Korean J. Ichthyol. 11(2), 126~133, 1999

Ultrastructure of Egg Micropyles and Zona Radiata in Three Aquacultural Teleosts

Jong-Man Yoon

Department of Marine Biomedical Science, College of Ocean Science and Technology, Kunsan National University

Ultrastructure of the zona radiata and the micropyle of ripe eggs in the rainbow trout (*Oncorhynchus mykiss*), catfish (*Silurus asotus*) and loach (*Misgurnus anguillicaudatus*) were examined by light, scanning and transmission microscopes. The egg micropyle of rainbow trout and catfish consists of a funnel-shaped vestibule and a tapered canal transversing the zona radiata. The micropyle of rainbow trout and catfish showed the similar structure with flat pit and long canal. The micropylar wall showed the clockwise spiral structure in rainbow trout. The micropyle of the loach showed the type with a hollow pit leading into a short canal and the micropylar wall showed the counterclockwise spiral structure. There are numerous and various size pores at the surface area around the opening in every experimented fish. Interconnecting ridges were observed in the unfertilized eggs of every fish.

Key words : Zona radiata, ultrastructure, micropyle, rainbow trout, loach, catfish

Introduction

Ripe eggs of oviparous teleosts are covered with thick envelopes termed zona radiata or chorion. In most teleosts, the eggs usually have one micropyle and the canal of which is as narrow as the diameter of the sperm head. Only the first spermatozoon reaching the micropyle can come directly into contact with the zona radiata of the egg (Brummett and Dumont, 1979; Hart and Donovan, 1983; Iwamatsu *et al.*, 1993; Yoon *et al.*, 1996). However, average 7 micropyles are restricted to a 100~200 μm region at the animal pole and penetrate the zona radiata of eggs in the white sturgeon, *Acipenser transmontanus*. Their outer opening measures 15 μm in diameter (Cherr *et al.*, 1982). The zona radiata ultrastructure surrounding the distal and proximal micropyle openings, the size of the openings and the shape of the canal differ between species. The micropyles and the surface pattern of fish eggs form important characteristic criteria for identification of different teleosts (Dumont and Brumett, 1980; Iwamatsu and Ohta, 1981; Hart and Donovan, 1983; Guraya, 1986; Kim *et al.*, 1993; Kim, 1998). This study was carried out to investigate the ultrastructural characteristics of the surface pattern and the micropyles of eggs in the rainbow trout (*Oncorhynchus mykiss*), catfish (*Silurus asotus*) and loach (*Misgurnus anguillicaudatus*) were observed by light, scanning and transmission microscopes.

Materials and Methods

Ripe female rainbow trout (*Oncorhynchus mykiss*), catfish (*Silurus asotus*) and loach (*Misgurnus anguillicaudatus*) were collected from the rearing tank (Kunsan National University, Kunsan) under the normal environmental conditions. Mature and unfertilized eggs were released from the ovarian lumen into freshwater. Unfertilized eggs was prepared by squeezing 2~3 testes in 50 ml Ringer's solution in a glass beaker. For transmission electron microscopy studies, the egg specimens were fixed in 2.5% glutaraldehyde, buffered with 0.1 M PBS, pH 7.2, for 2 hrs at 4°C and postfixed in 2% osmium tetroxide in the same buffer for 2 hrs at room temperature, dehydrated by graded series of ethanol, and embedded in Epon 812. Semithin sections of unfertilized egg, and testes stained with 1% toluidine blue dye were used to locate the cortical granules, and then semithin sections dyed were examined by light microscope (Olympus, Japan). Subsequently, ultrathin sections were obtained from the same block by ultramicrotome (No. 2088, LKB, Bromma, Sweden), and The sections were picked up on copper grids and double-stained with aqueous 5% uranyl acetate and lead citrate solution, and examined by a transmission electron microscope (ISI-LEM 2000, Jeol, Japan) operated at 70 kV.

For SEM observations, 2.5% glutaraldehyde-fixed eggs and spermatozoa were attached to coverslips, washed in 0.1 M phosphate-sucrose buffer, postfixed in 2% osmium tetroxide, and dehydrated by graded series of ethanol and iso-amyl acetate. The samples were then critical point dried with CO_2 in a Balzers CPD 030, and coated with 25 nm gold-palladium in a ion coater (Hitachi, Japan). The observations were made using by a scanning electron microscope (Hitachi, Japan) operated at 20 kV.

