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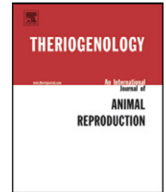

Expression and distribution of cell adhesion-related proteins in bovine parthenogenetic embryos: The effects of oocyte vitrification

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The objective was to investigate expression of cell adhesion-related proteins (E-cadherin, β-catenin, and the cytoskeletal protein F-actin) in bovine parthenogenetic embryos derived from vitrified-warmed oocytes. Bovine oocytes at metaphase II were randomly allocated into three groups: (1) untreated (control); (2) exposed to vitrification solution without freezing (toxicity); and (3) vitrified and warmed by the open-pulled straw method (vitrification). After parthenogenetic activation, in the vitrification group compared with the control, the timing of compaction was delayed in (108–120 vs. 96–108 hours, respectively), and the percentage of blastocysts that developed from eight-cell embryos was lower (32.08% vs. 61.03%; $P < 0.05$). To investigate whether vitrification delayed embryo compaction by affecting adhesion junction formation and function, immunostaining and quantitative reverse transcription polymerase chain reaction were done to characterize distribution patterns (E-cadherin, β-catenin, and the cytoskeletal protein F-actin) and expression levels of cell adhesion-related proteins (β-catenin). Distribution of β-catenin in eight-cell embryos from the vitrification group changed dramatically compared with the control and toxicity groups. Relative expression of β-catenin at the mRNA and protein levels was lower ($P < 0.05$) than that of the fresh and toxicity groups. However, expression and distribution of E-cadherin were similar among groups. In conclusion, abnormal distribution and decreased expression of β-catenin in bovine parthenogenetic eight-cell embryos derived from vitrified-warmed oocytes were associated with embryo compaction and reduced competence for subsequent embryo development.

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1. Introduction

Highly efficient oocyte cryopreservation is very important for the success of assisted reproduction technology in humans and for preservation of genetic material in domestic or endangered species [1,2]. However, mammalian oocyte cryopreservation remains difficult, at least in part because of their large size and low surface:volume ratio, which makes it difficult for cryoprotectants to move across the plasma membrane [3]. Although the birth of healthy calves from cryopreserved oocytes has been reported, the developmental potential of vitrified-warmed bovine oocytes is extremely low after IVF [4–6]. The decreased blastocyst rate caused by oocyte cryopreservation might be a result of many factors [7,8], including instability of plasma membrane proteins [9,10], change of ultrastructural consequences [11], loss of cytoplasmic mRNA [12], and DNA damage [13].

Cell adhesion molecules, a group of cell surface proteins [14], have key roles in trophectoderm epithelium differentiation and blastocyst morphogenesis [15]. In the mouse embryo, E–cadherin-mediated adhesion initiates embryo

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compaction at the eight-cell stage, largely dependent on protein kinase C activities [15]. Embryos deficient in E-cadherein mRNA and proteins had lower blastocyst rates [16]. Catenins were first identified as molecules tightly associated with the cell adhesion molecule E-cadherin [17] and were termed α-, β-, and γ-catenin, based on their molecular sizes of 102, 88, and 80 kDa, respectively [18,19]. β-Catenin becomes plasma membrane-associated when it interacts with E-cadherin and mediates zonula adherens formation during preimplantation [20,21]. In addition, E-cadherin/β-catenin complex can also partner with α-catenin to form an E-cadherin/β-catenin/α-catenin complex [22]. This complex, derived from maternal and zygotic genes, mediates adhesion of early blastomeres at two-, four-, and early eight-cell stages, but an increasing amount of the complex is accumulated and stored in a nonfunctional form ready to be used for compaction [23]. F-actin association is essential for adherens junction development, remodeling, and function [24]. Alteration in expression of any of these molecules might result in abnormal embryo compaction and decreased embryo development. However, it is unclear whether expression of these molecules in the embryo is affected by oocyte cryopreservation.

