

# Structure of the Ovary and Ultrastructural Study of Vitellogenesis in the Oocytes in Female *Gomphina veneriformis* (Bivalvia: Veneridae) in Eastern Korea

Ki-Ho Choi<sup>1</sup>, Chang Ho Chung<sup>2</sup>, Sa-Heung Lee<sup>3</sup>, Gab Man Park<sup>4</sup>, Moon-Sul Choi<sup>5</sup> and Ki-Young Lee<sup>5</sup>

<sup>1</sup>West Sea Fisheries Research Institute, National Fisheries Research and Development Institute, Incheon 400-420, Korea

<sup>2</sup>CMAS Korea, Seoul 138-151, Korea

<sup>3</sup>Department of Fisheries Science, Graduate School, Kunsan National University, Kunsan 573-701, Korea

<sup>4</sup>Department of Parasitology, Kwandong University College of Medicine, Gangnung 210-710, Korea

<sup>5</sup>Department of Marine Biotechnology, Kunsan National University, Kunsan 573-701, Korea

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## ABSTRACT

The structure of the ovary, ultrastructure of oocytes and morphological characteristics of vitellogenesis during oogenesis in female *Gomphina veneriformis* were investigated in clams collected from coastal waters of Samchok, Gangwon-do, Korea. In the previtellogenic oocytes, the Golgi complex was involved in the formation of a number of vacuoles. In the early vitellogenic oocytes, lipid droplets appeared among the Golgi complex, endoplasmic reticulum, and mitochondria in the cytoplasm of the oocyte were involved in the formation of lipid droplets. Coated vesicles, resulting from endocytosis appeared at the basal region of the early vitellogenic oocyte. The uptake of nutritive materials in the coated vesicles formed by receptor-mediated endocytosis appeared through the formation of coated endocytotic pits on the oolemma. In the late vitellogenic oocytes, large yolk granules were formed by a combination of small yolk granules. In the mature oocyte, a mature yolk granule in composed of three components: crystalline core, electron lucent cortex, and a limiting membrane. According to cytological and histological observations, vitellogenesis occurred by way of endogenous autosynthesis and exogenous heterosynthesis. Autosynthesis involved the combined activities of the Golgi complex, mitochondria, rough endoplasmic reticulum, whereas heterosynthesis involved endocytotic incorporation of extraovarian precursors at the basal region of the early vitellogenic oocyte. The follicle cells which was attached to oocytes, were involved in the development of the previtellogenic and early vitellogenic oocytes as a kind of nutritive cells containing a number of glycogen particles and lipid droplets in the cytoplasm.

**Keywords:** *Gomphina veneriformis*, ultrastructure, vitellogenesis, follicle cells, oogenesis

## Introduction

The equilateral venus, *Gomphina veneriformis*, is one of the most important edible bivalves in East Asian countries including Korea, China and Japan. In Korea, this species is found mainly in silty sand at

the subtidal zones in the coastal waters of Samchok, Gangwondo, Korea (Yoo, 1976; Kwon *et al.*, 1993). Due to past over-harvesting, it has been identified as a fisheries resource that should be managed using a more reasonable fishing regimen. For the reproduction and propagation of a living natural resource, it is important to understand some basic formation on the reproductive physiology associated with vitellogenesis during oogenesis, such as reproductive mechanism of the species.

There have been several studies on the genus *Gomphina* species, particularly, regarding *Gomphina*

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Corresponding author: Chung, Chang-Ho

Tel: +82 (2) 420-7494 e-mail: cmas045@hanmail.net

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*melanaegis*, several studies have been conducted on aspects of reproduction, including spawning season, artificial fertilization and egg development (Lee, 1976) and gonadal development and reproductive cycle (Lee et al., 1999) and regarding *G. veneriformis* on aspect of reproduction, including spermatogenesis and sperm ultrastructure (Park et al., 2002) and germ cells and the accessory cells during spermatogenesis (Chung et al., 2010).