Results and Discussion

Rainbow trout egg

The micropyle consists of a funnel-shaped vestibule and a tapered canal transversing the zona radiata in rainbow trout (Fig. 1). The micropyle of the rainbow trout egg showed the type of a flat pit and long canal (Riehl, 1980). This micropyle could classified to the type II which was distinguished by Guraya (1986), and measured approximately 180 μm at the distal opening, 20 μm at the proximal opening and tapers from 8.2 μm to 1.3 μm as it penetrates the zona radiata (Figs. 1B and C). The 16 μm micropyle proximal opening in pink salmon (*Oncorhynchus gorbuscha*) egg is surrounded by an area of protrusion and the funnel-shaped canal tapers to 2 μm at its terminal structure (Stehr and Hawkes, 1979). Since the diameter of the inner micropylar aperture in the egg this fish is slightly larger than the size of its sperm head, the block to polyspermy is considered to be mechanical and guaranteed by the morphological design of the micropyle (Iwamatsu and Ohta, 1981; Hart and Donovan, 1983; Yoon *et al.*, 1996). The proximal opening of micropyles of eggs in the white sturgeon, *Acipenser transmontanus* measures 15 μm in diameter. The micropylar final canal contacting the oolemma tapers to 1.2 μm in the diameter (Cherr *et al.*, 1982). The surface of the tapered micropylar canal is covered with numerous short microvilli (MV) and short projections, approximately 0.5 ~ 2.5 μm in height (Fig. 1B). The projections are impressions of the egg microvilli in the envelope membrane. It is thought that these structures represent folds in the zona radiata and serve as reservoirs of extravitelline material needed for the rapid and marked expansion of this layer at fertilization. Interconnecting ridges were present in the surface of unfertilized egg (Figs. 4B, 5B, arrowhead) (Ohta *et al.*, 1983; Iwamatsu *et al.*, 1993). Numerous short microvilli with irregular slug-like shapes covered the surface in *Fundulus heteroclitus* (Brummett and Dumont, 1979) and *Oryzias latipes* (Iwamatsu and Ohta, 1981). There were observed adhesive materials attracting the spermatozoa around micropyle of the egg surface (Fig. 1B) (Dumont and Brumett, 1980; Iwamatsu and Ohta, 1981; Yoon *et al.*, 1996). The inner surface of the egg immediately underlying the zona radiata was similar to the structure of the bamboo basket (Fig. 5). The spiral pattern of the folds in the micropylar wall was observed in rainbow trout. The micropylar wall showed the clockwise spiral structure (Fig. 1C). The rotation direction of spermatozoa entering the micropyle in medaka (*Oryzias latipes*) were did not correspond to the counterclockwise spiral structure of the micropylar wall (Iwamatsu *et al.*, 1993). Immediately upon fertilization by a sperm, the cortical granules begin to fuse with the vitelline membrane so as to release their contents into the zona radiata. The cortical granule material released by the egg interacted with the vitelline membrane material to produce the fertilization membrane (zona radiata) (Tegner and Epel, 1973; Kim *et al.*, 1993).

The helicoidal structure in the zona radiata was seen in rainbow trout (Fig. 4C). The zona radiata was an three-layered structure: the outer homogeneous layer, the intermediate multilamellar and less dense layer, and the inner deep dense layer(Kim *et al.*, 1993). The perivitelline space surrounding unfertilized oocytes contained an extracellualr matrices comprising granules and microvilli (Fig. 8B). This matrix was identical to the granule previously described in the extracellular space of the corona radiata and zona pellucida(Dandekar and Talbot, 1992). A region of egg surface beneath the micropyle showed numerous large irregular massess and globular cortical granules 30 seconds after insemination (Figs. 9B, C).

