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Electron Microscopic Observations on Micropyle after Sperm Penetration in Rainbow Trout, *Oncorhynchus mykiss*

Jong-Man Yoon*, Ku-Young Chung+, Dong-Suck Reu‡, In-Deok Lew¶, Soon-Chang Roh§, and Gye-Woong Kim§§

Animal Resources Research Center, Kon-Kuk University, Seoul 133-701; +Department of Animal Resources, Sang-Gi University, Won-Ju 220-702; ‡Department of Biology, Chong-Ju University, Chong-Ju 360-764; ¶Department of Food Engineering, Chung-Ju National University, Chung-Ju 380-702; §Department of Animal Science, Kon-Kuk University, Chungju, 380-701; §§Department of Animal Science, Kong-Ju National University, Yesan-kun, 340-800, Korea

The time-course process by which spermatozoa penetrates through the micropyle apparatus into the egg cytoplasm of rainbow trout, *Oncorhynchus mykiss*, was examined with transmission and scanning electron microscopy. In the unfertilized egg, the egg surface beneath the inner opening of the micropylar canal did not differ distinctly from the rest of the animal pole area. A spermatozoon attached to the micropyle opening 20 seconds after insemination. In the initial stages of penetration, the spermatozoon still within the micropylar canal attached perpendicularly at its apical tip to the egg surface, then the sperm head was rapidly engulfed by the folded egg surface with its many microvilli. A large fertilization cone with microvillus-free surface appeared on the egg surface sur-rounding the penetrating spermatozoon. The head portion of the penetrating spermatozoon was completely wrapped by the egg surface with only the tail portion visible externally 30 seconds after insemination. The fertilization cone displayed the tail portion of the penetrating spermatozoon on the central portion of its surface 60 seconds after insemination. 150 seconds after insemination, breakdown of the cortical granules elevation were initiated at the animal pole, then completed at the vegetable pole area. The spermatozoon disappeared from the outer surface of the egg before the fertilization cone completely retracted 250 seconds after insemination. In result, the block to polyspermy to permit entry of a single sperm is considered to be mechanical by the morphological design of the micropyle and fertilization cone.

KEY WORDS: Micropyle, Scanning Electron Microscopy, Fertilization Cone, Cortical Granules, Block to Polyspermy

Morphological observations on the sperm-egg fertilization in fish have been reported by several investigators (Kessel-Shih, 1976; Ojima and Makino, 1978; Lee and Yamazaki, 1989; Focarelli *et al.*, 1991). Studies have been done on fish spermatogenesis or spermiogenesis in *Tandanus tandanus* (Davis, 1977), *Clarias gariepinus* (Van Oordt *et al.*, 1987), *Lepidogalaxias salamandroides* (Leung, 1988), and *Murex brandaris* (Amor and Durfort, 1990).

*To whom correspondence should be addressed.

There have also been a few ultrastructural studies of teleost fertilization (Brummett and Dumont, 1979; Iwamatsu and Ohta, 1981; Kim *et al.*, 1993; Iwamatsu *et al.*, 1993).

We have carried out transmission and scanning electron microscopic observations on micropyle and cortical granules of unfertilized egg and initial stages of sperm penetration into the egg of 3-year-old rainbow trout (*Oncorhynchus mykiss*) before and following artificial insemination from December 1993 to January 1994.

Materials and Methods

Adult female and male rainbow trout, *Oncorhynchus mykiss*, were collected from the rearing tank (Department of Animal Science, Kon-Kuk University, Seoul) under the normal environmental conditions from December 1993 to January 1994. A total of 50 fishes were sampled. Mature, unfertilized eggs were released from the ovarian lumen into freshwater. To add spermatozoa to unfertilized eggs,a sperm suspension (10-15 $\times$ 10^8 spermatozoa/ml of semen) was prepared by squeezing 2-3 testes in 50ml Tyrode's solution in a glass beaker. The values of GSI (gonadosomatic index, gonad weight<g> $\times$ 100/body weight<g>) were employed to monitor gonadal maturation. All results are expressed as mean ± S.E treated by SPSS computer program.