Although several studies have reported on the reproductive ecology of *G. veneriformis*, there are still disagreement in the available knowledge regarding its reproductive biology. Little information is available on the ultrastructural studies of germ cell differentiation and the functions of the follicle cells during oogenesis in female *G. veneriformis*. The results of ultrastructural changes in oocytes of this species will provide important information on its reproductive mechanisms such as vitellogenesis. Therefore, the purpose of the present study is to describe ultrastructural changes in oocytes with follicle cells and vitellogenesis during oogenesis of *G. veneriformis* using cytological and histological methods.

## Materials and Methods

### 1. Sampling

Female specimens of *Gomphina veneriformis* were collected monthly at the subtidal zones on the coastal waters of Samchok, Gangwondo, Korea, from January to December, 2007 (Fig. 1). The clams were then transported to the laboratory where they were maintained in seawater at 20°C.

### 2. Ultrastructure of germ cells and follicle cells during oogenesis

For transmission electron microscope observations, excised pieces of the gonads were cut into small pieces and 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 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°C. Specimens were then dehydrated in

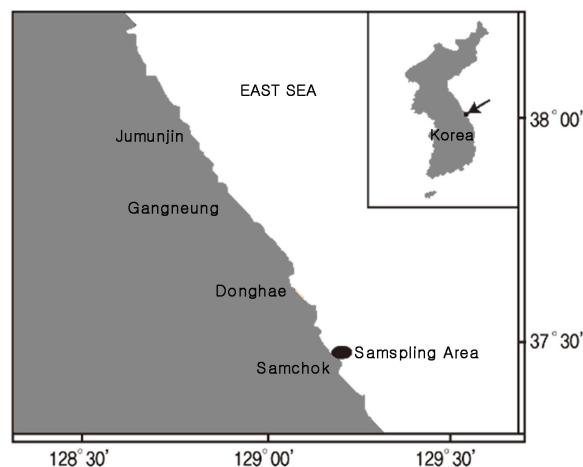


Fig. 1. Map showing the sampling area.

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.

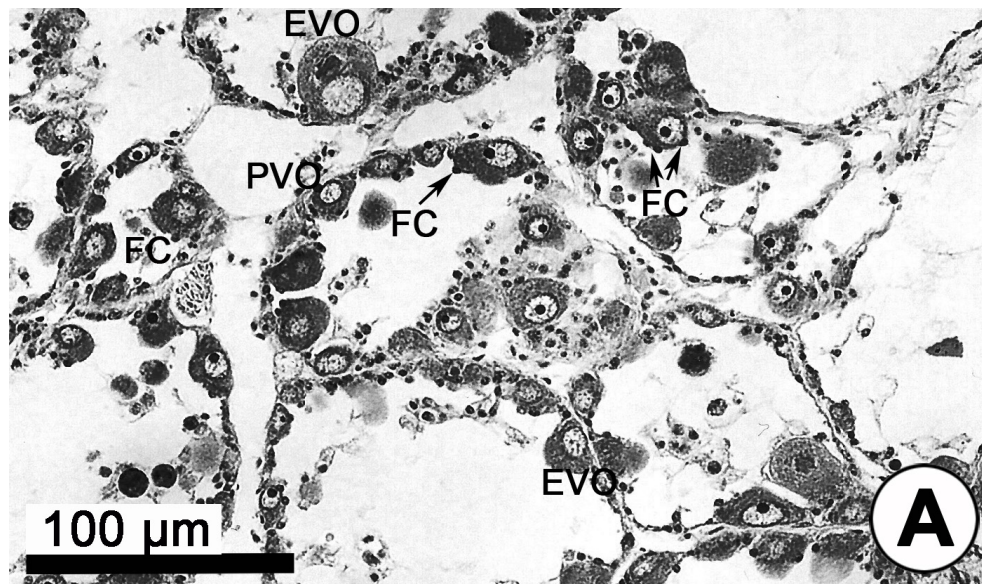
### 3. Histologic analysis

For light microscopic observations of the ovarian cycle, histological preparations were made for analysis of the gonadal phases, and the tissues were subjected to standard histological procedures (dehydrated in alcohol and embedded in paraffin) and sectioned at 5-7 μm using a rotary microtome. Sections were then mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin, and inspected under a light microscope.

