42. Triploid Rainbow Trouts induced by Polyethylene Glycol

By Takayoshi UEDA,*¹ Michiko KOBAYASHI,*¹ and Ryozo SATO**²

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The trend of the application of the cell technology to the study of fishes has come to the fore. The artificial productions of triploids, all female or male fishes, homozygous diploids, and clones, *etc.* were made by the chromosomal technology. On the other hand, the technique of nuclear transplantation has been a trial of, but the method with micropipettes is far from the generalization. Then, we have been searching the chemical methods in order to transplant the nuclear to the fish egg.

In this study, triploid rainbow trouts which resulted from the dispermy were induced by the fertilization using polyethylene glycol treated sperms.

Materials and methods. Eggs and seminal fluid were obtained from one female and one male rainbow trout *Salmo gairdneri* at the Nikko Branch of the National Research Institute of Aquaculture, Fishery Agency (Tochigi Prefecture). 0.2–5 ml of seminal fluid was put into a centrifugal 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 3 ml of A-solution (750 mg NaCl, 220 mg KCl and 470 mg HEPES 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 1 ml of polyethylene glycol (PEG)-solution (10 g PEG 4,000 dissolved in 10 ml of A-solution) for 1 min at room temperature. After 2 ml of A-solution was added, the tube was placed for 2 min at room temperature. After the tube was swinged gently, the top clear part of the fluid was removed. And then, the sperms were washed with A-solution three times. After the removal of the supernatant, the PEG treated sperms suspended in 3 ml of A-solution were used for the fertilization (group II). The chromosome slides of parents were made by the fin culture method and those of embryos by the direct and the culture methods (Ueda 1986).

Results. The karyotype of the maternal trout ($2n$, 60), as shown in Fig. 1, consisted of 44 meta- (M) and submetacentrics (SM), 2 subtelocentrics (ST), and 14 acrocentrics (A). Therefore, NF (nombre fondamental) was 104. The karyotype of the paternal trout ($2n$, 61), as shown in Fig. 2, consisted of 43 M and SM, 1 ST, 17 A. Therefore, NF was 104. In the group I, 5 specimens with 60 chromosomes (44 M·SM+2 ST+14 A, NF=104), 1 specimen with 60 chromosomes (44 M·SM+1 ST+15 A, NF=104), 5 specimens with 61 chromosomes (43 M·SM+2 ST+16 A, NF=104), and 4 specimens with 61 chromosomes (43 M·SM+1 ST+17 A, NF=104) were recognized (Fig. 3). In the group II, 4 specimens with 60 chromosomes (44 M·SM+2 ST+14 A, NF=104), 4 specimens with 60 chromosomes (44 M·SM+1 ST+15 A, NF=104), 1 specimen with 61 chromo-

*¹) Dept. of Biol., Fac. of Ed., Utsunomiya Univ., Utsunomiya 321, Japan.

**²) Nikko Branch, Natl. Res. Inst. of Aquacult., Fishery Agency, Nikko 321-16, Japan.

somes (43 M•SM+2 ST+16 A, NF=104), 5 specimens with 61 chromosomes (43 M•SM+1 ST+17 A, NF=104), 1 specimen with 90 chromosomes (66 M•SM+2 ST+22 A, NF=156), 2 specimens with 91 chromosomes (65 M•SM+2 ST+24 A, NF=156), 1 specimen with 91 chromosomes (65 M•SM+3 ST+23 A,

Fig. 1

Fig. 2

Figs. 1-2. 1: The karyotype of maternal rainbow trout. $2n=60$, M and SM=44, ST=2, A=14, NF=104 (XX-type). The arrows indicate 2 ST. 2: The karyotype of paternal rainbow trout. $2n=61$, M and SM=43, ST=1, A=17, NF=104 (XY-type). The arrow indicates 1 ST.

Fig. 3. The metaphase figures of the embryos in the groups I and II. The arrows indicate ST. a: $2n=60$, 44 M•SM+2 ST+14 A, NF=104 (XX-type). b: $2n=60$, 44 M•SM+1 ST+15 A, NF=104 (XY-type). c: $2n=61$, 43 M•SM+2 ST+16 A, NF=104 (XX-type). d: $2n=61$, 43 M•SM+1 ST+17 A, NF=104 (XY-type).

NF=156), 1 specimen with 92 chromosomes (64 M•SM+1 ST+27 A, NF=156), 1 specimen with 92 chromosomes (64 M•SM+2 ST+26 A, NF=156), and 1 specimen with 92 chromosomes (64 M•SM+3 ST+25 A, NF=156) were recognized (Figs. 3-5).

Fig. 4. The metaphase figures of the embryos in the group II. The arrows indicate ST. a: $3n=90$, 66 M•SM+2 ST+22 A, NF=156 (XXY-type). b: $3n=91$, 65 M•SM+2 ST+24 A, NF=156 (XXY-type). c: $3n=91$, 65 M•SM+3 ST+23 A, NF=156 (XXX-type). d: $3n=92$, 64 M•SM+1 ST+27 A, NF=156 (XYY-type). e: $3n=92$, 64 M•SM+2 ST+26 A, NF=156 (XXY-type). f: $3n=92$, 64 M•SM+3 ST+25 A, NF=156 (XXX-type).

Discussion. In the strain of rainbow trout which the parents belong to, the chromosomal polymorphisms, $2n=60, 61, 62, 63$ and 64 , and the XX/XY-sex chromosome system (ST was X and one A was Y chromosomes) were observed (Ueda and Ojima 1984a). The karyotypes of the parents were consistent with those of the previous study (Ueda and Ojima 1984a). The gametes with 30 (X) chromosomes were formed from the female with 60 chromosomes, and those with 30 or 31 (X or Y) chromosomes from the male with 61 chromosomes (Ueda and Ojima 1984b). In the groups I and II, the diploid specimens with 60 or 61

Fig. 5. The karyotype of the embryo in the group II. $3n=92$, M and $SM=64$, $ST=1$, $A=27$, $NF=156$ (XYY-type). The arrow indicates 1 ST.

chromosomes were observed, as was expected. Judging from the chromosomal constitution, the individuals with 90, 91 or 92 chromosomes in the group II were triploid. It must be certain that the triploid specimens with 92 chromosomes and/or just one ST (XYY) were produced by the dispermy. Because the triploid individuals which resulted from the dispermy induced by the fertilization with PEG treated sperms, PEG, the common chemical for cell fusion, may have a good effect on the fusion of rainbow trout sperms. And further, the nuclear sticking to genetical inactivated sperm may be transplanted to the fish egg. On the other hand, it was possible that as a little PEG remaining after washing acted on one egg membrane, the egg permitted two sperms to enter.

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