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Cytoplast source influences development of somatic cell nuclear transfer (SCNT) embryos in vitro but not their development to term after transfer to synchronized recipients in dromedary camels (*Camelus dromedarius*)

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ABSTRACT

Studies were conducted to evaluate the adequate time for exposure of donor nucleus to oocyte cytoplast before its activation and the effect of oocyte source on the development of SCNT embryos in camels. A higher number of embryos cleaved and developed to blastocyst stage ($P < 0.05$) when couplets were activated between 1 and 2 h-than that of those activated at 0.5 h or more than 2 h post-fusion. A reduced number of reconstructed embryos cleaved ($55.2 \pm 7.6\%$) and developed to the blastocyst stage ($20.5 \pm 5.5\%$) when in vitro matured oocytes collected from the slaughterhouse were used as donor cytoplasts, compared to in vitro (71.3 ± 1.3 and $36.7 \pm 7.3\%$) or in vivo matured (91.7 ± 8.3 and $35.4 \pm 6.0\%$) oocytes obtained from live animals ($P < 0.05$), respectively. However, no differences were observed between the different types of oocyte sources on the establishment of pregnancies and delivery of offspring's. In conclusion, couplets activated 1–2 h post-fusion had higher in vitro developmental potential and oocytes collected from live animals were better in supporting the cleavage and blastocyst production in vitro than oocytes collected from slaughterhouse ovaries, however, all sources of oocytes can be utilized as donor cytoplasts and have the potential to support development of full-term calves after transfer into synchronized recipients.

1. Introduction

Since the production of the first cloned mammal by somatic cell nuclear transfer (SCNT) in 1997 [1], cloned offspring's have been produced successfully in many species including camel [2]. Cloning by SCNT can be used to produce racing champions, males of high genetic merit or the winners of camel beauty contest, called Beauty Queens. Recently, a cloned Bactrian camel calf was produced by the technique of interspecies SCNT using dromedary camel as a source of cytoplasts as well as a surrogate for carrying the pregnancy of cloned embryos to term [3], opening the doors for application of this technology to preserve critically endangered wild Bactrian camels. The potential application of SCNT in camels are constrained by the low availability of oocytes, and low pregnancy rates after the transfer of reconstructed embryos. Ovaries from slaughterhouses are the cheapest and most abundant source of oocytes and are used, in most of the domestic animal species, for the production of SCNT embryos. However, due to the non-availability of camels for slaughter in UAE, we rarely get ovaries from this source.

In addition to the state of the donor nucleus, a suitable recipient cytoplast is important for the success of SCNT. The nucleus of a somatic cell is reprogrammed from a differentiated nucleus to a totipotent embryonic nucleus in the enucleated ooplasm. Therefore, for improvements on the efficiency of SCNT in this species, we do not only require an understanding of the factors that result in improved reprogramming of the donor nucleus but also an understanding of how the source of oocytes influences donor nucleus reprogramming, for proper utilization of the limited number of oocytes available. Most of the studies on SCNT have focused on nuclear donor cell like their origin [4], stage of differentiation [5–7], age of donor [8] and cell culture conditions and length [9–13], however, fewer studies have evaluated the effect of cytoplast source on the development of nuclear transfer embryos. Many studies have shown that the events of early embryogenesis are

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almost completely dependent on maternal transcripts and oocyte proteins [14–17], suggesting that the recipient cytoplast is as important as the donor cell in the success of SCNT, due to its significant contribution in the reprogramming of donor nucleus.

Duration of exposure of the donor nucleus to oocyte cytoplasm before activation has been seen to affect embryonic development in vitro. Some studies have reported that prolonged exposure of donor cell nucleus to the oocyte cytoplasm before activation to be beneficial for embryo development in cattle [18,19], and mice [20], while others have reported that excessive exposure of the donor cell nucleus to oocyte cytoplasm results in lower rates of in vitro development in bovine SCNT embryos [21]. Given the conflicting data on the subject, the present study was designed to study the effect of: 1) exposure time of donor cell nucleus to oocyte cytoplast and 2) the source of oocytes and their type of maturation (in vivo vs in vitro) on development of reconstructed embryos in order to optimise the protocols for SCNT in dromedary camel (*Camelus dromedarius*).

2. Materials and methods

All the chemicals and media were purchased from Sigma unless otherwise indicated. Fetal calf serum (FCS) was purchased from Gibco. Female dromedary camels aged between 5 and 14 years, maintained at our center, were used as oocyte donors and recipients for NT embryos. All the camels were in good physical condition and weighed approximately 400–450 kg. They were supplied with hay and water ad libitum and were also fed a diet of mixed concentrates once daily. All procedures were performed in accordance with the government of United Arab Emirates' animal care and use guidelines.