## RESULTS

### 1. Position and structures of the ovary

The general morphology of the ovary of *G. veneriformis* similar to that of other bivalves. The ovary is located between the subregion of the mid-intestinal glands in the visceral cavity and the reticular connective tissue of the foot. The ovary is a



**Fig. 2.** A photomicrograph of the structure of the ovary in female *Gomphina veneriformis*. Oogonia, previtellogenic Oocytes and early vitellogenic oocytes appeared in the lumen of the follicle in the early stage of oogenesis. Abbreviations: EVO, early vitellogenic oocyte; FC, follicle cell; FW, Follicular wall; OG, oogonium; PVO, previtellogenic oocyte.

diffused organ consisting of branching follicles, in which differentiating oocytes in a variety of stages develop. Oogonia and oocytes are distributed in a centripetal pattern from the follicular wall to the lumen of the follicle. The oogenic follicles are surrounded by a matrix of the vesicular connective tissue cells (VCT cells), which are encircled by a connective tissue compartment and hemocoel. In particular, as shown in Fig. 2, each previtellogenic or vitellogenic oocytes and a few follicle cells (which is attached to each oocyte) are found in the follicle. Several previtellogenic oocytes and vitellogenic oocytes are distributed along the inner wall of each follicle in all stages of differentiation, whereas the outer wall of the follicle is characterized by a discontinuous layer of thin squamous myoepithelial cells that forms a partial barrier between vitellogenic oocyte and the hemocoel (Fig. 3B). More specifically, VCT cells, which are attached to the outer wall of the follicle appear near the vitellogenic oocyte. As gonadal maturation progresses, the external view of the mature ovary and testis is showed the same color, therefore, it is hard to distinguish their sexes by external features. However, it is easy to distinguish their sexes by

histological observation of gonadal tissue sections.

