Small Ruminant Research 116 (2014) 32–36

Contents lists available at ScienceDirect

Small Ruminant Research

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

Short communication

The effects of meiotic stage on viability and developmental capability of goat oocytes vitrified by the Cryoloop method

Guo Bo Quan, Wei Juan Li, Zhi Gang Lan, Shuai Shuai Wu, Qing Yong Shao, Qiong Hua Hong*

Yunnan Animal Science and Veterinary Institute, Jindian, Panlong County, Kunming City, Yunnan Province, China

ARTICLE INFO

Article history:
Received 28 March 2013
Received in revised form
21 September 2013
Accepted 8 October 2013
Available online 25 October 2013

Keywords:
Goat oocyte
Germinal vesicle stage
Meiosis II
Cryoloop
Vitrification

ABSTRACT

In order to evaluate the effects of meiotic stage on survival of vitrified goat oocytes, the Cryoloop method was used to cryopreserve goat immature germinal vesicle (GV) and meiosis II (MII) oocytes following in vitro maturation (IVM). The oocytes collected from local slaughter house were divided into two parts. In the first part, the immature cumulus oocyte complexes (COCs) were further divided into three groups: (1) untreated (control), (2) exposed to the vitrification and dilution solutions but without being plunged into liquid nitrogen (toxicity), or (3) vitrified by the Cryoloop method (vitrification). In the second part, the MII oocytes produced by IVM of immature COCs were also divided into three groups as the above immature COCs. Oocytes survival was assessed by morphological appearance, nuclear maturation, and developmental capability following parthenogenetic activation (PA). The experiment was repeated three times. Our data indicated the rates of oocytes with normal appearance and developmental capability in the toxicity or vitrification group were decreased as compared to the control oocytes. In the toxicity group, the rate of GV oocytes with normal morphology was 76.25% ± 3.37% and significantly less than that of MII oocytes (92.18% ± 1.94%, $P < 0.05$). However, the cleavage rate of MII oocytes was not significantly different from that of GV oocytes in the toxicity group (68.23% ± 1.71% vs 67.59% ± 3.51%, $P > 0.05$). Additionally, the percentage of GV oocytes developing to blastocyst was significantly less than that of MII oocytes in the toxicity group (13.84% ± 2.81% vs 29.78% ± 4.17%, $P < 0.05$). The rate of vitrified/thawed GV oocytes with normal morphology was 60.37% ± 2.91% and significantly less than that of MII oocytes (82.91% ± 3.01%, $P < 0.05$). Additionally, the cleavage rate of GV oocytes was also significantly less than that of MII oocytes in the vitrification group (42.81% ± 2.94% vs 57.91 ± 1.06%, $P < 0.05$). However, the blastocyst rate of vitrified/thawed GV oocytes was not significantly different from that of MII oocytes (8.51% ± 1.46% vs 12.41% ± 3.74%, $P > 0.05$). In conclusion, although vitrification can greatly damage the structure and development capability of goat oocytes, the Cryoloop method can result in acceptable levels of survival and development of goat oocytes. Additionally, compared to immature GV goat oocytes, mature MII oocytes may be more tolerant to the vitrification process.

Abbreviations: GV, germinal vesicle; MII, meiosis II; IVM, in vitro maturation; PA, parthenogenetic activation; IVF, in vitro fertilization; ATP, adenosine triphosphate; SSV, solid surface vitrification; PBS, phosphate buffered saline; COCs, cumulus oocyte complexes; FBS, fetal bovine serum; FSH, follicle-stimulating hormone; LH, luteotrophic hormone; 17β-E2, 1,3,5-estratrience-3,17β-diol; EGF, epidermal growth factor; ITS, insulin–transferrin–selenium; IVC, in vitro culture; EAA, essential amino acid; NEAA, non-essential amino acid; BSA, bovine serum albumin; EGS, estrus goat serum; EG, ethylene glycol; DMSO, dimethyl sulfoxide.

* Corresponding author. Tel.: +86 0871 65017507.
E-mail address: qionghuahong@163.com (Q.H. Hong).

0921-4488/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.smallrumres.2013.10.005

1. Introduction

Successful cryopreservation of mammalian oocytes has great significance on female genetic bank and reproductive biotechnology of livestock (Prentice and Anzar, 2011). Additionally, oocyte cryopreservation can facilitate international exchange of excellent female germplasm, as it can avoid injury and sanitary risks involved in live animal transportation (Pereira and Marques, 2008; Prentice and Anzar, 2011). However, despite this extensive research over the past 20 years, there is no freezing protocol which can guarantee satisfactory survival and developmental rates after freezing and thawing regardless of the studied species (Kuleshova and Lopata, 2002; Van der Elst, 2003).

Currently, most of investigators focused on cryopreservation of mature MII oocytes. However, the shape and physiological characteristics of MII oocytes may determine their extreme sensitivity to low temperatures. Cryopreservation can seriously damage mammalian oocyte's structure, physiological function, and ability of in vitro fertilization (IVF) and development. Additionally, cryopreservation can induce oocyte parthenogenesis, premature extrusion of corticle granules finally causing zona hardening, microtubular spindle injury, and condensed chromosomes resulting in atypical haplotypes (Fabbri et al., 2001; Kuwayama, 2007; Stachecki and Cohen, 2004). Cryopreservation may injure the integrity of mammalian mitochondria which can maintain the adenosine triphosphate (ATP) level of oocyte and is essential to mediate fertilization, post-fertilization events, and the first cell division (Sanchez-Partida et al., 2011; Wilding et al., 2001). The alternative strategy is to cryopreserve immature GV oocytes. Compare with MII oocytes, GV oocytes may be more tolerant to cryoinjury caused by cryopreservation owing to absence of spindle and the genetic materials enclosed in nucleus (Moawad et al., 2012; Prentice and Anzar, 2011). However, several reports showed that immature oocytes may be more sensitive to freezing than matured oocytes (Agca et al., 2000; Ledda et al., 2001; Mara et al., 2013; Rojas et al., 2004).