For transmission electron microscopic studies, the living 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 and fertilized egg, and testes stained with 1% toluidine blue dye were used to locate the cortical granules and sperm cells. Subsequently, ultrathin sections were obtained from the same block by ultramicrotome (No.2088, LKB, Bromma, Sweden) with a diamond knife, and the sections were picked up on copper grids and double-stained with aqueous 5% uranyl acetate and lead citrate solution, and examined in 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 isoamyl 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

Ultrastructures of gametes

Sperm type

Spermatozoa, approximately 20 μm in major axis, have their parachute-shaped and spheroidal head (Figs. 1, 2 and Table 1). These cells have a intensely and homogeneously stained nucleus, about 1.9 μm in diameter and a mean width of 1.2 μm (Fig. 1 and Table 1). There are seen two large mitochondria in the middle- piece under the sperm head (Fig. 1). Mitochondria (about 145 nm long) of rainbow trout spermatozoa are arranged into a spheroid-shaped sheath that surrounds the axial elements of the midpiece. Mitochondrial membrane is oriented toward the plasma membrane of the sperm head and becomes detached from the fibrous sheath. The outer dense fiber is attached to the mitochondrial sheath in the midpiece and the axoneme is surrounded by the fibrous sheath in the principal piece. The interior of the mitochondria consists of an electron dense matrix and the mitochondrial membrane that exhibits cristae-like infoldings.

The axoneme has nine peripheral doublets and two centrioles. Two centrioles are quite separate and the central pair and sheath complex of the flagellum is inserted into the base of the distal centriole. The spermatozoa of rainbow trout have a spheroid-shaped head. However,the spermatozoa of carp (Saad *et al.*, 1988) and Korean loach (Yoon *et al.*, 1993) have round-shaped head. The rainbow trout sperm, as in the

