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Small Ruminant Research 56 (2005) 103–111
Small Ruminant Research
www.elsevier.com/locate/smallrumres

# Cryopreservation and short-term culture of isolated caprine primordial follicles

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Received 11 June 2003; received in revised form 23 March 2004; accepted 23 March 2004

**Abstract**

Cryopreservation of primordial follicles in ovarian tissue has been extensively described in several species. However, until now no information is available concerning cryopreservation of isolated caprine primordial follicles. The aim of this work was to assess the toxicity of dimethylsulphoxide (DMSO) and propanediol (PROH), in different concentrations (1.5 and 3 M), on isolated caprine primordial follicles and verify the viability of these follicles after freezing/thawing procedures. Follicular viability was tested by trypan blue staining before and after 24 h of in vitro culture. Before culture the percentage of viable primordial follicles after the toxicity test and cryopreservation were similar to fresh follicles (control). On the other hand, after 24 h of in vitro culture the percentage of viable follicles cryopreserved in 1.5 M DMSO or PROH was significantly inferior than non-cryopreserved follicles, exposed or not to cryoprotectants. In conclusion, this study showed that isolated primordial follicles can be successfully cryopreserved in 1.5 DMSO or PROH, resulting in more than 60% viable follicles after a short-term culture. It is also concluded that in vitro culture is an essential parameter for the evaluation of follicular viability after cryopreservation.


*Keywords:* Primordial follicles; Immature oocyte; Cryopreservation; In vitro culture; Caprine

## 1. Introduction

Cryopreservation of male and female germ cells is an important technique, largely applied in germplasm banks to preserve genetic material of valuable animals and endangered breeds or species. While spermatozoa have been successfully cryopreserved for some time, oocyte cryopreservation is still a challenge.

Whittingham (1977) first reported that mice M II oocytes can generate live young after cryopreservation. Since this success, the preservation of mature

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doi:10.1016/j.smallrumres.2004.03.007

oocytes under low temperatures have been attempted in several species. Nevertheless, birth of offspring have been limited to a few trials (rabbits: Vicent et al., 1989, cows: Fuku et al., 1992, humans: Porcu et al., 1997). The reasons to explain this limited success include damage to the meiotic spindle, the zona pellucida, the cellular membrane or the ooplasm caused by exposure to low temperatures. These injuries can result in fertilization problems or defective embryos (Candy et al., 1994). Although these problems are quite common when fully-grown oocytes are frozen, cryopreservation of immature oocytes included in primordial follicles present different morphologic features and could be an interesting alternative for preservation female of germ cells. Primordial follicles are the first follicles formed in the ovaries and are composed of an immature oocyte surrounded by one layer of flattened follicular cells (Liu et al., 2000; Gosden et al., 2002). The oocytes inside these follicles are smaller than the fully-grown M II oocyte (only 1% of the volume of the latter). Also, these oocytes are undifferentiated (Liu et al., 2001), have fewer cytoplasmic organelles, and a total absence of a zona pellucida and cortical granules (Gosden et al., 2002). Moreover, these oocytes are arrested in the prophase of the first meiotic division with its nucleus in the germinal vesicle stage, and for this reason they are less susceptible to cytogenetic errors. All these features are potentially good when a given cell is to be cryopreserved (Carroll and Gosden, 1993; Hovatta et al., 1996; Newton et al., 1996; Oktay et al., 1997; Newton, 1998). According to the literature (Hovatta et al., 1996; Newton et al., 1996) the survival rate of the primordial follicles following the cryopreservation are of 70–80% and are thus a good source of female germ cells, to be exploited in germplasm banks (Liu et al., 2001).

Several studies regard the cryopreservation of primordial follicles inside ovarian tissue have been performed in different species (mice: Candy et al., 1997; Gunasena et al., 1997, rats: Sugimoto et al., 1996, cows: Paynter et al., 1999, ewes: Gosden et al., 1994; Salle et al., 1998, 1999, 2002; Baird et al., 1999; Demirci et al., 2001, 2002, humans: Hovatta et al., 1996; Gook et al., 2000, and non-human primates: Candy et al., 1995), while work done out with isolated follicles are very few (Jewgenow et al., 1998). In all these trials, the tissue was usually cryopreserved with cryoprotectant agents, such as dimethylsulphoxide (DMSO) or propanediol (PROH), at the concentration of 1.5 M. However, studies comparing the efficiency of other cryoprotectant concentrations are very rare. Also, no information regarding the cryopreservation or the toxic effects of cryoprotectant agents for caprine primordial follicles after isolation from the ovarian tissue is available. Therefore, the aim of the present work was to evaluate the viability of isolated caprine primordial follicles after exposition to or cryopreservation in DMSO or PROH at the concentration of 1.5 M or 3 M, as well as following in vitro culture. DMSO and PROH 3 M were used to verify if in higher concentrations cause a strong follicular degeneration.

## 2. Materials and methods

### 2.1. Source and preparation of ovarian tissue

Caprine ovaries ($n = 10$) were obtained at a local slaughterhouse from adult cross breed goats ($n = 5$), during the absence of rain. The ovaries were trimmed of adhering tissue and washed in 70% ethanol and then twice in phosphate buffered saline (PBS). The ovaries were then placed into tubes containing 20 ml PBS and transported to the laboratory within 1 h after collection in a thermo flask filled with water at 20 °C.

### 2.2. Isolation of ovarian primordial follicles from caprine ovaries

Primordial follicles were isolated from the ovaries using a specific mechanical procedure developed by Lucci et al. (1999). Briefly, the ovarian tissue was cut into small fragments using a tissue chopper (The Mickle Laboratory Engineering Co., Gomshal, Surrey, UK) adjusted to make 75 μm serial sections. The ovarian fragments were then placed in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (modified MEM—MEM$^+$), and mechanically dissociated using Pasteur pipettes. The suspension was then successively filtered through 500 and 100 μm nylon mesh filters. The suspension containing isolated primordial follicles was divided into 11 aliquots (1 ml each). One aliquot was used to analyze the viability of