## 2. Ultrastructure of oocytes and follicle cells during oogenesis

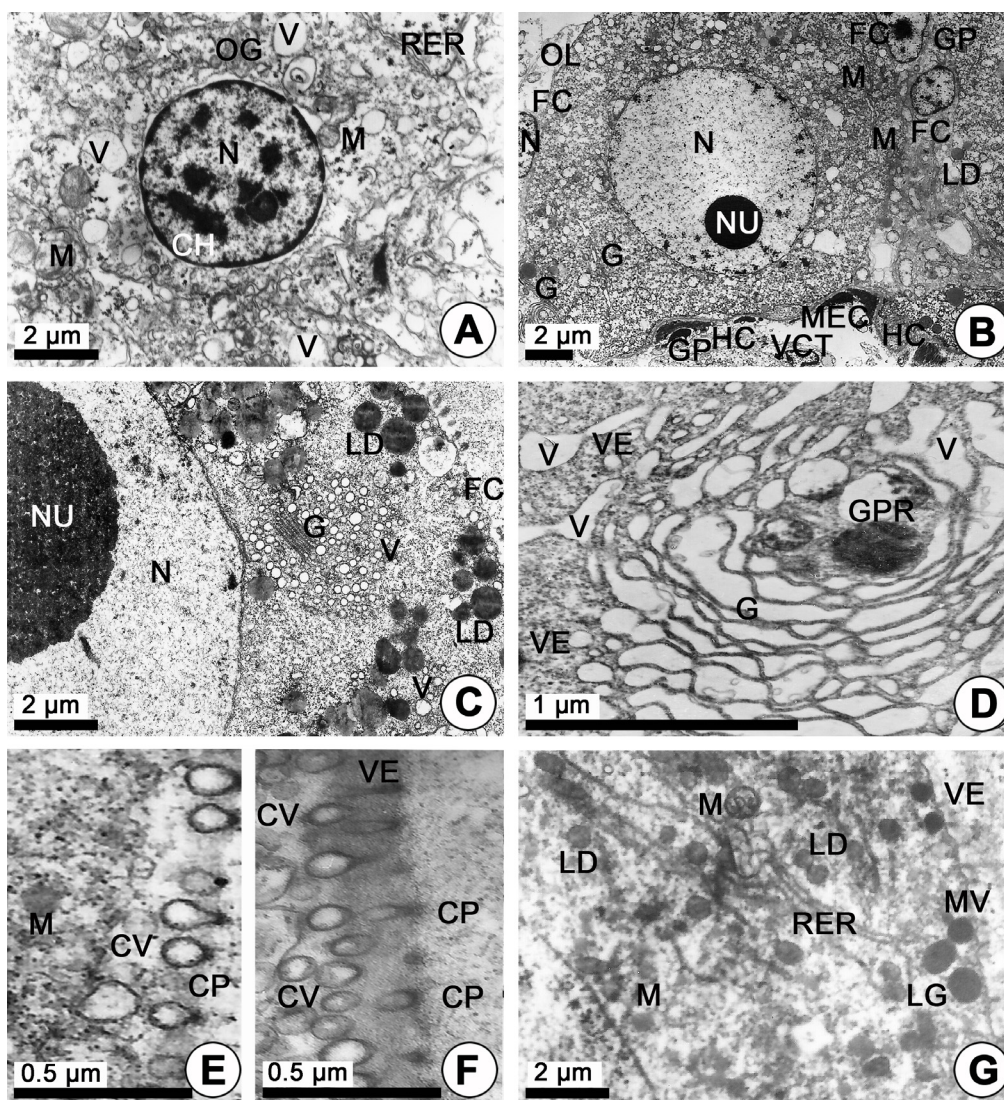
Based on ultrastructural observations, ovarian activity and morphological characteristics of germ cells during oogenesis can be classified into four phases (Eckelbarger & Davis, 1996): (1) oogonia, (2) previtellogenic oocytes, (3) vitellogenic oocytes, and (4) mature oocytes. Ultrastructural characteristics in each phase of the oocytes are as follow:

### 3. Oogonia

The oogonia (about 10-11  $\mu\text{m}$  in diameter) are characterized by a high nuclear-cytoplasmic ratio. They were either found individually or formed a cluster on the germinal epithelium. Each oogonium contained a large nucleus with chromatin materials, a few mitochondria, and the Golgi complex were present in the cytoplasm of oogonium. They were either found individually or formed a cluster in the follicle (Fig. 3A).

### 4. Previtellogenic oocytes

As the oogonium entered into the first prophase of



**Fig. 3.** Electron micrographs of oogenesis in female *Gomphina veneriformis*. **A**, An oogonium, with a large nucleus (N), several mitochondria (M) in the cytoplasm; **B**, A previtellogenic oocyte (PVO), with a nucleolus (NU) in a large nucleus (N), the mitochondria and rough endoplasmic reticulum (RER) and an attached follicle cell (FC) and large quantities of glycogen particles (GP); **C**, An early vitellogenic oocyte, with a nucleus and several lipid droplets (LD) and attached follicle cells (FC); **D**, An early vitellogenic oocyte, with the Golgi product (GPR) in the Golgi complex (G); vacuoles (V) and vesicles (VE); **E, F**, early vitellogenic oocytes, with occurrences of the coated vesicles (CV) through the coated endocytotic pits (CP) formed by endocytosis at the cortical region near the oolemma; **G**, An early vitellogenic oocyte, with rough endoplasmic reticulum, mitochondria and lipid droplets in the cytoplasm.

