Animal Cells and Systems
Vol. 14, No. 4, December 2010, 343–349

Taylor & Francis
Taylor & Francis Group

Ultrastructural studies of vitellogenesis in oocytes and follicle cells during oogenesis in female *Protothaca* (*Notochione*) *jedoensis* (Bivalvia: Veneridae)

Hee-Woong Kang[a], Ki-Ho Choi[a]*, Je-Cheon Jun[b], Ki-Young Lee[c] and Kwan-Ha Park[d]

[a]*West Sea Fisheries Research Institute, NFRDI, Incheon 400-420, Korea;* [b]*Aquaculture Research Institute, NFRDI, Busan 619-705, Korea;* [c]*Department of Marine Biotechnology, Kunsan National University 573-701, Korea;* [d]*Department of Aquatic Life Medicine, Kunsan National University, Gunsan 573-701, Korea*

(*Received 25 April 2010; received in revised form 30 July 2010; accepted 7 September 2010*)

Ultrastructural studies of vitellogenesis in oocytes and follicle cells during oogenesis in female *Protothaca* (*Notochione*) *jedoensis* were investigated by histological and transmission electron microscope observations. In early vitellogenic oocytes, combined activities of the Golgi complex, mitochondria and rough endoplasmic reticulum in the cytoplasm are associated with autosynthetic vitellogenesis. Furthermore, at this time, many coated vesicles at the basal region of the oolemma of the oocyte lead to the formation of vesicles through endocytosis in the cytoplasm. Through the formation of the coated pits on oolemma during vitellogenesis, the uptake of extrafollicular precursors (nutritive materials) occurs in coated vesicles by endocytosis. Therefore, it is assumed that these exogenous materials are involved in heterosynthetic vitellogenesis. During late oogenesis, exogenous yolk precursors (yolk granules), lipid droplets and proteinaceous yolk granules are present in the cytoplasm of late vitellogenic oocytes. In mature oocytes, small yolk granules appear intermingled and form large mature yolk granules. Thus, two processes of vitellogenesis occur in oocytes by way of endogenous autosynthesis and exogenous heterosynthesis. The follicle cells attached to the oocytes appear to play an integral role in vitellogenesis in this study.

Keywords: *Protothaca (Notochione) jedoensis*; oogenesis; vitellogenesis; follicle cells

Introduction

The jedo venus clam, *Protothaca* (*Notochione*) *jedoensis*, is one of the commercially important edible bivalves in East Asian countries including Korea, China, and Japan (Kim et al. 2003; Min 2004). In Korea, this species is mainly found in silty sand at the subtidal zones in coastal waters of Boryeong, Choongcheongnam-do, Korea (Yoo 1976; Kwon et al. 1993). Because of past over-harvesting, it has been denoted as a fisheries resource that should be managed using a more reasonable fishing regimen. For basic studies of reproduction and propagation of a living natural resource, it is important that we understand its vitellogenesis with regard to germ cell differentiation during oogenesis, and the functions of follicle cells in female *P.* (*N.*) *jedoensis*.

To date, several studies have been carried out on *Protothaca* spp. in Korea and other countries, particularly on aspects of reproduction, including the reproductive cycle of *P. grata* (Pizarro and Cruz 1987), gametogenic development of *P. asperrina* (Ewart et al. 1988), spermatozoon ultrastructure of *P. pectorina* (Matos et al. 1997), and sexual maturation and the sex ratio of *P. jedoensis* (Kim et al. 2003). Although the reproductive ecology of this species has been studied, there are still gaps in our knowledge on reproductive biology. Little information is available on vitellogenesis in oocytes and the follicle cells during oogenesis, To date, there have been some studies on bivalve oogenesis in *Patinopecten yessoensis* (Chung et al. 2005), *Chlamys (Azumapecten) farreri farreri* (Chung 2008) and *Sinonovacula constricta* (Chung et al. 2008). However, ultrastructural study of oogenesis in the oocyte of this species was not carried out. Therefore, a study is needed on oogenesis in developing oocytes and follicle cells for the study of the reproductive mechanism in vitellogenesis.

