

Comparison of oviductal cell and granulosa cell co-culture system for development of IVM/IVF produced *Camelus dromedarius* embryos

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Preliminary results in our laboratory showed that the In Vitro maturation and fertilization of dromedary camel oocytes collected from slaughterhouse material was possible. The aim of this study was to compare the efficacy of development of embryos produced by IVM-IVF to the Blastocyst stage in co-culture media with oviductal cells (OCS) or Granulosa cells (GCS).

Materials and methods: Ovaries were collected from slaughtered camels and transported to the laboratory within 2 hours in saline at 37°C. Cumulus-oocytes complexes (COC) were collected by aspiration from 4 to 10 mm follicles and washed in PBS. A total of 1598 oocytes were collected, 18% from this total showed degenerated cytoplasm and/or absence of cumulus cells. COC were matured by incubation at 38.5°C, under 5% CO₂ and maximum of humidity (>95%) for 36h in TCM199 medium supplemented with 10% fetal calf serum (FCS), 10 ng/ml epidermal growth factor (EGF), 1 µg/ml follicle stimulating hormone (FSH) and 500 µM cysteamine. In vitro fertilization of in vitro matured COC's was performed using ejaculated semen with 0.5×10^6 spermatozoa/ml in modified TALP solution after centrifugation and washing on Percoll discontinuous gradient. Sperm penetration was confirmed in 106 (68%). Normal fertilization (presence of one male and one female pronucleus) was observed in 52% of fertilized oocytes whereas 34% of the oocytes showed polyspermy. Fertilized denuded oocytes (819) were co-cultured with dromedary oviduct (OCS) (n= 417) or granulosa cells (GCS) (n=402) in presence of TCM199 supplemented with 10% FCS.

Results: Rate of in vitro maturation was confirmed by Metaphase II apparition in 63% (56/88) of the oocytes. Rate of first cleavage (2-16 cells) was similar in the two co-culture systems (oviduct: 32% vs. granulosa: 33%). However, significantly more embryos were obtained from oocytes in OCS (61%), than those in GCS (45%) ($P < 0.05$). The percentage of fertilized oocytes developing to the early morula stage after 7 days was 19% and 12% for 417 oocytes in OCS and 402 oocytes in GCS, respectively. Development to the blastocyst stage was attained in the OCS only by 10% of the fertilized oocytes.

Conclusion: this research shows that in vitro embryo production from recovered oocyte is possible in the dromedary using ejaculated semen and oviduct cells co-culture. Studies are underway in our laboratory in order to evaluate the reasons for the low hatchability of embryos produced in vitro and it's implication on pregnancy rate after transfer.

Keywords: Camel, IVF, IVM, co-culture