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SEM Study of Sperm Penetration in Polyspermic Urodele Amphibian (*Ambystoma mexicanum*) Eggs

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유미양서류 *Ambystoma mexicanum*란의 다수정 현상에 대한 주사 전자현미경적 연구

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요 약

유미 양서류의 일종인 액솔로틀 (*Ambystoma mexicanum*)의 정자가 난자 속으로 들어가는 일련의 과정을 관찰하였다.

액솔로틀의 난은 전형적인 다수정 현상을 보이는 것으로 나타났으며, 수정이 일어날 때 정자의 꼬리를 포함한 모든 부분이 난자의 표면을 뚫고 들어간다. 각각의 정자가 들어가는 점은 난자 표면에 명확한 부분 즉, 정자 침입점으로 구별된다. 그러나, 수정이 시작되는 단계에서는 난자의 표면에 별다른 붕괴현상이 관찰되지 않으며, 일단 수정이 끝난 후에는 정자 침입점은 긴 미세융모로 덮이게 된다.

INTRODUCTION

The urodele amphibian egg is typically polyspermic. As many as 12 (or more) sperm penetrate a single axolotl egg without generating developmental abnormalities. More usually, however, natural spawnings yield urodele eggs with approximately three sperm-penetration each (Fankhauser, 1932). The sperm penetration sites are marked on the surface of the egg by a distinct crater the "sperm pit". Compared with typical anuran eggs the

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urodele sperm entrance site is much more distinct. No similar sperm pit exists on anuran eggs (Elinson and Manes, 1978). Anuran eggs are probably always monospermic. An electrical block to polyspermic acts in anuran eggs to prevent multiple sperm penetration. Shortly after the first sperm touches the egg surface the egg membrane quickly responds by generating an efflux of chloride ions which causes a depolarization througout the egg surface. For example, in *Rana pipiens* the membrane potential changes from −28 mV to +6 mV in less than one second (Cross and Elinson, 1980; Schlichter and Elinson, 1981). For *Xenopus laevis* eggs, a rapid depolarization from −19 mV to +8 mV has also been reported recently (Grey *et al.*, 1982).

Urodele eggs display no such rapid block to polyspermy. The urodele egg also appears to lack cortical granules, as compared to anuran eggs. Whether sperm penetration in urodele (*Ambystoma mexicanum*) eggs is similar to anuran eggs was examined in this report. The sperm penetration process was observed and comparisons were made to the fertilization reaction in anuran eggs.

METHODS AND MATERIALS

Axolotl eggs were obtained from natural matings at the Indiana University Axolotl Colony. As eggs were shed from the female's cloaca they were collected and their development timed. It was possible to collect eggs at 10 min intervals. Since eggs are fertilized as they pass through the cloaca, collecting eggs at frequent intervals provided a method for estimating the time of fertilization.

At appropriate intervals eggs were manually de-jellied with watchmaker's forceps and fixed for scanning electron microscopy (Youn *et. al.*, 1981). The procedures of the fixation is as follows. Eggs were fixed overnight at 4°C in a 2.5% glutaraldehyde solution buffered with 0.1 M cacodylate (pH 7.2). The fixed eggs were rinsed in 0.1 M cacodylate buffer and then post-fixed at 4°C in cacodylate-buffered (0.1 M, pH 7.5) OsO_4 for 3 hours. Dehydration in a gradient alcohol series was followed and the samples were critical point dried. After mounting on aluminium stubs specimens were coated with gold-palladium and examined under the SEM. Photographs were taken on Polaroid type 55 positive-negative film.

RESULTS AND DISCUSSION

The axolotl sperm distinct undulating membrane (Fig. 1) which appears to penetrate the egg surface completely (Figs. 2-5). Multiple sperm penetrations in a relatively localized area of the egg are possible. The photograph in Fig. 3 shows 4 separate penetration sites in a relatively close proximity to one another. Occasionally, sperm entrance occurs in the region of the vegetal pole, which is a substantial distance from the location of the female