To understand the reproductive mechanism in vitellogenesis, the processes of yolk formation by endogenous autosynthesis and exogenous heterosynthesis in *P.* (*N.*) *jedoensis* should be studied in detail. Such information will provide important knowledge of the reproductive mechanism in vitellogenesis of this species. Therefore, the present study aims to describe the ultrastructures of developing oocytes and the follicle cells associated with vitellogenesis during oogenesis in *P.* (*N.*) *jedoensis* using cytological methods.

*Corresponding author. Email: badaboy@nfrdi.go.kr

ISSN 1976-8354 print/ISSN 2151-2485 online

DOI: 10.1080/19768354.2010.525809
http://www.informaworld.com

Materials and methods

Sampling

Female specimens of *P.* (*N.*) *jedoensis* (Lischke) were collected monthly from the intertidal and subtidal zones of Simpo, Korea, from January to December, 2006. The clams were then transported to the laboratory where they were maintained in seawater at 20°C.

Photomicroscope observation

Tissue specimens for the optical microscope were prepared in Bouin fixative for 24 hours. Serial paraffin sections of thickness 5–8 μm were prepared. Double staining was performed with Hansen's haematoxylin and 0.5% eosin. Connective tissue and a muscular tissue were divided by Mallory's method. The form and size of the germ cell were examined and observed under the light microscope.

Transmission electron microscope observation

For production of tissue specimens for TEM, fixation was performed in 2.5% glutaraldehyde–2% paraformaldehyde (0.1 M cacodylate buffer, pH 7.5) for 2 h. A full rinsing was carried out by three repetitions at around 30 minutes interval with 0.1 M cacodylate buffer (pH 7.5), and fixation was done in 2% osmium tetroxide (0.2 M cacodylate buffer, 7.5) for 90 minutes. Tissue fragments after fixation were dried via ethanol, transposed with propylene oxide, and embedded in Epon-812 mixed solution. Ultrathin sections were produced by a Sorvall MT-2 ultramicrotome. Electron staining of ultrathin sections was done with uranyl acetate and lead citrate. Ultrathin sections were observed via a JEM 100 CX-2 electron microscope (100 kV).

Results

Position and morphology of the ovary

The general ovary morphology of *P.* (*N.*) *jedoensis* is similar to that of other bivalves (Chung et al. 2005, 2008; Chung 2007, 2008). The ovary is irregularly arranged from the subregions of the mid-intestinal glands in the visceral cavity to reticular connective tissues of the foot. The ovary is a diffused organ consisting of branching follicles contained differentiating oocytes in a variety of stages. Oogonia and various oocytes are distributed in a centripetal pattern from the follicular wall to the lumen. Some follicle cells, which are attached to each oocyte in the different stages, are found in the follicular walls (Figure 1). As ovarian maturation progresses, the sexes of the clams can be easily distinguished macroscopically; the mature ovary is yellow brown in color.

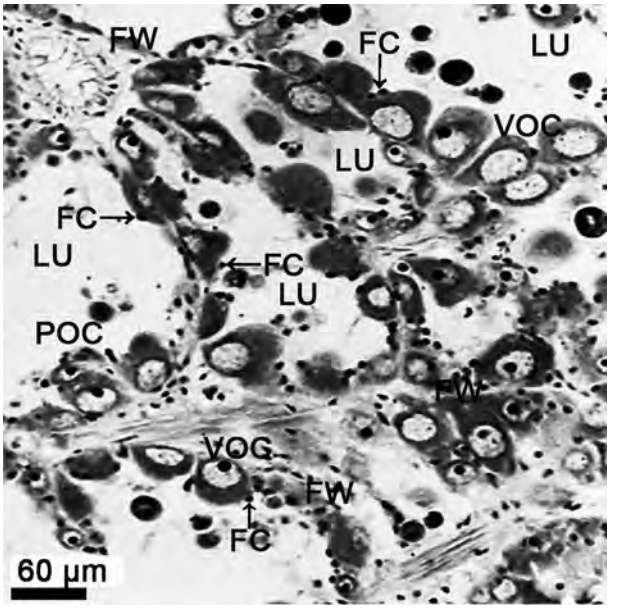

Figure 1. A photomicrograph of the ovarian structure in the early active stage in female *Protothaca* (*Notochione*) *jedoensis*. Note a number of previtellogenic oocytes (POC) and early vitellogenic oocytes (VOC) containing a few follicle cells (FC, arrow) in the lumina (LU) of the oogenic follicles with follicular walls (FW).

