Small Ruminant Research 112 (2013) 141–146

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Short communication

Developmental potential of vitrified goat oocytes following somatic cell nuclear transfer and parthenogenetic activation

Kanokwan Srirattana, Nucharin Sripunya, Anawat Sangmalee, Sumeth Imsoonthornruksa, Yuanyuan Liang, Mariena Ketudat-Cairns, Rangsun Parnpai*

Embryo Technology and Stem Cell Research Center, School of Biotechnology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

ARTICLE INFO

Article history:
Received 4 May 2012
Received in revised form 24 October 2012
Accepted 25 October 2012
Available online 20 November 2012

Keywords:
Cryotop
Goat oocyte
Somatic cell nuclear transfer
Vitrification

ABSTRACT

Oocyte cryopreservation is an alternative tool in assisted reproductive technology. The objective of this study was to determine the developmental potential of vitrified goat oocytes after somatic cell nuclear transfer (SCNT). *In vitro* matured goat oocytes were vitrified by Cryotop method. The survival rate of vitrified oocytes at 1 h after thawing determined by fluorescein diacetate staining was 92.1% which was significantly lower than that of fresh oocytes (99.3%). Live oocytes from both vitrified and fresh groups were used as recipient cytoplasts for SCNT. No significant difference in fusion rate was found between vitrified (97.6%) and fresh (93.2%) oocytes. The cleavage rates of vitrified oocytes in the SCNT and parthenogenetic activation (PA) embryos (29.6% and 27.9%) were significantly lower than those from fresh SCNT and PA oocytes (80.9% and 91.1%). The developmental rates to 8-cell stage of SCNT and PA embryos from vitrified oocytes were also lower than that of fresh oocytes. There was no significant difference among the groups in the development to morula stage (11–27%). However, the blastocyst rate of PA embryos derived from fresh oocytes (12.5%) was significantly higher than the other groups (1.2–3.3%). Although high survival rate of vitrified oocytes was obtained, the cleavage, 8-cell, and blastocyst formation rates of SCNT and PA embryos from vitrified oocytes were still lower than those of fresh oocytes.

1. Introduction

Dairy goat is one of livestock species that provides milk and meat. It is also ideal for the transgenic production of therapeutic recombinant proteins. Somatic cell nuclear transfer (SCNT) is an important technology both for producing cloned or transgenic animals and for research purposes. Oocyte cryopreservation is a tool for preservation of female genetic resources and for minimizing limitations associated with oocyte production and recovery. Cryotop vitrification is an effective method involves placing oocytes or embryos in a small volume of cryoprotectant solution on a plastic sheet of Cryotop device and most of the loading solution is removed by aspiration before plunging directly into liquid nitrogen (LN_2). Therefore, the final volume is approximately 0.1 μl, which is the advantage of this technique because the cooling and warming rates are very fast, the chilling injury at vitrification step and recrystallization at warming step are avoided (Kuwayama et al., 2005). The Cryotop method has been successfully used to cryopreserve oocytes in bovine (Dinnyés et al., 2000; Chian et al., 2004; Inaba et al., 2011), sheep (Succu et al., 2008), buffalo (Gasparrini et al., 2007; Muenthaisong et al., 2007; Liang et al., 2011, 2012a,b), pig (Galeati et al., 2011) and rabbit (Jiménez-Trigos et al., 2012) and embryos in bovine, buffalo (Laowtammathron et al., 2005) and goat (Morató et al., 2011). Vitrification of immature goat oocytes has been performed using several different methods for

* Corresponding author. Tel.: +66 44 22 3164; fax: +66 44 22 3164.
E-mail address: rangsun@g.sut.ac.th (R. Parnpai).

0921-4488/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.smallrumres.2012.10.011

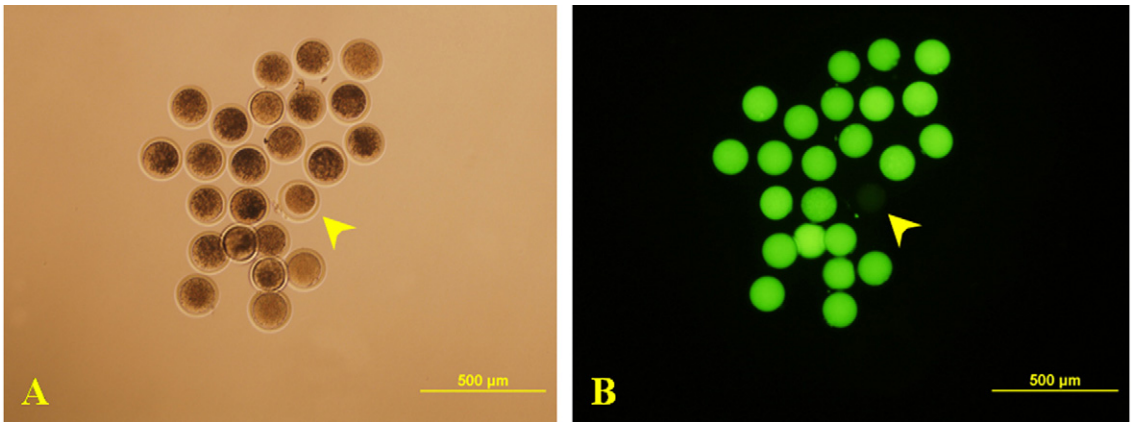

Fig. 1. Fluorescein diacetate staining of oocytes in bright field (A) and UV light (B). Arrow head indicates dead oocytes. Magnification 40×.

