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Follicular Layer of Oocytes of *Micropercops swinhonis* (Pisces: Perciformes)

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In the goby *Micropercops swinhonis*, the follicular layer of full-grown oocytes consists of an outer layer (theca cell) and an inner layer (granulosa cell). As the oocyte grows, columnar cells of inner granulosa layer secrete mucin to their cytoplasm and then surround the oocyte. Such granulosa cells appear to be cuboidal cells in the early vitellogenesis, yolk vesicle stage, to be replaced by columnar cell secreting mucins (adhesive materials) in the middle vitellogenesis, yolk granule stage. The enveloping layer of the oocyte has a muco-follicle layer filled with mucins. The mucins are an amorphous and electron-dense substance. Interestingly, the oocyte enveloping layer becomes thickened towards the animal pole as vitelogenesis proceeds. A zona radiata of about 7.8~11.5 μm thick is present below the muco-follicle layer. The zona radiata is composed of an one-layered electron-dense externa and a three to five-layered electron-less interna.

Key words : *Micropercops swinhonis*, oocyte envelope, enveloping layer, zona radiata

Introduction

Oviparous bony fishes lay eggs that are heavier than water (demersal eggs), or buoyant (pelagic eggs). Most stream fishes are demersal eggs, which may be adhesive (at least temporarily adhesive), and deposited in clumps, or they may be attached to some substrate. In many fishes taxa the functions of the oocyte envelopes were closely related to eggs properties: the attachment of the eggs to the substratum in stream bed, and sticks or debris; the retention of water or pressure; the protection of embryo; the chorionic respiratory system; the process of water hardening (Blaxter, 1969; Wourms, 1976; Laale, 1980; Groot and Alderdice, 1985; Riehl and Greven, 1990; Erickson and Pikitch, 1993: Thiaw and Mattei, 1996; Riehl and Patzner, 1998). In addition, the development and the structure of the oocyte envelopes has been used for the systematic purposes and the identification of eggs, or the evaluation of environmental factors that determine their habitat and spawning characteristics (Laale, 1980; Groot and Alderdice, 1985; Riehl and Greven, 1993; Britz *et al.*, 1995; Thiaw and Mattei, 1996; Park and Kim, 1997, 2001a,b).

The goby *Micropercops swinhonis* is a small freshwater fish belonging to the family Odontobutidae. The primary habitat of *M. swinhonis* appears to be weedy patches in slowly flowing bodies of water, and their fertilized eggs are attached to the plant stem or the surface of pebbles (Kim and Kim, 1996; Kim, 1997). Park *et al.* (1998) reported the development of the oocyte and its enveloping layers in *M. swinhonis* and that the eggs are sticky and sink (demersal), and the outer surface of the oocyte has bullet-shaped neutral mucin deposits. However, there was no information on fine structures and development of oocyte envelope (zona radiata) covering the oocyte in this species.

Therefore, the purpose of the present study is to describe the developmental features and the

fine structures of the oocyte envelopes, and the relationship between spawning substrates and the egg envelopes in *M. swinhonis*.

Materials and Methods

Females of *M. swinhonis* were collected from Go-san stream at Samrye-eub, Chollabuk-do in Korea during the spawning season, the late April to the late May, 2001. Ovaries holding immature and mature eggs were dissected from the abdomen.

Tissues for light microscopy were fixed in 10% neutral buffered formalin. These were dehydrated through a standard ethanol series to 100%, cleared in chloroform and then embedded in wax (Paraplast, Oxford). Blocks were sectioned at 5 μm. Sections were deparaffinized and stained using standard techniques. Stains used to described the adhesive layers were as follows: Harris haematoxylin and eosin (general morphology), alcian blue-periodic acid Schiff's (AB-PAS, acid and neutral mucins)

For the transmission electron microscopy (TEM), adult gravid females were anaesthetized with MS222. Their ovaries were excised and prefixed in 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.2. Postfixation was performed in 1% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series, specimens were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed with JEOL-1200EX transmission electron microscope.

