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Oocytes from small and large follicles exhibit similar development competence following goat cloning despite their differences in meiotic and cytoplasmic maturation

Min Yang [a], Justin Hall [a], Zhiqiang Fan [a], Misha Regouski [a], Qinggang Meng [a], Heloisa M. Rutigliano [a,b], Rusty Stott [a,b], Kerry A. Rood [a,b], Kip E. Panter [c], Irina A. Polejaeva [a,*]

[a] Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, Utah, USA
[b] School of Veterinary Medicine, Utah State University, Logan, Utah, USA
[c] USDA ARS Poisonous Plant Research Laboratory, Logan, Utah, USA

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ABSTRACT

Reduced developmental competence after IVF has been reported using oocyte derived from small follicles in several species including cattle, sheep, and goats. No information is currently available about the effect of follicle size of the cytoplast donor on *in vivo* development after somatic cell nuclear transfer (SCNT) in goats. Oocytes collected from large (≥3 mm) and small follicles (<3 mm) were examined for maturation and *in vivo* developmental competence after SCNT. Significantly greater maturation rate was observed in oocytes derived from large follicles compared with that of small follicles (51.6% and 33.7%, $P < 0.05$). Greater percent of large follicle oocytes exhibited a low glucose-6-phosphate dehydrogenase activity at germinal vesicle stage compared with small follicle oocytes (54.9% and 38.7%, $P < 0.05$). Relative mRNA expression analysis of 48 genes associated with embryonic and fetal development revealed that three genes (MATER, IGF2R, and GRB10) had higher level of expression in metaphase II oocytes from large follicles compared with oocytes from small follicles. Nevertheless, no difference was observed in pregnancy rates (33.3% vs. 47.1%) and birth rates (22.2% vs. 16.7%) after SCNT between the large and small follicle groups). These results indicate that metaphase II cytoplasts from small and large follicles have similar developmental competence when used in goat SCNT.

1. Introduction

Somatic cell nuclear transfer (SCNT) has nearly 20 years of history and has been successfully used for cloning of various livestock species [1–4]. One of the greatest benefits of this technology is its ability to provide a cell-mediated platform for livestock genetic engineering. Somatic cells can be genetically modified *in vitro* and screened to identify the cells containing the gene of interest, which then are used to generate transgenic animals via nuclear transfer. Despite the nearly two-decade history, SCNT efficiency remains low with typically only 1 to 4% of manipulated embryos developing to term.

Due to the low efficiency of SCNT, a large number of cytoplast donors are required for the procedure. A local abattoir is the best option to obtain goat ovaries for oocyte collection on a regular basis. However, goat ovaries from abattoirs are often derived from prepubertal goats. In

* Corresponding author. Tel.: +1 435 797 3718; fax: +1 435 797 2118.
E-mail address: irina.polejaeva@usu.edu (I.A. Polejaeva).

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prepubertal goats, most of the oocytes come from the follicles with a diameter smaller than 3 mm [5] making it difficult to release the cumulus-oocyte complexes (COCs) by traditional aspiration. For this reason, oocytes are routinely obtained by slicing the surface of the ovary, resulting in oocytes with a heterogeneous diameter, different COC morphology, and varying stages of development. Low SCNT efficiency is typically attributed to the quality and competence of the cytoplast donor, which is generally accepted as a key determining factor for the success of embryonic development [6]. The size of the follicles from which the oocytes are collected is one of the factors that influence the maturational and developmental competence using assisted reproductive technologies [7,8]. The existing data indicate that significantly less oocytes derived from follicles with a diameter of less than 3 mm reached metaphase II after IVM either in adult or prepubertal goats [9,10]. Furthermore, in cattle [11], sheep [12], and buffalo [13], oocytes from large follicles have greater blastocyst development rate after IVF than oocytes from small follicles. However, to our knowledge, no reports have been published on the effect of the follicle size of the cytoplast donor on the efficiency of goat cloning.