example; conventional straw (Kharche et al., 2005; Sharma et al., 2006), hemistraw, Cryoloop, and Cryotop methods (Rao et al., 2012). However, fewer vitrified immature goat oocytes (27.5%) reached metaphase II (MII) stage when compared with fresh oocytes (74.2%; Kharche et al., 2005). Previous studies reported that the immature oocytes are more sensitive to vitrification than matured oocytes (Rojas et al., 2004; Dobrinsky and Johnson, 1994). The low developmental rate of vitrified immature oocytes may come from impaired intracellular communication between the oocyte and the cumulus cells and disruption of microfilaments (Rojas et al., 2004). In SCNT process, oocytes at MII stage have been used as recipient cytoplast in difference species (Campbell et al., 2005) including goat (Baguisi et al., 1999). Due to the limitation of oocyte obtained from live and slaughterhouse animals, oocyte vitrification is an alternative way to solve this problem. Begin et al. (2003) reported that viability of vitrified matured goat oocytes by solid surface vitrification (SSV) were lower than that of fresh oocytes. But, no significant difference in survival rate was found when the SSV method was compared with the Cryoloop method. Although matured goat oocytes have been cryopreserved by SSV and Cryoloop methods, no studies have been conducted on vitrification by Cryotop followed by SCNT. Therefore, the objective of this study was to determine the developmental potential of SCNT embryos derived from vitrified goat oocytes.

2. Materials and methods

2.1. Animals

Animal care and procedures were conducted according to the guidelines of the Ethics Committee of the Laboratory Animal Care of Suranaree University of Technology.

2.2. Chemicals

All chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA) unless otherwise stated.

2.3. Oocyte collection and in vitro maturation

Estrous cycles of mixed-breed goats aged 8–12 months old used as donor were synchronized by insertion of an intravaginal progesterone-releasing device (CIDR®, Pfizer Animal Health, Auckland, New Zealand) for 12 d. Two days before CIDR removal, an intramuscular (i.m.) injection of 30 mg follicle stimulating hormone (FSH, Folltropin®, Bioniche, Ontario, Canada) was performed every 12 h for 8 times. At the second FSH injection, each goat received an i.m. injection of 0.4 mg synthetic prostaglandin ($PGF_{2\alpha}$, Iliren®, Intervet International GmbH, Unterschleißheim, Germany) and at the sixth FSH injection, CIDR was removed. At 2.5 d after the last FSH injection, ovariectomies were performed. The cumulus-oocyte complexes (COCs) were collected by aspiration from 2 to 8 mm diameter follicles using a 21-gauge needle attached to a 10 ml syringe and then cultured in *in vitro* maturation (IVM) medium under a humidified atmosphere of 5% CO_2 in air at 38.5 °C for 21 h. The cumulus cells were then removed by repeat pipetting in 0.2% hyaluronidase. The MII oocytes (extruded the first polar body) were separated into fresh and vitrified groups.

2.4. Oocyte vitrification

Groups of 5 MII oocytes were washed in base medium (BM) consisting of TCM199-Hepes supplemented with 20% fetal bovine serum (FBS, Gibco, MD, USA) and then placed in BM supplemented with 10% dimethylsulfoxide (DMSO) and 10% ethylene glycol (EG) for 1 min. After that, oocytes were transferred to BM supplemented with 20% DMSO, 20% EG and 0.5 M sucrose for 30 s, and then placed on Cryotop device (Kitazato Supply, Tokyo, Japan) and immediately submerged into LN_2 (Liang et al., 2012a).

Vitrified oocytes were warmed by placing the tip of the Cryotop into 3 ml of BM supplemented with 0.5 M sucrose at 38.5 °C for 5 min, then transferred to BM for another 5 min, and cultured in BM under a humidified atmosphere of 5% CO_2 in air at 38.5 °C for 1 h.

2.5. Evaluation of oocyte viability

The oocyte viabilities from both vitrified and fresh groups were examined by fluorescein diacetate (FDA) staining (Mohr and Trounson, 1980). Briefly, the oocytes were treated with phosphate buffered saline (PBS) supplemented with 2.5 μg/ml FDA and 5 mg/ml bovine serum albumin (BSA) for 2 min in the dark. The oocytes were then washed in PBS supplemented with 5 mg/ml BSA for 3 times before visualization under ultraviolet light from a fluorescence microscope (IX71, Olympus, Tokyo, Japan). Live oocytes that expressed bright green fluorescence (Fig. 1) were used in the next step.

2.6. Embryo production by somatic cell nuclear transfer and parthenogenetic activation

The donor cell preparation, SCNT, parthenogenetic activation (PA) and *in vitro* embryo culture were performed according to the methods of a previous report with some modifications (Srirattana et al., 2010). Live oocytes from both vitrified and fresh groups were used for SCNT and PA. Briefly, an individual ear fibroblast from a male goat was transferred into an enucleated goat oocyte and fused with electrical stimuli (26 V, 15 μs, 2 DC pulses) in Zimmermann fusion medium (Zimmermann and Vienken, 1982). The success of the fusion was examined 1 h after