meiosis, they developed into previtellogenic oocytes. Previtellogenic oocytes (20-25  $\mu\text{m}$  in diameter) were small and oval in shape, having a large nucleolus in the nucleus (approximately 4-5  $\mu\text{m}$ ). With cytoplasmic growth, a number of mitochondria, the rough endoplasmic reticulum, and several vacuoles were

concentrated around the perinuclear region in the cytoplasm. The nuclei and cytoplasm of the previtellogenic oocytes increased in volume during the previtellogenic phase. The nucleus and oocyte diameters were 4-5  $\mu\text{m}$  and 15-25  $\mu\text{m}$ , respectively. At this time, follicle cells measuring 4-5  $\mu\text{m}$  in diameter

were attached to each of the previtellogenic oocytes. The follicle cells possessed a dense marginal chromatin in the nucleus, and contained rough endoplasmic reticulum, mitochondria, large quantities of glycogen particles and lipid droplets in the cytoplasm.

Thus, previtellogenic oocytes were distributed along the inner wall of each follicle, whereas the outer wall of the follicle is characterized by a discontinuous layer of thin squamous myoepithelial cells that forms a partial barrier between previtellogenic oocyte and the hemocoel (Fig. 3B). At this stage, more specifically, vesicular connective tissue cells (VCT cells), which are attached to the outer wall of the follicle appear near the previtellogenic oocyte. At this time, VCT cells contained large quantities of glycogen particles and a few lipid droplets in the cytoplasm (Fig. 3B).

### 5. Vitellogenic oocytes

As the further development of previtellogenic oocytes proceeded, the oocytes developed into early vitellogenic oocytes. Each early vitellogenic oocyte (about 25-35  $\mu\text{m}$ ) was partially surrounded by follicle cells that maintained intimate contact with its smooth oolemma. In the early vitellogenic oocytes, especially, with the initiation of yolk formation, several lipid droplets and mitochondria appeared in the cytoplasm (Fig. 3C). The Golgi products in the Golgi complex were present in the cytoplasm, with numerous vacuoles, and vesicles appearing near the Golgi complex (Fig. 3D). When the early vitellogenic oocytes began to form microvilli on the oolemma, the initial contours of the microvilli were oval or slightly elongated in shape. Several coated vesicles were present due to endocytosis, and appeared at the basal region of the oolemma of the oocyte. Uptake of nutritive material in the coated vesicle occurred through the formation of the coated pits on the oolemma during vitellogenesis (Figs. 3E, F). At the same stage, lipid droplets that were surrounded by mitochondria and well-developed endoplasmic reticulum appeared near the cortical layer (Fig. 3G). In the late vitellogenic oocytes (approximately 40-55  $\mu\text{m}$ ), cortical granules appeared at the cortical region.

In this phase, in particular, a number of proteinaceous yolk granules, lipid droplets, and mitochondria appeared in the cytoplasm, but follicle cells that were attached to the oocytes gradually lost their intimate association with the surface of the oocyte, therefore, the number of follicle cells gradually decreased. However, microvilli appeared along the vitelline envelope where the follicle cells had withdrawn (Fig. 4A). At the same stage, various sizes of yolk granules containing large quantities of glycogen particles were mixed with proteinaceous or lipid yolk granules near the nucleus (Fig. 4B).

