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Effect of liquid helium vitrification on cytoskeleton of immature cattle oocytes

Xian Fei Guo[a,b], Xue Li Yu[a,b,*], Fan Zhang[a,b], Hua Wu[a,b], Xu Zhe Pei[a,b], Xiao Xia Li[a,b], Ying Hua Li[a,b]

[a] College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471023, China
[b] Henan Provincial Key Laboratory for Grass-Feeding Animal, Henan University of Science and Technology, Luoyang 471023, China

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ABSTRACT

The developmental potential and the changes in cytoskeleton structures in immature oocytes of cattle resulting from liquid helium (LHe) vitrification was evaluated in this study. Immature oocytes were randomly divided into three groups: fresh oocytes (negative control), oocytes vitrified in liquid nitrogen (LN group, positive control), and oocytes vitrified in LHe (LHe group). In Experiment 1, the proportions for normal morphology, maturation, cleavage, and blastocyst were greater in the LHe group than in the LN group (88.3% compared with 79.1%, 51.7% compared with 43.3%, 42.6% compared with 33.0%, and 11.0% compared with 4.7%, respectively; $P < 0.05$), and the rates of oocyte development were greater in the control group (100%, 72.8%, 64.3%, and 40.3%) than in the vitrified groups ($P < 0.05$). In Experiment 2, the effect of vitrification by LHe and LN on cytoskeleton of cattle oocytes was examined. The cytoskeleton had varying degrees of damage, and the negative influence of LHe vitrification on the cytoskeleton was less than that of LN vitrification ($P < 0.05$), and the vitrified group had greater cytoskeleton degeneration than the control group ($P < 0.05$). In conclusion, LHe vitrification reduced the negative effect of cryoinjury on cytoskeleton structure and improved the viability of immature oocytes of cattle compared with LN vitrification.

1. Introduction

Oocyte cryopreservation is a powerful method employed in reproductive biotechnologies to store animal oocytes for extended periods (Sripunya et al., 2010). Most recent investigations have focused on cryopreservation of mature oocytes, however, which induces disruption of spindle, chromosomes, and microfilaments (Shaw et al., 2000; Men et al., 2002), and migrated cortical granules (Fuku et al., 1995; Hyttel et al., 2000). Cryopreservation of immature oocytes is a potential alternative approach because the meiotic spindle is absent, and genetic material is confined within the nucleus (Massip, 2003). Immature oocytes are, however, extremely sensitive to cryopreservation because of damage to the cytoplasmic membrane, gap junction integrity, and communication between cumulus cells and oocytes (Arav et al., 1997; Diez et al., 2005). An improved method to cryopreserve immature oocytes must, therefore, be developed.

Vitrification of oocytes by liquid nitrogen (LN) has been successfully performed using several types of cryodevices, such as open pulled straws (OPSs), cryotops, and electron microscopy grids (Martino et al., 1996; Vajta et al., 1998; Morato et al., 2008b). The

* Corresponding author at: College of Animal Science and Technology, Henan University of Science and Technology, No. 263 Kaiyuan Road, Luolong District, Luoyang 471023, China.
E-mail address: yxl4282333@126.com (X.L. Yu).

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developmental potential of vitrified-warmed mammalian oocytes remains low, however, because of possible damage to the cytoskeleton (Diez et al., 2012; Moussa et al., 2014). The cytoskeleton is a complex network structure composed of microfilaments, microtubules, and intermediate filaments (Goode et al., 2000). These components are all distributed in the oocyte cytoplasm and have important roles in maturation or early embryo development, such as in maintenance of cell shape, intracellular transport, and cytokinesis (Mo et al., 2014). Low temperatures induce damage to the cytoskeleton as evidenced by abnormal spindle microtubule configuration, disrupted chromosomes, and disorganized microfilaments (Morato et al., 2008a). These cellular changes could further impair the survival and developmental ability after vitrification and warming.

