Short Paper

Extension of the Fertilization Envelope
in the Japanese Mitten Crab

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In crabs, the extension of the fertilization envelope has been known mainly in studies on the formation of the funiculus between the egg and the ovigerous seta.¹⁻³⁾ Researchers generally assume that the extension of the funiculus is caused by the physical movement of the maternal pleopod after the fertilization envelope has been attached to the seta.^{1,3,4)} In the present study on the Japanese mitten crab, *Eriocheir japonicus*, we found another type of extension of the newly formed fertilization envelope in artificially fertilized (*in vitro*) eggs.

The techniques and basic knowledge of artificial fertilization (*in vitro*) used in the present study have been presented in earlier papers.^{5,6)} Mature Japanese mitten crabs were collected from the estuary of the Shiodomari river in Hakodate, Japan, in June 1991 during the crab's spawning season. The medium used for sperm dilution, insemination, and incubation was sea water (salinity 34.5‰). The sperm was rinsed with sterilized sea water five times by centrifugation. The rinsed sperm was diluted to 1,250,000 spermatozoa/ml, which is the minimum density for 100% fertilization.^{*1} The eggs and sperm used in this study were taken from one copulated female. After insemination, the eggs were rinsed three times. Different numbers (1, 10, 20, 50, 100, 424, 2500) of fertilized eggs were incubated separately in sterile culture dishes (60×15 mm) with 10 ml of sterile sea water at 20°C. To compare with the effect of fresh medium, the used medium (medium used for incubation for 7 hours at a density of 2,500 eggs/10 ml) was used to incubate a single egg. The culture dishes were kept in an orbital shaker (18 cycles/min, orbit="figure-eight" pattern) to prevent the eggs from sticking to the culture dishes. Thirty-one hours after

Fig. 1. Diameters of fertilization envelopes observed 31 h after fertilization (32-cell stage) displaying an increase with high egg density (eggs/10 ml) and with used medium (medium used for 2,500 eggs/10 ml for 7 h). Mean value $\pm$ (SD). n = number of observed eggs.

Fig. 2. Fertilization envelopes incubated in different egg densities and different media.

All the eggs were observed 31 h after fertilization (32-cell stage). a, single egg/10 ml in fresh medium. b, 10 eggs/10 ml in fresh medium. c, 20 eggs/10 ml in fresh medium. d, 50 eggs/10 ml in fresh medium. e, 100 eggs/10 ml in fresh medium. f, 424 eggs/10 ml in fresh medium. g, 2,500 eggs/10 ml in fresh medium. h, single egg/10 ml in used medium (medium used for 2,500 eggs/10 ml for 7 h). Arrow indicates the fertilization envelope. Star indicates the egg body. Scale bar = 300 μm.

artificial insemination (32-cell stage), the eggs were randomly selected for measurement of the diameters of their fertilization envelopes by micrometer. All observations were performed with light microscopy.

The fertilization envelopes of the fertilized eggs started to extend immediately after insemination. No further extension was observed beyond 1 hour after insemination. Figures 1 and 2 display an increase in the diameters of the fertilization envelopes with eggs incubated with fresh medium at higher densities. The minimum diameters were 330–340 μm at a density of single egg/10 ml and the maximum diameters were 380–390 μm at a density of over 100 eggs/10 ml. However, in the case of eggs incubated in single egg/10 ml with the medium used, the diameters of the fertilization envelopes were about 48 μm longer on average than those of the eggs incubated individually in fresh medium, and were almost the same as those of the eggs incubated at a density of over 100 eggs/10 ml with fresh medium.

These results strongly imply that the extension of the fertilization envelope is related to a change in the quality of the medium, and this change may be induced by some substance produced by the eggs.

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*1 Lee, unpublished data.