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Vitrification of in vitro matured goat oocytes and the effect on in vitro fertilization

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Abstract

To study the impact of vitrification of in vitro matured caprine oocytes on in vitro fertilization (IVF), ovaries were collected from a local abattoir and goat oocytes aspirated. The oocytes were subjected to in vitro maturation (IVM) for 27 h. Twenty-seven hours post IVM oocytes were cryopreserved in a vitrification solution (VS) consisting of propylene glycol (40%, w/v) and trehalose (0.25 mol l^{-1}) in PBS with 4% (w/v) BSA. Some oocytes were exposed to propylene glycol without cryopreservation to check the degree of damage induced by propylene glycol. In the vitrified group, 94.3% of the oocytes were viable for IVF. 16.1% of the oocytes were damaged after thawing. From the vitrified group, 75.8% of the oocytes were subjected to IVF, while for 18 h. Cytological studies following fixing of the IVF oocytes revealed 17.37% of the oocytes showing a pronucleus in the control group, compared to 8.08 and 7.86% in the vitrified and cryoprotectants exposed groups, respectively. This study revealed that goat oocytes can be successfully cryopreserved using a simple vitrification method. However, the fertilizing ability of oocytes was severely affected by both vitrification and exposure to cryoprotectants.

Keywords: Caprine; Vitrification; IVM; Fertilization rate; Cryoprotectant

1. Introduction

Embryo culture, IVF, cloning and other related reproductive technologies in farm animals require developmentally competent and viable oocytes. In vitro maturation of oocytes recovered from slaughterhouse ovaries has become a prevalent source of oocytes for such procedures. Oocytes have a short fertile life which is a major limitation in the implementation of many in vitro technologies. Effective cryopreservation of oocytes would greatly enhance the availability at the time of need from low cost sources such as abattoir animals to high genetic potential animals. However, only limited success has been achieved in the cryopreservation of mammalian oocytes, especially in domestic livestock (Niemann, 1991; Parks and Ruffing, 1992).

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Cryopreservation can be done by conventional slow and rapid freezing, but this method is a tedious process, which involves many steps during freezing and thawing and it is costly also. Vitrification of oocytes is an alternate approach. Vitrification entails the solidification of liquid by an extreme increase in viscosity during very rapid cooling (Fahy et al., 1984). Successful vitrification has been reported for equine (Hurtt et al., 2000), ovine (Schiewe et al., 1990) and bovine (Massip et al., 1986) embryos, and for porcine (Arav et al., 1990), bovine (Hochi et al., 1998), ovine (Dattena et al., 2000) and buffalo oocytes (Dhali et al., 2000). Live birth of piglets (Misumi et al., 2003) and calves (Fukui et al., 1992) have been obtained, although the number of viable offspring obtained from cryopreserved oocytes remains low. Although pregnancies have been produced from vitrified thawed bovine oocytes (Hamano et al., 1992), fertilization rates have been reported to be significantly lower in non-vitrified oocytes (Fukui et al., 1995). Lim et al. (1992) reported the post thaw survival and cleavage rate following IVF after two steps of freezing to be lower than that in in vitro maturing and matured oocytes.

Cryopreservation of oocytes could generate a readily available source of oocytes for research and allow experiments to be performed at a convenient time. This study was designed to investigate the effect of vitrification and thawing of caprine oocytes after 27 h of IVM on their subsequent in vitro fertilization capacity.

2. Materials and methods

All the chemicals and media used were from Sigma–Aldrich Corporation, USA, unless otherwise specified, the plastic wares from Griener, Germany and micro filters of 0.22 and 0.45 μm from Millipore Corporation, USA.

2.1. In vitro maturation (IVM)

Goat ovaries were collected from the local abattoir and transported at 32–35 °C in 0.9% saline to the laboratory, within 2–3 h following slaughter. Oocytes were collected from non-atretic follicles (>4 mm in diameter) by the follicular puncture method (Teotia et al., 2001). Excellent to good cumulus enclosed oocytes were selected for further experimentation in this study. Cumulus oocyte complexes (COCs) were matured in vitro over a granulosa cell monolayer at 38 ± 1 °C in an atmosphere of 5% CO_2 and 95% RH for 27 h (Tyagi et al., 1997).

2.2. Oocytes exposed to a cryoprotectant

Twenty-seven hours post IVM, a group of oocytes ($n = 305$), were exposed to the cryoprotectants, propylene glycol (40%, w/v) for 10 min without cryopreservation. After exposure, oocytes were checked under an inverted phase contrast microscope (Olympus, Japan) for structural damage due to the propylene glycol.

2.3. Vitrification and thawing of oocytes

The vitrification solution (VS) consisted of propylene glycol (40%, w/v) and trehalose (0.25 mol l^{-1}) in PBS, supplemented with 4% (w/v) BSA. Twenty-seven hours post IVM, COCs were washed in a maturation media (tissue culture media (TCM)) with 10% (v/v) estrus goat serum (EGS). All matured COCs were transferred in one step to 50 μl droplets (approximately 15–20 oocytes in a drop) of the VS. These COCs were equilibrated for 10 min in the VS and then loaded into 0.25 ml French straw (15–20 oocytes) in a sucrose l^{-1} solution separated by air bubbles on either side of the VS containing the matured oocytes. The straws were sealed with the aid of hot forceps and plunged directly into liquid nitrogen (LN_2).

The straws were thawed after 7 d by transferring them to a water bath at 37 °C for 20 s. Oocytes were then transferred to a culture dish containing sucrose l^{-1} in PBS, to remove the cyroprotectant. The oocytes were allowed to equilibrate for 1 min and fresh PBS was added drop by drop to reach a final sucrose concentration of 0.125 mol l^{-1} within 10 min.

2.4. In vitro fertilization (IVF) of vitrified oocytes

All vitrified and thawed oocytes were placed in fresh washing media. The oocytes were washed in the washing media 4–5 times, followed by 2–3 washes in the fertilization tyrode, albumin, lactate and pyruvate (TALP). These selected washed oocytes ($n = 235$) were used for in vitro fertilization for a period of 18 h, according to the method of Teotia et al. (2001). Eighteen hours after IVF, all the oocytes were thoroughly washed with