Ultrastructure of germ cells during oogenesis

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

Oogonia

The oogonia are about 10 μm in diameter and characterized by a high nuclear-cytoplasmic ratio. They were either found individually or formed a cluster on the follicular wall. Each oogonium contains a large nucleus with chromatin and several mitochondria and vacuoles are present in the cytoplasm. They are either found individually or formed a cluster in the oogenic follicle (Figure 2).

Previtellogenic oocytes

Oogonia develop into previtellogenic oocytes by the first prophase of meiosis. Previtellogenic oocytes are small and oval in shape, containing a large nucleolus in the nucleus. A number of mitochondria, rough endoplasmic reticula, and several vacuoles appear in the

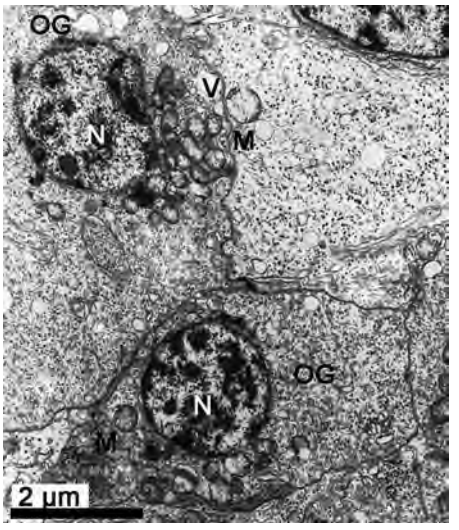

Figure 2. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. Oogonia (OG). Note a large nucleus (N), and several mitochondria (M) and vacuoles in the cytoplasm.

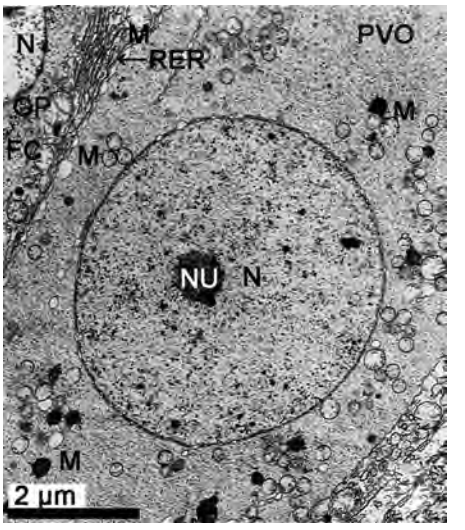

Figure 3. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. A previtellogenic oocyte (PVO) and a follicle cell (FC). Note a nucleolus (NU) in a large nucleus (N), and mitochondria in the cytoplasm of the previtellogenic oocyte (PVO), and well-developed rough endoplasmic reticula (RER, arrow) and glycogen particles (GP) in the cytoplasm of the follicle cell (FC) attached to the oocyte.

cytoplasm of the previtellogenic oocyte. The nuclei and oocytes are 4–5 μm and 20–27 μm in diameter, respectively. The nucleus contains a nucleolus and low electron dense chromatin, and the Golgi complex, mitochondria and a number of vacuoles appear in the cytoplasm (Figures 3 and 4). At this time, follicle cells measuring about 5 μm in diameter are attached to each of the previtellogenic oocytes, possessing a dense marginal chromatin in the nucleus, and contain a number of rough endoplasmic reticula, mitochondria and glycogen particles in the cytoplasm (Figure 3). The Golgi products appear among the vacuoles and vesicles which are formed by the Golgi complex in the cytoplasm (Figure 5).

