

REPRODUCTIVE BIOTECHNOLOGIES IN WILD FELIDS

Dra. Lucía N. Moro

Laboratorio de Biotecnología Animal, Facultad de Agronomía, Universidad de Buenos Aires, Argentina. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

The extinction of animals is one of the most alarming consequences caused by environmental degradation, and deprives us of unique genetic materials. The *Felidae* family does not escape to this problem and it is for this reason that reproductive biotechnologies become very useful tools for the conservation of these species.

Due to the limited availability of oocytes from wild cats, domestic cat oocytes (DC) were used as an experimental model to develop different reproductive techniques. Thus, our principal objective was to adapt the existing biotechnologies to reproduce and preserve endangered felid species by using DC oocytes.

In our first experiment, we evaluated different conditions of maturation and embryo culture in DC embryos generated by ICSI. Once the ICSI technique was working in the DC, we generated interspecific embryos by injecting cheetah (Ch, *Acinonyx jubatus*) and leopard (Leo, *Panthera pardus*) spermatozoa in DC oocytes. In this experiment, we obtained similar blastocyst rates in both the DC homoespecific ICSI and the interspecific ICSI, without the need of chemical activation after sperm injection.

After that, we studied the somatic cell nuclear transfer (SCNT) in the DC and interspecific SCNT (iSCNT) by using DC oocytes felids and bengal (Be, an hybrid between DC and asian leopard), cheetah y tigre (Ti, *Panthera tigris*) cells as nuclear donors. The aim was to develop new strategies to improve this technique in the Dc and wild felids. Moreover, the effect of embryo aggregation was also assessed by culturing ZP free reconstructed embryos in microwells, individually (1X), or 2 clones of the same species together (2X, aggregated embryos). In this experiment, we obtained embryos until the blastocyst stage of all the groups. Aggregation improved embryonic development in all the species and the quality of Ti2X and Gd2X blastocysts. Furthermore, the relative expression of genes related to pluripotency and early differentiation (*OCT4*, *NANOG*, *SOX2* and *CDX2*) was studied in Dc, Be and Ch clone blastocysts. This analysis found that aggregated cat embryos normalized their relative levels of gene expression as those of the IVF control.

In conclusion, the ICSI technique represents a method directly applicable to wild felids reproduction, especially when semen samples are of poor quality. With respect to SCNT and iSCNT, DC oocytes were able to reprogram wild felid cells and to generate blastocysts. Furthermore, clone aggregation has shown to improve embryo development in all the groups and to normalize the relative gene expression only in the DC but not in interspecific embryos. The SCNT is an alternative technique to generate animals that are not in good reproductive conditions or that have died and their cells were cryopreserved.