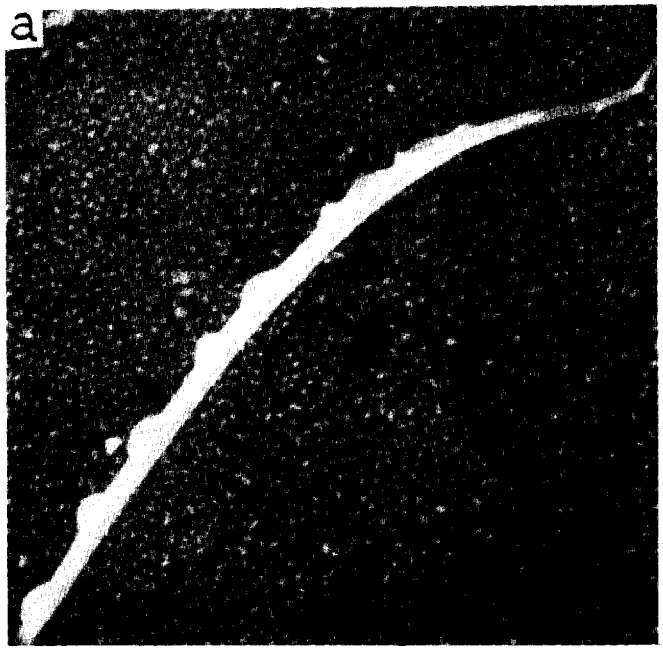

Fig. 1. The axolotl sperm tail displays an undulating membrane at high magnification (1,600 X).

pronucleus (animal pole). Perhaps the long interval (approx. 6 hrs, 20°C) between sperm attachment and the first cleavage division has evolved to accomodate sperm penetrations which occur at great distances from the location of the egg nucleus.

The pigmentation alterations associated with the sperm entrance sites provide a distinct spot on the egg surface so called "sperm pit". Under dissecting microscope the sperm pit is easily visualized on the animal hemisphere as a dark spot (Fig. 2). On the vegetal hemisphere surface, or on the surface of albino eggs the sperm entrance site can be seen as a distinct and clearly defined depression (or "pit"). Within two hrs (20°C) after sperm entrance the intensity of the dark spot diminishes. Within 3~4 hrs after penetration the site of entry is almost impossible to locate as a dark spot. Only on exceptional eggs can any remnant of the dark spot be visualized by the time of the first cleavage division and furrow formation have begun (Fig. 2).

The reaction of the egg surface to sperm penetration is broad. At the exact site of penetration, however, the surface appears to be devoid of microvilli which are present over the rest of the egg surface (Fig. 4). As sperm penetration proceeds, no alteration in the egg surface was observed (Fig. 5). It was not possible to precisely calculate the time required for sperm penetration, since artificially inseminated eggs were not employed for these studies. Even if they were, it would not be possible to exactly establish the time required for penetration since eggs which displayed several sperm penetrations exhibited varying lengths of sperm tails among the multiple sperm seen entering the egg. Never-

