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Endoplasmic reticulum stress inhibition is a valid therapeutic strategy in vitrifying oocytes ☆

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ABSTRACT

The aim of this study is to determine the link between oocyte cryopreservation and endoplasmic reticulum (ER) stress; whether ER stress inhibition improves the efficiency of oocyte vitrification is also explored. Oocytes from mice were exposure to tauroursodeoxycholic acid (TUDCA, an ER stress inhibitor) or TM (tunicamycin, an ER stress inducer) with or without vitrification. The expressions of X-box binding protein-1 (XBP-1) protein and caspase-12 protein, viability of vitrified-warmed oocytes, and their subsequent embryo competence were measured. The levels of XBP-1 protein and caspase-12 protein expression in vitrified-warmed oocytes were significantly higher than those of fresh control oocytes. TUDCA improved the viability of vitrified-warmed oocytes and their subsequent embryo competence. Mouse oocyte cryopreservation is associated with ER stress, and ER stress inhibition improves the efficiency of oocyte vitrification.

Introduction

Freezing of oocytes for fertility preservation is an option for women who do not have a partner and for women do not wish to preserve embryos because of religious or ethical concerns [26]. Clinical applications for oocyte cryopreservation also include ovum donation programs [6,22], minimization of ovarian hyperstimulation syndrome risk, oocyte accumulation in low-responder patients [21], and surplus oocyte storage after controlled ovarian stimulation when embryo cryopreservation is not feasible [4].

Metaphase II oocyte cryopreservation by vitrification has for the two last decades, been at the forefront of research in assisted reproduction technologies. Because the oocyte is the largest mammalian cell, it has been difficult to crypreserve with the use of the classic slow freezing protocol, owing to its sensitivity to ice crystal formation because of the cytoplasmic volume and its water charge [25]. Oocyte vitrification techniques that associate very high cooling rates and high cryoprotectant concentrations have been shown to avoid the formation of ice crystals and to improve oocyte survival rate after warming (odds ratio 2.46, 95% confidence interval 1.82–3.32) and fertilization rate (odds ratio 1.50, 95% confidence interval 1.07–2.11) [5,14,23]. Up to now, over 10,000 pregnancies and infants born after fertilization of cryopreserved oocytes have been reported worldwide [24]. The main problem raised by vitrification is the possible toxic effects, because of the use of high concentration of cryoprotectants. The underlying mechanism of cryoinjury to oocytes is not well elucidated yet [1,7,9,19].

The endoplasmic reticulum (ER) is the site for biosynthesis of lipids, membrane proteins, and secretory proteins [16]. Several stimuli, including mutations, chemical treatment or environmental stress, that disrupt ER homeostasis, affect proper protein folding and lead to the accumulation of unfolded and misfolded proteins in the ER lumen, followed by induction of ER stress [15]. ER stress, in turn, leads to the activation of a series of adaptive pathways known as unfolded protein response (UPR) to maintain ER homeostasis [2]. In cases where ER stress is prolonged or too severe to resolve, apoptosis is induced [27]. Previous studies reported that oocyte cryopreservation caused ultrastructural alterations to organelles, such as smooth endoplasmic reticulum swelled [10]. Therefore, in this study, we designed experiments to determine whether cryopreservation induces ER stress. Tauroursodeoxycholic

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acid (TUDCA), a bile acid acting as a potent chemical chaperone that inhibits ER stress in vitro, was employed to determine whether ER stress inhibition relieves injury to oocytes caused by cryopreservation [31]. Tunicamycin (TM), a chemical reagent, is generally used to induce ER stress [33], so we explore whether TM aggregates ER stress induced by oocyte vitrification. Its possible underlying mechanism is also evaluated.

Materials and methods

Animals

Female ICR mice (10 weeks of age) (n = 100) were used for oocyte collection. The mice were housed under temperature-controlled conditions (22 ± 2 °C). Food and water were available at all times under photoperiod of 12 h of light and 12 h of dark. The Animal Care Committee of Hospital for Maternity and Child Care of Jinan City approved all the experimental procedures carried out in the study.

Oocyte collection

Female mice were super-ovulated by an intraperitoneal injection of 10 IU pregnant mare's serum gonadotropin and 48 h later followed by an intraperitoneal injection of 10 IU human chorionic gonadotropin. Cumulus-oocyte-complexes (COCs) were collected from the ampullar region of the oviduct 14 h after HCG injection. Cumulus cells were dispersed by incubating the COCs for 1 min in a solution containing hyaluronidase, and the cells were then removed by pipetting gently. The denuded oocytes were then washed with HEPES-buffered human tubal fluid supplemented with 1.0 mg/mL bovine serum albumin (BSA) at room temperature before being subjected to cryopreservation experiments. Only morphologically normal metaphase II (MII) stage oocytes as judged by the presence of a first polar body were included in the study.