The switch from maternal to embryonic genome control in cattle appears to occur at the eight- to 16-cell stages [25]. Depletion of active maternal mRNA in the oocyte cytoplasm or interruption of the shift from the maternal to embryonic control might result in a permanent arrest of embryo development at the eight- to 16-cell stages before compaction [26]. Therefore we focused our interest on eight-cell stage embryos. In this study, the effects of vitrification on bovine oocyte development and embryo compaction were examined. Because oocyte vitrification delayed compaction timing in eight-cell embryos, distribution patterns and expression levels of three cell adhesion-related molecules were examined to determine whether vitrification affected embryo compaction by modulating adhesion molecules in preimplantation embryos.

2. Materials and methods

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

2.1. Oocyte collection and in vitro maturation

Bovine (*Bos taurus*, age 3–6 years) ovaries were transported from the abattoir to the laboratory in physiological saline solution at 26 °C to 30 °C within 2 hours after slaughter. Antral follicles (2–8 mm in diameter) were manually aspirated using an 18-gauge needle attached to a 10-mL syringe. Oocytes with at least four layers and compact cumulus cells (COCs) were selected for *in vitro* maturation (IVM). Oocytes were washed three times in HEPES-buffered TCM-199 medium and then washed twice in $NaHCO_3$-buffered TCM-199. Fifty COCs were transferred to 0.75-mL maturation medium (M199 with 10 mg/mL oFSH [Ovagen, Auckland, New Zealand], 10 mg/mL oLH [Ovagen], 1 mg/mL estradiol [Ovagen], and 10% fetal bovine serum [FBS; Gibco]) in 4-well plates (Nunclon). The COCs were cultured for 22 hours at 38.5 °C in a humidified atmosphere with 5% CO_2.

2.2. Oocyte vitrification and warming

After IVM, cumulus cells were removed by incubation in hyaluronidase at 38 °C for 5 minutes and then disrupted by repeated pipetting for 1 minute. Cumulus-free normal metaphase (M)II oocytes with the first polar body were selected and randomly allocated to the following experimental groups:

(1) Control group: oocytes underwent no treatment, but were cultured until parthenogenetic activation.
(2) Toxicity group: oocytes were first exposed to 10% ethylene glycol (EG) and 10% dimethyl sulfoxide (DMSO) in Dulbecco's phosphate-buffered saline (DPBS) containing 20% FBS for 30 seconds; EDFSF30 solution (15% EG and 15% DMSO in FSF solution, which consisted of DPBS medium with 300 g/L Ficoll, 0.5 M sucrose, and 20% FBS) for 25 seconds; then rinsed in 0.25 M sucrose solution for 3 minutes followed by 0.15 M sucrose solution for 3 minutes. Oocytes were then washed twice in maturation medium. Surviving oocytes were transferred to fresh culture media and incubated for 1 hour at 38.5 °C in 5% CO_2 before parthenogenetic activation.
(3) Vitrification group: oocytes were first pretreated in 10% EG with 10% DMSO in DPBS containing 20% FBS for 30 seconds, then transferred to EDFSF30 in the narrow end of the pulled straw, and held for 25 seconds. The straws were then immediately plunged into liquid nitrogen, and five to six oocytes were then loaded into each open pulled straw.

When warming oocytes, the tip of the open pulled straw was placed into 0.25 M sucrose solution (at approximately 38 °C–39.8 °C), and oocytes were expelled using a mouth pipette. Then, oocytes were then rinsed in sucrose solutions according to the procedure mentioned in the toxicity group. Surviving oocytes were cultured for 1 hour, followed by parthenogenetic activation.

2.3. Parthenogenetic activation

Before activation, oocytes were washed three times in HEPES-buffered TCM-199 with 10% FBS (H199) and then activated by the following treatments: (1) incubation for 5 minutes in 7% ethanol in H199 at room temperature; or (2) cultured for 4 hours in 2 mM 6-DMAP in M199. Fifteen oocytes were transferred to development culture drops of 60 μL Charles Rosenkrans 1 [27] with BSA (3 mg/mL) and cultured at 38.5 °C in 5% CO_2 for up to 48 hours before determining rates of activation and cleavage. Cleaved embryos were cultured for an additional 5 days in Charles Rosenkrans 1 with 5% FBS.

2.4. Cell counting

Blastocysts were fixed and stained for differential cell counting as described by Thouas et al. [28]. All blastocysts

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