Loach egg

The micropyle of the loach egg showed the type with a hollow pit leading into a short canal (Figs. 2C, 10B). The micropyle of the loach egg may belong to the type I which was distinguished by Guraya (1986). The micropylar depth becomes very shallow due to a reduction in the thickness of the inner layers of the zona radiata. The spiral pattern of the folds in the micropylar wall was observed in loach (Fig. 2C). The micropylar wall showed the counterclockwise spiral structure (Figs. 2C, 11A). This spiral direction did not correspond to that of rainbow trout (Fig. 1) and corresponded to that of medaka (Iwamatsu *et al.*, 1993). There were numerous microvilli having trapping materials attracting the spermatozoa in the vicinity of the outer opening of a micropyle (Fig. 11B). The possibility that there is an intimate relationship between sperm behavior and the structure of the micropyle has recently been reported by some investigators (Brummett and Dumont, 1979; Iwamatsu *et al.*, 1993; Yoon *et al.*, 1996).

Catfish egg

The micropyle in the egg of consists of a funnel-shaped vestibule and a tapered canal transversing the zona radiata in catfish (Fig. 3). The micropyle showed the structure with flat pit and long canal (Fig. 3B). This micropyle could classified to the type II which was distinguished by Guraya (1986). The structure of micropyle in this fish species corresponds to that of micropyle in medaka and rainbow trout (Iwamatsu *et al.*, 1993). The micropyle canal in the eggs measures approximately 24 μm at the distal opening, 5μm at the proximal opening. The inner diameter of the micropyle tapers from 5 μm to 1.8 μm as it penetrates the zona radiata (Fig. 3C). The micropyle of the catfish egg appears more complex than that of loach.

Received September 1, 1999
Accepted October 18, 1999

References

Brummett, A.R. and J.N. Dumont. 1979. Initial stages of sperm penetration into the egg, *Fundulus heteroclitus*. J. Exp. Zool. 210 : 417~434.

Cherr, G.N., W.H. Clark, Jr. 1982. Fine structure of the envelope and micropyles in the eggs of the white sturgeon, *Acipenser transmontanus*. Devel. Growth Different. 24 : 341~352.

Dandekar, P. and P. Talbot. 1992. Perivitelline space of mammalian oocytes: Extracellular matrix of unfertilized oocytes and formation of a cortical granule envelope following fertilization. Mol. Reprod. Dev., 31 : 135~143.

Dumont, J.N., A.R. Brummett. 1980. The vitelline envelope, chorion and micropyle of *Fundulus heteroclitus* eggs. Gamete Res., 3 : 24~44.

Guraya, S.S. 1986. The cell and molecular biology of fish oogenesis, Karger. pp. 141~147.

Hart, N.H. and M. Donovan. 1983. Fine structure of the chorion and site of sperm entry in the egg of *Brachydanio*. J. Exp. Zool., 227 : 277~296.

Iwamatsu, T. and T. Ohta. 1981. Scanning electron microscopic observations on sperm penetration in teleostean fish. J. Exp. Zool., 218 : 261~277.

Iwamatsu, T., S. Ishijima and S. Nakashima. 1993. Movement of spermatozoa and changes on micropyles during fertilization in medaka eggs. J. Exp. Zool., 266 : 57~64.

Kim, D.H, D.S. Reu, W.J. Kim and Y.K. Deung. 1993. A comparative study on the ultrastructures of the egg envelope in fertilized eggs of angelfish (*Pterophyllum eimekei*) and zebrafish (*Brachydanio rerio*). Korean Soc. Electron Microscopy, 23(3) : 115~128.

Kim, D.H. 1998. Comparative ultrastructure of fertilized egg envelopes in teleost. Doctor thesis, Chongju University, pp. 55.

Ohta, H., K. Takano, T. Izawa and K. Yamauchi. 1983. Ultrastructure of the chorion and the micropyle of the Japanese eel, *Anguilla japonica*. Bull. Japan. Soc. Sci. Fish., 49(3) : 501.

Riehl, R. 1980. Micropyle of some salmonids and coregonids. Env. Biol. Fishes, 5 : 59~66.

Stehr, C.M. and J.W. Hawkes. 1979. The comparative ultrastructure of the egg membrane and associated pore structures in the starry flounder, *Platichthys stellatus* (Pallas), and pink salmon, *Oncorhynchus gorbuscha* (Walbaum). Cell. Tiss. Res., 202 : 347~356.

Tegner, M.J. and D. Epel. 1973. Sea urchin sperm-egg interactions studied with the scanning electron microscope. Science, 179 : 685~688.