Oocyte vitrification and warming procedures

The oocytes were randomly divided into fresh groups and vitrification groups (n = 100 per group). The fresh groups include group C0 (not exposure to either TUDCA or TM), group CI (exposure to 50 μM TUDCA in Dulbecco's phosphate buffered saline for 5 min), and group CII (exposure to 1 μM TM in Dulbecco's phosphate buffered saline for 5 min). The vitrification and warming procedures were similar to those reported by Huang et al. [12]. The vitrification groups were divided into three groups. The vitrification solutions contained either no supplements (group V0), or TUDCA alone (group VI) or TM alone (group VII).

Group B: equilibration solution was prepared with 7.5% (v/v) ethylene glycol (EG) + 7.5% (v/v) 1,2-propanediol (PROH) in Dulbecco's phosphate buffered saline (DPBS); vitrification solution was prepared with 15% (v/v) EG + 15% (v/v) PROH in DPBS.

Group C: equilibration solution was prepared with 7.5% (v/v) EG + 7.5% (v/v) PROH + 50 μM TUDCA in DPBS; vitrification solution was prepared with 15% (v/v) EG + 15% (v/v) PROH + 50 μM TUDCA in DPBS.

Group D: equilibration solution was prepared with 7.5% (v/v) EG + 7.5% (v/v) PROH + 1 μM TM in DPBS; vitrification solution was prepared with 15% (v/v) EG + 15% (v/v) PROH + 1 μM TM in DPBS.

Briefly, the oocytes were suspended in an equilibration solution for 3 min. The oocytes were then transferred to the vitrification solution for 45–60 s at room temperature. 4–5 oocytes were loaded onto a McGill Cryoleaf (MediCult Company, Denmark) which was then immediately plunged into liquid nitrogen for 1 week. For warming, the McGill Cryoleaf was immersed directly in a 37 °C-warming solution (containing 1.0 M sucrose in 10% FBS-supplemented DPBS) for 1 min. The warmed oocytes were transferred to 0.5 M and 0.25 M sucrose in 10% FBS-supplemented DPBS for 3 min, respectively, and then washed twice with washing medium (10% FBS in DPBS) before they were transferred to pre-equilibrated human tubal fluid (HTF) containing 9.0 mg/mL BSA at 37 °C in 5% CO_2 in humidified air.

Western blot analysis

Following warming and incubation for 1 h at 37 °C in 5.0% CO_2 incubator, the oocytes were collected in 60 mmol/L tris–Cl (pH 6.8), 1% SDS, 10% glycerol, and 0.7 mol/L β-mercaptoethanol. Western blotting was performed on lysates. Samples were boiled and were subjected to electrophoresis on 8% or 13% polyacrylamide gels and then transferred to Immobilon-P nylon membranes (Millipore Corp). After blocking in 5% nonfat milk (BioRad) and 0.05% Tween-20 in PBS, blots were incubated with antibodies. Primary antibody was anti-rabbit monoclonal antibody (P0372, Sigma). Secondary antibody consisted of a dilution of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and was detected with Super Signal Ultra Chemiluminescent Substrate (Pierce) on Kodak BioMax Light film (Eastman Kodak).

Evaluation of oocyte viability

After warming and incubation for 1 h at 37 °C in 5.0% CO_2 incubator, survival of the oocytes was assessed morphologically based on plasma membrane integrity and discoloration of the ooplasm after the oocytes were recovered from the warming procedure [12].

Sperm preparation

For sperm collection, the male mice were killed by cervical dislocation. Both epididymides were excised and sperm were collected from the cauda region of each epididymis. The contents of each cauda epididymis were squeezed out by a pair of forceps and then transferred immediately into a drop (0.4 mL) of HTF. The sperm were incubated in the pre-equilibrated HTF containing 9.0 mg/mL BSA for 90 min at 37 °C in 5% CO_2 in humidified air to induce capacitation.

Insemination with intracytoplasmic sperm injection (ICSI), fertilization, and embryo culture

Oocytes were used for ICSI. ICSI was performed by using an Olympus light microscope equipped with Narishige micromanipulators and a Piezo system (Prime Tech, Japan). After sperm capacitation, 5 μL of sperm suspension was mixed in a droplet consisted of 5 μL of 12% PVP solution under paraffin oil. Prior to ICSI, oocytes were transferred from HTF medium into a droplet of Hepes buffered HTF medium under paraffin oil. The sperm head was injected into the oocyte by using a Piezo drive unit [17]. After injection, the oocytes were washed thoroughly with mHTF medium and then transferred into a droplet containing 50 μL of cleavage medium (SAGE Media, USA) under paraffin oil for culture for 6 h at 37 °C in 5% CO_2 in humidified air. Then the oocytes were washed and placed in 20-μL microdrop of Embryo Maintenance media (Cooper Surgical/SAGE) under mineral oil for further developmental culture at 37 °C in 5% CO_2 in humidified air. Fertilization of the oocytes was confirmed by the extrusion of second polar body at 6 h and the presence of two blastomeres at 24 h after insemination. The cleaved embryos were cultured until 120 h after insemination