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Artificial Androgenesis Induced with Gamma Irradiation in Masu Salmon, *Oncorhynchus masou*

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Abstract

Mature eggs of masu salmon (*Oncorhynchus masou*) were exposed to gamma irradiation with a wide range from 10^2R to 10^5R and inseminated with normal unirradiated sperm. During the subsequent development, the survival rate fell with the increase of gamma doses, but, beyond a certain limit, this unfavorable condition was recovered and reached a peak at $5–6\times10^4$R, followed by the second drop in the survival rate. These facts demonstrated the presence of a "Hertwig effect" between the survival rate and the irradiation doses. The second fall in the survival rate may suggest a harmful effect of extremely high doses of irradiation to egg cytoplasm. The embryos that survived in the groups irradiated with $3–8\times10^4$R showed "haploid syndrome" in their morphological appearance and the measurement of the nuclear size of cartilage cells suggesting the haploidy of those embryos. From these data, it was concluded that the gamma irradiation of $5–6\times10^4$R could be the most effective agent to destroy genetic materials in the nucleus without injurious influences on the cytoplasm to induce androgenetic haploid development.

Radiation induced parthenogenesis was first described in frogs by Hertwig in 1911[1] and subsequent investigations were mainly done on amphibia. Such works in fish were reported in loach, carp, sturgeon,[2] flatfish and trout[3] but most of the research was restricted to gynogenesis. Experiments reported here examined the effective dose of cobalt-60 gamma irradiation to induce androgenetic development with favorable survival in masu salmon (*Oncorhynchus masou*). The presence of a "Hertwig effect" in gamma irradiation to the eggs was also ascertained in the present species.

Materials and Methods

Present work was performed with masu salmon, *Oncorhynchus masou*, which had been raised at the Mori branch of the Hokkaido Fish Hatchery, Mori is 45 km north of Hakodate. For the gamma irradiation of eggs, five females and three males of mature fish ranging from 27.5 cm to 34.5 cm in the fork-length were transported by car from the Mori hatchery to the Radio Isotope Research Center, Faculty of Engineering, Hokkaido University, Sapporo, 260 km north of Hakodate.

Mature eggs were obtained by the incision method, rinsed with isotonic solution and divided into 14 groups including 150 eggs each. Thirteen groups of them were exposed to cobalt–60 gamma irradiation for 30 minutes to give the following

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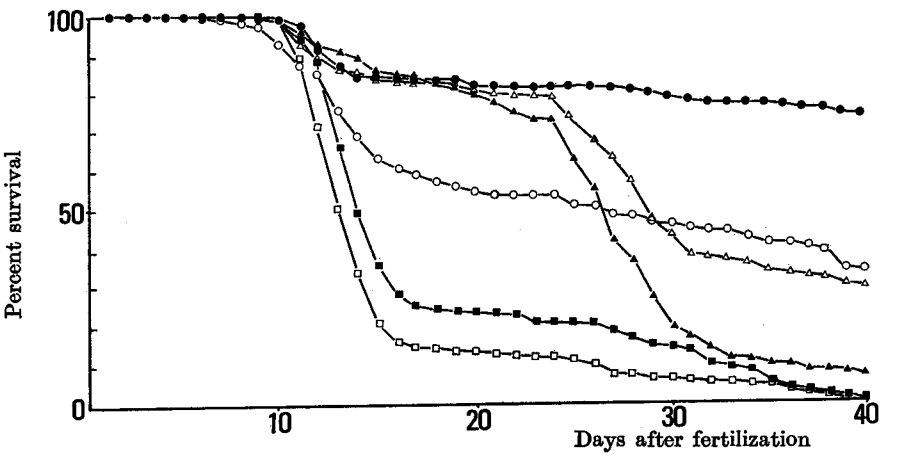
doses of 10^2, 10^3, 5×10^3, 10^4, 2×10^4, 3×10^4, 4×10^4, 5×10^4, 6×10^4, 7×10^4, 8×10^4, 9×10^4 and 10^5 roentogen (R) each. The rest of the group was not irradiated and served as the control. During the exposure to gamma irradiation, the eggs were cooled with cracked ice packed in a vinyl sac. Control eggs were also cooled for the same period without irradiation. After gamma irradiation, the eggs of irradiated and unirradiated groups were inseminated with normal masu salmon sperm by the dry method at the same time. These inseminsted eggs were transferred to the laboratory of Faculty of Fisheries, Hokkaido University, Hakodate and incubated at about 10°C during September 20, to Novemeber 18, 1978.

