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### Effect of fetal bovine serum and reproductive fluids on bovine preimplantation embryo development

The European Society of Human Reproduction and Embryology estimates that, worldwide, one in six couples will suffer some form of infertility during their reproductive lifetime. This has led to an exponential increase of the use of Assisted Reproductive Technologies (ARTs). Moreover, they are routinely used for livestock to generate animals of high genetic value and for the preservation of endangered species. Despite ARTs represent one of the most outstanding achievements of the 20th century, they are not exempt of risks. In humans, higher rates of preterm births, and syndromes such as Beckwith-Wiedemann and Angelman have been reported in children born by ARTs. Similarly, in cattle and sheep, the Large Offspring Syndrome (LOS) was observed after *in vitro* embryo production. Since the supplementation of the culture media with fetal bovine serum (FBS) was thought to be partially responsible for LOS, culture media development moved towards a chemically defined composition, which is far from physiological. Hence, the aim of this research stay was to evaluate the potential benefits of the addition of reproductive fluids during *in vitro* embryo production to mimic the *in vivo* environment, in order to produce more *in vivo*-like embryos.

We evaluated the number and quality of the bovine blastocysts produced *in vitro* under two different culture conditions, a chemically defined medium or a medium supplemented with reproductive fluids. No significant differences were observed in blastocysts rates between the two culture conditions, in their subsequent survival to vitrification, or in their total cell number. However, a significant increase in the proportion of inner cell mass, was observed in blastocysts produced in the presence of reproductive fluids.

Additionally, blastocysts cultured in the presence or absence of FBS from oocytes obtained by *Ovum Pick-Up*, were vitrified for further analysis by single-cell sequencing, in order to unravel the molecular alterations causing LOS.

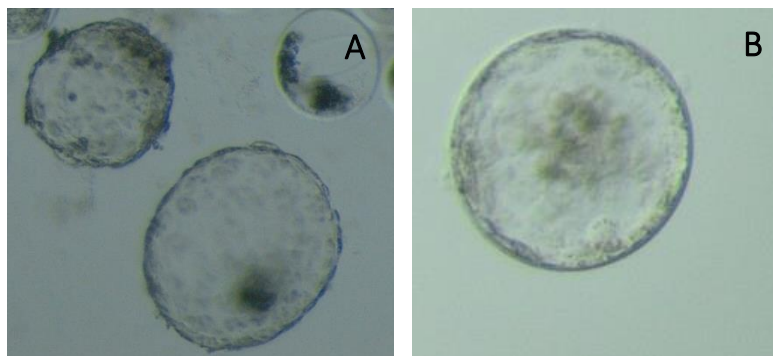


Image 1. A) Bovine blastocysts at day 8 of development produced *in vitro* in culture medium supplemented with reproductive fluids. B) Bovine blastocysts at day 6 of development produced *in vitro* in chemically defined culture medium, from oocytes obtained by *Ovum Pick-Up*.