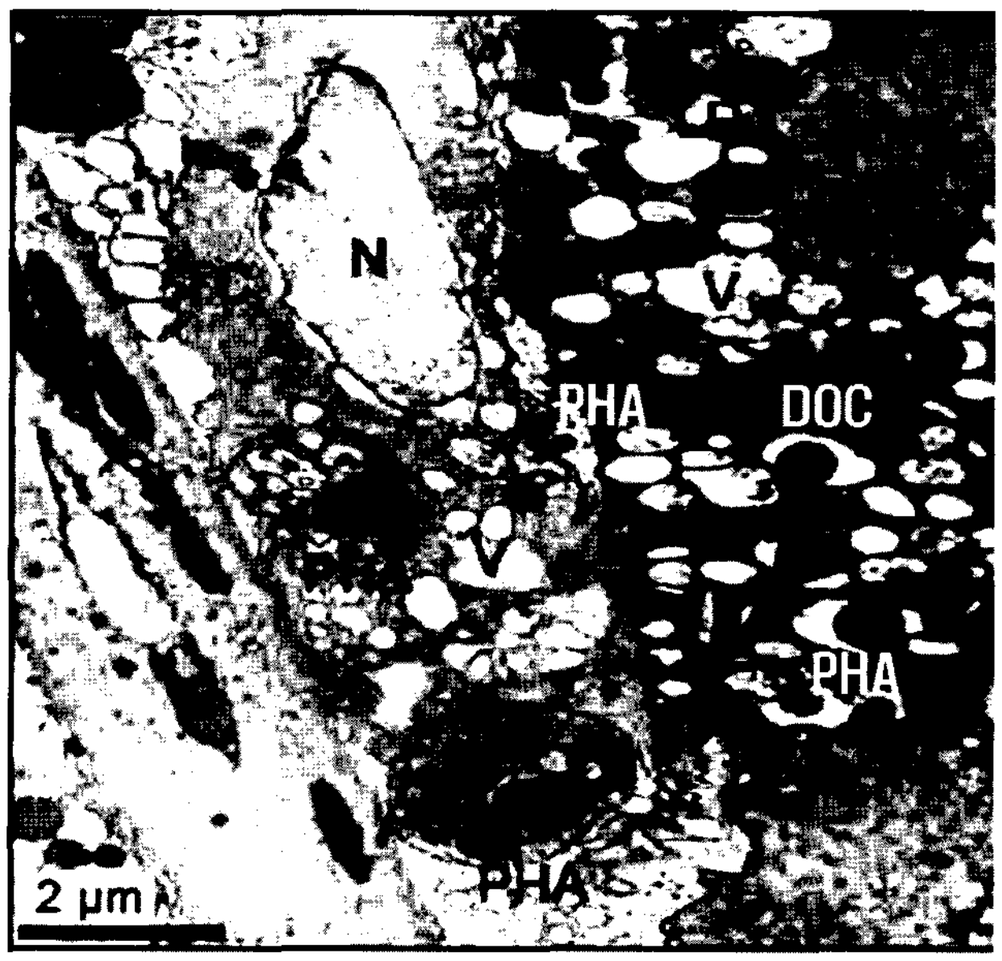

Fig. 5. Electron micrographs of oocyte degeneration in female *Spisular sachalinensis*, A degenerating oocyte, and the attached follicle cells, with phagosomes (lysosomes), a number of vacuoles, degenerating yolk granules in the degenerating oocyte, and a few phagosomes (lysosomes), lipid droplets and vacuoles in the cytoplasm of the follicle cell. Abbreviations: DOC, degenerating oocyte; FC, follicle cell; LD, lipid droplet; N, nucleus; PHA, phagosome; V, vacuole.

(lysosomes), a number of vacuoles, and a small number of lipid droplets appeared in the cytoplasm of the follicle cell, whereas glycogen particles decreased in the cytoplasm of the cells, it were attached to the degenerating oocyte (Fig. 5).

DISCUSSION

In general, the follicle cells at the periphery of the oogenic follicle (or acinus) initially appears close to the 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).

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 figure appeared in the ooplasm of the degenerated oocytes in *S. sachalinensis*. 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, that 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).

Regarding reproductive energy allocated to the production of gametes, some authors (Morvan and Ansell, 1988) stated that continuous production and resorption of gametes can be regarded as an adaptation to environmental temperature and food availability. If the energy allocated to the production of gametes is too large, nutritive reserves might not be sufficient to allow all eggs to reach the critical size and maturity for

spawning and fertilization. In this case, the products of gamete atresia can be resorbed and the energy reallocated to still developing oocytes or used for other metabolic purposes by marine mollusks (Dorange and Le Pennec, 1989; Mortavkine and Varaksine, 1989).

In *M. edulis*, after spawning, gamete resorption is common in the acini of the ovary. It is supposed that *M. edulis* resorbs gametes in follicles to utilize the high nutritive reserves in developing oocytes for other metabolic activities (Pipe, 1987) as observed in other bivalves (Dorange and Le Pennec, 1989; Motavkine and Veraksine, 1989). Therefore, it is assumed that *S. sachalinensis* has a similar reproductive mechanism to resorb and utilize high nutritive substances rather than releasing non-viable gametes.

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