Theriogenology 83 (2015) 366–376

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Developmental competence of ovine oocytes after vitrification: Differential effects of vitrification steps, embryo production methods, and parental origin of pronuclei

S.M. Hosseini, V. Asgari, S. Ostadhosseini, M. Hajian, H.R. Ghanaei, M.H. Nasr-Esfahani*

Department of Reproduction and Development, Reproductive Biomedicine Centre, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

ARTICLE INFO

Article history:
Received 4 July 2014
Received in revised form 26 September 2014
Accepted 27 September 2014

Keywords:
Oocyte
Vitrification
IVF
Intracytoplasmic sperm injection
Parthenogenetic activation
SCNT

ABSTRACT

Despite many advances in the field of oocyte cryopreservation, the adverse effects of cryopreservation on oocyte competence are still an open challenge in most mammalian species. Using ovine *in vitro*–matured oocytes, the differential effects of vitrification steps, embryo production methods, and parental origin of pronuclei were systemically investigated to unravel (1) the most critical stage (if any) of oocyte vitrification, (2) the most suitable method (if any) of embryo production for a vitrified oocyte, and (3) differential contributions of male or female pronuclear formation to the poor quality of vitrified oocyte. Although cryoprotectants used during vitrification had some inevitable adverse effects on oocyte competence, the damages caused by low temperature *per se* (chilling injury) were the main cause of poor quality of vitrified oocytes. When vitrified oocytes underwent either IVF or intracytoplasmic sperm injection (ICSI), embryo development rates were substantially lower than those of fresh ones. In contrast, when vitrified oocytes underwent either parthenogenetic activation (PA) or SCNT, embryo development rates were very similar to those of fresh ones. Evaluation of nuclear morphology after IVF, ICSI, PA, and SCNT oocytes revealed that vitrification had no apparent effect on the female (IVF, ICSI, and PA) and somatic (SCNT) pronuclear formation rates but significantly reduced male pronuclear formation after either IVF or ICSI compared with fresh counterparts. Quantitative analysis of transcripts revealed comparable mRNA abundances of CNX43, HSP90, GMNN, NPM, and OCT4 between vitrified and fresh oocytes, whereas CCNB, ATP1A1, and PAP transcripts were significantly lower in vitrified versus fresh oocytes. Although underlying mechanisms of poor quality of vitrified oocytes are multifactorial, the ability to obtain equivalent development after PA and SCNT, but not IVF and ICSI, in vitrified versus fresh oocytes may argue that the cytoplasm of vitrified oocyte has the necessary components to support *in vitro* embryonic development of the maternal, even adult somatic cell, chromosomes but fails to do so with sperm chromosomes.

1. Introduction

In cryopreservation of mammalian germ cells, oocyte vitrification is of indispensable central value because of its numerous potential application for preserving human

* Corresponding author. Tel.: +983112600446; fax: +983112605525.
E-mail address: mh.nasr-esfahani@royaninstitute.org (M.H. Nasr-Esfahani).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.09.031

fertility [1] and multiplication of elite or endangered species [2]. Indeed, oocyte cryopreservation facilitates oocyte donation programs and maintains the chance of fertility for at-risk patients [3]. Moreover, the successful establishment of embryonic stem cell lines from parthenogenetic activation (PA) and SCNT embryos derived from fresh [4,5] and vitrified [6] oocytes has increased the importance of oocyte cryopreservation in a cryobank setting.

The vitrification is a multistep process in which oocytes are first exposed to increasing concentrations of permeable and nonpermeable cryoprotectants to avoid intracellular/extracellular ice crystallization during ultrarapid cooling of oocyte below the water and/or cryoprotectant glass transition temperatures. Oocyte warming, on the other hand, occurs in the presence of decreasing concentrations of nonpermeable cryoprotectants to control hypoosmotic shock during oocyte rehydration [7,8]. The ability of oocyte to develop into a viable embryo strongly depends on cellular and molecular aspects of oocyte competence [9], and therefore, an interesting question of oocyte cryobiology is to understand how different steps of a vitrification process may affect different elements of the oocyte that are intimately involved in oocyte competence to develop into a viable embryo. Such studies may help to distinguish the most critical stage of oocyte cryopreservation technique which has important implication for development of an optimized oocyte-tailored cryopreservation protocol.