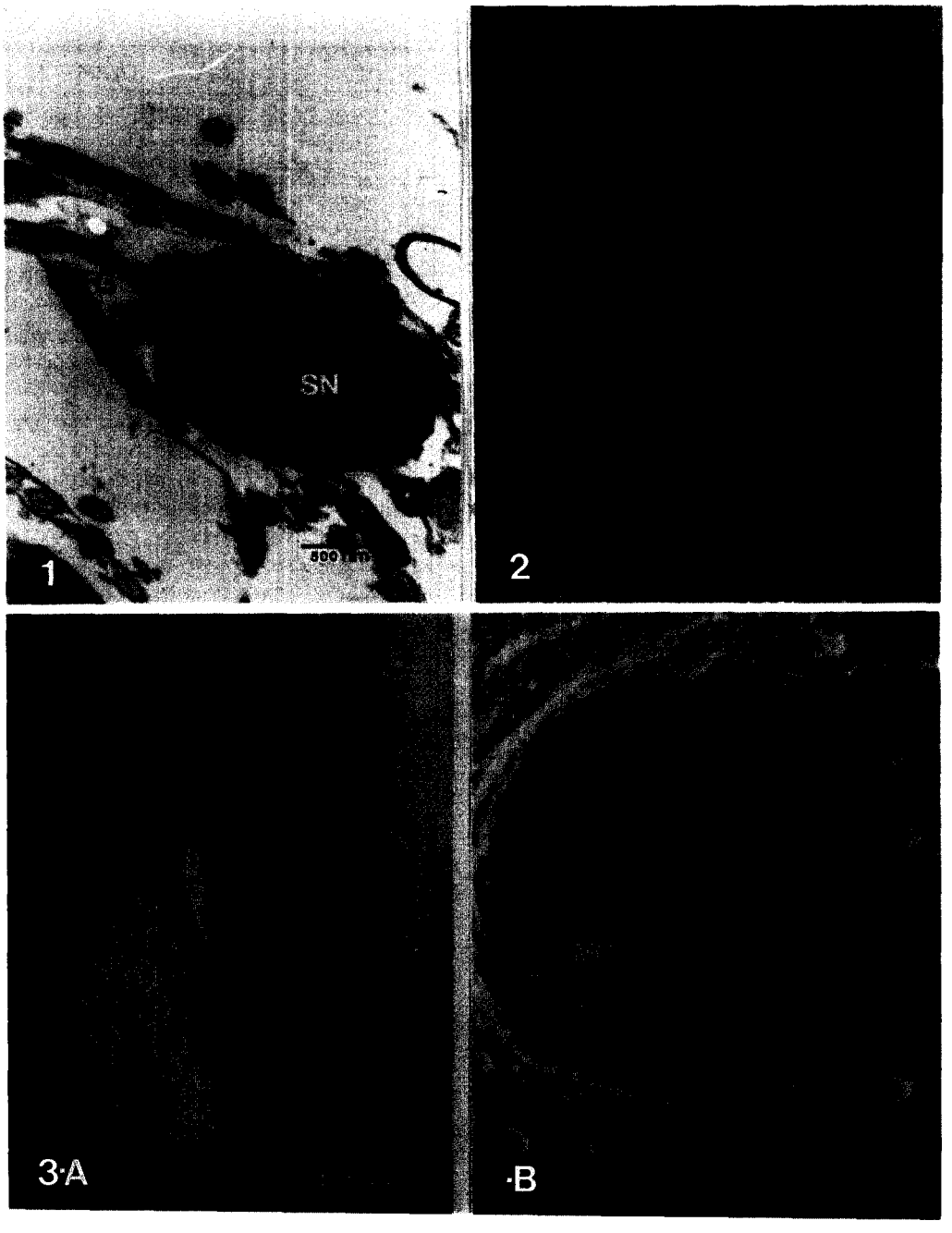


Fig. 1. Longitudinal section of the freshly collected spermatozoon. The sperm head is smooth. The flagellum is straight and inserted into the sperm head. FG: flagellum. SN: sperm nucleus. × 34,500.

Fig. 2. Scanning electron micrograph of spermatozoa. Note most of lobules are already packed with spermatozoa. Their spheroid-shaped heads have a mean width of 1.4 μm. × 6,000.

Figs. 3-A, B. A micropylar apparatus (arrow in -A) and the egg surface beneath the inner opening (B) of the micropyle of an unfertilized egg. The shallow funnel-like structure of the outer opening. The wider distal opening leads into a canal which tapers to an aperture just large enough to permit entry of a single sperm. MC:micropylar canal. A: × 200, B: × 13,000.

Table 1. Summary of properties of testis, milt and sperm of rainbow trout (*Oncorhynchus mykiss*)

TESTIS			MILT		SPERM	
GSI (%)	COLOR	SHAPE	DENSITY (sperms ml-)	OSMOLARITY (mosmol kg^{-1})	LENGTH OF HEAD (μm)	MOTILITY (%)
4.86±0.07	milky white	long tubular	$10\text{-}15 \times 10^8$	285±5.18	2.0±0.03	75

case of other teleosts (Leung, 1988; Saad *et al.*, 1988; Yoon *et al.*, 1993), has no acrosome. An egg micropyle is located in the egg surface of rainbow trout.

The gonadosomatic index (GSI) are approximately 4.86% during the breeding season, in December and January. The semen collected in the breeding season, in December, showed milky color and sperm concentration is $10\text{-}15 \times 10^8$ per ml of semen (Table 1). The motility ratio of spermatozoa shows 75%. The osmolarity of rainbow trout milt ranged from 246 to 323 mosmol kg^{-1}.

Egg type

A micropyle in rainbow trout is situated at the animal pole of egg (Fig. 3-A). The spiral structure of the micropylar wall turns the clockwise direction in rainbow trout (Fig. 3-A), while that counterwise in Japanese medaka (Iwamatsu *et al.*, 1993). The structure and development of micropyles, which situated the animal pole of the oocyte, greatly vary in different teleosts. The micropyle ultrastructure has been used successfully in fish phylogenetic studies (Brummett and Dumont, 1979; Iwamatsu and Ohta, 1981; Hart and Donovan, 1983; Guraya, 1986). The micropylar apparatus in the egg of rainbow trout consists of a funnel-shaped vestibule of diameter of approximately 180 μm and a taped micropylar canal traversing the zona radiata (Fig. 3-A) (Guraya, 1986). Their outer opening measures approximately 8.2 μm in diameter. The micropylar canal tapers twice, ultimately terminating at the oolemma with an inner opening of diameter of about 1.2 μm. The outer diameter of the canal is about 7.6 μm and inner diameter 3.6 μm (Fig. 3-B). The micropyle of the rainbow trout egg appear smaller than that in crucian carp, tilapia and ayu (Guraya, 1986). The micropyle of this fish is similar with that of chum salmon (Oh, 1995). This is due to the width of sperm head in other fish (Yoon *et al.*, 1994). Generally, the width of the sperm head in coldwater fish is smaller than that in warm-water fish.

SEM studies of the funnel-shaped micropyle in mature eggs have revealed that the wider distal opening leads into a canal which tapers to an aperture just large enough to permit entry of a single sperm (Brummett and Dumont, 1979; Iwamatsu and Ohta, 1981; Hart and Donovan, 1983; Guraya, 1986; Wolenski and Hart, 1987; Kim *et al.*, 1993). Since the diameter of the inner micropylar aperture in the egg of rainbow trout is slightly larger than the size of its sperm head, the block to polyspermy is considered to be mechanical by the morphological design of the micropyle (Brummett and Dumont, 1979; Iwamatsu and Ohta, 1981; Hart and Donovan, 1983; Guraya, 1986; Wolenski and Hart, 1987). Thus, micropyle of teleost egg faciliates fertilization (Guraya, 1986).