Results

1. General morphology and developement of oocyte envelope

In *M. swinhonis* the oocyte envelope becomes developed from perinucleolus stage, which the oocyte has nucleoli located at the periphery of the germinal vesicle (large nucleus) and is surrounded by a thin, single layer of squamous epithelial cell (Plate 1A and B). As oocyte grows, the oocyte increases in size and is accumulated yolk materials (vitellogenesis stage). At the beginning of the vitellogenesis, early yolk vesicle stage, the follicular layer become bilaminar by the addition of a single cuboidal cell layer (inner follicular layer) immediately below the outer squamous layer (outer follicular layer) (Plate 1A and C). By this time, a zona radiata is formed between the inner follicular layer and ooplasm. As the oocyte grow, the yolk vesicles increase in size and number, and move to the periphery of the oocyte (late yolk vesicle stage) (Plate 1A and C). At this stage the zona radiata is divided into two distinct layers; an outer thin zone, 0.5 μm, staining strongly and an inner thicker and paler zone which stained weakly in AB-PAS reaction. The zona radiata shows the striated appearance.

As vitellogenesis proceeds, the cytoplasm of the oocyte becomes occupied by many dense yolk granules which are a limiting membrane (early yolk granule stage) (Plate 1A and C). During later yolk granule stage, the yolk granules fuse with each other to forms several yolk masses (late yolk granule stage) (Plate 1A and C). At early yolk granule stage the follicular bilayer, an outer squamous cell layer and an inner cuboidal cell layer, increase in height (Plate 1D). Some of inner cuboidal cells become columnar cell (Plate 1C and D). A few of columnar cells have AB-PAS positive mucins suggesting neutral mucins in their cytoplasm (Plate 1D). As oocyte grows, the follicular cells and the zona radiata increase greatly in size, thickness, height, or number. By this time, towards the animal pole, the oocyte becomes larger and thicker, and then its the inner follicular layer is becoming thicker than opposition part, vegetable pole (Plate 1G). At later yolk granule stage, most cuboidal cells of the inner follicular layer are replaced by columnar cells filled with mucins (Plate 1D to G). The inner follicular layer became thicker. By the end of this stage, the cytoplasmic mucins of the columnar cells coalesce into basally located bullet-shaped structures (Plate 1F). These structures were strongly positive to AB-PAS. Subsequentially the columnar cells lost their cellular integrity and remained as bullet-shaped structures. In the animal pole, the bullet-shaped structures disappear and are replaced by mucins (Plate 1G).

2. Fine structure of the oocyte envelope

In *M. swinhonis*, the full-grown oocyte consists of the outer and inner follicular layer, oocyte envelope (zona radiata) and the yolk materials. The follicular layer consists of theca cells, the outer layer, and granulosa cells, the inner layer, which are separated by the basement membrane (Plate