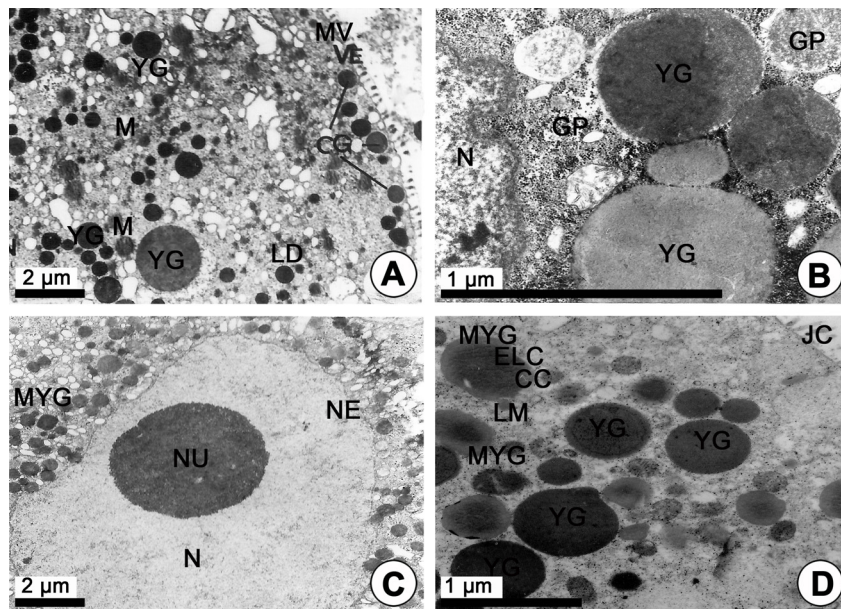
### 6. Mature oocytes

In the mature oocytes (approximately 60-70  $\mu\text{m}$ ), the thick vitelline envelope of the mature oocyte was slightly separated from the germinal epithelium. In the cytoplasm of the mature oocyte, small yolk granules containing several different components continuously mixed with each other and became larger, mature yolk granules (Fig. 4C). The mature yolk granules were composed of three components: (1) a crystalline core, (2) an electron-lucent cortex, and (3) a limiting membrane. The vitelline envelope of the mature oocyte was approximately 0.45  $\mu\text{m}$  thick and was surrounded by a jelly coat (Fig. 4D).

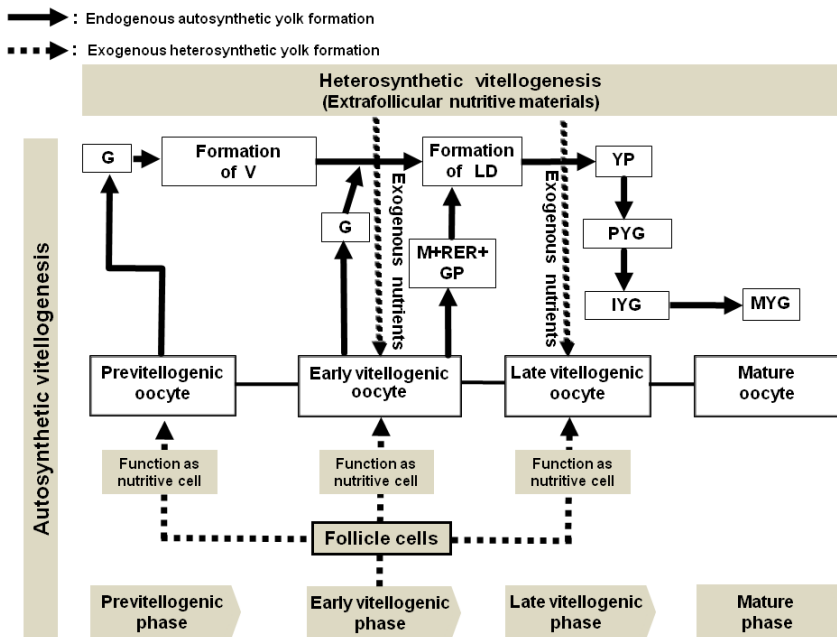
## Discussion

### 1. Vitellogenesis of the oocytes

As shown in Fig. 5, in previtellogenic oocyte, the Golgi complex forms vacuoles and vesicles. Especially, in the early vitellogenic oocyte, several coated vesicles are present due to endocytosis, that appear at the basal region of the oolemma of the oocyte. Uptake of nutritive material in the coated vesicle occurs through the formation of the coated pits on the oolemma during vitellogenesis. Although many authors suggested the formation of lipid droplets during vitellogenesis of oocytes in several species, no clear morphological evidence has yet been provided for the processes involved in lipid droplet formation (Pipe, 1987, Dorange and Le Pennec, 1989, Gaulejac *et al.*, 1995). In the present study, however, lipid droplets appeared among the Golgi complex,