The inner micropylar aperture is differentiated as a circular cluster of 10-20 microvilli-like projections (Figs. 8-A, 8-B and 8-C)(Guraya, 1986). The cortical granules are absent in the ooplasm directly below the sperm entry site (Guraya, 1986). These cortical granules consist of monolayer structure in rainbow trout, while those multilayer in Korean loach and Israeli carp (Lee *et al.*, 1989; Yoon *et al.*, 1991; Park *et al.*, 1993).

These cortical granules or alveoli are situated between oolemma and zona radiata in the ovulated ripe egg (Fig. 8-B)(Guraya, 1986).

The whole inner surface is minutely rugged or rough in structure. The micropyle apparatus spiraled inward with several layers on the inner surface of the canal (Fig. 3-B). The outer surface of the zona radiata, attaching filaments, appeared to have a network structure (Figs. 3-A, 4-A, and 5-

B)(Iwamatsu and Ohta, 1981). The microvilli secrete numerous adhesive materials having trapping materials (arrows) attracting the spermatozoa around micropyle of the egg surface (Figs. 5A and 5-B).

Time-course ultrastructural changes of the fertilized eggs before and after insemination

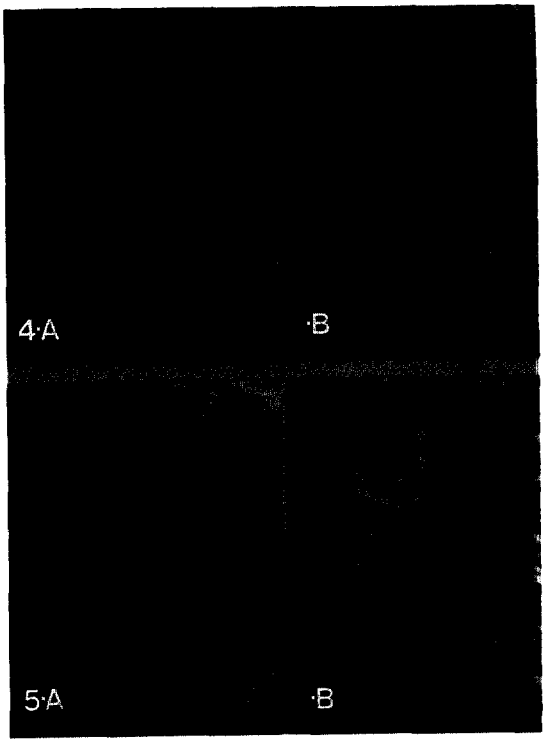
Ten seconds after insemination

The unfertilized egg surface is surrounded by finger-like projections, called microvilli, extended from the plasma membrane through the vitelline membrane. These are considered that many sperm adhere to the egg surface immediately after insemination (Figs. 4-A, 4-B, 5-A, and 5-B). The interconnecting ridges of microvilli in fertilized egg (Fig. 7-A) appear to be longer than those in unfertilized egg (Fig. 5-A and 5-B)(Kessel-Shih, 1976).

Immediately after rainbow trout are placed together, numerous spermatozoa standing in a row adhere to the surrounding micropyle (Fig. 4-A). The sperm head, midpiece and flagellum are clearly defined (Fig. 4-B). The materials attracting sperm are considered to be secreted from microvilli around the micropyle (Fig. 5-B).

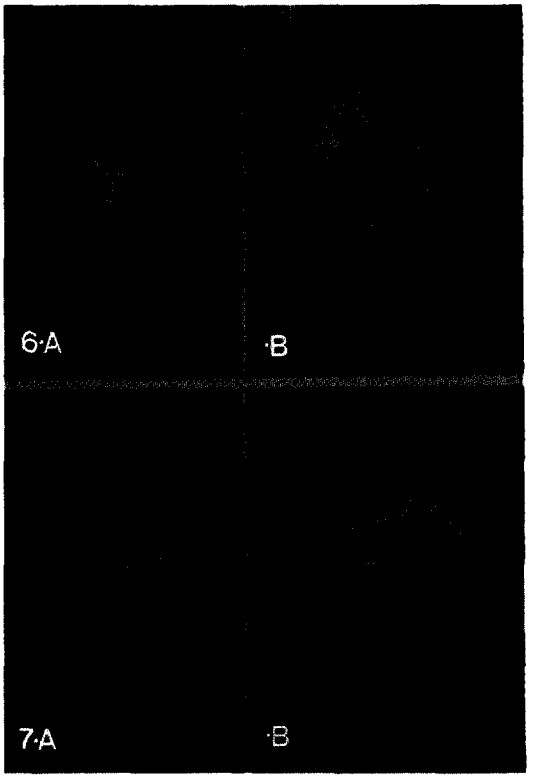
Twenty seconds after insemination

Five spermatozoa attached perpendicularly to the inner surface of the micropyle (Fig. 6-A and 6-B). The wrinkled appearance of the inner surface