Vitellogenic oocytes

With cytoplasmic growth, previtellogenic oocytes develop into vitellogenic oocytes. As a matter of convenience, the vitellogenic oocyte can be divided into two vitellogenic oocytes: early and late vitellogenic oocytes. With the initiation of yolk formation, lipid droplets that are surrounded by mitochondria and a number of well-developed endoplasmic reticula appear near the cortical layer in early vitellogenic oocytes (Figure 6). When the early vitellogenic oocytes begin to form microvilli on the oolema, the initial contours of the microvilli on the oolema are oval or slightly elongated in shape. Several coated vesicles are present by way of endocytosis, and they appear at the basal region of the oolemma of oocytes. The uptake of nutritive materials in the coated vesicle occurs through the formation of coated pits on the oolemma during vitellogenesis (Figure 7). In late vitellogenic oocytes, a number of lipid droplets and glycogen particles appear at the perinuclear region (Figure 8). At this time, yolk precursors, a number of proteinaceous yolk granules, lipid droplets and mitochondria appear in the cytoplasm of the oocyte, but follicle cells that are attached to the oocytes gradually lose their intimate association with the surface of the

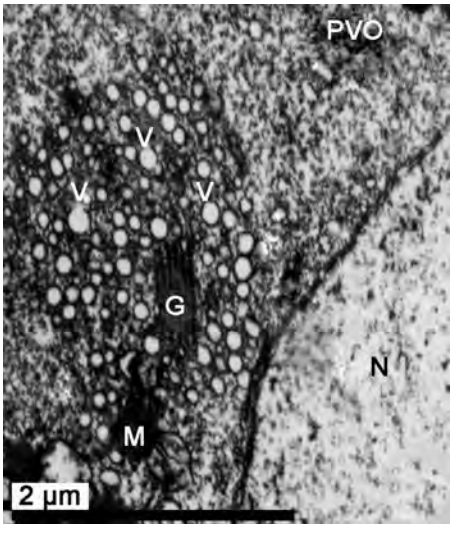

Figure 4. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. An early vitellogenic oocyte (EVO). Note the early vitellogenic oocyte (EVO) containing a large nucleus (N), and several mitochondria (M) and a number of vacuoles (V) in the cytoplasm.

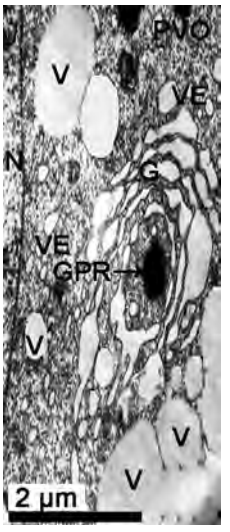

Figure 5. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. An early vitellogenic oocyte (EVO). Note the Golgi product (GPR, arrow) near the vacuoles (V) and vesicles (VE) which is formed by the Golgi complex (G).

oocyte. In particular, several lipid droplets, myelin-like organelles and vacuoles are present in the follicle cells. Thereafter, microvilli appear along with the vitelline envelope where the follicle cells have withdrawn (Figure 9), while various sizes of proteinaceous yolk granules combine and become larger yolk granules in the cytoplasm of the oocyte (Figure 10).

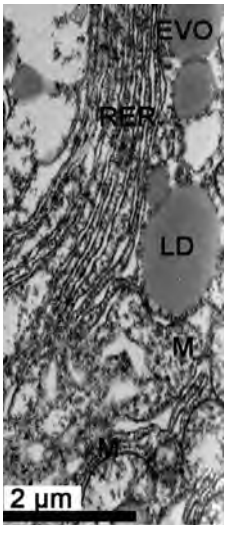

Figure 6. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. An early vitellogenic oocyte (EVO). Note lipid droplets (LD) between well-developed rough endoplasmic reticula (RER) and mitochondria (M) in the cytoplasm of an early vitellogenic oocyte (EVO).

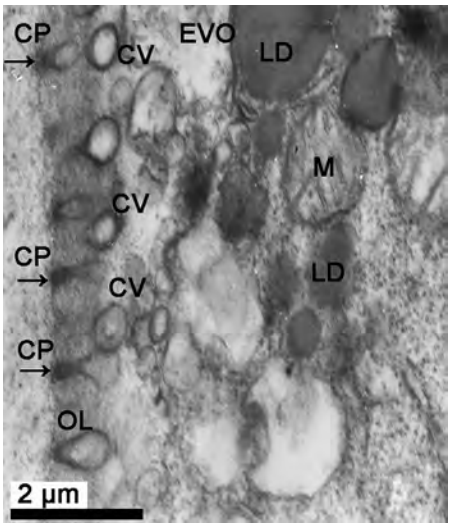

Figure 7. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. An early vitellogenic oocyte (EVO). Note the coated vesicles (CV) occurring through the coated endocytotic pits (CP, arrow) formed by endocytosis at the cortical region near the oolemma (OL).