At present, a limited number of investigations have been done on vitrification of small ruminant oocytes. In ovine, poor developmental rates are obtained following vitrification of immature (Isachenko et al., 2001; Moawad et al., 2012) and mature (Maalouf et al., 2009; Succu et al., 2007; Zhang et al., 2009) oocyte. Compared with sheep, the reports about vitrification of goat oocytes were less. Begin et al. (2003) evaluated the efficiency and toxicity of two vitrification methods including both solid surface vitrification (SSV) and Cryoloop vitrification on goat MII oocytes. Their data indicated the two vitrification methods resulted in acceptable levels of survival and cleavage of goat oocytes. Kharche et al. (2005) found the conventional plastic straw vitrification may be detrimental for IVM and IVF of goat oocytes. Taru Sharma et al. (2006) used propylene glycol and trehalose as main protectants to assess the effect of vitrification by the conventional 0.25 ml plastic straw on goat MII oocytes. Their study showed that goat oocytes can be successfully cryopreserved using this vitrification method. However, the fertilizing ability of oocytes was severely damaged by both vitrification and exposure to cryoprotectants (Taru Sharma et al., 2006).

According to our knowledge, there is no investigation comparing the sensitivity of goat immature GV oocytes or mature MII oocytes to vitrification. In this study, in order to further evaluate the tolerance of goat oocytes to vitrification, the Cryoloop method was used to cryopreserve GV or MII goat oocytes. The morphology appearance, IVM, and in vitro development of oocytes following PA were assessed.

2. Materials and methods

Unless otherwise stated, all chemicals were analytical reagent grade and purchased from Sigma Chemical Co., USA. All plastic ware used in this study were purchased from Nunc, Denmark.

2.1. Source of ovaries and collection of oocytes

Ovaries of Yunling black goat were collected from a local slaughterhouse in Kunming city and transported to our laboratory in pre-warmed (37 °C) sterile saline containing 960 mg/l penicillin and 50 mg/L streptomycin within 2–3 h after slaughter. The ovaries were rinsed once with 75% ethanol and immediately with the same sterile saline four times before transference to a 100 mm culture dish containing 20 ml of the holding medium. The holding medium was phosphate buffered saline (PBS) plus 36 mg/L sodium pyruvate, 1000 mg/L glucose, 960 mg/l penicillin, 50 mg/L streptomycin, and 3 mg/ml bovine serum albumin (BSA). The antral follicles of 1–6 mm in diameter on the ovarian surface were punctured under a microscope (Olympus SZX16, Japan) using a blade. Only COCs with homogeneous cytoplasm surrounded by more than three layers of compact cumulus cells were selected and washed 5–6 times in holding medium at 38.5 °C on a heating stage before use. In this study, the experiments of goat GV and MII oocytes were done every time simultaneously. The washed COCs were divided into two parts. In the first part, the immature COCs were further divided into three groups: (1) untreated (control), (2) exposed to the vitrification and dilution solutions but without being plunged into liquid nitrogen (toxicity), or (3) vitrified by the Cryoloop method (vitrification). In the second part, the MII oocytes produced by IVM of immature COCs were also divided into three groups as the above immature COCs (Fig. 1).

2.2. IVM of immature oocytes

The collected COCs were washed three times in the maturation medium on a heating stage at 38.5 °C. The maturation medium consisted of M199 (Gibco), 10% (v/v) fetal bovine serum (FBS), 10 μg/ml follicle-stimulating hormone (FSH) (Follatropin-V: Bioniche, Canada), 10 μg/ml luteotrophic hormone (LH) (Lutrophin-V: Bioniche, Canada), 1.0 μg/ml 1,3,5-estratrience-3,17β-diol (17β-E_2), 10 ng/ml epidermal growth factor (EGF), and 1% insulin–transferrin–selenium (ITS). For IVM, the COCs (20 oocytes per droplet) were transferred into the droplet (50 μl) of the maturation medium covered with mineral oil in the culture dish and equilibrated at 38.5 °C for 2 h in a humidified atmosphere of 5% CO_2 in air before use. The oocytes were cultured for 27 h. After IVM, the cumulus cells surrounding oocytes were removed by treating oocytes with 0.5% (w/v) hyaluronidase in the maturation medium for 5 min in an incubator with 5% CO_2 under humidified air at 38.5 °C. The denuded oocytes were washed three times using the maturation medium, after which only morphologically normal oocytes exhibiting homogeneous ooplasms and clearly visible polar body were selected for PA or vitrification.

2.3. PA and in vitro culture of MII oocytes

After IVM, the denuded MII oocytes were chemically activated using ionomycin and 6-dimethylaminopyridine. Briefly, the oocytes (15–20 oocytes per droplet) were firstly pretreated with 5 μM ionomycin in the culturing medium for 5 min. Then the oocytes were cultured in the culture medium containing 2 mM 6-dimethylaminopyridine for 4 h. The culture medium was synthetic oviduct fluid (SOF) plus 2% essential amino acid (EAA), 1% non-essential amino acid (NEAA), 1 mM glutamine,