Yoon, J.M., K.Y. Chung, D.S. Reu, I.D. Lew, S.C. Roh and G.W Kim. 1996. Electron microscopic observations on micropyle after sperm penetration in rainbow trout, *Oncorhynchus mykiss*. Korean J. Zool., 2(2) : 173~181.

Figures

Figs. 1A, B. and C. (A) A micropylar apparatus and the egg surface beneath the inner opening of the micropyle of an unfertilized egg in rainbow trout. (B) The shallow funnel-like structure of the outer opening. (C) The wider distal micropylar opening leads into a canal which tapers to an aperture just large enough to permit entry of a single sperm. MC : micropylar canal. Scale bar=150 μm (A), 8.6 μm (B) and 2 μm (C).

Figs. 2A, B and C. Scanning electron microscophs of the micropylar region of an unfertilized egg of loach. (A) The canal opening situated in the bottom of the depression is surrounded by a slightly raised lip. (B) The shallow funnel-like structure of the outer opening. (C) The conical depression or vestibule is devoid of chorionic fibrils. MC : micropylar canal. Scale bar=30 μm (A), 20 μm (B) and 4.3 μm (C).

Figs. 3A, B and C. Scanning electron microscophs of the micropyle in egg envelope of catfish. (C) The surface surrounding the outer opening have many small pores. M : micropyle. Scale bar=150 μm (A), 30 μm (B) and 12 μm (C).

Figs. 4A, B and C. (B) High-power view of the boxed area in 4A. Note the interconnecing ridges in the outer surface of the zona radiata. (C) The zona radiata has numerous pore canals. ES:egg surface, H:hexagon wall of envelope, S:subfollicular space, ZR : zona radiata. Scale bar=10 μm (B) and 17.6 μm (C).

Figs. 5A and B. Note the microtrabecular appearance of branches and holes. (A) The inner surface of the zona radiata revealed a bamboo basket organization. The filaments form generally a tight meshed network. Note the presence of a homogeneous fine network of filaments. (B) At high magnification note that filaments appear as a bead on a string structures (arrowheads). GF : gelatin fibre, IEM : interconnecting extracellular matrices. Scale bar=7.5 μm (A) and 2.5 μm (B).

Figs. 6A and B. (A) The secondary zona radiata shows a spongy surface. (B) The inner surface of the secondary zona radiata has a very fine microtrabecular appearance. The filaments form a large meshed network. Scale bar=10 μm (A) and 3.0 μm (B).

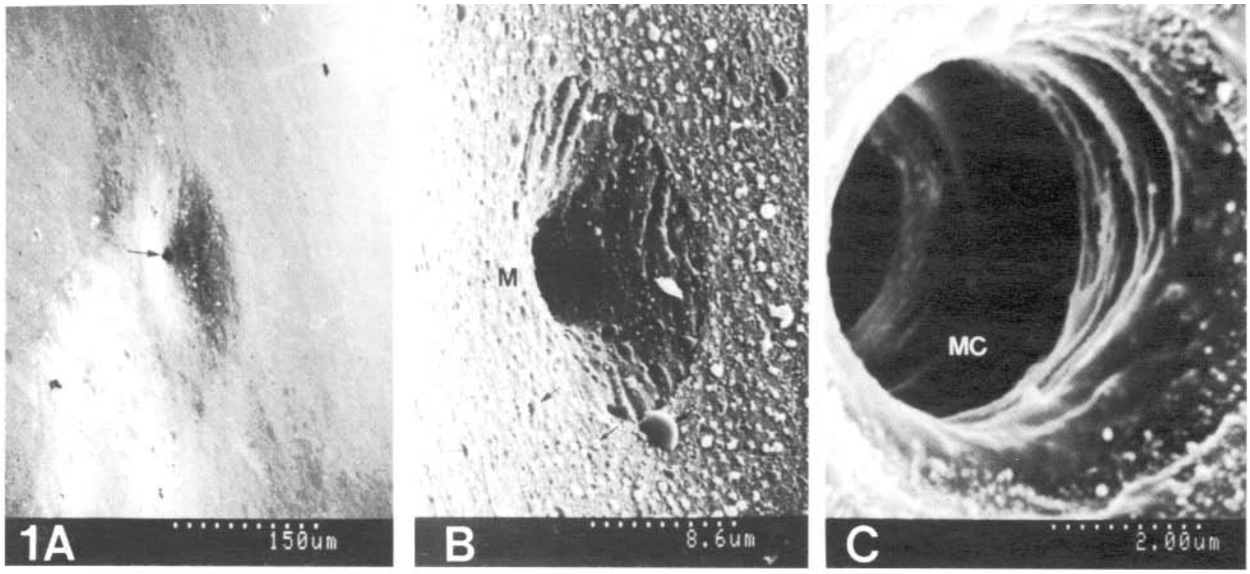
Figs. 7A and B. (A) The zona radiata divided into three layers; the inner deeply dense, the intermediate less dense and the outer homogeneous. Three margin lines of the zona radiata in the fertilized oocytes is different from that of mature oocytes. (B) A higher magnification of Fig. 7A. Scale bar=4 μm (A) and 1 μm (B).

