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MEIOSIS RESUMPTION AND EARLY MITOSIS AFTER SOMATIC CELL NUCLEAR TRANSFER IN NON-ENUCLEATED GOLDFISH OOCYTES.

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Introduction

The cryopreservation of genetic resources is of major interest for the security of biodiversity and for the sustainability of agronomic industry. Cryopreservation of somatic cells, which carry the genome of both parents, is one mean to regenerate functional breeders, but this requires the mastering of nuclear transfer. In mammals, somatic cell nuclear transfer consists in injecting the somatic nuclei into an enucleated mature recipient oocyte, in order to obtain clone which bear the genome of interest from the donor animal. However, in fish species, the oocyte structure makes the enucleation step difficult to achieve (presence of the chorion and bulk nutritive reserve), so a non-enucleated oocyte is often used for nuclear transfer. To obtain a real clone and not hybrid embryos, the oocyte chromatin must therefore be spontaneously excluded from the developing embryo. This phenomenon has been described in several studies but the mechanism involved is still unknown. Deciphering this phenomenon requires a high understanding of the first developmental steps that are meiosis resumption and early mitosis. The aim of the study is to understand the interplay between the maternal chromatin and the injected one upon nuclear transfer during these entire critical steps, in order to identify and use means to improve the success rate of nuclear transfer in fish.

Methods

Fin somatic cell and mature oocytes were obtained from 2 years old goldfish (*Carassius auratus*) females. For nuclear transfer, the whole fin cell was injected under the micropyle. The egg activation was induced 30min after cell injection. Somatic and maternal chromatin during meiosis resumption and early mitosis was characterized by immunofluorescence (hoechst and vybrant labelling for chromatin identification and α -tubulin for spindle organization).

Results and Discussion

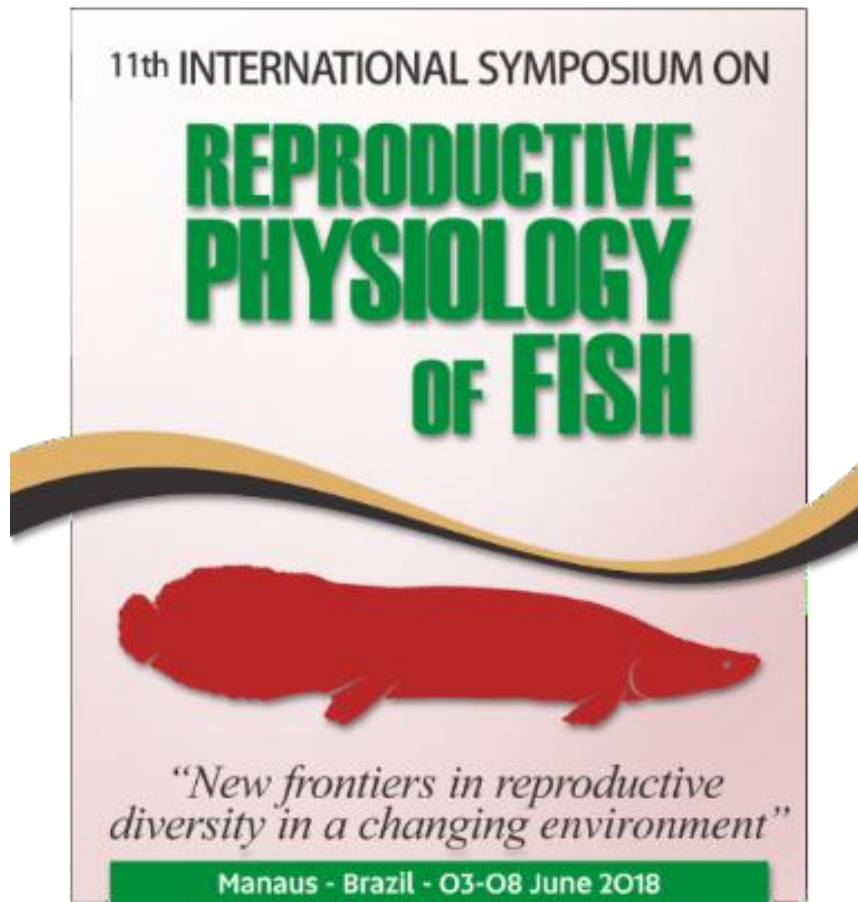
We observed that a high proportion of clone underwent a normal polar body extrusion after activation (60% vs 96 % for the controls). We hypothesize that in most cases, maternal chromatin achieved correctly its meiosis resumption and that the maternal chromatin behavior was not disturbed by nuclear transfer. However, these observations do not augur early mitosis fitness in clone. Indeed, we observed several spindle defects in clone cells: metaphase misalignment, abnormal segregation, and multicentrosomal spindle, associated with DNA fragmentation.

Conclusion

Oocyte non-enucleation for SCNT procedure did not drastically alter the meiosis resumption process, but it could be involved in the perturbation of mitosis during clone early development.

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PROGRAM AND ABSTRACTS



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