Mature oocytes

In the cytoplasm of the mature oocytes (about 60–70 μm in diameter), small yolk granules continuously mix with each other and become larger mature yolk granules. A mature yolk granule is composed of three components; (1) a crystalline core, (2) an electron-lucent cortex, and (3) a limiting membrane (Figure 11).

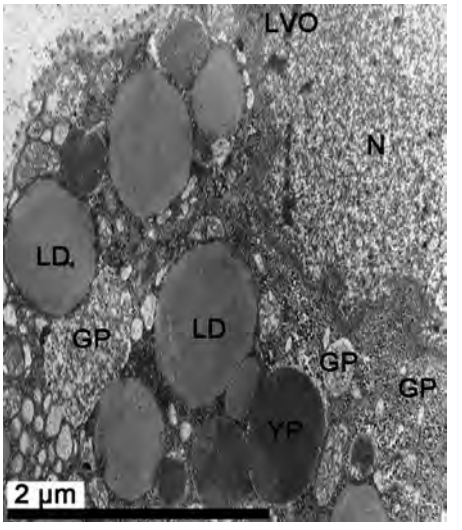

Figure 8. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. A late vitellogenic oocyte (LVO). Note a number of lipid droplets (LD) and glycogen particles (GP) in the cytoplasm near the nucleus (N).

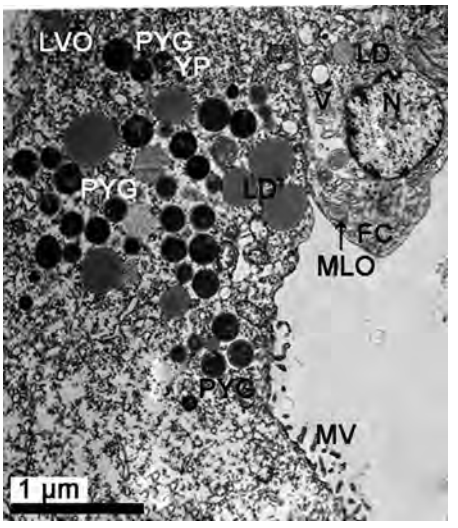

Figure 9. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. A late vitellogenic oocyte (LVO) and follicle cells (FC). Note a number of proteinaceous yolk granules (PYG), lipid droplets (LD), microvilli on the vitellogenic envelope of the oocyte, and follicle cells (FC) containing lipid droplets (LD) and myelin-like organelles (MLO, arrow) in the cytoplasm.

Residual oocytes

After spawning, undischarged oocytes are degenerated and reabsorbed. In particular, degenerating yolk granules, which are combined with myelline-like organelles which are formed by various lysomes, appear in the cytoplasm of degenerating oocytes (Figure 12).

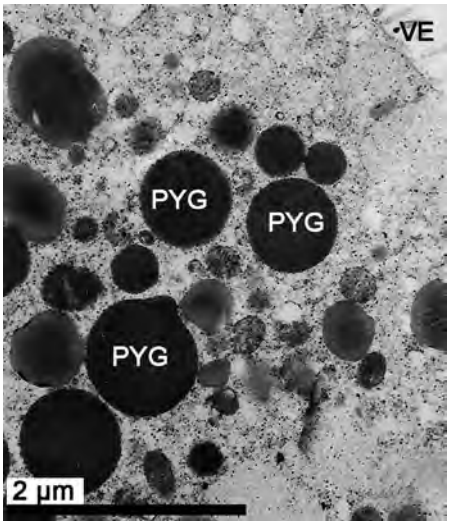

Figure 10. Electron micrograph of oogenesis in female *Protothaca* (*Notochione*) *jedoensis*. A late vitellogenic oocyte (LVO). Note proteinaceous yolk granules (PYG) in the cytoplasm of the oocyte.