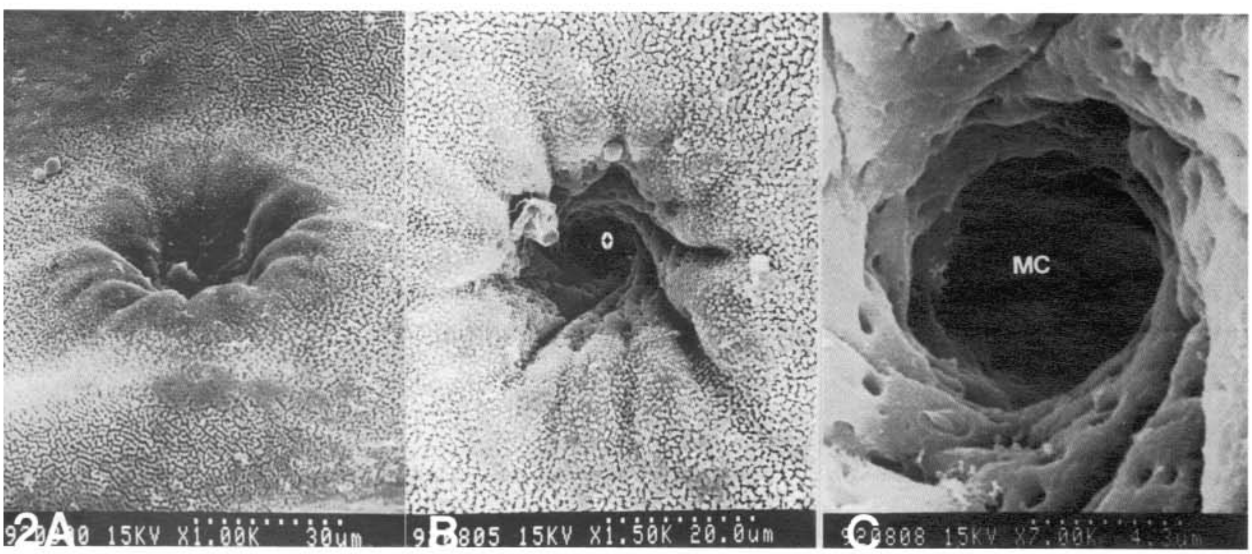
Figs. 8A and B. (A) Scanning electon micrograph of the vitelline membrane showing the irregular and circular microvilli and cortical granules in rainbow trout. (B) A higher magnification of the vitelline membrane. MV : microvilli. Scale bar=1,200 μm (A) and 300 μm (B).