Figs. 4-A, B. Micropyle of eggs 10 seconds after insemination. Note numerous spermatozoa in the vicinity of the outer opening of a micropyle. A: × 1000, B: × 3,500.

Figs. 5-A, B. Note the microvilli secreting adhesive materials having trapping materials (arrows) attracting the spermatozoa around micropyle of the egg surface. A: × 2,500, B: × 7,000.



Figs. 6-A, B. Spermatozoa attached perpendicularly to the egg surface 20 seconds after insemination. Note four spermatozoa adhering to the inner surface of the canal. A: × 2,000, B: × 6,000.

Figs. 7-A, B. Egg surfaces engulfing spermatozoa 30 seconds after insemination. A spermatozoon just after attachment by its apical tip to the egg surface showing its bent tail of a penetrating spermatozoon. A: × 1,000, B: × 5,000.

in micropyle is illustrated in Fig. 6-B.

Thirty seconds after insemination

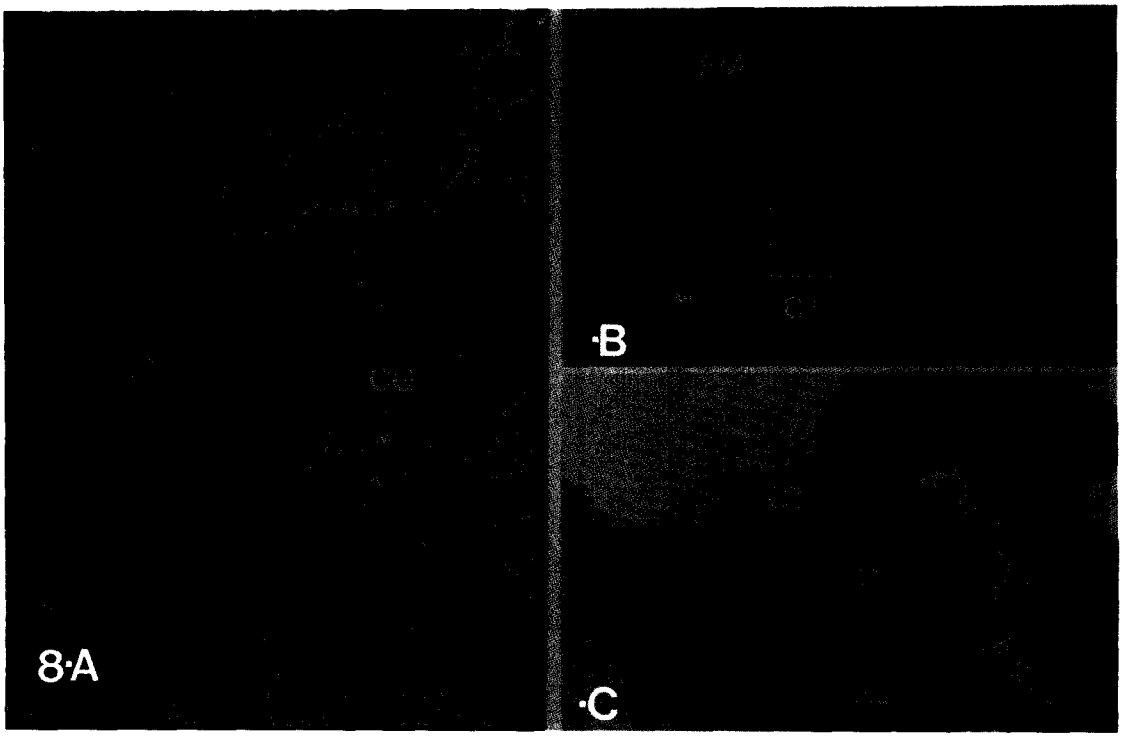
The head portion of the penetrating spermatozoon is completely wrapped by the egg surface with only the tail portion visible externally (Figs. 7-A and 7-B). The diameter of the inner opening of the micropyle indicates that only one sperm can gain access to the egg (Guraya, 1986). No sperm is attached to the egg surface in this region. Large cortical granules that have discharged their contents are observed around the animal pole area (Iwamatsu and Ohta, 1981). There are structural changes of cortical granules from spheroid type to irregular type (Figs. 8-A, 8-B and 8-C). The fertilization membrane rises by the action of a cortical protease (Tegner and Epel, 1973). The region of the zona radiata around the animal pole become thinner and elevated from the egg surface as soon as the cortical alveolar contents are secreted, as demonstrated by Iwamatsu and Ohta (1981), and Guraya (1986). These changes are consistent with the expansion of the vitelline layer in the formation of the fertilization membrane. The cortical reaction producing block to polyspermy has covered the top half of this egg. The fertilization membrane is about half formed (Tegner and Epel, 1973; Iwamatsu and Ohta, 1981; Brummett and Dumont, 1979).