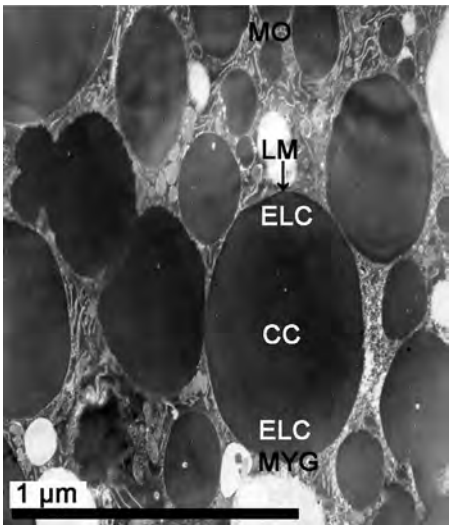

Figure 11. Electron micrograph of a mature oocyte and a degenerating oocyte in female *Protothaca* (*Notochione*) *jedoensis*. (11) Mature yolk granules in a mature oocyte. Note a number of mature yolk granules (MYG) being composed of three parts: crystalline core (CC), electron lucient cortex (ELC), and a limiting membrane (LM, arrow).

Discussion

Vitellogenesis in oocytes

In this study, as shown in Figure 13, the Golgi complex, which is composed of Golgi sacs, Golgi vacuoles and Golgi vesicles, formed various-sized vacuoles and vesicles in the cytoplasm of the previtellogenic oocyte. In early vitellogenic oocytes, a number of lipid droplets

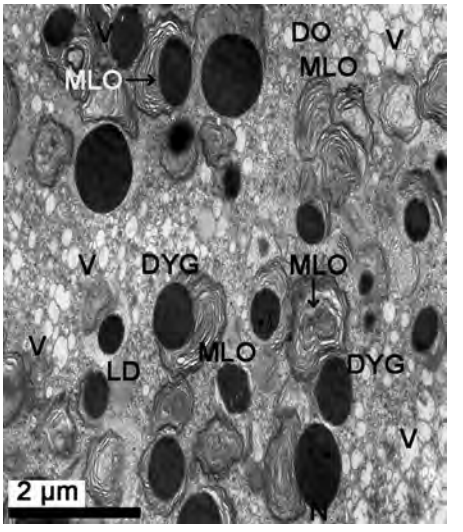

Figure 12. Electron micrograph of a mature oocyte and a degenerating oocyte in female *Protothaca* (*Notochione*) *jedoensis*. The degenerating oocytes (DO). Note a number of degenerating yolk granules (DYG) surrounded with myelin-like organelles (MLO, arrow) in the cytoplasm of the degenerating oocyte (DO).