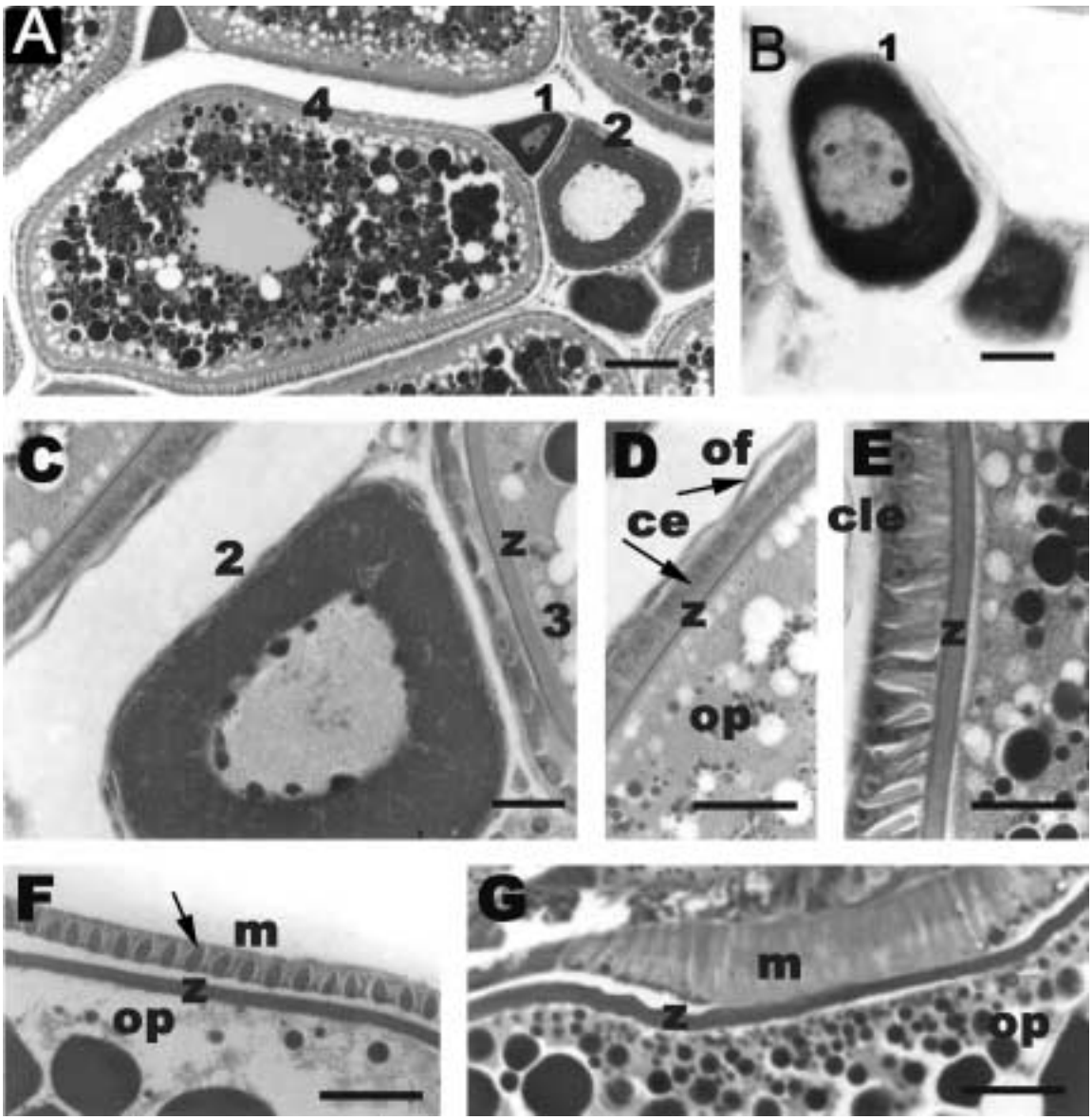

Plate 1. A schematic diagram of light microscopy of oocytes in *Micropercops swinhonis*.
A, Developmental stages of oocytes (Bar = 25 μm). Harris hematoxylin and eosin; B, perinucleolus stage. Harris hematoxylin and eosin (Bar = 10 μm); C, Early yolk vesicle stage. There is early yolk granule stage having cuboidal granulosa cells. Harris hematoxylin and eosin (Bar = 10 μm); D, Early yolk granule stage. The enveloping layer consists of an outer follicular layer, an inner follicular layer (cuboidal cell) and a two-layered zona radiata. Harris hematoxylin and eosin (Bar = 5 μm); E, Late yolk granule stage. Early cuboidal cells are replacey by columnar cell. Harris hematoxylin and eosin (Bar = 14 μm); F, Late yolk granue stage. The columnar cells secret mucins in their cytoplasms and the mucin deposits are bullet-shaped. Harris hematoxylin and eosin (Bar = 30 μm); G, The inner follicular layer are filled with mucin deposits. Harris hematoxylin and eosin (Bar = 30 μm).
Abbreviations: 1, perinucleolus stage; 2, early yolk vesicle stage; 3, late yolk vesicle stage; 4 yolk granule stage; ce, cuboidal cell; cle, columnar cell; m, mucus; of, outer follicular cell; op, ooplasm; z, zona radiata.

