POSTER 48

SOMATIC CELL NUCLEAR TRANSFER IN RAINBOW TROUT (*Oncorhynchus mykiss*): WHAT MAKES IT DIFFERENT FROM FERTILIZATION WITH A SPERMATOZOA ?

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Introduction

Somatic cells are a highly convenient diploid support for biodiversity conservation: they carry the genome of both parents, and they can be collected whatever the age or sex of the donor. In fish, cryobanking of somatic cells is highly valuable because eggs and embryos cannot be cryopreserved. With those cells, the regeneration of the donor genotype will however rely on the nuclear transfer technology in which the donor cell nucleus is injected into a recipient oocyte. Somatic cell nuclear transfer (SCNT) has been performed in fish since the 1960s, mainly in Cyprinidae (carp, goldfish and zebrafish), Adrianichthydae (medaka) and Cobitidae (loach). Although survival rates after nuclear transfer are low, SNCT is a promising technology of reconstruction. No study has been reported in Salmonidae, despite their importance in aquaculture.

Methods

We used rainbow trout oocytes, arrested at metaphase II as recipients and rainbow trout somatic cells isolated from caudal fin as nucleus donors. Nuclear transfer was performed on non-enucleated oocytes, in order to reduce the development rate variability induced by enucleation. Oocytes were incubated in Fish Ringer (300 mOsm/kg) in order to keep them inactivated and to maintain their quality during the whole injection process. The donor cell was delivered through the micropyle, which is the route that the spermatozoa use to enter the oocyte, using a 15µm needle. Development was then initiated by transferring oocyte in fresh water.

Results and Discussion

All steps sought to imitate as much as possible the fertilization process. Injecting the egg through the micropyle required its visualization. We observed that the position of the lipid globules is quite independent from the micropyle position. Therefore, the micropyle had to be found based on the slight changes in light diffraction rather than by the egg internal structures underneath. After egg activation with water, some vitellus coagulation was induced, likely because of alteration of egg plasma membrane upon needle puncture during injection. However, no other suitable medium for egg activation could be found. We also demonstrated that early development (up to 8 days post fertilization) could be estimated only once the chorion is removed. In all, although all those necessary steps were mastered, no development could be induced on more than 100 SCNT. Interestingly, control oocytes which were injected with medium only, before normal fertilization were also unable to initiate any development. Those results suggest that the penetration of the needle and/or the medium used are deleterious to the oocytes. This had never been observed in other species.

Conclusion : This first SCNT attempt shows that Salmonidae are quite refractory to this technology, and a better understanding of the alterations induced at the animal pole is required. **Acknowledgements**: Financial support of “Investissements d’Avenir “ANR-11-INBS-0003 (CRB-Anim 2013-2019) and COST AQUAGAMETE FA 1205.

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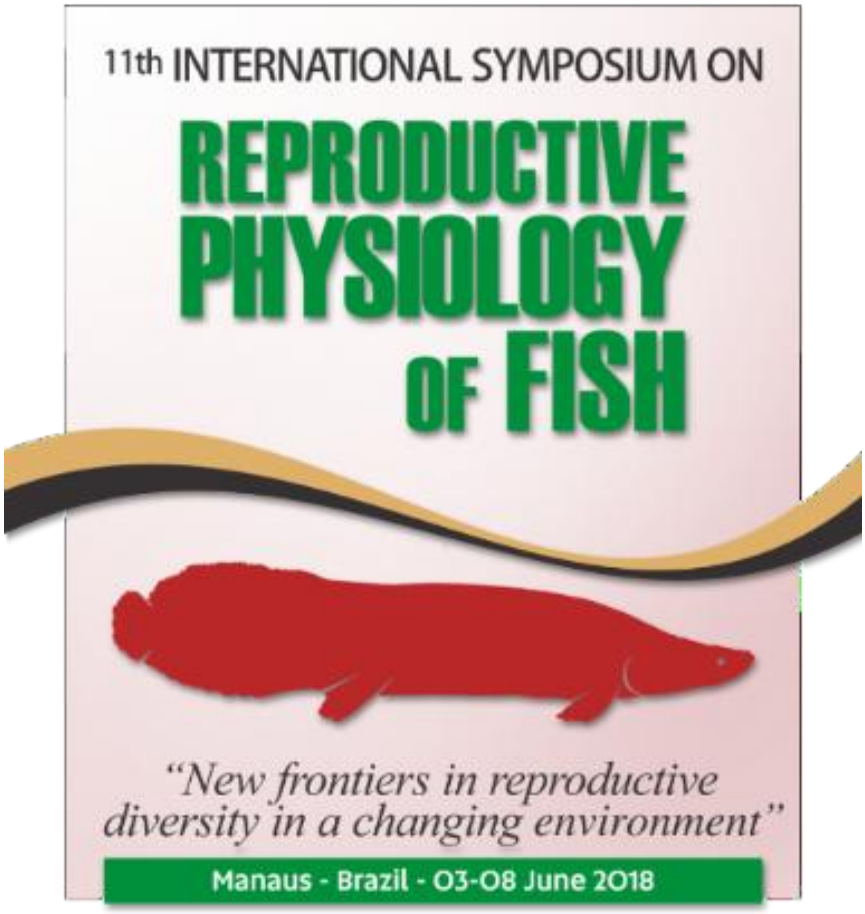
ABSTRACTS



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