Cryobiology 72 (2016) 86–92

Contents lists available at ScienceDirect

Cryobiology

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

Cryopreservation method affects DNA fragmentation in trophectoderm and the speed of re-expansion in bovine blastocysts

Yasushi Inaba [a, 1], Satoshi Miyashita [a, b, 2], Tamás Somfai [a, *], Masaya Geshi [a], Satoko Matoba [a], Osamu Dochi [b], Takashi Nagai [a, c, d]

[a] NARO Institute of Livestock and Grassland Science, Tsukuba, Ibaraki 305-0901, Japan
[b] Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
[c] College of Agriculture and Life Sciences, Seoul National University, Seoul 151-742, Republic of Korea
[d] Food and Fertilizer Technology Center, Taipei 10648, Taiwan

ARTICLE INFO

Article history:
Received 15 February 2016
Accepted 16 March 2016
Available online 17 March 2016

Keywords:
Bovine
Blastocyst
Freezing
Vitrification
Viability
Trophectoderm
DNA fragmentation

ABSTRACT

This study investigated re-expansion dynamics during culture of bovine blastocysts cryopreserved either by slow-freezing or vitrification. Also, the extent and localization of membrane damage and DNA fragmentation in re-expanded embryos were studied. Frozen-thawed embryos showed a significantly lower re-expansion rate during 24 h of post-thawing culture compared to vitrified embryos. Vitrified embryos reached the maximum level of re-expansion rate by 12 h of culture whereas frozen embryos showed a gradual increase in re-expansion rate by 24 h of culture. When assayed by Hoechst/propidium iodide staining there was no difference in the numbers and ratio of membrane damaged cells between re-expanded frozen and vitrified embryos; however, the extent of membrane damage in blastomeres was significantly higher in both groups compared with non-cryopreserved embryos (control). TUNEL assay combined with differential ICM and TE staining revealed a significantly higher number and ratio of TE cells showing DNA-fragmentation in frozen-thawed re-expanded blastocysts compared to vitrified ones; however, vitrification also resulted in an increased extent of DNA fragmentation in TE cells compared with control blastocysts. In frozen-thawed blastocysts increased extent of DNA fragmentation was associated with reduced numbers and proportion of TE cells compared with vitrified and control embryos. The number and ratio of ICM cells and the extent of DNA fragmentation in ICM did not differ among control, frozen and vitrified groups. In conclusion, compared with vitrified embryos, blastocysts preserved by slow-freezing showed a delayed timing of re-expansion which was associated with an increased frequency of DNA fragmentation in TE cells.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Cryopreservation of bovine embryos has become a routine technique in animal husbandry worldwide. To date, the two main methods of cell cryopreservation are 1) traditional slow-freezing and 2) vitrification [31]. In previous studies vitrification methods have been proven to result in higher embryo survival [20,22,26] compared with slow-freezing. Nevertheless, slow-freezing has remained the dominating method for the cryopreservation of cattle embryos due to its relatively easy performance and the feasibility of embryos frozen in straws for transfer under on-farm conditions.

Post-warming survival of blastocyst stage embryos is most often evaluated by their ability to re-expand. However, to our knowledge the dynamics of re-expansion after freezing or vitrification during *in vitro* culture has not been investigated. Such information is essential to determine the optimum timing for viability evaluation after thawing or warming. Although ultra-structure of frozen/thawed and vitrified/warmed bovine blastocyst show differences compared to their fresh counterparts [8,12,40], little is known on the ultra-structural differences between frozen/thawed and vitrified/warmed bovine embryos. Also, cellular alterations during cryopreservation reducing embryo viability need further

* Corresponding author. NARO Institute of Livestock and Grassland Science, Ikenodai 2, Tsukuba, Ibaraki 305-0901, Japan.
E-mail address: somfai@affrc.go.jp (T. Somfai).
[1] National Livestock Breeding Center, Tokachi Station, Otofuke, Hokkaido 080-0572, Japan.
[2] National Livestock Breeding Center, Miyazaki Station, Kobayashi, Miyazaki 886-0004, Japan.

http://dx.doi.org/10.1016/j.cryobiol.2016.03.006
0011-2240/© 2016 Elsevier Inc. All rights reserved.

clarification.