2A). Capillaries develop in the theca connective tissue (Plate 2A).

The granulosa cells of the inner layer display intense secretory activity resulting in the development of adhesive layer that fills the cytoplasm (Plate 2A to C). Fragments of cytoplasm containing rough endoplasmic reticulum, smooth endoplasmic reticulum, Golgi complex, and mitochondria are present. The adhesive layers are an amorphous and electron-dense substance (Plate

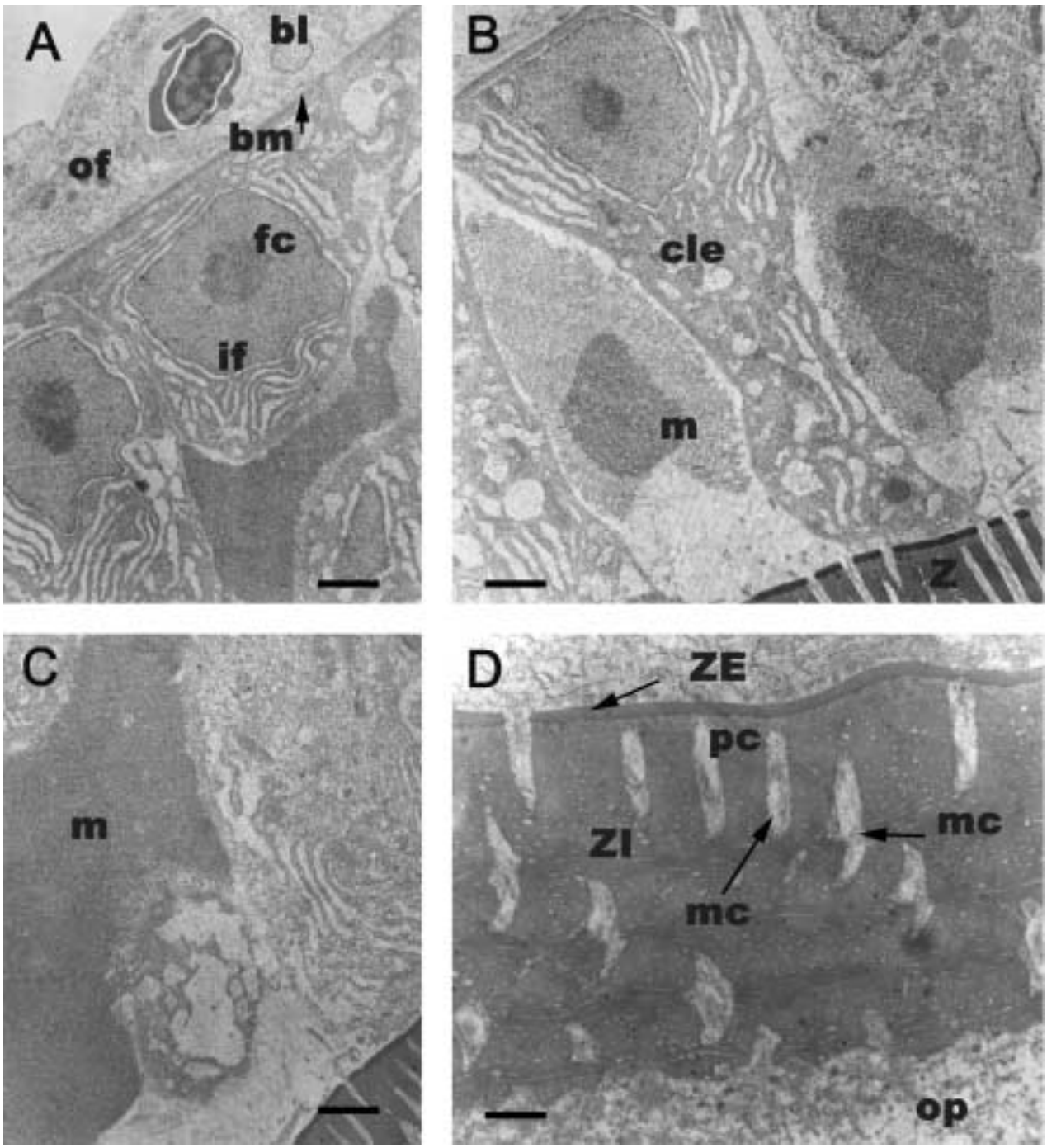

Plate 2. A schematic diagram of transmission electron microscopy of oocytes in *Micropercops swinhonis*.
A, The follicular layer divide into an inner and outer layer by the basement membrane (Bar = 4 μm); B, Early yolk granule stage. Columnar cells secret small amount of mucus to their cytoplasm (Bar = 4 μm); C, In late yolk granule stage, columnar cells become large and most of their cytoplasm are filled with mucin (Bar = 4 μm); D, A zona radiata consists of externa and interna (Bar = 1.3 μm). **Abbreviations**: bm, basement layer, cle, columnar cell, fc, follicular cell; if, inner follicular layer; m, mucus; mc, microvillus; of, outer follicular layer; op, ooplasm; pc, pore canal; Z, zona radiata; ZE, zona radiata externa; ZI, zona radiata interna.

2B and C). As the vitellogenesis progresses the adhesive layer become longer and thicker toward the animal pole (Plate 2B and C).

The zona radiata is double−layered, consisting

of an electron-dense externa and a less dense interna (Plate 2D). The thickness of the zona radiata varies, between 7.8 and 10.5 μm. The zona radiata externa is the electron-dense outer layer and more or less homogeneous. This layer is very thin, about 0.3~0.5 μm. No structures exist on the zona radiata externa. The zona radiata interna, inner layer, is less electron-dense. The zona radiata interna consists of three to five layers which exhibit heterogeneous electron-density. This