MIMICKING THE MATERNAL ENVIRONMENT TO OPTIMIZE THE IN VITRO PRODUCTION OF EMBRYOS IN WILD AND DOMESTIC MAMMALS

Pascal Mermillod[1], Carmen Almiãna[1], Agostinho Neto[1], Yann Locatelli[1,2]

[1]INRA, UMR 7247, INRA, CNRS, Université de Tours, IFCE, Physiologie de la Reproduction et des Comportements, 37380 Nouzilly, France

[2]MNHN, Réserve de la Haute Touche, Obterre, France

The preservation of endangered wild species and domestic breeds represents a challenging perspective regarding quick decline of biodiversity in the world. A wide range of reproductive biotechnologies are now available to facilitate the conservation and genetic management of mammals threatened with extinction (1). The maternal environment offers optimized conditions for gametes maturation, fertilization and embryo development, based on a constant dialogue between somatic and germinal tissues, guarantee for the success of reproduction. Bypassing this dialogue through in vitro technologies, such as in vitro production of embryos (IVP), leads suboptimal regulation of early events with negative impact of reproductive success and offspring health. Since IVP techniques offer a wide range of applications for the spreading and amplification of valuable genotypes, it seems important to understand the mechanisms of this dialogue and find some ways to reproduce it in vitro in view to optimize the output of the technique. In deer species, it has been clearly shown that the presence of oviduct epithelial cells (OEC) can improve the rate and quality of embryo development of embryos produced from oocytes collected from slaughtered females in red deer (2,3) or from living females by laparoscopic ovum pick up (LOPU) in sika deer (4). In bovine, we showed that this beneficial effect of OEC relies on a molecular dialogue with early developing embryos, modulating the gene expression profiles of both partners (5). In pigs, we showed that oviduct fluid can regulate the polyspermy, which is a major problem for IVP in this species (6,7). Recently, extracellular vesicles such as exosomes emerged as a new paradigm in cell communication, beyond the traditional ligand – receptor pathways, triggering intracellular signaling cascades. These membrane vesicles of 50-150 nm can transport proteins, mRNA and ncRNA from secreting cell to target cells and modify their properties. We found exosomes in OF, containing proteins, and different RNAs, including microRNA (8) and we showed that these exosomes are able to reproduce the effects of OEC on embryo development in cattle (8) as well as the effect of OF on pig IVF (Unpublished data). In conclusion, mimicking the maternal environment in vitro by using cells or fluid from the oviduct can improve the IVP success, at least a part of this effect may be mediated by extracellular vesicles. Further studies of these vesicles will allow to set up optimized IVP conditions allowing the efficient production of high viability embryos

1. Comizzoli, P., Mermillod, P. & Mauget, R. Reproductive biotechnologies for endangered mammalian species. *Reprod. Nutr. Dev.* **40,** 493–504 (2000).
2. Comizzoli, P. *et al.* Successful in vitro production of embryos in the red deer (Cervus elaphus) and the sika deer (Cervus nippon). *Theriogenology* **55,** 649–659 (2001).
3. Locatelli, Y. *et al.* Successful use of oviduct epithelial cell coculture for in vitro production of viable red deer (Cervus elaphus) embryos. *Theriogenology* **64,** 1729–1739 (2005).
4. Locatelli, Y. *et al.* Assessment LOPU-IVF in Japanese sika deer (Cervus nippon nippon) and application to Vietnamese sika deer (Cervus nippon pseudaxis) a related subspecies threatened with extinction. *Theriogenology* **78,** 2039–2049 (2012).
5. Schmaltz-Panneau, B., Locatelli, Y., Uzbekova, S., Perreau, C. & Mermillod, P. Bovine Oviduct Epithelial Cells Dedifferentiate Partly in Culture, While Maintaining their Ability to Improve Early Embryo Development Rate and Quality. *Reprod. Domest. Anim. Zuchthyg.* **50,** 719–729 (2015).
6. Batista, R. I. T. P. *et al.* Combination of oviduct fluid and heparin to improve monospermic zygotes production during porcine in vitro fertilization. *Theriogenology* **86,** 495–502 (2016).
7. Cordova, A. *et al.* Development rate and gene expression of IVP bovine embryos cocultured with bovine oviduct epithelial cells at early or late stage of preimplantation development. *Theriogenology* **81,** 1163–73 (2014).
8. Almiñana, C. *et al.* Oviduct extracellular vesicles protein content and their role during oviduct-embryo cross-talk. *Reprod. Camb. Engl.* **154,** 153–168 (2017).