The survival rate was checked daily in each group during this experimental period and the remaining embryos were fixed with Bouin's solution at 40 days after insemination when control embryos began to hatch. Fixed embryos were embeded in paraffin block and sectioned at 7 μ thickness. The sectioned samples were stained with Delafield's hematoxylin and eosin for the measurement of the cartilage cell nuclei of embryos with a microscope Olympus model OSM-D_2 to check the haploidy.

Results

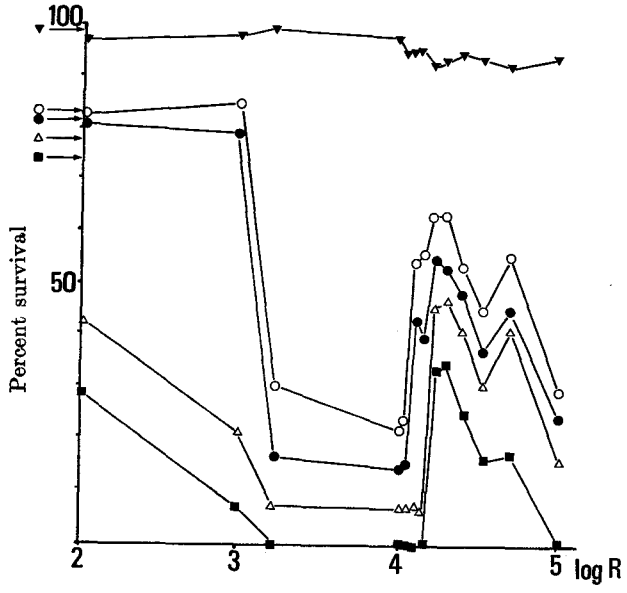
Survival curves for the experimental groups (10^2, 10^3, 10^4, 5×10^4, 10^5R) were shown in Text-Fig. 1. All groups showed very little change in the survival rate with high levels from 98.8% to 92.2% during early development from fertilization to about 10 days after. Heavy mortalities appeared in the next 10 to 15 days after fertilization. The control unirradiated group also showed a high level of survival during early development followed by a slight decrease resulting with the survival rate to be around 80% up to the time of normal hatching. Experimental groups irradiated with 10^2R and 10^3R showed almost the same survival curves as



Text-Fig. 1. Survial rate of irradiated and control group during the development from fertilization to 40 days after fertilization. ●; control, △; 10^2R, ▲; 10^3R, □; 10^4R, ○; 5×10^4R, ■; 10^5R.

that of the control until 25 days after fertilization, followed by the slight survival of 43% and 21% respectively at 30 days after fertilization. In 10^4R group,the survival level quickly dropped down to about 20% at 15 days and no viable embryo was found at 40 days after fertilization. Similar results were observed in the group exposed to a high dose of 10^5R. The embryos in the 5×10^4R group demonstrated a relatively moderate decrease in the survival rate and resulted in about 40%, even at 40 days after fertilization.

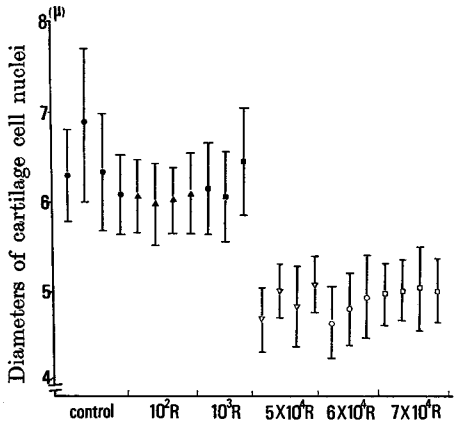
Relationships between the doses of gamma irradiation to eggs and the viability of resultant embryos were given in Text-Fig. 2. Although irradiated groups of 5×10^3, 10^4 and 2×10^4R showed a very low survival rate, the groups irradiated with increasing beyond 3×10^4R were gradually recovered in their survival rates, reaching a peak at the irradiation of $5–6\times10^4$ R. These facts clearly demonstrated the presence of a "Hertwig effect" between the dosage of gamma irradiation and the survival rate.



Text-Fig. 2. The relationship between the survival rate and the irradiation doses. ▲; survival rate at 10 days after fertilization. ○; at 15 days, ●; at 20 days, △; at 30 days, ■; at 40 days. Arrow indicates the survival rate of the control group at each days.

