POSTER 129

GERM STEM CELL TRANSPLANTATION PROCEDURE FOR THE REGENERATION OF ISOGENIC TROUT LINES.

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Introduction

Isogenic fish lines are clonal families, homozygous at all DNA loci. Isogenic trout lines were generated at INRA and they are currently highly valuable models to investigate the genetic and environmental determinism of traits such as disease resistance, stress tolerance, feed efficiency, sex-ratio or gamete quality. The present study was aimed to evaluate a procedure to regenerate these important genetic resources, which are fragile and, if lost, *cannot be reproduced*.

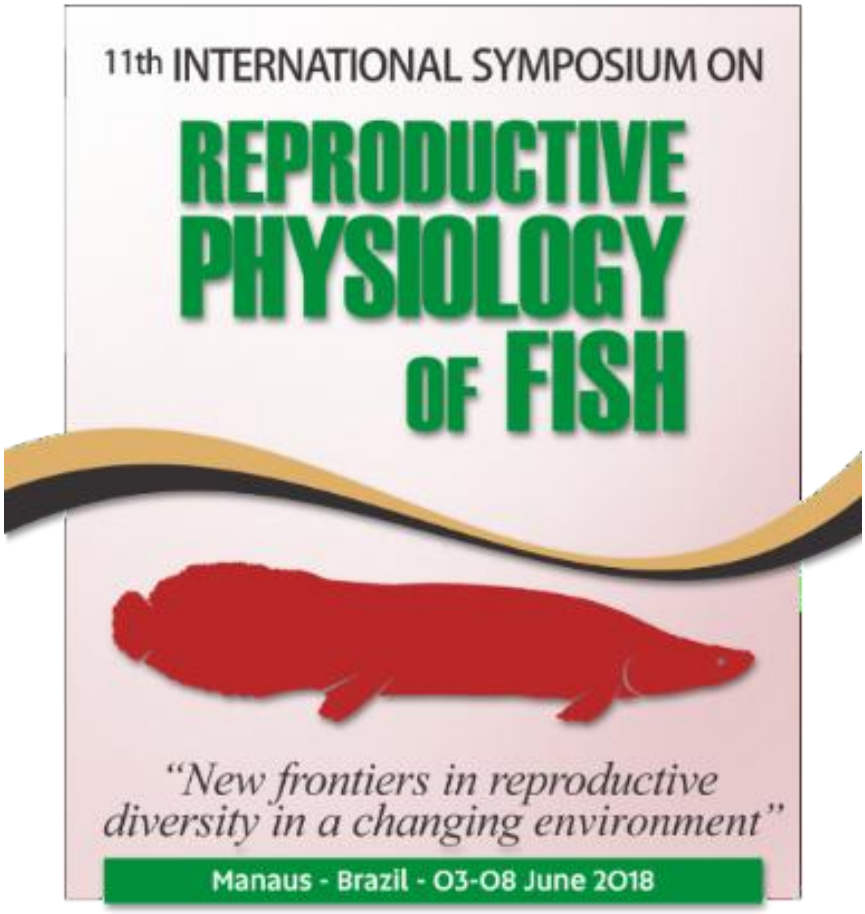
Methods

Isogenic lines were produced using two rounds of endomitotic and meiotic gynogenesis and are all-female populations. Purified undifferentiated spermatogonia were obtained from isogenic neomales (sex-reversed isogenic homozygous females) by centrifugal elutriation. Spermatogonia were injected in the abdominal cavity of hatching *triploid* embryos (2 exp). Male and female recipients were grown until sexual maturation then crossed to analyze their offspring. To discriminate the progeny derived from the *donor*, a "golden" strain (homozygous for a dominant yellow color variant) was used as *recipient*, while isogenic donors present the wild-type "black" coat. DNA genotyping was performed using a panel of diagnostic microsatellite markers.

Results and Discussion: 55% of the transplanted triploid embryos had survived to hatching. The presence of donor cells inside male and female gonads was confirmed by genotyping (70% of positive recipient). In 7- month-old females, histological evaluations showed that oocytes were blocked in the diplotene stage in triploid fry and developed beyond this stage only in individuals transplanted with diploid germ cells. One year post transplantation, 70% of the males matured and sperms were used individually to fertilize 600 eggs from another wild-type trout line. Interestingly 100% of all their progenies were female (SDY negative) and showed the recessive "black" coat, suggesting that triploid recipients produce *functional* spermatozoa derived from the donor transplanted spermatogonia only. Furthermore, all progenies harbored specific alleles derived from the isogenic donor (n=20 per cross). One year later, 77 % of the transplanted female ovulated (1780+/-640 ovules/kg body weight). Ovules from 12 transplanted females were fertilized with fresh or frozen sperm from the transplanted males. The use of cryopreserved sperms produced fewer offspring. 100% of all progenies exhibited the recessive "black" phenotype of the donor line, showing that crossing gametes obtained from male and female transplanted triploid recipients generated progeny from the isogenic donor line only. The first DNA genotypings on the offspring confirmed that one specific isogenic line was generated.

Conclusion: High rate of isogenic oocytes and spermatozoa production can be achieved by transplantation of purified isogenic spermatogonia into triploid recipients, and their crossing regenerate the donor isogenic line in one generation. (This study was supported by the UE AquaExcel2020 project).

PROGRAM
AND
ABSTRACTS



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