layer is thicker than the zona radiata externa, and occupies most of the zona radiata in thickness, about 7.3~10.0 μm. Just beneath the zona radiata, the ooplasm exists. The zona radiata has microvilli and pore canals in its membrane (Plate 2D). Due to these structures, the zona radiata is showed to be striated in light microscope. The ooplasmic microvilli project through the pore canals of the zona radiata toward the inner follicular layer.

Discussion

A Korean gobiid fish, *Micropercops swinhonis*, spawns in April and May (Kim and Kim, 1996; Kim, 1997). During this breeding period, the oocytes of *M. swinhonis* become surrounded by a mucous envelope (Park *et al.*, 1998). The adhesive structures, which fasten the teleostean eggs to various substrates, are not a rare phenomenon (Blaxter, 1969; Laale, 1980; Riehl and Greven, 1990, 1993; Abraham *et al.*, 1993; Park *et al.*, 1998, Park and Kim, 2001a, b). In many teleost fishes, various adhesive structures of the oocyte are attached at the zona radiata externa (Kim and Park, 1996; Park and Kim, 1997, 2001a, b). The zona radiata interna develops after the zona radiata externa and is secreted from the oocyte (Riehl and Greven, 1993). The adhesive structures may be produced by the following materials: the follicular epithelium in the goby, *Pomatoschistus minutus* and some *Silurus* species (Kobayakawa, 1985; Abraham *et al.*, 1993); additional layers produced by the follicular epithelium in some *Clupea* (Gillis *et al.*, 1990); the ovarian wall in the stickleback, *Puntungia tymensis* (Riehl and Greven, 1993); a special follicular epithelium in the perch, *Perca fluviatilis* (Riehl and Greven, 1993). The adhesive materials covering the oocyte were neutral mucins primarily of mucoproteins and mucopolysaccharides, or gelatin (Yorke and McMillan, 1979; Laale, 1980; Abraham *et al.*, 1993; Thiaw and Mattei, 1996; Park *et al.*, 1998).