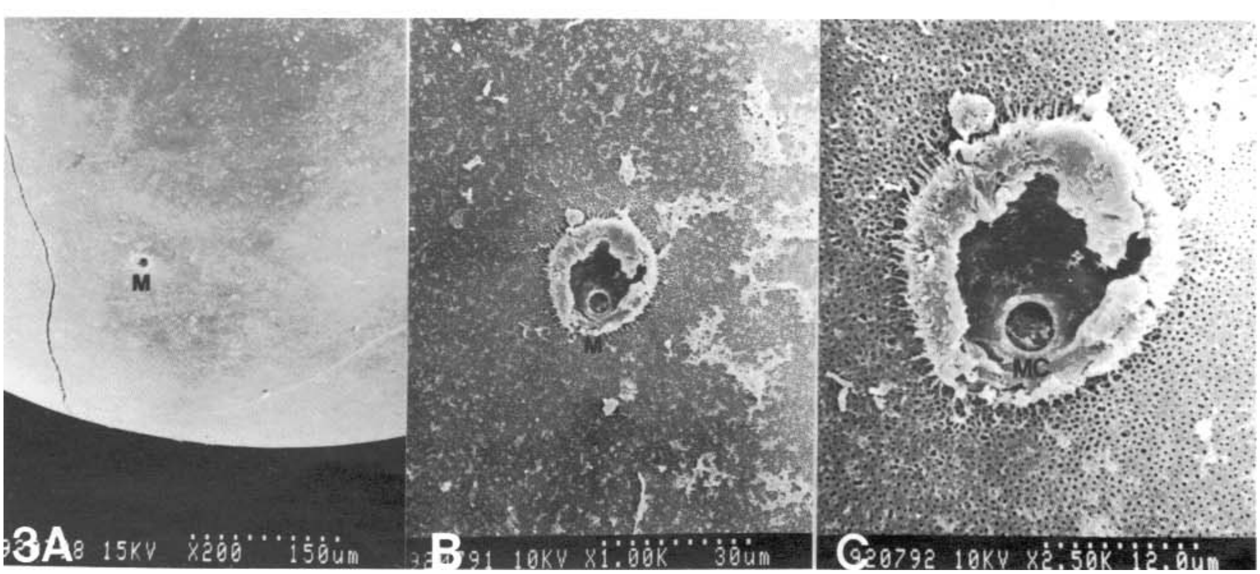
Figs. 9A and B. (A) A region of egg surface beneath the micropyle showing numerous large irregular massess and globular cortical granules 30 seconds after insemination. (B) A higher magnification of Fig. 9A. (C) Note the breakdown of the cortical granules beneath the fertilization membrane. CG:cortical granules, IM : irregular masses, MV : microvilli. Scale bar=150 μm (B) and 120 μm (C).

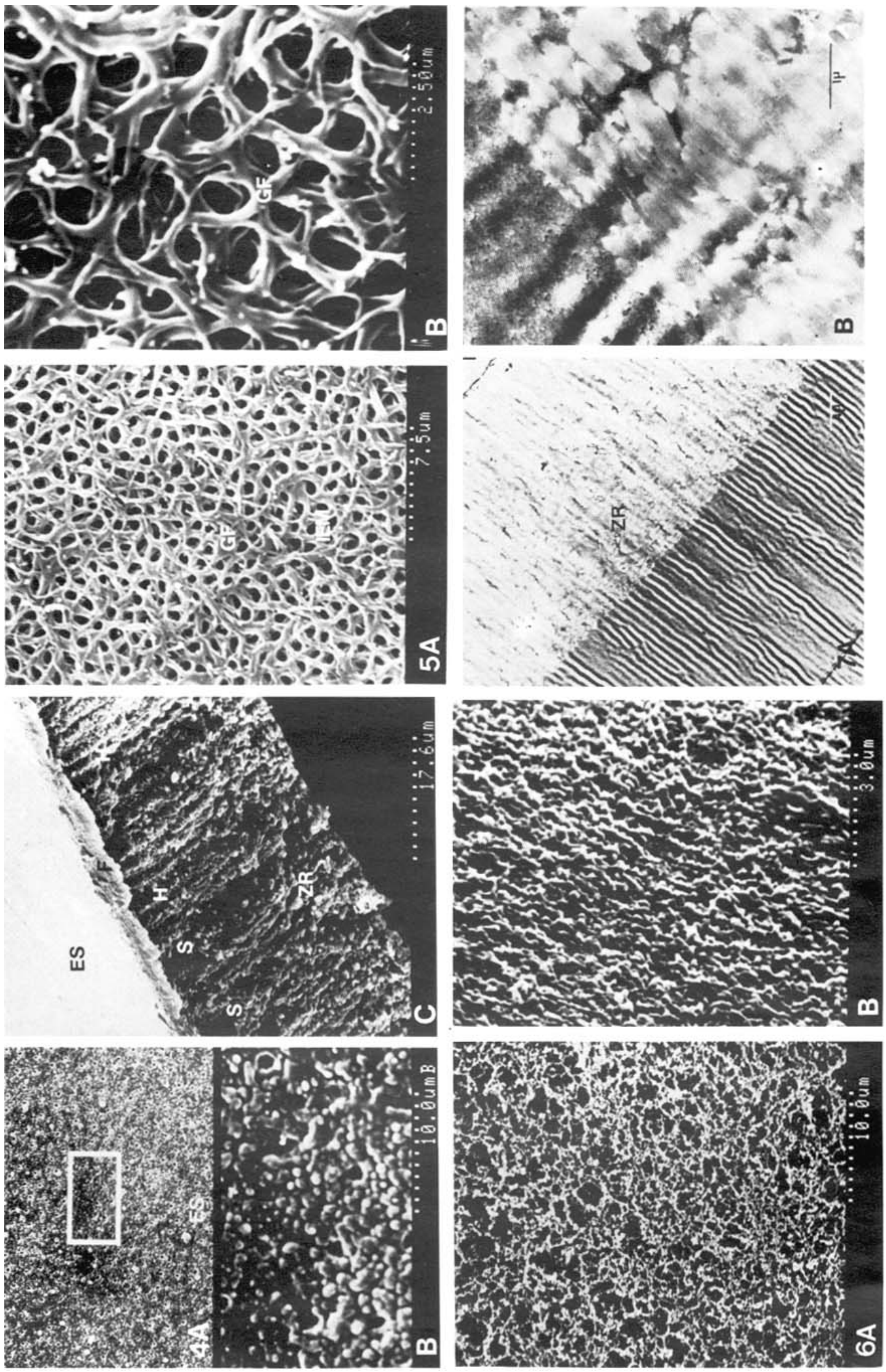
Figs. 10A and B. Photomicrographs (LM) of vegetal pole (A) and animal pole (B) of a mature egg showing a micropyle in loach. There was a polarity in the distribution of different sized cortical granules in the egg of *Misgurnus anguillicaudatus*, showing to the distinct gradient in the structural organization of the egg cortex from the site of sperm entry to the vegetal pole. ×400 (A), ×320 (B).

