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## Ultrastructural Study of Oogenesis of the Female Razor Clam *Solen grandis* on the West Coast of Korea

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### **Introduction**

The razor clam, *Solen grandis* Dunker is present along the coasts of Korea, China and Japan. In particular, it is abundant in the intertidal area of the south and west coasts of Korea where tidal flats are well developed. In Korea, the razor clam is one of the most important marine resources for human consumption. Recently, due to reclamation of tidal areas along the west coast, marine pollution of tidal areas along the west coast, and reckless overharvesting of this clam, its standing stock has reduced for a decade. Therefore, it is necessary to manage the population of the clam with a proper fishing regime that will maintain an optimal population size in aqua farm. So far, regarding reproductive ecology of the razor clam in Korea and Japan, there have been several studies on reproductive cycle (Chung et al., 1986; Chung and Kim, 1989; Chung and Park, 1998.), etc. However, little information is available on vitellogenesis during oogenesis. The purpose of the present study is to understand vitellogenesis during the oogenesis.

### **Materials and Methods**

Specimens of the razor clam, *Soeln grandis*, were collected monthly from the intertidal zone of Yoobudo, west coast of Korea from January to December, 2001 (Fig. 1) A total of 48 clams from 98.4 to 114.6 mm in shell length were used for the present study

For electron microscopic observations, excised pieces of 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 1 % osmium tetroxide solution in 0.2 M phosphate buffer solution (pH 7.4) for 1 hour at 4°C. Specimens then were 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 a LKB ultramicrotome at a thickness

of about 800-1000 Å. Tissue sections were mounted on collodion-coated copper grids doubly stained with uranyl acetate followed by lead citrate, and observed with a JEM 100CX-2 (80 kv) electron microscope.

### **Results and Discussion**

Oogenesis occurs in the oogenic follicles of the ovary and can be divided into five successive phases: (1) oogonium, (2) previtellogenic oocyte, (3) vitellogenic oocyte, and (4) mature oocyte phases.

**Oogonial phase:** The stem cells, which constituted the boundaries of the follicles, gave rise to oogonia, characterized by a high nuclear-cytoplasmic ratio. Oogonia are small, oval in shape, and 10-11µm in diameter and contained a large nucleus with chromatin nucleolus, and have several small mitochondria and endoplasmic reticulum in the cytoplasm.

**Previtellogenic phase:** The oogonium differentiated into the previtellogenic oocyte with a remarkable nucleolus in the nucleus. At the previtellogenic oocyte, the nucleus and cytoplasm increased in volume, and several small mitochondria and vacuoles were present in the perinuclear region of the cytoplasm, while the microvilli were not present on the vitelline envelope of the oocyte.

**Vitellogenic phase:** In the early vitellogenic oocyte, the well-developed Golgi apparatus and mitochondria were present near the nucleus, and numerous small vesicles and large vacuoles were scattered from the perinuclear region to the vitelline envelope of the oocyte. Numerous lipid droplets were present in the vacuoles and vesicles, which were formed by the Golgi apparatus near the nuclear envelope and were dispersed toward the cortical layer near the vitelline envelope. At this stage, round or oval microvilli on the vitelline envelope begin to appear. With the initiation of the formation of lipid granules and proteid yolk formation (vitellogenesis), small vesicles and lipid granules and lipid granules and mitochondria were located around the cortical layer. They were mixed each other and became larger ones around the nuclear envelope, and dispersed toward the cortical layer. In the late vitellogenic oocyte, an accumulation of cortical granules occurred in the cortical layer autotynthetically, and proteid substances were present near the annulus lamellae and several well-developed rough endoplasmic reticula. proteid yolk granules, which formed by the cortical granules and lipid granules in the cytoplasm, were dispersed from the cortical layer to perinuclear cytoplasm. At this time, an amphinucleolus in the nucleus appeared, especially, exogenous lipid granular substances and lots of glycogen particles in the germinal epithelium passed into the ooplasm of the oocyte through the microvilli of the vitelline envelope.

**Mature phase:** in the mature oocyte, mature proteid yolk globules appeared in the cytoplasm at the same time many small yolk globules were fused to each other, and became larger mature yolk globules in size. The mature yolk globule in a mature oocyte was composed of three parts: 1) main body, 2) superficial layer, and 3) limiting membrane.

At this stage, the type of the microvilli, some of which bifurcate, protruded and extended just beyond the outer border of the vitelline envelope. The thick vitelline envelope of the mature oocyte was covered with relatively thick jelly coat.

#### References

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