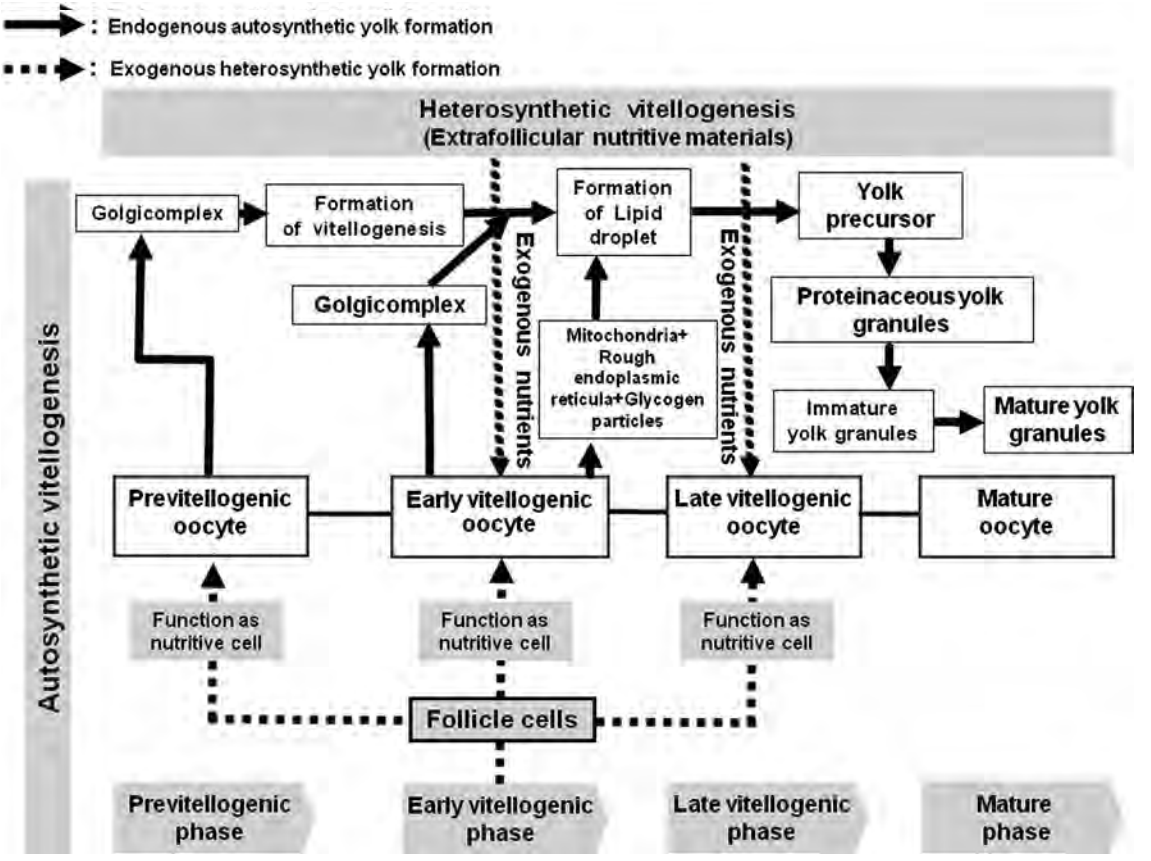

Figure 13. A schematic diagram of the processes of autosynthetic and heterosynthetic vitellogenesis during oogensis in female *Protothaca* (*Notochione*) *jedoensis*.

(regarded as materials for endogeneous autosynthetic yolk formation) appeared near the Golgi complex, a number of mitochondria and endoplasmic reticula. Therefore, it is assumed that the various cell organelles mentioned above in this species are involved in endogeneous autosynthetic yolk formation (Pipe 1987; Gaulejac et al. 1995; Chung et al. 2005). At this developmental stage, several coated vesicles appeared through endocytosis, and were present at the basal region of the oolemma in early vitellogenic oocytes. In particular, the uptake of nutritive materials (extrafollicular nutritive materials) in coated vesicles occurred through the formation of coated pits on the oolemma during vitellogenesis (Chung 2007). In this study, heterosynthesis occurred through the incorporation of extrafollicular precursors into oocytes by endocytosis prior to the formation of the vitelline envelope. From these findings, it is assumed that vitellogenesis in *P.* (*N.*) *jedoensis* occurs through the processes of endogenous autosynthetic and exogenous heterosynthetic yolk formations (Figure 13). Thus, the processes of yolk formation by endogenous autosynthesis and exogenous heterosynthesis in *P.* (*N.*) *jedoensis* are similar to those of *C. virginica* (Eckelbarger and Davis 1996), *M. edulis* (Pipe 1987) and *Sinonovacula constricta* (Chung et al. 2008).

Functions of follicle cells attached to oocytes

Regarding the number of follicle cells attached to an oocyte, Jong-Brink et al. (1983) reported that oocyte-follicle cell relationships can be divided into three types according to the number and arrangement of 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 this study, as shown in Figure 1, *P.* (*N.*) *jedoensis* oocytes were surrounded by a small number of follicle cells during early and late oogenesis. Accordingly, this species can be classified into the third type of oocyte-follicle cell relationship.

In many bivalves, several studies reported that the follicle cells appeared at the periphery of the follicle and subsequently migrated to the early and late vitellogenic oocytes (Pipe 1987; Gaulejac et al. 1995;

Eckelbarger and Davis 1996). Usually, these cells initially appeared close to previtellogenic oocytes, and then progressively attached to them. Glycogen particles, a small number of vacuoles, and lipid droplets are visible in the cytoplasm of follicle cells. In this study, some of the general phenomena in follicle cells attached to previtellogenic oocytes appeared from the early stage of development. At the adherence zone of follicle cells and vitellogenic oocytes, lipid droplets, myelin-like organelles (or myelin figure) and vacuoles appeared in the cytoplasm of late vitellogenic oocytes. Therefore, it is assumed that follicle cells function as nutritive cells for oocyte development, which is associated with heterosynthetic vitellogenesis (Figure 13).

