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Effect of cryopreservation by slow cooling and vitrification on viral contamination of IVF embryos experimentally exposed to bovine viral diarrhea virus and bovine herpesvirus-1

A. Bielanski *, A. Lalonde

Animal Diseases Research Institute, Ottawa, ON, Canada

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Abstract

The objective was to determine the effect of cryopreservation by conventional slow controlled cooling (0.5 °C/min) and by vitrification on the presence of bovine viral diarrhea virus (BVDV) and bovine herpesvirus-1 (BHV-1) infectivity associated with frozen-thawed Day 7 bovine embryos. In this study, Day 7 embryos generated by in vitro fertilization (IVF) were exposed in vitro for 1.5 h to BVDV (N = 393) and BHV-1 (N = 242) and subsequently tested before and after cryopreservation for the presence of infectivity. Exposure of embryos to viral agents resulted in 72% of them infected prior to cryopreservation. Stepwise exposure of embryos to cryoprotectants, as well as their removal, substantially reduced the proportion of contaminated embryos (46% vs. 72%, $P < 0.05$). Overall, both freezing methods reduced the percentage of infectious embryos compared with that of embryos similarly exposed to viruses but not cryopreserved (31% vs. 72%, respectively; $P < 0.001$). The percentage of embryos with infectious viruses was not significantly higher after vitrification than after slow cooling (38% vs. 22%). In addition, after cryopreservation, a higher percentage ($P < 0.002$) of embryos exposed to BHV-1 (42%) remained infectious than did embryos exposed to BVDV (24%). In conclusion, cryopreservation reduced the proportion of infected embryos but did not render all of them free from infectious pathogens.

Keywords: BHV-1; BVDV; Cryopreservation; IVF embryos; Viral contamination

1. Introduction

In recent years, freezing of mammalian embryos has become a common practice. The international trade of domestic animal genetics is principally based on shipment of frozen embryos and semen. Traditionally, embryos have been frozen by slow cooling and more recently by the application of ultrafast cooling (vitrification) [1–5].

Aseptic cryopreservation and storage of embryos are of concern to regulatory authorities in prevention of disease transmission by embryo transfer (ET) and assisted reproductive technologies (ARTs). Semen and embryos can be infected with many microorganisms, which retain their viability for long intervals at low temperatures achieved in liquid nitrogen [6]. The application of cryopreservation to embryos allows sufficient time for verification of the sanitary status of the donors as a measure for controlling transmission of infectious diseases. Whenever cryopreserved germplasm (semen or embryos) is used or moved from one place to another, the potential risks of disease transmission must be considered.

* Corresponding author. Tel.: +1 613 228 6698; fax: +1 613 228 6669.
E-mail address: bielanskia@inspection.gc.ca (A. Bielanski).

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Oocytes and embryos used for ART can become contaminated in a number of ways [7,8]. First, gametes have the opportunity to become infected when the infectious agent is present in blood and/or in the reproductive tract of an infected donor. Second, the danger of contamination may be considerably increased when oocytes and embryos are harvested from asymptomatic and persistently infected donors and also when contaminated semen or supplements of biological origin are used for IVF and embryo culture [7]. Finally, embryos can become infected during cryopreservation and storage because of inappropriately sealed containers and through contaminated liquid nitrogen (LN_2) [9–11].

Most pathogenic bacterial agents associated with semen survive temperatures below the freezing point [12–15]. However, there is no information regarding the effect of the cryopreservation process on infectivity of viral pathogens associated with embryos generated for ET. Therefore, in this study, we examined the effect of two cryopreservation methods on post-freeze contamination of embryos experimentally exposed to bovine viral diarrhea virus (BVDV) and bovine herpesvirus-1 (BHV-1; also named infectious bovine rhinotracheitis virus, or IBRV).

2. Materials and methods

2.1. Experimental design

Day 7 bovine embryos produced by IVF were exposed in vitro to BVDV and BHV-1 and then rinsed once to remove unbound viral particles. Subsequently, embryos were allocated into groups for the following experiments: (1) testing for the presence of viral agents without exposing them to freeze-thaw solution and cryopreservation (positive controls); (2) testing for the presence of viral agents after their exposure to freeze-thaw solutions without cooling; and (3) testing for the presence of viral agents after vitrification and slow cooling. Furthermore, embryos from IVF pools, not exposed in vitro to viruses or cryopreservation, were also tested for the presence of BVDV and BHV-1 (negative controls).

2.2. Embryo production

Except where otherwise indicated, chemicals were obtained from Sigma Chemical Co (St. Louis, MO, USA). Bovine fetal serum and bovine serum albumin used were free of BVDV and BHV-1 antibodies.

Bovine ovaries, collected immediately after slaughter at a commercial abattoir, were rinsed and washed twice in fresh phosphate-buffered saline (PBS) containing 100 mg/mL streptomycin, 100 IU/mL penicillin, and 25 μg/mL Fungizone (GIBCO/Invitrogen Canada Inc, Burlington, Ontario). Cumulus-oocyte complexes (COCs) were aspirated from ovarian follicles (3 to 6 mm in diameter) using a 21-gauge needle and a 10-mL syringe. Oocytes surrounded by multilayers of compact follicular cells were washed three times in maturation medium composed of tissue culture medium (TCM-199) with Earle's salts supplemented with 10% fetal bovine serum (FBS; free of BVDV), 1 μg/mL estradiol, 35 μg/mL follicle-stimulating hormone (FSH-P, Folltropin; Bioniche Animal Health, Belleville, ON, Canada), 10 IU/mL human chorionic gonadotropin (hCG; Ayerst Laboratories, Philadelphia, PA,USA), 0.4 mM glutamine, 0.2 mM sodium-pyruvate, and 50 μg/mL gentamicin. The oocytes were matured in 700 μL of the maturation medium in 4-well culture dishes (cat. no. 176740; Nunclon, Roskilde, Denmark) at 38.5 °C and in 5% CO_2 in air with 100% humidity for 24 h. After maturation, the oocytes were washed twice in modified Tyrode's solution (TALP-HEPES) and once in fertilization medium (IVF-TALP) before being transferred to fertilization wells. The 500 μL fertilization medium (IVF-TALP) contained hypotaurine (10 mM), penicillamine (20 mM), epinephrine (1.0 mM), heparin (10 mg/mL), and 0.6% bovine serum albumin.

A discontinuous Percoll (Sigma Chemical Co, St Louis, Mo, USA) density gradient (45% and 90% Percoll) was used to prepare sperm for IVF. Briefly, frozen-thawed pooled semen from six commercial bulls (free of BVDV) was added on top of 45% Percoll gradient in a 15-mL centrifuge tube. The tube was centrifuged at 700 × *g* for 20 min at room temperature. The supernatant was removed, and sperm pellet was washed once by centrifugation in 3 mL Sperm-TALP (calcium-free Tyrode's salts, albumin, lactate, and sodium pyruvate). Fertilization of oocytes was carried out with approximately 1×10^6 motile sperm/mL at 38.5 °C in 5% CO_2 in air. Twenty-two hours postinsemination, the cumulus cells were removed from the oocytes by vortexing for 90 sec in 2 mL Sperm-TALP. Subsequently, presumptive zygotes were washed twice in Sperm-TALP and once in the modified synthetic oviductal fluid (SOF) before being transferred to 500 mL SOF and incubated for 7 d under mineral oil at 38.5 °C in 5% CO_2, 5% oxygen, and 90% nitrogen.

2.3. Embryo freezing and thawing

To avoid the presence of potential bovine viral contaminants or antibodies to viral agents, horse serum