All the embryos that survived in the groups irradiated with the doses of 5–6 $\times10^4$R represented grossly abnormal morphology so called "haploid syndrome" for example a short broad body, severe microcephaly, edema, distortion of tails or heads and lack of eyes (Plate I, Figs. 2–7). The embryos described above never hatched. In the group irradiated with 10^5R, no embryonic body had been formed. However, remaining embryos of the groups irradiated with 10^2R and 10^3R seemed to be as normal as those of the control in their external appearance.

In order to confirm the haploidy of the surviving embryos having abnormal appearance without any contribution of maternal genome, measurements of the nuclear size were carried out on 20 cartilage cells in the embryos of the experimental groups irradiated with 10^2R, 10^3R, 5×10^4R, 6×10^4R and 7×10^4R. The results are shown in Text-Fig. 3. There was a significant difference in the nuclear size of cartilage cells between embryos of controls, 10^2R and 10^3R groups and those of irradiated groups with higher doses of irradiation. The former embyros had a normal appearance having 6.88–5.99 μm in the mean nuclear size. The latter had abnormal appearances designated as "haploid syndrome" and the nucleus ranged from 5.09 to 4.64 μm in mean size. These results indicate that the embryos of the irradiated groups with $5–7\times10^4$R were haploid resulting from the androgenetic development of genetically inactive eggs when inseminated with normal sperm.



Text-Fig. 3. Nuclear size of cartilage cells in unirradiated, low-dose irradiation and high-dose irradiation groups. Vertical bars indicate confidence limits at 99.5%. ●; control, ▲; 10^2R, ■; 10^3R, ▽; 5×10^4R, ○; 6×10^4R, □; 7×10^4R.

Discussion

The present study demonstrated the presence of a "Hertwig effect" in the androgenesis of masu salmon (*Oncorhynchus masou*) and revealed that gamma irradiation of 5×10^4R or 6×10^4R was most effective to induce androgenetic haploid development with a satisfactory survival rate in the resultant embryos. This result is in agreement with that of other research done on loach[4]. No species specificity may consist in the doses to induce androgenesis among fish species.

Requisite doses to induce androgenesis demonstrated here are lower than those to induce gynogenesis.[2)3)4)] Moreover, the best survival rate reported in the present androgenetic haploid embryos is much lower than that reported in gynogenetic embryos[2)3)4)]. The "Hertwig effect" in artificial gynogenesis is generally explained by the low survival rate due to the distrubance of embryonic mitoses owing to incomplete destruction of sperm chromosome and by the successful haploid embryogenesis of the eggs activated with genetically inactive but motile sperm due to complete destruction of the sperm nucleus with higher doses of irradiation over 10^5R.[3)4)5)] Such explanations can also interpret the mechanism of the radio-induced androgenesis only due to paternal haploid gamete penetrated into genetically inactive eggs, although the worst survival rate in the groups irradiated with the highest dose of 10^5R can not be expounded. The only explanation is that

extremely high doses of gamma irradiation like 10^5R probably destroys not only the nucleus of the eggs but also the egg cytoplasm including enzyme protein indispensable for the subsequent embryogenesis. Such a harmful influence on the egg cytoplasm may also explain the lower survival rate in androgenetic haploid embryos.

Arai and Yamazaki[6] reported in the previous paper that the maternal, probably cytoplasmic, LDH isozyme phenotype persisted during earlier stages and the new appearance of paternal isozymic subunit occurred at the tail bud stage in the embryogenesis of salmonid species. It is difficult, however, to determine the exact persisting period of maternal cytoplasmic LDH isozyme during development, because of the presence maternal genome responsible for the production of the same isozyme. Androgenetic studies will provide informative evidences on such problems and also on the exact times of paternal gene activation during embryogenesis. Moreover, abnormal embryogenesis will be analyzed by using such androgenetic embryos as those induced in nucleo-cytoplasmic heterogenous hybrids, because it has been reported in amphibia[7] that some nucleocytoplasmic incompatibility may often result in abnormal embryogenesis of a lethal hybrid.

Some investigators have pointed out possible applications of artificial parthenognesis in fish breeding to get genetically identical offsprings.[3)5)8)] For the purpose of fish breeding, artificial gynogensis may be better than androgenesis, because the survival rate of gynogenetic haploid embryos may be more favorable owing to no injurious irradiation effect on the egg cytoplasm. Furthermore, there may be an easier possibility of artificial diploidization in gynogenesis by applying cold shocks to eggs after fertilization.[3)5)8)9)]