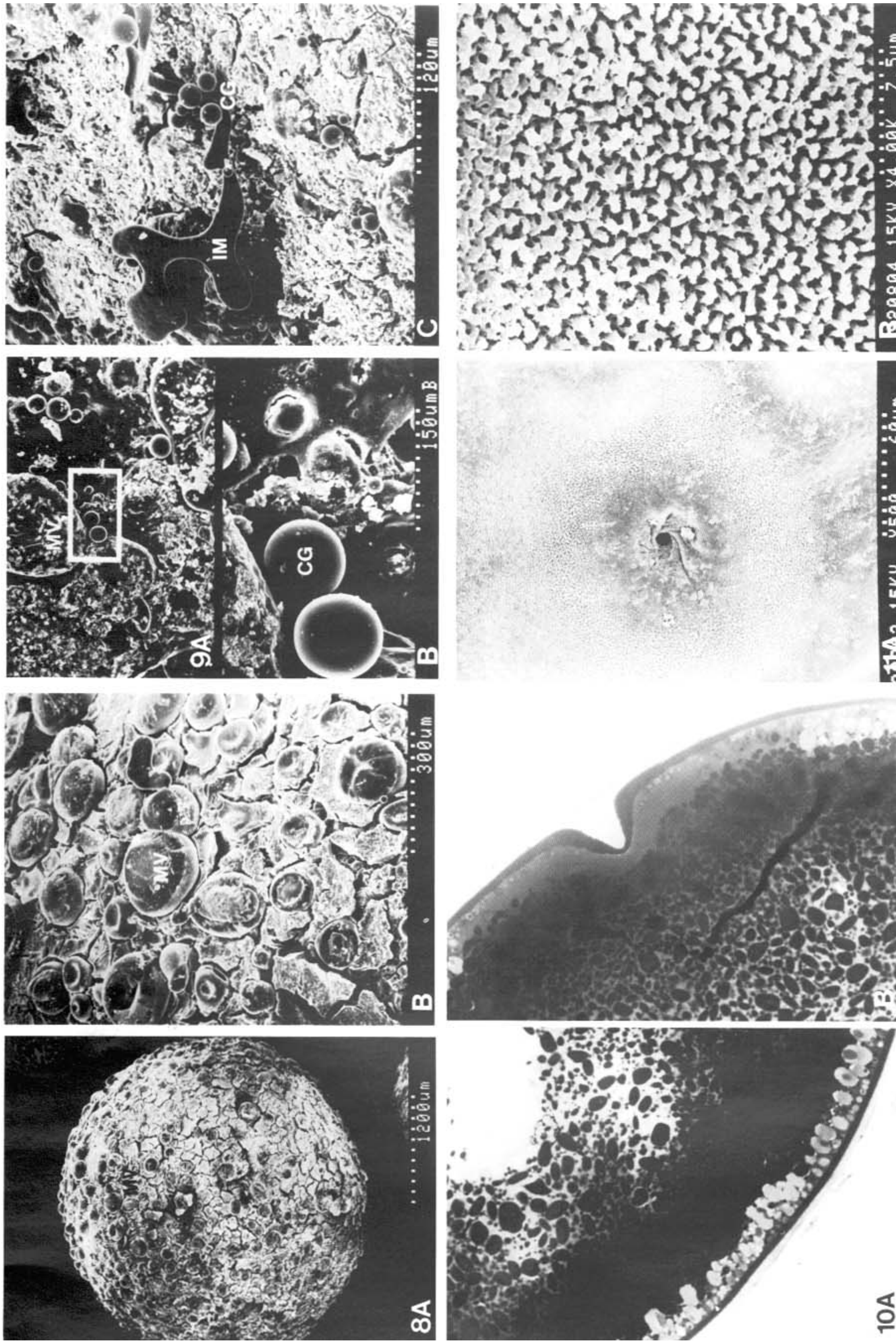
Figs. 11A and B. (A) A micropyle and the egg surface in the vicinity of the outer opening of the micropyle of an unfertilized egg in loach. The shallow funnel-like structure of the outer opening. (B) Note the microvilli having trapping materials attracting the spermatozoa in the vicinity of the outer opening of a micropyle. Scale bar=60 μm (A) and 7.5 μm (B).