Membrane damage and DNA fragmentation are anomalies in embryos frequently caused by cryopreservation [6,16,29,30] and, therefore, they can be considered as candidate factors reducing embryo competence. On the other hand the extent and location of these anomalies in cryopreserved embryos and their relationship with post-thawing/warming re-expansion remains unknown.

The objectives of the present study were to investigate the frequency and speed of re-expansion during post-thawing/warming culture of bovine blastocysts cryopreserved either by slow-freezing or vitrification and to assess the extent and localization of membrane damage and DNA fragmentation in re-expanded embryos for the first time.

2. Materials and methods

2.1. Oocyte collection and in vitro maturation (IVM)

Collection and IVM of bovine follicular oocytes were performed as described previously by Imai et al. [14]. Bovine ovaries of unknown age were collected at a local slaughterhouse, transported to the laboratory, and then washed in Dulbecco's phosphate-buffered saline (D-PBS). The ovaries were then stored in D-PBS supplemented with 50 μg/mL gentamicin (Sigma-Aldrich Co., St. Louis, MO, USA) at 15 °C for approximately 15 h until test results showed that each donor animal was negative for bovine spongiform encephalopathy (BSE) in accordance with the Abattoir Law of Japan [38]. Cumulus-oocyte complexes (COCs) were aspirated from small follicles (3–6 mm in diameter) using a 5-mL syringe with a 19-gauge needle and used for IVM. The maturation medium was 25 mM HEPES-buffered TCM199 (Medium 199, Gibco Invitrogen, Life Technologies, Auckland, New Zealand) supplemented with 5% (v/v) calf serum (CS, Gibco Invitrogen). Collected COCs were evaluated by their cumulus cell morphology and cytoplasmic color. COCs with more than one layer of cumulus cells were selected, washed twice with IVM medium, and deposited in 100-μL droplets (20 COCs/droplet) of IVM medium covered by paraffin oil (Paraffin Liquid; Nacalai Tesque Inc., Kyoto, Japan). COCs were cultured for 22 h in 35-mm Petri dishes (Nunclon Multidishes, Nalge Nunc International, Rochester, NY, USA) at 38.5 °C in 5% CO_2 in air with saturated humidity.

2.2. In vitro fertilization (IVF) and culture (IVC)

IVF was carried out as reported previously by Imai et al. [14]. Briefly, at the end of IVM, frozen semen from a proven Japanese Black bull stored in a 0.5-mL straw was thawed in a 37 °C water bath for 30 s, transferred to 3 mL of 90% Percoll solution in 10-mL test tubes(As One, Osaka, Japan), and centrifuged at 710*g* for 10 min. Then, the pellet was re-suspended and centrifuged at 500*g* for 5 min in 6 mL of sperm-washing medium composed of Brackett and Oliphant solution (BO) [3] supplemented with 10 mM hypotaurine (Sigma-Aldrich Co.) and 4 U/mL heparin (Novo-Heparin Injection 1000, Aventis Pharma Ltd., Tokyo, Japan). The pellet was subsequently re-suspended in sperm-washing medium and BO solution supplemented with 20 mg/mL bovine serum albumin (BSA, crystallized and lyophilized, Sigma-Aldrich Co.) to achieve the final concentrations of 3×10^6 spermatozoa/mL, 5 mM hypotaurine, 2 U/mL heparin, and 10 mg/mL BSA. Droplets (100 μL) of this suspension prepared in 35-mm plastic dishes (Nunclon Multidishes) and covered by paraffin oil served as fertilization droplets. The COCs were removed from the IVM medium, washed twice in BO supplemented with 10 mg/mL BSA, placed in the fertilization droplets (20 COCs/droplet), and cultured for 6 h at 38.5 °C in 5% CO_2 in air with saturated humidity. The start of IVF was considered 0 h of fertilization. At the end of insemination, putative zygotes were completely denuded from cumulus cells and spermatozoa by gentle pipetting with a fine glass pipette in pre-incubated IVC medium. Then, 15–20 embryos were placed separately in culture drops. IVC was performed in 100-μL drops of Charles Rosenkrans 1 medium [33] with amino acids [13] supplemented with 5% CS covered with paraffin oil. Embryos were cultured at 38.5 °C in 5% CO_2 in air with saturated humidity.