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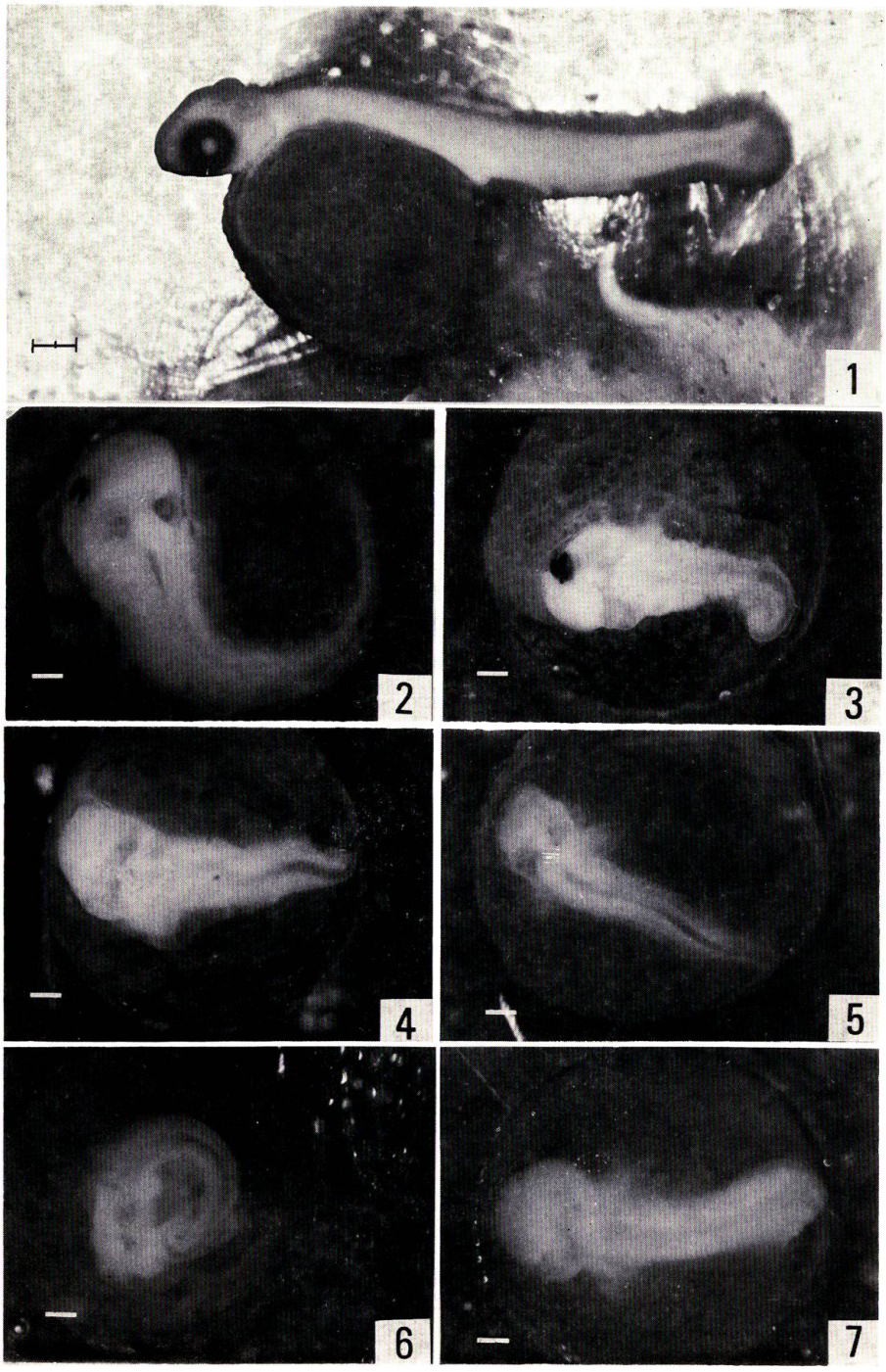
Explanation of PLATE I

Scale shows 1.0 mm in Fig. 1. and 0.5 mm in Figs. 2–7.

Fig. 1. Masu salmon alevin from unirradiated control group at 40 days after fertilization. Scale indicates 0.5 mm length.
Figs. 2–7. Photographs of masu salmon embryo sampled from irradiated group with 5×10^4 R. Scale indicates 0.5 mm length.
Fig. 2. Embryo having abnormal appearance characterized by an enlarged broad head.
Fig. 3. Embryo having an extremely short body, edema in the trunk and abnormal head and tail.
Fig. 4. Embryo having a microcephaly with no eyes.
Fig. 5. Embryo having an extremely severe microcephaly.
Fig. 6. Embryo with torsion of body.
Fig. 7. Embryo having an enlarged and broad head on a short body.



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