

*The effects of the treatment of spermatozoa  
with acid sea water on re-insemination  
of sea urchin eggs,  
Hemicentrotus pulcherrimus.*

by Akira KOENUMA

*Department of Biology, Faculty of Liberal Arts and Science, Shinshu University.*

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It has been well known that the polyspermic fertilization takes place frequently in the insemination of the sea urchin eggs treated with various reagents. In sea urchin eggs, it has been accepted that activated eggs are not fertilizable even if the fertilization membrane has been removed. This suggests that some sort of sperm block mechanism may be present on the surface of the activated egg. This sperm block mechanism may be made invalid by the treatment of the eggs with Ca-Mg-free media (ISHIDA, J. and E. NAKANO '50, SUGIYAMA, M. '51, NAKANO, E. '54).

According to AMOROSO and PARKES, polyspermy occurred in the egg of a rabbit, when it was inseminated with X-irradiated spermatozoa ('47). The occurrence of the polyspermic division of eggs by the insemination with modified spermatozoa was reported in a echiuroid worm, *Urechis uncinatus*, and in a sea urchin, *Hemicentrotus pulcherrimus* (KOENUMA, A. '56, '59). But in sea urchin eggs the effect of modified spermatozoa was not evident. Perhaps, the rapid formation of the fertilization membrane in sea urchin egg may have prevented the penetration of spermatozoa. If sea urchin eggs treated with or without Ca-Mg-free-medium, of which fertilization membranes are removed mechanically after the first insemination are inseminated with modified spermatozoa, their effect will come out apparently. This work was carried out under such an expectation.

#### Material and Methods

The eggs used in the experiments were those of the sea urchin, *Hemicentrotus pulcherrimus*, which were obtained near the marine biological station of Misaki, Kanagawa. The unfertilized eggs used in the experiments were collected by the spawning caused by the treatment of a female animal with KCl solution. The sperm used was obtained from the gonads removed from male animal.

The insemination was carried out by the transference of the eggs into

a sperm suspension with a small volume of sea water. The undiluted or "dry" sperm was taken as an arbitrary standard and various concentrations of sperm suspensions were made from this by dilution. The sperm concentrations used in the experiments were  $10^{-3}$  and  $10^{-4}$ . In the insemination with modified spermatozoa, the sperm was treated for 5 minutes with acid sea water, pH 6.5, by means of the dilution 10 times denser than the used concentration, and then it was diluted by normal sea water to the used one.

Experiments were carried out in a room temperature which was between  $9^{\circ}\text{C}$ . and  $12^{\circ}\text{C}$ .

Eggs were inseminated and observed in following cases:

- (1) Eggs were inseminated with  $10^{-4}$  intact sperm suspension.
- (2) Eggs were deprived of their fertilization membranes by sucking and blowing the eggs into and out of a narrow pipette 2 minutes after the insemination with  $10^{-4}$  intact sperm suspension.
- (3) Eggs which were inseminated with  $10^{-4}$  intact sperm suspension and deprived of their fertilization membranes by the method mentioned above were re-inseminated with  $10^{-3}$  intact sperm suspension.
- (4) Eggs were inseminated with  $10^{-4}$  modified sperm suspension.
- (5) Eggs were re-inseminated with  $10^{-3}$  and  $10^{-4}$  intact sperm suspensions after a series of treatments, where eggs were inseminated at first with  $10^{-4}$  intact sperm suspension, and deprived of their fertilization membranes by the previous method, then immersed with 1/2 M NaCl-KCl mixture, 27:3, pH 7.8, for 5 minutes and finally washed with normal sea water.
- (6) Eggs were re-inseminated with  $10^{-3}$  and  $10^{-4}$  modified sperm suspensions after a series of treatments mentioned above.
- (7) Fertilized eggs of which fertilization membranes were removed by sucking and blowing method were re-inseminated with  $10^{-3}$  modified sperm suspension without the treatment of Ca-Mg-free medium.
- (8) Eggs which were activated by the insemination with  $10^{-4}$  modified sperm suspension and deprived of their fertilization membranes by sucking and blowing method, were re-inseminated with  $10^{-3}$  intact sperm suspension without the treatment of Ca-Mg-free medium.
- (9) Eggs which were activated by the insemination with  $10^{-4}$  modified sperm suspension and deprived of their fertilization membranes by the method above were kept intact after the treatment of Ca-Mg-free medium.

The inseminated eggs were observed at first cleavage.

Statistical test was carried out by the examination of two hundred eggs in each experiment. In the table only the percentage figures are given.

### Results and Discussion

The results of the experiments are given in the table 1. In this table, undivided eggs, normally divided eggs and polyspermic eggs represent the eggs which did not divide, divided into two cells and divided into 3 or 4 cells at first cleavage respectively. In all the experiments almost every egg formed fertilization membrane by first insemination.

