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GERM STEM CELLS TRANSPLANTATION IN FISH: AN INNOVATIVE BIOTECHNOLOGY FOR THE FAITHFULL REGENERATION OF CRYOPRESERVED GENETIC RESSOURCES Collected FROM SELECTED LINEs of AGRONOMIC INTEREST

Jean-Jacques Lareyre*, Anne-Sophie Goupil, Ahmed Maouche, Alexandra Depincé, Lionel Goardon, Marjorie Bideau, Nicolas Dechamp, Edwige Quillet, Francine Krieg, and Florence Le Gac

*INRA UPR1037, Laboratory of Fish Physiology and Genomics, BIOSIT, Campus de Beaulieu, 35042 Rennes, France
jean-jacques.lareyre@inra.fr

Interactions between the nuclear and mitochondrial genomes are important for animal performance traits. The mitochondrial genome is transmitted to the offspring by the female only, through its accumulation into the oocytes. Unfortunately, fish oocytes and embryos cannot be cryopreserved, which results in the absence of an appropriate procedure allowing the conform regeneration of selected fish lines. The present study was aimed to set up a standard and practical biotechnology based on germ stem cell (GSC) grafting that could be easily implemented in fish farms to conserve and regenerate the whole genetic characteristics (mitochondrial and nuclear genomes) of original and/or selected populations in fish.

Highly purified germ stem cell (GSC) and total testicular cell fractions were obtained from sex-reversed females (named neomales) belonging to a wild type (i.e. black skin) homozygous isogenic trout line. The cell fractions were injected independently in the abdominal cavity of triploid trout embryos homozygous for the dominant “golden” mutation (yellow skin). Male and female triploid trout embryos become sterile unless their gonads are colonized by diploid transplanted germ cells. Using diagnostic genetic markers, we showed that the percentage of successfully transplanted male and female recipients was high (about 80%) and similar whatever the cell fraction. Interestingly, we observed that grafted females ovulated during the egg-laying season of the recipient fish line (November instead of January for the donor fish line). Egg production from 2 years old grafted females reached normal values (2200 eggs/kg body weight) for both cell fractions, but egg quality indicators (eggs size and percentage of hatched embryos) tended to be improved after using the total testicular cell fraction. In contrast, milt production and sperm counts of precocious one year old males were highly variable regardless of the GSC fraction used but remained sufficient to fertilize thousands of eggs. Genotyping showed that milt contained spermatozoa derived from donor GSC only. Progenies were generated using eggs and milt collected from grafted female and male recipients, respectively. As expected, all fry were genetically identical to the GSC donor fish line with a black colored skin and a female genetic sex.

In conclusion, this study demonstrates that total testicular cells can be transplanted into triploid recipient trout embryos to efficiently and faithfully regenerate valuable genetic resources in farmed fish.

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