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Short communication

Vitrification of pre-pubertal ovine cumulus–oocyte complexes: Effect of cytochalasin B pre-treatment

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Abstract

The aim of this study was to evaluate the effect of cytochalasin B (CCB) pre-treatment before vitrification on ability of immature oocytes from lamb ovaries to progress until metaphase II (MII) stage after vitrification/warming procedure. Cumulus–oocyte complexes (COCs) were obtained from ovaries of lambs, from 80 to 90 days old, collected from a local slaughterhouse. Before vitrification, COCs were randomly distributed in two experimental groups corresponding to the incubation with or without 7.5 μg/ml CCB for 30 min. In order to study cryoprotectant and CCB pre-treatment toxicity (toxicity test), oocytes were exposed to cryoprotectants, with or without CCB pre-treatment, but without plunging into N_2 liquid. Vitrification solution was composed by 4.48 M EG plus 3.50 M DMSO supplemented with 0.25 M sucrose. Two-step addition was performed. After vitrification or toxicity test, COCs were matured in bicarbonate-buffered TCM 199 containing 10% foetal calf serum and 10 ng/ml epidermal growth factor. A sample of COCs was directly in vitro matured (control group). Rates of MII oocytes of toxicity groups both, with or without CCB pre-treatment were lower than control group (41.1–50.0 versus 79.9, respectively; $P < 0.05$). After vitrification, a lower number of oocytes progressed to MII stage in comparison with non-vitrification groups ($P < 0.05$). In vitrified groups both with or without CCB pre-treatment 8.0 and 12.7%, respectively, of immature oocytes reached MII stage by the end of in vitro maturation culture. No effect of CCB was observed, either in the toxicity or vitrified groups. In conclusion, no effect of CCB pre-treatment before vitrification

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was detected in this study with immature oocytes of pre-pubertal sheep. More studies are needed in order to increase ovine oocyte survival after vitrification.

Keywords: Cumulus–oocyte complex; Pre-pubertal; Vitrification; Cytochalasin B; Ovine

1. Introduction

Studies on vitrification of ovine oocytes are very scarce, mainly with immature oocytes obtained from lamb ovaries. In fact, only one paper on vitrification of immature oocytes was found (Isachenko et al., 2001), but this was performed with COCs obtained from ewe ovaries.

Until recently, attention has been focused on the cryopreservation of mature oocytes. However, at this stage, microtubular spindles are highly sensitive to cooling and cryoprotectant agents (CPA) including chromosomal abnormalities (Sathananthan et al., 1988; Aman and Parks, 1994; Men et al., 2003). The development of an effective method of cryopreservation for not fully matured oocytes, with their cumulus cells, would offer an alternative to storage of mature gametes or embryos (Isachenko and Nayudu, 1999). Immature oocytes could be stored immediately after collection and only matured and fertilised when required. This kind of oocyte is assumed to be less prone to microtubular and chromosomal damage (Massip, 2003). However, COCs are a complex structure and their integrity must be preserved to enable oocyte progression to MII.

Exposing oocytes to cryoprotective additives and/or cooling them from about 37 to 20 °C or below causes various cytoskeletal and chromosomal alterations (Massip, 2003). A cytochalasin B (CCB) pre-treatment was used to reduce injury to oocytes and embryos during vitrification (Isachenko et al., 1998; Dobrinsky et al., 2000; Fujihira et al., 2004). Information about CCB effect in oocyte vitrification is very scarce, and results are controversial and depend on the animal species used. Some works have found that cytochalasin increased oocyte survival after vitrification of immature porcine oocytes (Isachenko et al., 1998; Fujihira et al., 2004); nevertheless, other studies in bovine did not observe any effect in both immature and mature oocytes (Vieira et al., 2002; Mezzalira et al., 2002, respectively). However, and in disagreement with Mezzalira et al. (2002); Rho et al. (2002) reported that CCB pre-treatment enhanced stabilisation of microtubules during MII bovine oocyte vitrification, although no in vitro embryo development was reported. Cytochalasin as a cytoskeletal relaxant was considered to make the cytoskeletal elements less rigid (Fujihira et al., 2004). In MII oocyte, CCB reduced damage to microtubules and may enhance stabilisation of spindle microtubules during vitrification (Rho et al., 2002). In the case of germinal vesicle oocytes, no organised meiotic spindle is present, and this relaxant effect may preserve the functionality of the gap junctions between oocyte and granulosa cells, and permit a faster and more uniform penetration of the cryoprotectants (Vieira et al., 2002). To our knowledge, no study on the effect of CCB in the vitrification of sheep oocyte has been reported.

The aim of this study was to evaluate the effect of CCB pre-treatment before vitrification on ability of immature oocytes from lamb ovaries to progress until metaphase II stage after vitrification procedure.