Sixty seconds after insemination

A small fertilization cone (about 4 μm in average diameter) is identified in the central portion of the depressed inner micropyle (Figs. 9-A and 9-B). Most of the microvilli on the egg surface become slender. No supernumerary spermatozoa are found on the egg surface surrounding the sperm-penetrating region.

Ninety seconds after insemination

A large fertilization cone (about 6 μm in average



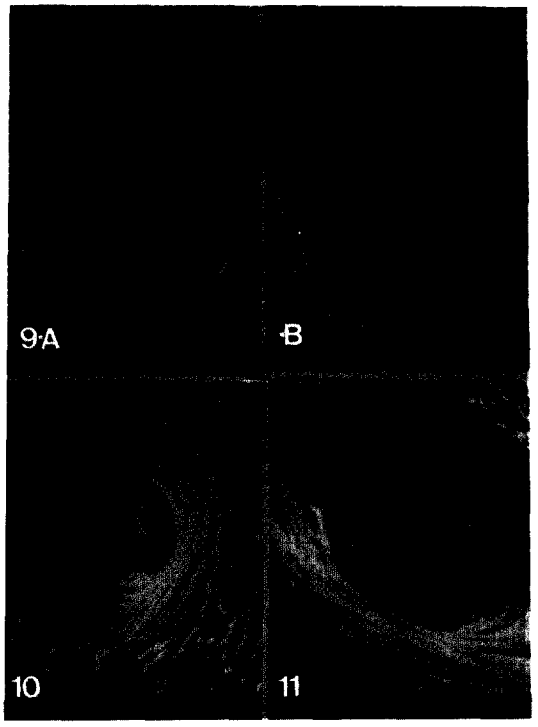
Figs. 8-A, B, C. A region of egg surface beneath the micropyle showing numerous large irregular masses and globular cortical granules 30 seconds after insemination. Note the breakdown of the cortical granules beneath the fertilization membrane. CG:cortical granules, CT:cortical tubules, FM:fertilization membrane, IM:irregular masses,OP: ooplasm. A: × 250, B: × 100, C: × 400.

diameter) with relatively smooth surface protrudes from the central portion of the depressed inner micropyle (Fig. 10). The production of the fertilization cone with the commencement of alveolar break-down is completed in the period from 30 to 90 seconds after insemination (Iwamatsu and Ohta, 1981). The fertilization cone is sometimes displayed the tail portion of the penetrating spermatozoon on the central portion of cone surface (Iwamatsu and Ohta, 1981). However, no sperm flagellum is identified in cone surface shortly before and after 90 seconds in rainbow trout.

The microvilli of the fertilized egg appear to be shorter and farther apart than those of an unfertilized egg (Fig. 10). No interconnecting ridges are present in fertilized egg. These structural changes are considered to be consistent with the expansion of the vitelline layer in the formation of the fertilization membrane (Tegner and Epel, 1973).

One hundred and twenty seconds after insemination

The larger fertilization cone protrudes from micropylar canal to micropylar vestibule (Fig. 11). The cone can be shown as a spheroid ball without microvilli. The height of the micropylar vestibule with the shallow funnel-like structure is similar to



Figs. 9-A, B. The egg surface 60 seconds after insemination showing small fertilization cones. Note the smoothness of the egg surface in the vicinity of the penetrating spermatozoon. Fine walelike folds but no sperm tail on the central surface of a fertilization cone. A: × 800, B: × 2,500.

Fig. 10. The egg surface 90 seconds after insemination. Note large and irregular fertilization cones in the vicinity of the outer opening of a micropyle. × 3, 500.

Fig. 11. Inner and outer opening of the micropyle 120 seconds after insemination. The larger fertilization cone can be seen as a blister-like bulge. The shallow funnel-like structure of the outer opening. × 6,000.