**Fig 4.** Electron micrographs of oogenesis in female *Gomphina veneriformis*. **A**, An late vitellogenic oocyte, with the cortical granules (CG), a number of yolk granules (YG), lipid droplets (LD), and mitochondria (M); **B**, A late vitellogenic oocyte, with a number of yolk granules (YG) and large quantities of glycogen particles (GP) in the cytoplasm; **C**, a mature oocyte, with a number of mature yolk granules; **D**, A mature oocyte, with a number of mature yolk granules (MYG) being composed of three parts: (1) crystalline core (CC), (2) electron lucent cortex (ELC), and (3) a limiting membrane (LM).



**Fig. 5.** A schematic diagram of the processes of autosynthetic and heterosynthetic vitellogenesis during oogenesis in female *Gomphina veneriformis*. Abbreviations: G, Golgi complex; GP, glycogen particles; LD, lipid droplet; IYG, immature yolk granules; M, mitochondria; MYG, mature yolk granules; PYG, Proteinaceous yolk granules; RER, rough endoplasmic reticula; V, vitellogenesis; YP, yolk precursor.

well-developed endoplasmic reticulum, and mitochondria in the early vitellogenic oocytes of *G. veneriformis*. Therefore, it is assumed that they may be involved in the formation of lipid droplets (Chung *et al.*, 2002, 2005, 2006; Chung, 2007). As shown in Fig. 5, vitellogenesis showed a potential for autogenous and heterogenous yolk formation. The process of yolk formation by both endogenous autogenesis and exogenous heterogenesis of *G. veneriformis* was similar to those of *Crassostrea virginica* (Eckelbarger and Davis, 1996), *Mytilus edulis* (Pipe, 1987), *Patinopecten yessoensis* (Chung *et al.*, 2005), *Meretrix lusoria* (Chung, 2007), *Chlamys (Azumapecten) farreri farreri* (Chung, 2008) and *Sinonovacula constricta* (Chung *et al.*, 2008).

## 2. Relationship of the oocyte-follicle cell with the ovarian development

Jong-Brink *et al.* (1983) distinguished three categories of oocyte-follicle cell relationships according to the number and arrangement of the follicle cells.

In the first type, the oocyte is completely surrounded by an increasing number of follicle cells; in the second type, the oocyte is surrounded by a small, distinct number of follicle cells; in the third type, a small number of follicle cells surround the oocyte only during the early stages of oogenesis. In the present study, the oocyte of *G. veneriformis* surrounded by a small number of follicle cells during the early and late stages of oogenesis. As shown in Fig. 2, this species can be classified into the third type of oocyte-follicle cell relationship. In the early stage of oogenesis, the follicle cells appeared at the periphery of the follicle and subsequently migrated to the early developing germ cells. The cells initially appeared close to the previtellogenic oocyte, and then progressively surrounded the oocyte. At this stage, a small number of vacuoles and myelin-like organelles were visible in the cytoplasm of the follicle cells. The attached follicle cells showed progressive cytological modifications as their cytoplasmic volumes increased. In the present study, a few follicle cells attached to a previtellogenic oocyte appeared from the early stage of oogenesis. However, the number of follicle cells attaching to oocytes gradually decreased with gonadal

maturation (Fig. 2). The follicle cells attaching to the previtellogenic oocyte, early vitellogenic oocyte and late vitellogenic oocyte contained large quantities of glycogen particles and lipid droplets in the cytoplasm of the follicle cell. Therefore, it is assumed that follicle cells are involved in nutrient supply to the oocytes for vitellogenesis during oogenesis.

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