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Improved parthenogenetic development of vitrified-warmed bovine oocytes activated with 9% ethanol plus 6-DMAP

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Abstract

The objective was to compare various activation protocols on developmental potential of vitrified bovine oocytes. Bovine oocytes matured in vitro for 23 h were vitrified with EDFSF30 in open pulled straws. After warming, they were cultured in vitro for 1 h, followed by parthenogenetic activation. Vitrified-warmed oocytes had a morphologically normal rate similar to that of controls (nonvitrified oocytes cultured in vitro for 24 h; 98.6% vs. 100%, $P > 0.05$). When vitrified-warmed oocytes were first activated with 7% ethanol for 5 min and then incubated in 6-dimethylaminopurin (6-DMAP) for 4 h, cleavage and blastocyst rates were 41.2% and 23.2%, respectively, which were lower than those of controls (77.5% and 42.0%, $P < 0.05$). Subsequently, we varied the ethanol concentration to increase the effectiveness of parthenogenetic activation. When either 5%, 6%, 7%, 8%, 9%, 10%, or 11% ethanol alone (for 5 min) or in combination with 6-DMAP (4 h) was used to activate vitrified-warmed oocytes, cleavage rates ranged from 22.3% to 61.1% and blastocyst rates ranged from 1.1% to 30.6%. These rates were optimized when oocytes were treated with 9% ethanol plus 6-DMAP; this was verified in experiments evaluating other activation protocols with 9% ethanol, calcium ionophore A23187, or ionomycin alone, or in combination with DMAP or cycloheximide (CHX). In conclusion, the oocyte activation protocol affected developmental capacity of vitrified bovine oocytes; 9% ethanol (5 min) followed by 6-DMAP (4 h) promoted optimal parthenogenetic activation.

Keywords: Bovine oocytes; Embryonic development; Oocyte cryopreservation; Parthenogenetic activation; Vitrification

1. Introduction

The parthenogenetic activation of oocytes is important in cloning research, as artificial activation of oocytes is an essential component of nucleus transfer protocols. Optimized activation protocols could enhance complete reprogramming of reconstructed embryos, which may increase success rates in cloning. Furthermore, parthenogenetic activation represents a valid tool to mimic the fertilization Ca^{2+} transients and oscillation in nucleus transplantation experiments and to investigate the comparative roles of paternal and maternal genomes in controlling early embryonic development.

Parthenogenesis of bovine oocytes can be induced with an electrical pulse [1,2], ethanol [3–5], calcium ionophore A23187 [6,7], cycloheximide (CHX) [4,8],

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1,4,5-inositol triphosphate [9], ionomycin [10,11], or strontium [12]. When ethanol was used in combination with CHX, the success rate for parthenogenesis was further enhanced [13]. Similarly, better results were obtained after oocytes were first treated with ethanol or ionomycin prior to treatment with 6-dimethylaminopurin (6-DMAP) [14]. Although mechanisms differed among these activating agents, their influence on fertilization Ca^{2+} transients and oscillation was similar.

Activation protocols mentioned above frequently are used on fresh mammalian oocytes. It is often difficult, however, to satisfy the experimental requirements for large numbers of fresh oocytes at a specific time and location. Therefore, cryopreservation of oocytes could be a practical solution.

Since offspring were first obtained from mouse oocytes cryopreserved by the slow freezing method of Whittingham [15], many experiments have reported successful cryopreservation of bovine oocytes [16–20]. The open pulled straw (OPS) method is one of the most effective technologies for cryopreservation of bovine oocytes and embryos [21]. Although bovine oocytes vitrified by the OPS method have been used to support somatic cell cloned embryos to develop to term in our laboratory, reconstructed embryos derived from oocytes [19] or parthenogenetic embryos from vitrified-warmed oocytes [22] activated by combination of A23187 and 6-DMAP treatments had lower rates of blastocyst formation (6.0% to 23.0% [19]) than those reported with fresh oocytes and various activation protocols (3.2% to 34.0% [12,23–25]). When fresh oocytes were treated with 7% ethanol plus 6-DMAP, the blastocyst formation rate was 25.6% [23]. However, parthenogenetic activation of vitrified bovine oocytes using the 7% ethanol plus 6-DMAP protocol has not been examined. Furthermore, it is not clear whether parthenogenetic activation of vitrified bovine oocytes can be improved by altering the concentration of ethanol.

In the current study, vitrified-warmed mature bovine oocytes were used to examine the effectiveness of the following protocols: activation with single agents, including ethanol, ionomycin, calcium ionophore A23187, CHX, and 6-DMAP, or activation with ethanol, ionomycin, and A23187 in combination with either 6-DMAP or CHX. The cleavage rate, blastocyst formation rate, and cell number of blastocysts were observed after parthenogenetic activation.

2. Materials and methods

Unless indicated otherwise, all chemicals and media were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and plastics from Corning, Inc. (Corning, NY, USA).

2.1. Preparation of the OPS straws

The OPS straws were made by the method described by Vajta et al. [21], with some modifications. Briefly, 0.25-mL plastic straws (I.V.M., L'Aigle, France) were heat softened and pulled manually. The pulled straw was cooled in air and then cut at the narrowest point, with 2.5 ± 0.5 cm left over. Straws with an outer diameter of 0.23 ± 0.01 mm (measured with a microforge; MF900; Marishige, Tokyo, Japan) and wall thickness of 0.02 mm were used.

2.2. Pretreatment and vitrification solutions

The pretreatment vitrification solution was 10% ethylene glycol (EG) +10% dimethyl sulfoxide (DMSO), which contained 10% (vol/vol) EG and 10% (vol/vol) DMSO in phosphate-buffered saline (PBS) containing 20% fetal bovine serum (FBS; Hyclone; Gibco BRL, Paisley, Scotland, UK). Vitrification solution EDFSF30 contained 15% (vol/vol) EG and 15% (vol/vol) DMSO in FSF solution, which consisted of Dulbecco phosphate-buffered saline (DPBS) medium containing 300 g/L Ficoll, 0.5 M sucrose, and 20% (vol/vol) fetal calf serum (FCS).

2.3. Oocytes and in vitro maturation

Ovaries from cows and heifers were collected at an abattoir and transported to the laboratory in physiologic saline at 35 °C. Cumulus-oocyte complexes (COCs) were aspirated from 2- to 6-mm follicles using a 10-mL syringe with an 18-gauge needle. The COCs with an intact compact cumulus (assessed with a stereomicroscope; Nikon SMZ645; Nikon, Tokyo, Japan) were selected and washed three times in hydroxyethyl piperazine ethanesulfonic acid (HEPES)-buffered TCM-199 medium and then twice in $NaHCO_3$-buffered TCM-199. The COCs were transferred into 0.5 mL maturation medium (M199 + 10 μg/mL Ovine follice-stimulating hormone (oFSH) [Ovagen, Auckland, New Zealand] + 10 μg/mL Ovine luteinizing hormone (LH) [Ovagen] + 1 μg/mL estradiol [Ovagen] + 10% FBS) in 4-well dishes (Nunc A/S, Roskilde, Denmark) and overlaid with paraffin oil. The COCs were then cultured for 23 h at 38.5 °C in a humidified atmosphere with 5% CO_2. After maturation, cumulus cells were partially removed using a 1-mL pipette, leaving two or three layers of cumulus cells