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Effect of meiotic status, cumulus cells and cytoskeleton stabilizer on the developmental competence of ovine oocytes following vitrification

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ABSTRACT

This study was conducted to determine the effect of meiotic status, cumulus cells and cytoskeleton stabilizer on ovine oocyte vitrification. Oocytes at various developmental stages including GV (germinal vesicle), GVBD (GV breakdown), MI (metaphase I) and MII (metaphase II) were vitrified using open pulled straw (OPS) method. After warming, the survival rates were determined based on the morphological appearance and 3′,6′-diacetyl fluorescein staining. The developmental potential of treated oocytes was evaluated by their ability to undergo successful in vitro fertilization (IVF) and support embryo development in culture after in vitro maturation. In the first experiments, we evaluated the effect of meiosis status on oocytes vitrification. Survival rates of oocytes after warming were not different among all groups. However, significantly higher proportion of cleavages and blastocysts were obtained from vitrified MII oocytes than those from vitrified immature oocytes. Next, we selected MII oocytes to determine the influence of cumulus cells on vitrification and the results showed that survival rates were not affected by the absence of cumulus cells. Furthermore, the cleavage rates and blastocyst rates were not different with or without cumulus cells. Lastly, we examined the effect of cytoskeleton stabilizer on MII oocyte vitrification. Compared with the vehicle treated controls, pretreatment with Taxol significantly improved the survival rates (81.91% vs. 66.00%), cleavage rates ((52.29% vs.34.25%) and blastocyst rates (9.72% vs. 4.86%). Pretreatment of MII oocytes with another cytoskeleton stabilizer Cytochalansin B had no effect on oocyte survival and in vitro embryo development. Collectively, the meiotic status affected the developmental potential of oocytes after vitrification. MII stage oocytes showed better resistance to cryopreservation compared with the oocytes at immatured stages. Taxol treatment prior to vitrification was beneficial to vitrified/warmed ovine matured oocytes.

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1. Introduction

Oocyte cryopreservation provides an efficient way to preserve female germ cells that benefits assisted reproduction technology (ART) and animal biotechnology (Atabay et al., 2004). The establishment of an ova bank relies on successful oocyte cryopreservation (Kharche et al., 2005). However, despite many years of research, the oocyte is still more difficult to cryopreserve than cleavage-stage embryos (Shaw et al., 2000). The sensitive nature of oocyte to cryopreservation may be attributed to several factors, including size, shape, permeability, and lipid content (Bhati et al., 2013). Several lines of evidences suggest that cryopreservation leads to spindle disorganization and microtubules depolymerization, therefore present a detrimental effect to oocyte development after warming (Aman and Parks, 1994). In addition, oocyte cropreservation may interrupt meiosis progression (Ledda et al., 2007). Such changes may result in increasing of chromosomes aneuploidy.

It has been recently reported that immature and mature oocytes respond differently to cryopreservation, indicating that meiotic status may affect oocyte survival and developmental competence after warming (Men et al., 2002; Magnusson et al., 2008). Several studies revealed that MII oocytes had better resistance to cryopreservation than immature oocytes in pig (Rojas et al., 2004) and in cattle (Men et al., 2002). However, there are controversial evidences available suggesting that bovine MII oocytes were less tolerant to vitrification than GV oocytes (Magnusson et al., 2008). The most obvious freezing damage in MII vitrified oocytes is meiotic spindle disorganization followed by microtubule depolymerization (Men et al., 2002; Rojas et al., 2004). Unlike matured oocytes, the use of immature oocytes for cryopreservation may circumvent some of the limitations associated with the cooling/warming of matured oocytes, specifically relating to the functional integrity of the meiotic spindle and ploidy of resulting embryos (Mandelbaum et al., 2004; Koutlaki et al., 2006; Ledda et al., 2007). However, little is known about the effect of the meiosis status on their developmental competence after vitrification in sheep.

It has been reported that bovine oocytes vitrified without cumulus cells had a higher survival rate after thawing and a significant higher percentage of embryos developed to the 8-cell stage compared with oocytes vitrified with cumulus cells (Chian et al., 2004). Similarly, the removal of cumulus cells before vitrification of immature ovine oocytes led to an increase in survival rate (Bogliolo et al., 2007). It has been suggested that cumulus cells may hamper penetration of cryoprotectants during the vitrification procedure, leading to the uneven intracellular distribution of cryoprotecntants, which therefore affects oocyte cryosurvival and developmental competence (Hyttel et al., 2000). However, the effect of the cumulus cells on matured ovine oocyte vitrification has not been carefully examined.

The growth, maturation and fertilization of oocytes requires an active movement and a correct localization of cellular organelles (Sun and Schatten, 2006). Components of the oocyte cytoskeleton are known to play vital roles during the MI to MII stage transition. For example, microtubules of the meiotic spindle are responsible for the movement of chromosomes during homologue segregation (Sun et al., 2001). However, the meiotic spindle of the oocyte is vulnerable to cryoinjury, and the impaired development of cryopreserved oocytes at MII has been attributed to damage to the spindle (Chen et al., 2003). Therefore, the beneficial effect of the cytoskeletal stabilizers like Cytochalasin B (CB) in porcine (Rojas et al., 2004; Gupta et al., 2007) and sheep (Shirazi et al., 2012) or Taxol in cattle (Morato et al., 2008), pig (Shi et al., 2006) and sheep (Zhang et al., 2009) on oocyte vitrification have been demonstrated. To our knowledge, the studies on comparing the effect of Taxol or CB treatment on ovine oocyte vitrification have not been reported.

Based on these observations, meiotic status and cumulus play important roles on vitrification efficiency and Taxol or CB could prevent damage to cytoskeleton. Thus, the present study was undertaken to investigate the effect of meiotic status, cumulus cells and cytoskeleton stabilizer (Taxol and CB) on the efficiency of ovine oocyte vitrification by open-pulled straw (OPS) method.

2. Materials and methods

2.1. Reagents

All chemicals and media were purchased from Sigma Chemical Company (St. Louis, MO), unless indicated otherwise.

2.2. Collection of oocytes and in vitro maturation

Ovaries were collected at local slaughter house and transported to the laboratory within 2 h in physiological saline at 35 °C. All of the animal experiments in this study were approved by Institutional Animal Care and Use Committee of China Agricultural University. Cumulus oocytes complexes (COCs) were collected from 2 to 6 mm follicles by aspiration using an 18-gauge needle. The oocytes with at least three layers of compact cumulus cells and evenly granulated ooplasm were cultured in the maturation medium in humidified atmosphere of 5% CO_2 at 38.8 °C for 24 h. The maturation medium contained 20% (v/v) heat-inactivated estrous sheep serum, 10 μg/mL FSH, 10 μg/mL LH, 10 ng/mL epidermal growth factor (EGF) and 1 μg/mL estradiol 17-β in TCM199 medium.

2.3. In vitro fertilization (IVF)

The oocytes were fertilized with sperm in synthetic oviduct fluid (SOF) medium containing 20% (v/v) estrous sheep serum and 10 μg/mL heparin (IVF medium). The sperm concentration was approximately 1×10^6/mL. The plates were incubated for 22 h at 38.8 °C in a 5% CO_2 humidified air atmosphere.

2.4. In vitro culture

At approximately 22 h post-insemination (hpi), presumptive zygotes (oocytes that were exposed to spermatozoa for fertilization to take place) were washed in SOF