1A
150um
M
B
8.6um
MC
C
2.00um


2A
15KV X1.00K 30um
O
B
805 15KV X1.50K 20.0um
MC
C
808 15KV X7.00K


M
3A
15KV X200 150um
M
B
10KV X1.00K 30um
MC
C
920792 10KV X2.50K 12.0um


4A
B
10.0umB
ES
S
S
H
ZR
C
17.6um
5A
GF
7.5um
B
GF
2.50um
6A
10.0um
B
3.0um
ZR
B
1µ


8A
B
MV
1200um
300um
9A
B
C
MV
CG
IM
150umB
120um
10A
B
11A
B
15KV X500 60um
20804 15KV X4.00K 7.5um

양식산 경골어류 3종의 난문과 방사대의 미세구조

윤 종 만

군산대학교 해양과학대학 해양생명의학과

무지개송어 (*Oncorhynchus mykiss*), 메기 (*Silurus asotus*) 그리고 미꾸리 (*Misgurnus anguillicaudatus*)의 성숙 난자의 방사대 (zona radiata) 및 난문의 미세구조적 특징을 광학, 주사 및 투과전자현미경을 이용하여 조사하였다. 무지개송어와 메기의 난에 있는 난문은 깔대기 형태의 전정과 방사대를 통과하는 끝이 작아지는 통로로 구성되어 있었다. 무지개송어와 메기의 난문은 편평한 구멍과 긴 통로로 구성된 구조를 나타내었다. 메기 난문의 구조는 무지개송어 난문의 구조와 일치되었다. 무지개송어의 난문을 구성하는 내부 벽은 시계방향(우선형)의 구조를 나타내었다. 미꾸리의 난문은 짧은 통로를 지닌 얕은 구멍을 가진 형태를 나타내었고, 시계방향의 반대방향으로 난문의 벽은 방향성을 나타내었다. 미꾸리의 이러한 나선형의 방향은 무지개송어의 방향과 일치하지 않는 것으로 나타내었다. 개구부 바로 주변부에 있는 표면은 실험에 이용된 3가지 어류에서 모두 여러 가지 크기의 작은 구멍이 존재하였다. 상호 연결부위 (ridge)가 모든 어류의 미수정된 난자에서 관찰되었다. 본 논문에서는 난성숙시 난막과 난문의 형태를 알아보기 위해서 3가지 양식어종 난자의 표면형태와 난문의 미세구조를 상호 비교하였다.