Short Paper

Triplid Rainbow Trout Induced by High-pH·High-calcium

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(Received February 24, 1988)

It has been reported that the polyethylene glycol (PEG) has a good effect on the fusion or adhesion of rainbow trout sperms, and the dispermy was induced by the fertilization with PEG treated sperm.¹⁾ On the other hand, the effect of high-pH·high-calcium on the tobacco leaf protoplast fusion has been found.²⁾ In this study of rainbow trout *Salmo gairdneri*, the effect of high-pH·high-calcium treated sperms on the induction of the dispermy and that of high-pH·high-calcium on the prevention of the second polar body extrusion were examined. Eggs and seminal fluid of rainbow trout were obtained from the Nikko Branch of the National Research Institute of Aquaculture, Fisheries Agency (Tochigi Prefecture) in December, 1987. In the strain of rainbow trout used here, the chromosomal polymorphisms, $2n=60, 61, 62, 63$ and 64 , were observed.³⁾ They had the same NF (nombre fundamental), 104.³⁾

Experiment 1: 0.5 ml of seminal fluid was put into a centrifuged tube (16 mm in diameter) and the centrifugation was made at 2,000 rpm for 3 min. As the control, after the removal of the supernatant, the sperms suspended in 5 ml of A-solution (750 mg NaCl, 220 mg KCl dissolved in 100 ml of the distilled water) were used for the fertilization (group I). On the other hand, after the removal of the supernatant, the sperms were treated with 5 ml of high-pH·high-calcium solution (750 mg NaCl, 220 mg KCl, 1,110 mg CaCl₂ dissolved in 100 ml of the distilled water, adjusted to be pH 10 with NaOH) for 20 min at room temperature. After the removal of the supernatant, the sperms were washed with A-solution three times. The sperms were suspended in the A-solution by gentle pipetting. Then, the tube was allowed to stand for 5 min at room temperature. The upper part of the suspension was collected on a blood corpuscles counting chamber, and the sperms were observed under the phase-contrast microscope. In this method without the lower part of the suspension, the single sperm was observed at 73.2% and the fusion or adhesion between two sperms was observed at 26.8%. And then these treated sperms were fertilized with normal eggs (group II). The survival rates at the eyed stage were 51.7% in the group I, and 6.3% in the group II (Table 1). The chromosome slides of the eyed embryos in each group were made by the short-term-culture method.⁴⁾ In the group I 26 diploids ($2n=60$ and $NF=104$; 8 specimens, $2n=61$ and $NF=104$; 12 specimens,

$2n=62$ and $NF=104$; 5 specimens, $2n=63$ and $NF=104$; 1 specimen) and 2 triploids ($3n=92$ and $NF=156$), and in the group II 8 diploids ($2n=60$ and $NF=104$; 3 specimens, $2n=61$ and $NF=104$; 4 specimens, $2n=62$ and $NF=104$; 1 specimen) and 4 triploids ($3n=91$ and $NF=156$; 2 specimens, $3n=92$ and $NF=156$; 2 specimens) were found (Table 1). We don't think that the appearance to triploids even in the control (group I) is particularly phenomenon, because spontaneous triploids in the family *Salmonidae* have been reported⁵⁻⁷⁾ and we sometimes found triploid rainbow trouts resulted from routine method of artificial fertilization. The incidence of triploids increased ($\chi^2=4.25$, $d.f=1$, $p<0.005$). It was considered that the dispermy was induced by the fertilization with high-pH·high-calcium treated sperms.

Experiment 2: Ten min after normal fertilization, the fertilized eggs were divided into two. One hand was untreated (group III), and the other hand was immersed in high-pH·high-calcium solution (780 mg NaCl, 28 mg KCl, 1,110 mg CaCl₂ dissolved in 100 ml of the distilled water, adjusted to be pH 10 with NaOH) for 20 min at room temperature (group IV). The survival rates at the eyed stage were 29.4% in the group III, and 14.7% in the group IV (Table 1). The chromosomes of the eyed embryos in each group were analyzed by the above mentioned method. In the group III 15 diploids ($2n=60$ and $NF=104$; 4 specimens, $2n=61$ and $NF=104$; 8 specimens, $2n=62$ and $NF=104$; 3 specimens) and 1 triploid ($3n=91$ and $NF=156$), and in the group IV 2 diploids ($2n=61$ and $NF=104$) and 8 triploids ($3n=90$ and $NF=156$; 5 specimens, $3n=91$ and $NF=156$; 3 specimens) were found (Table 1). The incidence of triploids increased ($\chi^2=14.79$, $d.f=1$, $p<0.01$). It was guessed that the effect of high-pH·high-calcium on the prevention of the second polar body extrusion induced the triploids.

References

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Table 1. The influence of high-pH·high-calcium on the survival rate at the eyed stage and on the incidence of triploids

Group no.	Sperm	Treatment Fertilized egg	Survival (%)	No. of specimen		3n (%)
				2n	3n	
I	—* ¹	—	51.7	26	2	7.1
II	+* ²	—	6.3	8	4	33.3
III	—	—	29.4	15	1	6.3
IV	—	+	14.7	2	8	80.0

*¹ Untreated. *² Treated.

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