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Program and Extended Abstracts

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In vitro embryo production in camelids: an overview

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Introduction

In vitro embryo production (IEVP) technology has been used to produce large number of embryos for transfer and for manipulations in a number of animal species. However, basic manipulations such as oocyte collection and their maturation, collection and preparation of semen for IVF procedures, culture and passaging of cells for SCNT, oocyte/cytoplast activation, culture media and conditions for embryo culture are some of the factors affecting the IVEP technology. Also, the interspecies differences in the maturation physiology do not allow the direct extrapolation of IEVP system from one species to another. In camelids, relatively limited information is available on development of this technology. The objective of the current presentation is to give an overview of the present status of in vitro embryo production in camelids.

Collection of cumulus oocyte complexes (COCs)

Ovaries collected from the slaughterhouse are usually processed as quickly as possible, however, storing them at room temperature for up to 12h does not seem to have any detrimental affects on the maturation rate of oocytes. (Wani and Nowshari, 2005). The COCs have been collected by follicular aspiration, ovarian slicing, mincing of ovaries or by excising the follicles and teasing them apart under a steriozoom microscope. A higher recovery rate of oocytes was achieved with the later method when compared with aspiration with a syringe and needle or an aspiration pump (Nowshari, 2005). Similarly, slicing of the dromedary ovaries was found to be effective compared with aspiration for collection of oocytes (Abdoon, 2001). In llamas, mincing the ovaries by a razor blade (Del Campo et al., 1994) yielded more oocytes per female than aspiration of follicles (Del Campo et al., 1992).

Ultrasound guided trans-vaginal ovum pick up (OPU) up has been used in llamas, alpacas and camels to aspirate the follicles from stimulated or non-stimulated ovaries. In llamas, ovarian superstimulation with eCG was associated with a slightly higher proportion of expanded COCs and oocytes in metaphase II, compared to
superstimulation with FSH (Ratto et al, 2005) whereas, in dromedary camels, eCG and FSH used together gives better results for superstimulation of the ovarian follicles. The best response to gonadotropins is obtained when treatment is initiated after elimination of the dominant follicle or synchronization of follicular waves.

**In vitro maturation of oocytes**

The majority of oocytes collected from slaughterhouse ovaries are in germinal vesicle stage, but at 20h of culture most of them had undergone germinal vesicle breakdown (Wani and Nowshari, 2005). In the initial studies on the maturation of dromedary camel oocytes a maturation time of 36h was considered to be optimum. However, studies on kinetics and ultra structure of oocytes during nuclear maturation suggest the optimal culture time to be around 30-32 h (Wani and Nowshari, 2005; Kafi et al., 2005). Also, the proportion of blastocysts obtained from the oocytes activated after 28 h of maturation were higher when compared with oocytes activated after that period of maturation in a recent study (Wani, 2008a). Abnormal chromatin configuration and degenerative changes have been observed after 40h of in vitro culture in camel oocytes. The COCs of llamas have also been initially cultured for 32–36h (Del Campo et al., 1994) but a recent study has shown that an incubation time of 28-30h resulted in higher maturation rates (Ratto et al., 2005). In Bactrian camel, 46.7% of oocytes achieved meiotic maturation after 24–26h of culture (Shorgan and Pang, 1993). Ultra structural studies during the maturation process of camel oocytes showed an increase in the perivitalline space of oocytes as the maturation process progressed until 24h, but no further increase occurred until 36h of culture (Kafi et al., 2005).

Tissue culture madiun-199 (TCM-199) is mainly being used for IVM of camelid oocytes; however, Ham’s F10 (Kafi et al., 2005) and CR1aa medium (Abdoon, 2001) have also been used in dromedary camels. In a study, TCM-199 was found superior to CR1aa or modified Connaught Medical Research Laboratories medium-1066 for dromedary oocyte maturation (Nowshari, 2005). No difference has been observed in the proportion of oocytes reaching M-II stage between the media supplemented with fetal calf serum, estrous camel serum or bovine serum albumin, however, a supplementation of 20 ng/mL of EGF to the maturation medium increased the oocyte maturation rate when compared with the media supplemented with 10 ng/mL, 50 ng/mL or no EGF groups (Wani and Skidmore, 2008).
Llama oocytes obtained by surgical aspiration 22h after buserelin administration had a maturation rate of 62% in the absence of hormones (Miragaya et al., 2002), while in alpacas, a maturation rates of 40–46% were obtained when COCs collected 18–24h after hCG administration were incubated for 26h (Gomez et al., 2002). Treatment with LH after ovarian superstimulation in Llama (Ratto et al., 2005) and GnRH in dromedary camels (unpublished data) permitted the recovery of expanded COCs most of which were in M-II stage.

**In vitro fertilization and intracytoplasmic sperm injection**

The production of embryos by IVM/IVF in camelids was first reported in llamas (Del Campo et al., 1994); however, the first offspring's were produced recently in dromedary camel (Khatir et al., 2006). In vitro production of camel embryos has been reported using fresh ejaculated (Khatir et al., 2006) and stored epididymal semen (Wani, 2008b) with a cleavage rate of 64% and 43-60% and a blastocyst production rate of 36% and 12-24%, respectively. In llamas, 32% oocytes cleaved after IVF with epididymal semen while the percentage of embryos reaching morula, early blastocyst and hatched blastocyst stages were 5.6%, 6% and 4.7%, respectively (Del Campo et al., 1994). All the above studies have shown that the chronology of embryo development in camelids is faster than in other species irrespective of the source of spermatozoa used in IVF.

Intracytoplasmic sperm injection of llama oocytes using ejaculated spermatozoa resulted into production of 16% morula stage embryos but no blastocysts were obtained (Miragaya et al., 2003). In dromedary camel, epididymal spermatozoa were injected into the IVM oocytes of abattoir origin and blastocyst production rate was similar to that of IVF (unpublished data).

**Chemical activation and nuclear transfer**

Recently a protocol for activation of camelid oocytes has been optimized (Wani, 2008a) in which, in vitro matured dromedary oocytes were activated with 5 µM ionomycine or 7% ethanol followed by exposure to 6-diethylaminopurine or roscovitine. It has been shown that activation of oocytes, after 28 h of maturation, with 5µM ionomycine for 3 min and subsequent culture in 6-DMAP for 4 h, gives optimal results and higher blastocyst production rates. Production of embryos by NT has been reported in llamas (Sansinena et al., 2003), in which adult fibroblast cells were used as the nuclear donors with a fusion rate of 62.5%, followed by cleavage rates of 32 and 40% in CR1aa and...
G1.2 medium, respectively. However, transfer of embryos surgically into the oviduct or non-surgically into the uterus did not result in any pregnancies. In preliminary studies on dromedary oocytes, a fusion rate of 70-80% was observed after enucleated zona free oocytes were fused with granulose cells, but a low blastocyst production rate was observed. No embryos were transferred to recipients in this experiment (Wani et al., unpublished data).

**Conclusion**

In vitro embryo production is not very efficient in camelids when compared with other domestic animal species but increased research activity and publications in recent years on developing this technique are promising. However, a very low availability of the slaughterhouse material in these species is the limiting factor for the development of these techniques. The ability to store ovaries for up to 12 h without a deleterious affect on in vitro oocyte maturation can be of tremendous help for their transportation over long distances, but more studies need to be concentrated on the collection of oocytes from live animals by ultrasound guided ovum pick up (OPU) like cattle or other domestic animal species.

**References**


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