The success rate of vitrification depends on three pivotal factors, namely, cooling rate, concentration of cryoprotectants, and sample volume (Lyu et al., 2013). Liquid helium (LHe), with its low boiling point (− 269 °C), may increase the cooling rate during vitrification. Normal morphology and post-thaw developmental competence of immature cattle oocytes were enhanced with LHe vitrification as compared with LN vitrification (− 196 °C) (Chen et al., 2014). The effect of LHe vitrification on cytoskeleton structure in immature cattle oocytes, however, remains unexplored.

The aims of the present study were, therefore, (1) to compare the development competence of immature cattle oocytes vitrified using LHe and LN and (2) to investigate the effect of LHe vitrification on cytoskeletal integrity of immature cattle oocytes.

2. Materials and methods

Unless otherwise indicated, all chemicals were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). The OPSs were prepared in the laboratory where the present research was conducted by using 0.25 mL French straws (IMV, L'Aigle, France).

2.1. Oocyte collection

Ovaries of cattle (Chinese yellow cattle, *Bos taurus*) were placed in physiological saline at 38.5 °C and transported from a local abattoir to the laboratory within 4 h. Cumulus oocyte complexes (COCs) were obtained from antral follicles (2–8 mm) through aspiration using an 18-g needle attached to a 10 mL syringe. Medium (1 to 2 mL of TCM-199 supplemented with 3% [v:v] FBS) was aspirated into the syringe before aspirating COCs and follicular fluid. Only COCs with three or more layers of cumulus cells and which had a homogeneous cytoplasm were selected for further processing (Diez et al., 2005).

2.2. Preparation of OPSs

After removing the cotton plug, the French straws were softened by heating over a flame and pulled manually until the inner diameter decreased from 1.7 mm to approximately 0.8 mm and the wall thickness of the central part decreased from approximately 0.15 mm to 0.07 mm. After cooling, the straws were cut at the narrowest location of the pulled portion. The outer diameter and wall thickness of the open pulled straw was measured by amplifying 50 times with a microforge and then was converted into actual size.

2.3. Vitrification

2.3.1. LN OPS vitrification

The LN OPS vitrification of immature oocytes was performed according to the method described previously (Vajta et al., 1998). All treatments before vitrification were performed at room temperature (25 °C–27 °C). The COCs were first equilibrated in holding medium (HM) consisting of TCM 199-Hepes + 20% (v/v) fetal bovine serum (FBS) for 5 min and initially dehydrated through exposure to vitrification solution-1 (VS1) for 1 min. The VS1 contained HM with 10% Dimethyl sulfoxide (Me_2SO, C_2H_6OS) and 10% ethylene glycol (EG). The oocytes were subsequently transferred in 20 μL droplets of HM containing 20% Me_2SO, 20% EG, and 0.5 M sucrose (vitrification solution-2; VS2). Straws with five oocytes each were loaded by touching the surface of an approximately 1.5 μL of VS2 droplet containing the oocytes; the narrow tip of the OPS was used in touching the droplet surface, and then the OPS was directly submerged in LN (Diez et al., 2005; Moawad et al., 2012). The time interval between the contact with VS2 and cooling did not exceed 30 s.

2.3.2. LHe OPS vitrification

The method used in LHe OPS vitrification was similar to that used in LN OPS vitrification, except that the procedures for plunging OPS into LHe. Because LHe was stored in Dewar (Cryofab, CMSH 60) and cannot be poured, the steps of plunging OPS into LHe were as follow. The COCs were aspirated into OPS, then the OPS with COCs was attached to the end of an iron wire (1.5 m in length and 3 mm in diameter) by using scotch tape and then immediately inserted into the LHe Dewar. Insertion of the iron wire was stopped when a large amount of gas was emitted from the Dewar. The OPS with oocytes was transferred from the LHe Dewar to the LN container after the OPS was immersed in LHe for a few seconds until the gas emission stabilized. The time interval between contact with VS2 and immersion into LHe did not exceed 30 s, and the time between retrieval of OPS from LHe and plunging the OPS in LN did not exceed 3 s. The OPS was removed from the iron wire by cutting the tape with a knife in LN, and the OPS containing COCs was preserved in LN (Fig. 1).