2.1. In vivo oocyte maturation

For oocyte retrieval by ovum pick-up, camels were superstimulated and prepared as described earlier [23]. On Day 1 camels were treated with 2000 IU of equine chorionic gonadotropin (Folligon; MSD Animal Health, Boxmeer, Netherlands), given as a single intramuscular injection and 400 mg follicle-stimulating hormone (FSH) (Folltropin; Bioniche, Ontario, Canada) injected twice daily with declining doses over 4 days. A single injection of buserelin (20 μg) was given at, 26 h before ovum pick-up, where most of the follicles had reached 1.3 and 1.8 cm in diameter. Follicles were aspirated into 50- or 15-ml conical tubes containing embryo-flushing media (IMV) supplemented with heparin (10,000 IU/L). Then COCs were isolated and washed free of any blood cells and other cellular debris (Fig. 1c).

2.2. In vitro oocyte maturation

Ovaries were collected from a local slaughterhouse and brought to the laboratory to a thermos flask containing warm normal saline solution (NSS) at 37 °C. On arrival to the laboratory, the temperature of the saline solution containing the ovaries ranged from 30 to 32 °C. Ovaries were processed and cumulus-oocyte complexes (COCs) were collected within 2 h of collection, as reported previously [22]. In brief, ovaries were washed 2–3 times with NSS, individual follicles were aspirated and pooled COCs (Fig. 1a) were cultured at 38.5 °C in an atmosphere of 5% CO_2 in air for 30 h. Maturation medium consisted of TCM-199 supplemented with 0.1 mg/mL L-glutamine, 10 μg/mL bFSH, 10 μg/mL bLH, 1 μg/mL estradiol and 10% FCS. COCs were also aspirated from visible follicles of live animals located in our center by ultrasound-guided ovum pick-up, and cultured in vitro (Fig. 1b) as described above for COCs from slaughterhouse origin. Donor animals were superstimulated in the same way as described above under the heading, In Vivo Oocyte Maturation, except that these animals were not injected with buserelin before ovum pick.

2.3. Preparation of recipient oocytes

Oocytes (in vitro and in vivo matured) were denuded from the surrounding cumulus cells by manual pipetting in the presence of hyaluronidase (1 mg/ml), and those with an extruded first polar body were selected for enucleation. Oocyte enucleation was performed as previously described [2]. In brief, matured oocytes were placed into the manipulation medium (Hepes-TCM-199 + 1% BSA) supplemented with 7.5 μg/ml of cytochalasin B and 5 μg/ml of Hoechst 33342 for 20 min before micromanipulation. The polar body, along with the metaphase II plate, were removed by aspiration with a 20-μm-inner diameter beveled pipette under an inverted microscope equipped with an Eppendorf micromanipulator (TransferMan NK2). The removed cytoplasm was exposed to UV light to confirm successful enucleation (Fig. 2a–c).

2.4. Preparation of donor cells

Ear skin biopsies were taken aseptically from two adult camels in sterile Dulbecco's phosphate buffer saline. Camel number 1 (SKFRC) was a racing champion; while camel number 2 (SKFBQ) was a beauty queen, winner of a camel beauty contest. After washing, tissue was cut into small pieces and cultured in tissue culture dishes (60 × 15 mm) containing DMEM supplemented with 10% FBS. Tissue explants were removed after the proliferation of fibroblasts was observed. Fibroblast monolayers were dissociated and passaged by exposing the cells to an enzymatic solution (0.25% trypsin and 0.05% EDTA) for 5 min. Fibroblast cells were frozen at passage two. The cells were thawed, passaged, and were used between 3rd to 6th passage as nuclear donors after serum starvation by culture in DMEM plus 0.5% FCS for more than 72 h.

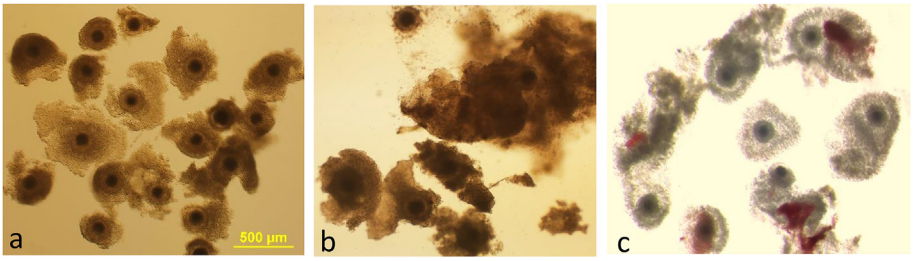

Fig. 1. Cumulus oocyte Complexes (COCs) harvested from: a, Ovaries of slaughterhouse origin; b, ultrasound guided ovum pick up (OPU) of super stimulated females without busereline administration and e, after busereline administration.