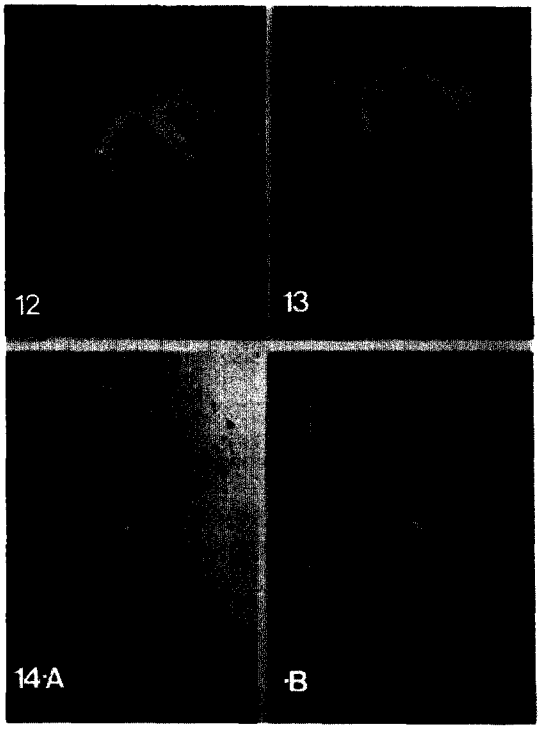


Fig. 12. Protrudent fertlization cones on egg surface 150 seconds after insemination. × 500.

Fig. 13. Protrudent fertlization cones on egg surface 200 seconds after insemination. × 500.

Figs. 14-A, B. The egg surface in the animal pole are a 250 seconds after insemination. The remnant of a retracting cone depressed in its center (white arrow heads) with adhesive materials (black arrow). × 200.

that of egg surface.

One hundred and fifty seconds after insemination

The fertilization cone shown as a blister-like bulge has the rough and irregular outer surface (Fig. 12). The height of cone is higher than that of egg surface.

Two hundred and fifty seconds after insemination

The protruded fertilization cone is shown as an upended bowling pin (Fig. 13). The egg surface in the vicinity of the protruded cone is much smoother in appearance.

The fertilization cone has diminished in size with the lapse of time. A retracting cone has a depressed central region (Fig. 14-A). The disappearance of the cone is similarly synchronized with completion of the cortical reaction at the vegetal pole region of the egg (Iwamatsu and Ohta, 1981).

The block to polyspermy in rainbow trout egg appears to be achieved by the morphological elimination of excess spermatozoa from the micropylar apparatus and the egg surface facing the inner opening of the micropyle, and the cortical reaction as described by many investigators (Brummett and Dumont, 1979; Iwamatsu and Ohta, 1981; Hart and Donovan, 1983; Guraya, 1986).

References

Amor, M.J. and M. Durfort, 1990. Atypical spermatogenesis in *Murex brandaris*. *Mol. Reprod. Dev.* **25:** 357-363.

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

Davis, T.L.O., 1977. Reproductive biology of the freshwater catfish, *Tandanus tandanus* Mitchell, in the Gwydir river, Australia I. Structure of the gonads. *Aust. J. Mar. Freshwater Res.* **28:** 139-158.

Focarelli, R., D. Rosa, and F. Rosati, 1991. The vitelline coat spikes: A new peculiar structure of *Mytilus galloprovincialis* eggs with a role in sperm-egg interaction. *Mol. Reprod. Dev.* **28:** 143-149.

Guraya, S.S., 1986. The Cell and Molecular Biology of Fish Oogenesis, Karger, India, 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.* **6:** 57-64.

Kessel-Shih, 1976. Scanning Electron Microscopy in Biology (A student' atlas on biological organization), pp. 334-337.

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 Microsc.* **23:** 115-128.

Lee, J-H, J-M Yoon, and H-Y Park, 1989. Histological changes of oocytes development and hormone levels in the Israeli carp (*Cyprinus carpio*) from February to May. *J. Aquacult.* **2:** 21-32.

Lee, T.H. and F. Yamazaki, 1989. Cytological observations on fertilization in the Chinese fresh-water crab, *Eriocheir sinensis*, by artificial insemination (in vitro) and incubation. *Aquaculture* **76:** 347-360.

Leung, L.K.P., 1988. Ultrastructure of the spermatozoon of *Lepidogalaxias salamandroides* and its phylogenetic significance. *Gamete Research* **19:** 41-49.