**Table 1.** Results of the inseminations in the eggs of sea urchin, *Hemicentrotus pulcherrimus*.

Treatments of eggs				Eggs at first cleavage		
first insemination	deprival of fertilization membranes	treatment with Ca-Mg-free medium	second insemination	Undivided eggs (%)	Nomally divided eggs (%)	Poly-spermic eggs (%)
10 <sup>-4</sup> n. sp.	—	—	—	2.8	96.5	0.4
10 <sup>-4</sup> n. sp.	+	—	—	54.4	43.1	2.4
10 <sup>-4</sup> n. sp.	+	—	10 <sup>-3</sup> n. sp.	64.1	34.5	1.4
10 <sup>-4</sup> mod. sp.	—	—	—	6.4	92.1	1.5
10 <sup>-4</sup> n. sp.	+	+	10 <sup>-3</sup> n. sp.	31.7	63.3	5.0
10 <sup>-4</sup> n. sp.	+	+	10 <sup>-4</sup> n. sp.	48.8	43.8	7.6
10 <sup>-4</sup> n. sp.	+	+	10 <sup>-3</sup> mod. sp.	40.9	38.0	21.1
10 <sup>-4</sup> n. sp.	+	+	10 <sup>-4</sup> mod. sp.	28.2	9.8	60.0
10 <sup>-4</sup> n. sp.	+	—	10 <sup>-3</sup> mod. sp.	48.9	21.7	29.4
10 <sup>-4</sup> mod. sp.	+	—	10 <sup>-3</sup> n. sp.	51.8	39.3	9.0
10 <sup>-4</sup> mod. sp.	+	+	—	31.6	66.6	1.8

n. sp. : normal intact sperm

mod. sp. : modified sperm

+ : received the treatment

— : did not receive the treatment

*The effect of the modified spermatozoa in the first insemination.*

It is seen in the table 1 that when the eggs were kept intact after the insemination, the modified spermatozoa and the intact spermatozoa produced almost common results. When the eggs, activated by intact spermatozoa and then deprived of their fertilization membranes were inseminated with intact spermatozoa, any increase of the polyspermic eggs did not occur. But when the eggs activated by modified spermatozoa and deprived of their fertilization membranes were inseminated with modified spermatozoa, the polyspermic eggs increased to some extent. These results may indicate a somewhat imperfect sperm block mechanism on the surface of the eggs which were activated by modified spermatozoa. In the eggs which were kept intact after the activation by modified spermatozoa, a result that any increase of polyspermic eggs did not occur may be explained by a rapid formation of the fertilization membranes.

*The effect of the modified spermatozoa in the re-insemination.*

In the table it is shown that when the activated eggs were re-inseminated with modified spermatozoa, the polyspermic eggs increased to some extent even in the case that the eggs were inseminated under the condition in which any increase of polyspermic eggs was not produced by the insemination of intact spermatozoa. This fact suggests that the penetrating power of the modified spermatozoa used in this experiment is larger than that of the normal intact spermatozoa.

It is also seen in the table that when the activated and denuded eggs

treated with Ca-Mg-free medium were re-inseminated with modified spermatozoa, there was another evidence that the penetrating power of the modified spermatozoa was larger than that of the intact spermatozoa.

Somewhat unexpected results that the increase of polyspermic eggs in the re-insemination with  $10^{-4}$  sperm suspension was larger than that in the re-insemination with  $10^{-3}$  sperm suspension may be explained by a fact that the undivided eggs in the table imply both unfertilized eggs and polyspermic eggs of which cleavages are inhibited by the penetration of too many spermatozoa into each egg.

The fact that polyspermy was produced by the insemination with modified spermatozoa has been reported by AMOROSO, E. C. and A. S. PARKES when a rabbit egg was inseminated with the spermatozoa treated with X-ray irradiation ('47).

When eggs of echiuroid worm and sea urchin were inseminated with spermatozoa treated with uranyl nitrate or acid sea water, other facts that the eggs exhibiting polyspermic cleavage increased in the insemination with modified spermatozoa were reported by KOENUMA, A. ('56, '59). But it was difficult to regard those facts as the decided evidences of the real polyspermy. The polyspermic cleavage of activated eggs does not always mean the real polyspermic eggs in the *Urechis*' eggs, for those eggs extrude the polar bodies after the penetration of spermatozoa, accordingly the failure of polar body extrusion in those eggs may be in a polyspermic appearance. In the case of sea urchin eggs, the increase of polyspermic eggs was so small that the effect of the modified spermatozoa was not evident.

Present results augmented the possibility of some interaction of spermatozoa on the formation of sperm block mechanism in fertilized sea urchin eggs, but the role of spermatozoa concerned must be complementary, for the sperm block mechanism has been clarified even if the eggs have been activated parthenogenetically (ISHIDA, J. and E. NAKANO '50, NAKANO, E. '54).

### Summary

The effects of the treatment of spermatozoa with acid sea water on re-insemination of the eggs of sea urchin, *Hemicentrotus pulcherrimus* were investigated. In the eggs activated by the insemination with modified spermatozoa, the increase of polyspermic eggs occurred in the re-insemination of the denuded eggs without the treatment of Ca-Mg-free medium. The increase of polyspermic eggs resulted by the re-insemination with modified spermatozoa, even if the eggs were inseminated under the condition where they were not fertilizable by normal intact spermatozoa. From these results it may be concluded that spermatozoa play a complementary role on the formation of sperm block mechanism in fertilized eggs and the penetrating power of the spermatozoa modified by acid sea water is larger than that of the normal intact spermatozoa.

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