2.3. Embryo freezing

Expanded blastocysts graded excellent according to the guidelines of the International Embryo Transfer Society [37] harvested on Day 7 (Day 0 = day of IVF) were used for freezing. They were frozen in D-PBS(+) supplemented with 1.5 M ethylene glycol (EG; Wako Pure Chemical Industries, Ltd., Osaka, Japan), 0.1 M sucrose (SUC; Nacalai Tesque Inc.), 4 mg/mL BSA (fraction V, Sigma-Aldrich Co.), and 20% CS (freezing medium). Embryos were transferred directly into the freezing medium, and then 1–3 embryos were loaded into a 0.25-mL straw (Clear straw, IMV Technologies Group, L'Aigle, France) and allowed to equilibrate for 15–20 min. The straws were then plunged into a −7.0 °C methanol bath of a programmable freezer (MPF-40, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), seeded at −7.0 °C, maintained at −7.0 °C for 10 min, cooled to −30 °C at a rate of −0.3 °C/min, and then plunged into liquid nitrogen (LN_2) [7]. After being stored for at least 1 week, the embryos were warmed by air thawing for 10 s and then immersed in a water bath at 30 °C for 20 s. After thawing, EG was removed from the embryos by immersion in D-PBS(+) supplemented with 20% CS at 38.5 °C for 10 min, and then the embryos were immediately transferred into fresh culture medium for *in vitro* evaluation of their developmental ability.

2.4. Embryo vitrification

Expanded blastocysts graded excellent according to the guidelines of the International Embryo Transfer Society [37] were cryopreserved by the Cryotop method as originally described by Ref. [19] with modifications [15]. Briefly, the embryos were placed in D-PBS(+) supplemented with 7.5% EG, 7.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich Co.), and 20% CS for 3 min and then transferred into a vitrification solution composed of D-PBS(+) supplemented with 16.5% EG, 16.5% DMSO, 0.5 M SUC, and 20% CS. One to 3 embryos were placed on a Cryotop® (Kitazato BioPharma Co. Ltd., Fujinomiya, Japan) sheet in a small volume of vitrification solution (<1 μL). After 1 min, the Cryotop was plunged into liquid nitrogen. After storage in liquid nitrogen for at least 1 week, the embryos were warmed by insertion of the Cryotop sheet into 2.5 mL of D-PBS (+) supplemented with 20% CS in 35-mm plastic dishes (Nunclon Multidishes) at 38.5 °C. After dilution for 3 min in the same dish at 38.5 °C, the embryos were immediately transferred into fresh culture medium for *in vitro* evaluation of developmental ability.

2.5. Evaluation of embryo viability

After dilution, embryos were briefly washed in Medium 199 supplemented with 20% fetal bovine serum (FBS; GIBCO Invitrogen) and 0.1 mM β-mercaptoethanol (Sigma-Aldrich Co.) and cultured for 24 h in the same medium (1–3 embryos/20 μL) covered with mineral oil at 38.5 °C in 5% CO_2 in air with saturated humidity [34]. Embryos showing re-expansion during post-thawing/warming culture were considered to be alive. The percentages of embryos showing re-expansion were determined in 4 h intervals during 24 h of additional culture after thawing/warming. In both frozen and

Download English Version:

https://daneshyari.com/en/article/10927648

Download Persian Version:

https://daneshyari.com/article/10927648

Daneshyari.com