In *M. swinhonis*, although the oocyte envelope was surrounded by mucous follicular cell (Park *et al.*, 1998), it was not sufficient to get detailed information of the oocyte envelope. Interestingly, it was evident in this study that toward to the animal pole the oocyte envelopes become larger and thicker. So the follicular layer toward the animal pore was 5 to 10 times thicker than the opposition of the animal pole, vegetable pole. These phenomenons are not well documented in other teleost fishes. A similar structure was known in the oocyte envelope of the sheatfish *Silurus glanis*, the muco-follicle cells of the jelly coat termed acorn bodies (Abraham *et al.*, 1993). However, the mucous follicle cell of *M. swinhonis* is different from the acorn bodies. Firstly, the muco-follicle cells of the jelly coat in *S. glanis* are not surrounded all around the oocyte but restricted at the acorn bodies. Whereas the follicular layer of *M. swinhonis* surrounds all around the oocyte, and toward the animal pore the follicular layer become thicker and larger. Secondarily, the transformation of the follicular layer occurs. In *M. swinhonis*, as vitellogenesis progresses, the cuboidal inner follicular cell, granulosa cell, is transformed to columnar cell, and at this time the columnar cell secretes mucous materials, adhesive structure. The cytoplasm of the columnar cell is filled with mucus, and finally the mucus producted formed muco-follicle layer surrounding the oocyte.

In TEM observation, the pore canals distributed throughout the zona radiata contribute to the transportation of nutrients from the granulosa cell to the developing egg body (Hurley and Fisher, 1966; Nagahama, 1983; Groot and Alderdice, 1985). In *M. swinhonis*, the zona radiata was 7.8 to 10.5 μm thick and consisted of three to five layers. In viviparous Goodeidae and Poeciliidae, the zona radiata was considerably thinned, 0.3 to 2.0 μm, and its layer was not so much, mostly 1 to 2 (Riehl and Greven, 1993). In the zona radiata, the reduction of the thickness and layer's number may be a response to the need for gaseous exchange between the embryonic and maternal tissues in viviparous vertebrates (Riehl and Greven, 1993). In oviparous fishes, whereas, the zona radiata is multi-layered and thick. In *Cynolebias melanotaenia*, the zona radiata was 3 layers and 4.5 μm in thickness (Wourms, 1976), and in *Oryzias latipes*, 3 layers and 12~15 μm

(Hart *et al.*, 1984). In addition, the thickness of the zona radiata in *Oncorhynchus* and *Salmo* was thickest, 28～62 μm and 31～50 μm, respectively (Groot and Alderdice, 1985; Riehl, 1991). In Korean spine loaches, *Niwaella multifasciata* and *Khichulchoia brevifasciata*, the thickness of the zona radiata is about 5.0 to 7.0 μm and 3.5 to 4.5 μm (Park and Kim, 2001b), respectively. In many fishes, the structures and features of the zona radiata are closely related to adaptation to spawning and egg development, and also environmental factors and systematic relationships (Ivankov and Kurdyayeva, 1973; Hirai, 1993; Britz *et al.*, 1995; Riehl and Patzner, 1998; Park and Kim, 2001a, b).

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좀구굴치 *Micropercops swinhonis*의 난여포층

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좀구굴치 성숙란의 난여포층은 외층인 theca cell과 내층인 granulosa cell로 구분되며 특히 원주형인 granulosa cell은 분비활동으로 인하여 세포질에 분비물이 축적되면서 난모세포를 둘러싸게 된다. 이러한 granulosa cell은 난황형성 초기인 난황포 시기에 입방형태를 보이지만 난황구 시기에는 원주상세포로 바뀌면서 점액을 분비하는 특징을 보여 주고 있다. 이러한 부착물질은 형태가 없으며 전자밀도가 높다. 또한 이러한 분비물을 가지는 난막은 난황형성이 더욱 진행됨에 따라 동물극 부근이 식물극보다 더욱 두꺼워지고 커지게 된다. 이러한 점막여포층 아래에는 약 7.8～11.5 μm 두께의 방사대가 존재한다. 방사대는 전자밀도가 낮은 외층과 3～5층의 여러 전자밀도층을 가지는 내층으로 구성되어 있다. 한편 점막여포층은 방사대에 존재하지 않고 있기 때문에 이 물질은 난세포질이 여포상피로부터 기원된 것으로 생각된다.