Oh, Y-S, 1995. Gonadal Sex Differentiation and Ultrastructures of Sperm in Chum Salmon (*Oncorhynchus keta*), Kon-Kuk Univ., Master thesis, pp. 23.

Ojima, Y. and S. Makino, 1978. Triploidy induced by shock in fertilized eggs of the carp (A preliminary study). *Pro. Jpn. Acad.* **54:** 359-362.

Park, H.-Y., S.-C. Roh, K.-R. Kang, S.-H. Lee, and J.-M. Yoon, 1993. Breeding and reproductivestudies on Korean native loach VI. Histological changes of pituitary gland and oocytes from March to May. *J. Anim. Sci. Tech.* **18:** 23-45.

Saad, A., R. Billard, M.C. Theron, and M. G. Hollebecq, 1988. Short-term preservation of carp(*Cyprinus carpio*) semen. *Aquaculture* **71:** 133-150.

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

Van Oordt, P.G. W.J., J. Peute, R. Van Den Hurk, and J.A.R. Viveen, 1987. Annual correlative changes in gonads and pituitary gonadotropes of feral African

catfish, *Clarias gariepinus*. *Aquaculture* **63:** 27-41.

Wolenski, J.S. and N.H. Hart, 1987. Scanning electron microscope studies of sperm incorporation into the zebrafish egg. *J. Exp. Zool.* **243:** 259-273.

Yoon, J.-M., G.-W. Kim, and H.-Y. Park, 1991. Studies on genetics and breeding in rainbow trout (*Oncorhynchus mykiss*). VI. Developmental stages of oocytes in reproductive cycles. *Korean J. Ichthyol.* **3:** 148-165.

Yoon, J.-M., G.-W. Kim, S.-C. Roh, and H.-Y. Park, 1993. Breeding and reproductive studies on Korean native loach. V. Ultrastructures changes of pituitary gland and oocytes from March to May. *J. Animal Scie. Tech.* **18:** 11-22.

Yoon, J-M, G-W Kim, C-K Park, and S-C Roh, 1994. Cell structures of spermatigenesis of rainbow trout (*Oncorhynchus mykiss*) in reproductive cycles. *Korean J. Elec. Microsc.* **24:** 55-66.

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정자 침입전후 무지개 송어의 난문에 대한 미세구조적 변화

윤종만 · 정구용[†] · 류동석[‡] · 류인덕[¶] · 노순창[§] · 김계웅[§§](건국대학교 동물자원연구센타, [†]상지대학교 동물자원학과, [‡]청주대학교 이공대학 생물학과, [¶]충주산업대학교 식품공학과, [§]건국대학교 자연과학대학, [§§]공주대학교 산업과학대학)

본 연구는 건국대학교 부설 양어장에서 사육중인 체중 700-1,200 g인 무지개 송어 암컷과 수컷을 공시어로 이용하였고, 주사 및 투과형 전자현미경을 이용하여 수정전후에 나타나는 난문의 형태 변화를 시간별로 조사하였다. 미수정된 난문의 외측부는 약 15-16 μm 이고, 내측부는 성숙된 난문의 내부 작경이 약 3-4 μm 인 깔때기 모양의 통로로서 돌출부에 의해서 둘러 싸여져 있다. 이 통로는 나중에 1.5 μm까지 수축되어 1 ml 당 10억마리 이상의 정자중 그 두부 직경이 약 1~1.2 μm 인 정자 1마리만이 간신히 침입하여 수정이 이루어지게 되는 적합한 크기를 가지고 있다. 정자가 외측부에 머물다가 두부가 내측부에 들어가는 시간이 20초 정도 걸리며, 정자두부가 들어가게 되고 꼬리가 보이는 과정까지 30초 이내의 시간이 걸린다. 이 때 난내부를 들여다 보면 수정막 바로 아래 층에 있던 표층과립의 파열이 시작된다. 정자의 꼬리가 완전히 들어가는 데 걸리는 시간이 60초 정도가 경과된다. 정자가 완전히 들어가면 난내부에 있던 표층과립이 난문을 통해서 난문 외측부로 완전히 융기하는 데 걸리는 시간이 60초로부터 150초 사이에 일어난다. 수정된지 250초가 경과되면 융기된 수정추가 다시 수축되어 개구부를 완전히 봉쇄하게 된다. 궁극적으로 1개의 정자만이 수정을 하게끔 허용하게 되고 더 이상의 정자가 침입하는 것을 방지하게 된다. 수정추의 형성은 결과적으로 다정자 침입의 억제 및 차단에 관여한다.