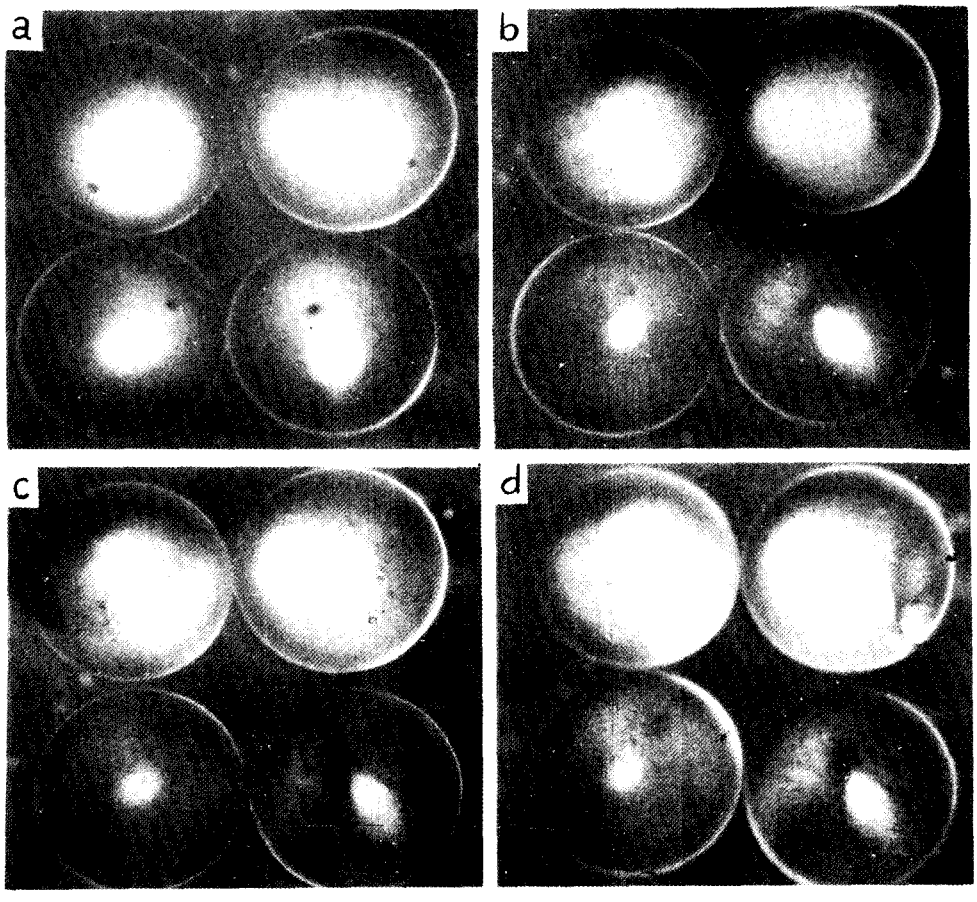

Fig. 2. Appearance of the sperm entrance site on the surface of newly fertilized axolotl eggs: (a) eggs photographed 30 min after passage through the cloaca; (b) 100 min later than (a); (c) 60 min later than (b); (d) approximately 6 hrs after insemination. The egg on the upper right displays the origin of the first cleavage furrow (large arrow). Small arrows indicate sperm entrance sites.

theless, by comparing photographs of several dozen eggs, an estimate of the time for the sperm to penetrate halfway was approximately 10 min (20°C).

Once penetration was complete long microvilli became concentrated at the center of the sperm pit (Fig. 6). Similar observations have been made by Picheral (1977b) for ***Pleurodele*** (urodele) eggs and by Elinson and Manes (1979) for ***Rana*** (anuran) eggs. Perhaps those microvilli are important for healing the wound created by sperm penetration. The similarity between the appearance of the microvilli in urodele and anuran eggs is somewhat unexpected. Cortical granules are known to be present in the cortex of the anuran eggs. Their breakdown following egg activation is considered to be important for altering the egg surface quickly after sperm penetration. Urodele eggs appear to lack cortical granules.

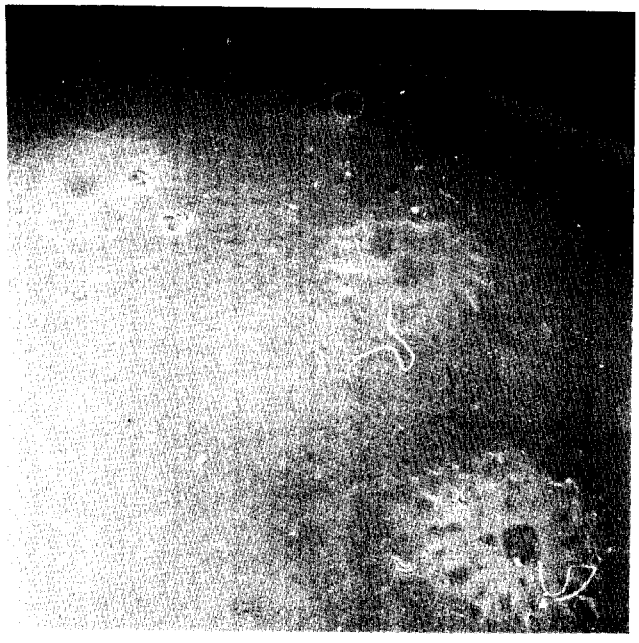

Fig. 3. Multiple sperm entrance sites on the surface of an axolotl egg (150 X). Fixed at approx. 20 min after fertilization.

