

Advances in In vitro production of camel embryos

Tibary A¹, Khatir H², Anouassi A²

College of Veterinary Medicine, Washington State University
Veterinary Research Center, Abu Dhabi, United Arab Emirates

In vitro production of embryos is an important reproductive biotechnology for the multiplication of genetically superior animals and the preservation of genetics. Substantial progress has been made in the last decade in camels. Intra-cytoplasmic sperm injection (ICSI) was attempted but most embryos were produced by In vitro fertilization (IVF). The first offsprings born by transfer of an embryo produced by IVF were reported in 2006. The first report of an offspring following transfer of a reconstructed embryo by somatic cell nuclear transfer (SCNT or cloning) was published in 2010. The present paper reviews the critical steps in achieving a reliable and sustainable system for in vitro production of transferrable embryos in camels. For IVF, these steps include collection and evaluation of cumulus-oocyte-complexes (COCs), in-vitro maturation, semen preparation for fertilization and embryo culture systems to the hatched blastocyst stage for in vivo transfer. For SCNT, in addition to these requirements, oocyte activation systems were developed.

Oocyte collection systems include postmortem aspiration or dissection of follicles and in vivo Transvaginal Ultrasound Guided Aspiration (TUGA) or surgical aspiration. The quality of COCs is highly dependent of the stage of follicular development. Postmortem oocyte collection is easy. Ovaries may be shipped from a long distance for processing but quality of oocytes is variable. Improvement of oocyte quality at harvest has been improved by hormonal stimulation prior to collection. Oocyte recovery rates with TUGA vary between 35 to 80%. TUGA can be used to collect in vivo matured oocytes. Technical issues with TUGA has lead to research efforts to try to collect oocytes after ovulation.

Several in vitro oocyte maturation systems were developed, including co-culture systems with granulose cells or oviductal epithelial cells or culture in semi-defined media supplemented with variable growth factors and hormones. High in vitro maturation rates (70 to 90%) are achieved using these systems.

IVF studies in our laboratory utilize fresh ejaculated but others have reported use of cauda epididymus semen. Motile spermatozoa are obtained by centrifugation on Percoll discontinuous gradient and capacitation and fertilization are achieved by incubation in modified Tyrode's solution. Cleavage rates following IVF range from 50 to 80%. Zygotes produced by IVF are cultured in defined media (mKSOMaa) for 6 to 8 days. Development rate to the hatched blastocyst stage varies from 30 to 60%. Pregnancy rates 15 days following transfer of embryos produced by IVM/IVF range between 30 and 40%. However pregnancy loss remains relatively high and birth rates from transferred of embryos produced by IVF is 20%.

Several research groups are involved in the study of embryos production by SCNT. The optimization of protocols for activation of in vitro matured oocytes has been studied. Ionomycin/6-DMAP, Calcium ionophore/6-DMAP and electrical stimulation/6-DMAP have

been tested for activation of dromedary oocytes. Reconstructed embryos have been obtained using various cell lines, however only a single birth has been reported to date. One of the major problems with reconstructed embryos is failure of hatching. Studies are under way to improve hatching using mechanical and enzymatic techniques.

In conclusion, recent research shows that in vitro production of camel embryos can be achieved after modifications of the protocols used in other species. In vitro maturation and fertilization system used by our research team seems to be satisfactory. The overall success rate of cloning by SCNT transfer is still limited and requires further research. Further research is also needed to study causes of the high pregnancy loss.