The aims of the present study were to investigate meiotic maturation of goat oocytes obtained from large and small follicles and compare the ability of cytoplast donors derived from small and large follicles to support embryonic and fetal development to term after goat SCNT. In addition, the enzymatic activity of glucose-6-phosphate dehydrogenase (G6PDH) was assessed in these two oocyte groups. Glucose-6-phosphate dehydrogenase is an enzyme synthesized in oocytes during oogenesis and folliculogenesis. The activity of G6PDH in oocytes has been previously correlated with several indicators of cytoplasmic maturation including cortical granule organization [14], activity of the mitochondria, cytoplasmic lipid content, and intracellular glutathione level [15–17], and therefore could be used as a marker of cytoplasmic maturation and developmental competence. Furthermore, we assessed gene expression levels of 48 genes in metaphase II (MII) oocytes derived from small and large follicle groups. The selected genes were previously implicated in embryonic and fetal development [18–21].

2. Materials and methods

All animal procedures were approved by and conducted according to the guidelines of the Utah State University Animal Care and Use Committee. All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.1. Oocyte collection

Ovaries from prepubertal domestic goats (*Capra aegagrus hircus*) were obtained from a local abattoir (Springville, UT, USA) and transported to the laboratory within 4 hours after collection. The ovaries were transported at 20 °C to 27 °C in saline containing 100-U/mL penicillin/streptomycin. On arrival to the laboratory, the ovaries were washed three times in saline and then three times in Dulbecco's phosphate-buffered saline (DPBS, Hyclone, Logan, UT, USA) containing 100-U/mL penicillin/streptomycin. Cumulus-oocyte complexes were recovered from ovaries in Modified TL-Hepes medium (Lonza, Walkersville, MD, USA) supplemented with 1% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 100-U/mL penicillin/streptomycin, and 30-μg/mL heparin using a modified slicing technique in which only the surface of the ovaries was sliced [22]. First, the surface of each large follicle (≥3 mm) was cut individually and rinsed with Modified TL-Hepes medium to release the oocytes from the large follicles. Ovaries were then moved to another dish to obtain the oocytes from remaining small follicles (<3 mm) by slicing the ovary surface. Oocytes with three or more layers of compact cumulus cells and homogeneous cytoplasm were used. The groups of oocytes derived from large and small follicles were treated separately throughout the process.

2.2. Brilliant cresyl blue (BCB) test

Brilliant cresyl blue staining was conducted to measure the G6PDH activity as previously described [23]. Immediately after collection, COCs were washed three times in modified DPBS (mDPBS, PBS supplemented with 1-g/L glucose, 36-mg/L sodium pyruvate, 0.5-g/L BSA, and 0.05-g/L gentamicin). The oocytes were treated with 26-μM BCB diluted in mDPBS at 38.5 °C in 5% CO_2 in air for 30 minutes and then washed two times in mDPBS. The blue color of BCB dye is reduced to colorless in the oocytes with high G6PDH levels. However, the blue color will remain in the cytoplasm of oocytes containing low levels of G6PDH [24]. We classified the oocytes into two groups according to their cytoplasm coloration. Oocytes with or without blue cytoplasmic coloration were designated as BCB+ (low G6PHD activity) and BCB− (high G6PDH activity).

2.3. In vitro maturation

The COCs were washed three times in maturation medium (TCM-199 [Gibco, Grand Island, NY, USA], containing 10% FBS, 10-μg/mL LH, 5-μg/mL FSH, 1-μg/mL estradiol-17β, and 0.05-g/L gentamicin) and then incubated in maturation medium for 22 hours at 38.5 °C in 5% CO_2 in air. The COCs were cultured in groups of 50 in 4-well plates containing 500 μL of maturation medium. After 22 hours of culture, cumulus cells were removed from oocytes by vortexing the COCs for 1 to 2 minutes in TL-Hepes containing 1-mg/mL hyaluronidase. Maturation status was assessed by the presence of a first polar body. Oocytes at this stage are termed MII oocytes.

2.4. Somatic cell nuclear transfer procedure

Skin ear biopsies were collected from a neonatal domestic goat for fibroblasts isolation. The cells were cultured in DMEM/high-glucose medium (Hyclone, Logan, UT, USA) supplemented with 15% FBS and 100 U/mL penicillin/streptomycin. The fibroblasts were grown to 80 to 90% confluence and used as nuclear donor cells for SCNT after 24 to 48 hours of serum starvation (0.5% FBS). The first polar body and metaphase plate were removed from an oocyte and a single donor cell was subsequently transferred into the perivitelline space of the enucleated oocyte. Fusion