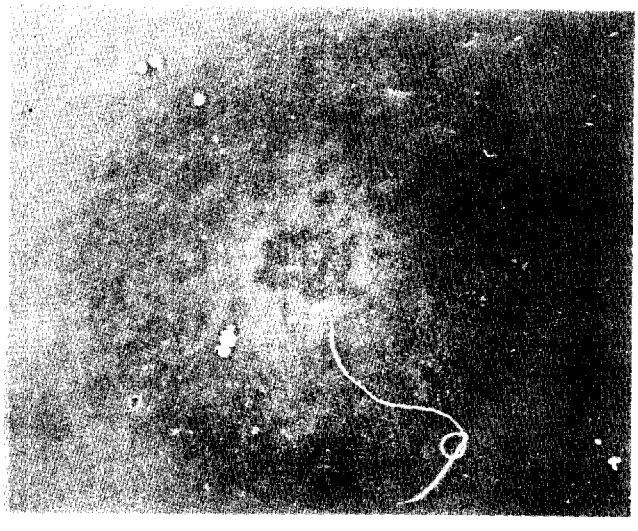

Fig. 4. Sperm entrance appears to cause a localized change in the egg plasma membrane which spreads beyond the actual penetration site (128 X).

The generation of the long microvilli at the sperm entrance site is apparently, therefore, unrelated to that of cortical granules.

Further ultrastuctural studies should perhaps be directed towards attempting to understand the mechanism which accounts for the healing of the wound site. Transmission electron micrographs may be required to reveal the nature of the membrane components which are involved in wound healing.

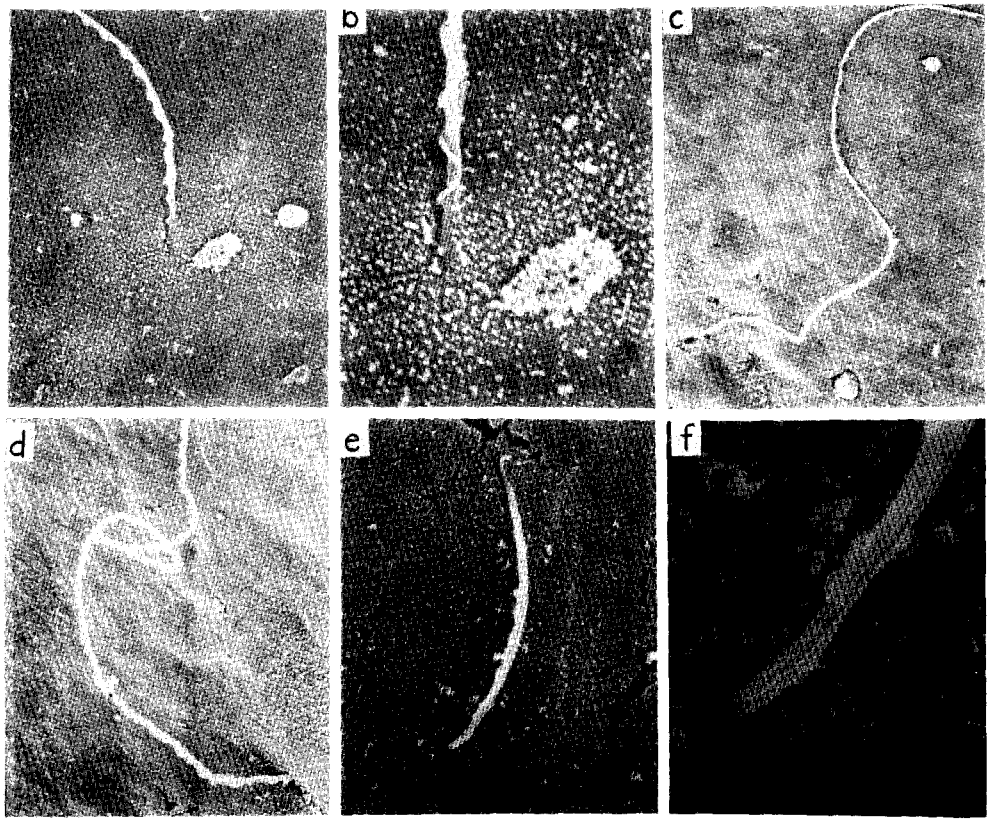

Fig. 5. Initially, no distinct alterations in the microvillus structure of the area around the sperm entrance site are observed: (a) 10 min after fertilization. (640 X); (b) higher magnification view of (a), 1,600 X; (c), (d), (e) represent additional views of the sperm entrance stie as penetration is underway. No evidence for a major disruption of the plasma membrane was observed. (f) higher magnification view of (e), 6,000 X