Apart from the technical steps, the method of embryo production may also affect developmental competence oocytes after cryopreservation. Because different cellular and molecular constituents of the oocyte have different degrees of susceptibility to cryodamages [3,10], the selection of a subsequent treatment for vitrified oocytes may substantially affect the final outcome. For example, low/failure IVF which frequently observed in vitrified oocytes occurs because of precocious cortical granule release, zona hardening, and cumulus cell detachment or loosening. Failed fertilization can be rescued by partial zona dissection [11,12] and intracytoplasmic sperm injection (ICSI) [13]. Moreover, owing the sensitivity of the oocyte DNA to cryodamages [14], the enucleated vitrified oocyte may serve a valuable source of cytoplasm for SCNT studies. To realize the potential application of vitrified oocytes, it is essential to determine the potential developmental differences in method of embryo production between vitrified and fresh oocytes. Such studies may also help to understand the effect of vitrification on oocyte capability to remodel sperm chromatin and the potential differences in the contributions of male and female pronuclei to the poor development of vitrified oocytes.

This study was set out to investigate the interactions between oocyte vitrification steps, embryo production methods, and parental origin of pronuclei were systemically investigated as an attempt to unravel (1) the most critical stage (if any) of oocyte vitrification, (2) the most suitable method (if any) of embryo production for a vitrified oocyte, and (3) differential contributions of male and female pronuclei to the poor quality of vitrified oocyte (see Fig. 1 for the experimental design).

2. Materials and methods

Unless otherwise specified, all chemicals and media were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Gibco (Grand Island, NY, USA), respectively.

2.1. Oocyte in vitro maturation

The procedure of sheep oocyte *in vitro* maturation was as described previously [15]. In brief, using a 20-ga needle attached to 2-mL syringe, the antral follicles (2–6 mm diameter) of abattoir-derived ovaries were aspirated to obtain

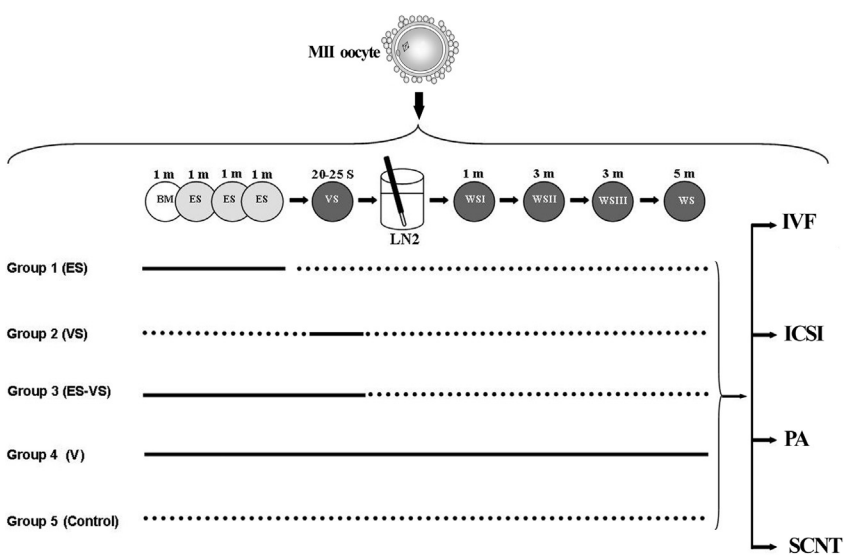

Fig. 1. Schematic representation of experimental design. Group 1 (ES): oocytes were only exposed to equilibration solution. Group 2 (VS): oocytes were only exposed to vitrification solution. Group 3 (ES-VS): equilibrated oocytes were transferred into vitrification solution. Group 4 (V): vitrified-warmed oocytes were used for a subsequent experiment. Group 5 (control): fresh oocytes. BM, base medium; ES, equilibration solution; ICSI, intracytoplasmic sperm injection; LN2, liquid nitrogen; PA, parthenogenetic activation; VS, vitrification solution; WS1-3, warming solution 1–3; WS: washing solution.