GOLDFISH EMBRYO DEVELOPMENT AFTER SOMATIC CELL NUCLEAR TRANSFER IN NON-ENUCLEATED OOCYTES: FIRST MITOSIS PROFILE, PLOIDY STATUS, AND GENETIC CONFORMITY

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Introduction:
Nuclear transfer has the potential to become one strategy for fish genetic resources management, by allowing fish reconstruction from cryopreserved somatic cells. Survival rates after nuclear transfer are still low however [5], both because of biological and technical constraints. Among them, the difficulty to enucleate the recipient oocyte in fish, together with the need to alleviate some of the alterations associated with the enucleation methods, led several authors to inject the donor nucleus into non-enucleated oocytes [1,2,4]. Strikingly, diploid clones were produced which exhibited genetic characteristics of the donor fish. The group of Wakamatsu demonstrated moreover that diploidized activated eggs were a good template for the development of somatic cell nuclear transplants [6], although the mechanism underlying a putative spontaneous erasure of the maternal genome remains to be explored. In a previous work, we developed a nuclear transfer method in goldfish where the recipient oocytes were at the metaphase II stage (non-activated) [3]. As also proposed by [5] and in several works in mammals, a metaphase II recipient should improve the reprogramming of the injected nucleus, by allowing better cell cycle synchronization between the donor and the recipient.

In the present work, we explored the development of embryos reconstructed after somatic cell nuclear transfer into non-enucleated non-activated oocytes in goldfish (Carassius auratus). We assessed whether such recipient could still allow spontaneous erasure of the maternal genome, as observed when nuclear transfer is performed on readily activated eggs [1,2,4]. We additionally question here the origin of the development defects in the reconstructed embryos.

Methods:
Nuclear transfer experiments were performed on goldfish as described in [3] using cryopreserved caudal fin cells as donor and non-enucleated non-activated oocytes as recipient. Egg activation was delayed for 30 min after nucleus injection. After activation, the reconstructed embryos developed in tap water at 20°C in plastic Petri dishes. Relationship between the first mitosis pattern and the survival rates over embryo development was analyzed under macroscopic magnification. The tubulin organization at the mid-blastula stage (6 hpf) was analyzed using conventional immunofluorescence labeling on whole embryos fixed in methanol. A method was further set up to analyze cell ploidy and microsatellite occurrence on very small samples, so that the ploidy status and the genetic origin of the embryos could be assessed on the same individuals as early as at 24 hpf (15-18 somites stage). More than 360 reconstructed embryos were analyzed in this work.

Results and Discussion:
Of the entire developing embryo observed at the 2 cell stages (70.7 % of the injected oocytes), only 41% displayed 2 neat blastomers, homogeneous in size. In these embryos, the first cell cycle was not significantly longer than in the control fertilized embryos, and they all reached the mid-blastula stage. Other embryos (40%) did not go through a clear 2 cell stage although they kept developing. At the 4 cell stage, they readily displayed 4 blastomers more or less regular in size. The remaining embryos (19%) either did not cleave before the 8 to 16 cell stage, or they had asymmetrical cleavages (odd blastomere number). Strikingly, these delayed and abnormal cleavages still produced live embryos at the mid-blastula stage: only 4% of them died before this stage. This indicates that abnormal cleavage did not prevent development, which is consistent with the absence of mitosis checkpoints before mid-blastula stage in fish. Interestingly, chromatin and tubulin organization at the mid-blastula stage was not significantly different between reconstructed embryos and fertilized controls. At 24 hpf, more than 68 % of the embryos normal at the 2 cell stages were still developing. Almost all the embryos which reached the hatching stage belonged to these initially normal embryos. Still, about 20% of the embryos originating from abnormal early cleavages were still developing at 24 hpf, but very few reached the hatching stage.

Although the reconstructed embryos were produced with non-enucleated oocytes, 44 % of them were diploids and only 22 % were triploids. The others were either haploids (13 %), aneuploids (20 %), or tetraploids (1 %). Among the diploids, 54 % were true clones as they displayed only the microsatellite sequences of the donor fish. The other embryos were true hybrids as they
bore a combination of the donor and the recipient microsatellite sequences.

**Conclusion:**

In this work, 1) we demonstrated for the first time that embryos reaching the mid-blastula stage after somatic cell nuclear transfer could be originating from various early cleavage patterns, and that abnormal early cleavage did not prevent normal development up to mid-blastula stage. This illustrates the great plasticity of fish early development, before the embryonic genome activation and various development checkpoints are set up at the mid-blastula stage. 2) Although nuclear transfer was performed before oocyte activation, in metaphase II cytoplasms still bearing the maternal genome, diploids were produced which were genetically identical to the donor fish. A spontaneous inactivation of the maternal genome operated in these embryos although one could have feared that only hybrids would be produced. This observation raises the question of the underlying mechanisms responsible for such enucleation.

**References:**