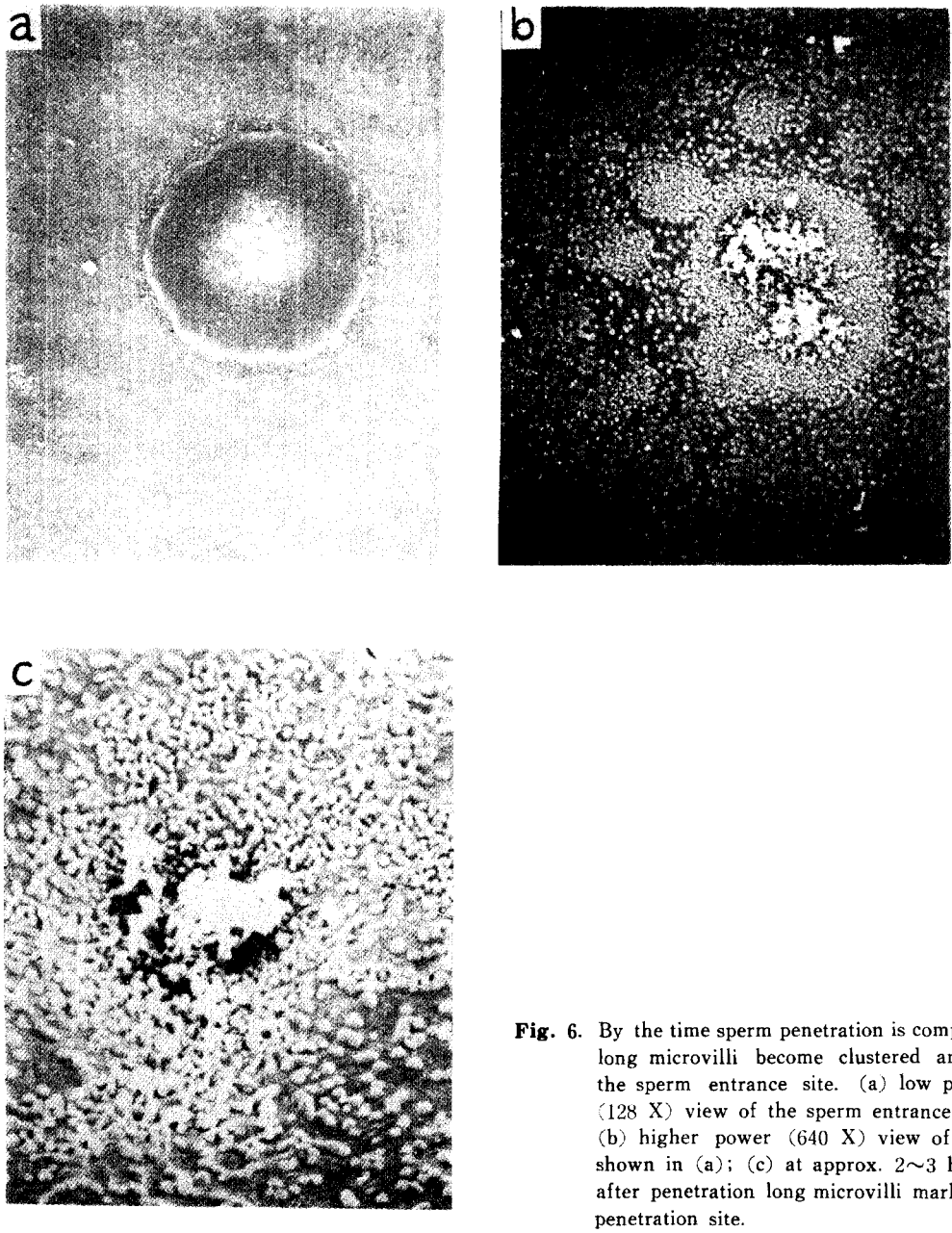

Fig. 6. By the time sperm penetration is complete, long microvilli become clustered around the sperm entrance site. (a) low power (128 X) view of the sperm entrance site; (b) higher power (640 X) view of site shown in (a); (c) at approx. 2~3 hours after penetration long microvilli mark the penetration site.

SUMMARY

A series of observation on sperm penetration in urodele (*Ambystoma mexicanum*) eggs are reported. The whole sperm including the tail appears to penetrate the egg surface. It

can be demonstrated that the *Ambystoma mexicanum* egg is typically polyspermic. Each sperm penetration point is marked by a distinct crater on the egg surface the so called sperm pit. Initially, no sign of disruption in the surface structure observed. Once sperm penetration was complete, the site of entry became covered with long microvilli.

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