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Equine *in vitro* fertilization in presence of porcine/bovine oviduct epithelial cells

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Several domestic and wild horses and donkeys are in a high risk of extinction. Embryo cryopreservation allows the preservation of both the female and the male genetics and embryo production is the fastest method to restore a breed. In equine, superovulation treatments are inefficient. ICSI (intracytoplasmic sperm injection) is well developed, but this technique is expensive, time-consuming and requires well-trained personal. Thus, the development of an efficient *in vitro* fertilization (IVF) technique would be interesting for embryo production. However, in the equine species, no efficient IVF technique is available. The aim of this study was to increase the developmental competence of the IVF zygotes performing the IVF in presence of porcine or bovine oviduct epithelial cells (POEC or BOEC) and pre-incubating the sperm with progesterone (P4).

Immature cumulus-oocyte complexes were collected from slaughtered mares in a local slaughterhouse and cultured for 27-29 hours in TCM199 supplemented with fetal calf serum and epidermal growth factor. Fresh or frozen sperm was diluted to 20×10^6 spermatozoa/ml and pre-incubated or not for 2 minutes with P4 (1 μ g/ml). Oocytes were inseminated at 2×10^6 spermatozoa/ml and co-incubation was performed in DMEM-F12 with POEC/BOEC during 18 hours. Then, zygotes were fixed and analyzed.

Our results show that 1) the percentage of zygotes obtained by IVF with POEC was 8.3% (2/24) when fresh sperm was incubated with P4 and 10.7% (3/28) without P4; 2) the percentage of zygotes obtained by IVF with POEC was 6.7% (2/30) when frozen sperm was incubated with P4 and 6.9% (2/29) without P4; 3) the percentage of zygotes obtained by IVF with BOEC was 0% (0/30) with fresh sperm without P4.

In conclusion, the IVF rate is better with fresh than with frozen sperm and with POEC than with BOEC. The pre-incubation of sperm with P4 does not improve the equine IVF rate.