Fates of the gametes

If the reproductive energy allocated to the production of gametes is too great, 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). In this study, after spawning, degenerating yolk granules in undischarged oocytes, which are combined with myelline-like organelles which are formed by various lysomes, appeared in the cytoplasm of degenerating oocytes. Consequently, degenerating yolk granules were degenerated and reabsorbed as found in other mollusks (Morvan and Ansell 1988; Dorange and Le Pennec 1989; Chung et al. 2005).

Acknowledgements

The authors are grateful to Dr William Heard of Florida State University and the referees for helpful comments and corrections to the manuscript. We also thank the English editor, Mrs Armagan Sabetian of Neo HARRISCO, for proofreading and corrections of the manuscript. This research was supported in part by funding from Research Projects (2009) of the Coastal Research Center, Kunsan National University and Research Projects (2008–2009) of species preservation, West Sea Fisheries Research Institute, NFRDI.

References

Chung EY. 2007. Oogenesis and sexual maturation in *Meretrix lusoria* (Röding, 1798) (Bivalvia: Veneridae) in western Korea. J Shellfish Res. 26:71–80.

Chung EY. 2008. Ultrastructural sdudies of oogenesis and sexual maturation in female *Chlamys* (*azumapecten*) *farreri farreri* (Jones & Preston, 1904) (Pteriomorphia: Pectinidae) on the Korea. Malacologia. 50:279–292.

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. J Shellfish Res. 24:913–921.

Chung EY, Ko CH, Kang HW, Choi KH, Jun JC. 2008. Ultrastucture of oocyte during oogenesis and oocyte degeneration associated with follicle cells in female *Sinonovacula constricta* (Bivalvia: Pharidae) in western Korea. Animal Cells and Syst. 12:313–319.

Dorange G, Le Pennec M. 1989. Ultrastructural study of oogenesis and oocytic degeneration in *Pecten maximus* from the Bay of St. Brieuc. Mar Biol. 103:339–348.

Eckelbarger KJ, Davis CV. 1996. Utrastructure of the gonad and gametogenesis in the eastern oyster, *Crassostrea virginica*. 1. Ovary and oogenesis. Mar Biol. 127:79–87.

Ewart JW, Carriker MR, Villalaz JR, Gomez JA, D'Crez L. 1988. Gametogenic development of the venerid clam *Protothaca asperrima* in the Bay of Panama. J Shellfish Res. 7:118–126.

Gaulejac DB, Henry M, Vicente N. 1995. An ultrastructural study of the marine bivalve *Pinna nobilis* (Linnaeus, 1758). 1. Oogenesis. J Molluscan Stud. 6:375–392.

Jong-Brink de M, Boer HH, Joosse J. 1983. Mollusca. In: Adiyodi KG, Adiyodi RE, editors. Oogenesis, oviposition, and oosorption, vol. 1. New York: John Wiley. p. 297–355.

Kim JH, Chung *EY*, Kim YH. 2003. Sexual maturation and the sex ratio of the jedo venus, *Protothaca jedoensis* (Bivalvia: Veneridae). Kor J Malacol. 19:9–17.

Kwon OK, Park GM, Lee JS. 1993. Coloured shells of Korea. Seoul: Academy Publishing Co. 288 pp.

Matos E, Matos P, Casal G, Azev C. 1997. Ultrastructure of the spermatozoon of *Protothaca pectorina* Lamarck (Mollusca: Bivalvia) of the north littoral of Brazil. Rev Brazil Zool. 14:779–783.

Min DK. 2004. Mollusks in Korea (revised supplementary edition). Busan: Hanguel Graphics. 566 pp.

Morvan C, Ansell AD. 1988. Stereological methods applied to the reproductive cycle of *Tapes rhomboids*. Mar Biol. 97:355–364.

Pipe RK. 1987. Oogenesis in the marine mussel *Mytilus edulis*: an ultrastructural study. Mar Biol. 95:405–414.

Pizarro JF, Cruz RA. 1987. Reproductive cycle of the clam *Protothaca grata* (Pelecypoda: Veneridae). Brenesia San Jose. 27:23–24.

Yoo JS. 1976. Korean shells in colour. Seoul: Ilgisa. 196 pp.