The effects of pre and post vitrification taxol treatment on bovine immature oocyte

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Summary

The aim of the present study was to find the effects of pre and post vitrification taxol treatment on bovine immature cumulus oocyte complexes (COCs). Bovine ovaries were collected from the local slaughterhouse in a container with 30-37°C physiologic saline and supplemented with antibiotics. The grade A aspirated COCs were assigned to 6 experimental groups; The 1st group was control (n=100) in which the COCs were subjected to *in vitro* maturation (IVM) in oocyte maturation medium (OMM). The 2nd group was the COCs (n=51) that were exposed to vitrification and warming solutions and then subjected to IVM. The 3rd group was the COCs which were incubated in OMM contained taxol (1 µM, n=51) for 20 min then transferred to IVM. The 4th group was the COCs (n=50) that were vitrified, warmed and transferred to IVM. The 5th group (n=57) was the COCs which were incubated in OMM containing taxol (1 µM for 20 min), vitrified (solutions with 1 µM taxol), warmed and transferred to IVM. The last group was the COCs (n=58) that were vitrified and warmed, then were incubated in maturation medium containing taxol (1 µM for 20) and, finally, were transferred to IVM. The COCs of all groups were incubated in OMM for 24 h under a humified (95%) condition with a temperature of 39°C and 5% CO_2. Oocyte maturation was not affected by exposing the COCs to vitrification solution or taxol groups compared to control ($P>0.05$). Vitrification reduced the oocyte maturation rate compared to control ($P<0.05$). Taxol treatment (before and after vitrification) did not improve the bovine oocyte maturation rate compared to control ($P<0.05$). The results have shown no beneficial effect of taxol on vitrification of bovine immature oocytes.

Key words: Immature oocyte, Vitrification, Open pulled straw, Bovine, Taxol

Introduction

New approaches based on reducing the volume of vitrification solutions and using the cytoskeleton stabilizers significantly improved oocyte vitrification. The high temperature exchange rate, due to a reduced volume of vitrification solution, decreased the detrimental effects of vitrification on oocytes (Kuwayama *et al*., 2005; Vajta and Kuwayama, 2006). The open pulled straw (OPS; (Liu *et al*., 2007)) is widely used for this purpose. On the other hand, the beneficial effects of the cytoskeletal stabilizers like cytochalasine B (Rojas *et al*., 2004; Silvestre *et al*., 2006; Gupta *et al*., 2007; Tharasanit *et al*., 2009), Taxol (Shi *et al*., 2006; Morató *et al*., 2008a, b, c; Zhang *et al*., 2009) and Cyclohexamide (Le Gal and Massip, 1999) on oocyte vitrification have been demonstrated. Cytochalasine B was successfully used for vitrification of immature (Silvestre *et al*., 2006; Tharasanit *et al*., 2009) and mature (Zhang *et al*., 2009) oocytes. Cyclohexamide was also used for vitrification of bovine mature and immature oocytes (Le Gal and Massip, 1999). Taxol is a cytoskeleton stabilizer that is used in high doses for the treatment of some cancers, but some studies have reported its effects on the re-polymerization of disrupted microtubules of somatic cells (Jordan *et al*., 1993) and oocytes (Morató *et al*., 2008c). It improved the vitrification of human immature and bovine, ovine and murine mature oocytes (Shi *et al*., 2006; Morató *et al*., 2008a, b, c;

Zhang *et al*., 2009).

There is no report in which the bovine immature oocytes were treated with taxol before or immediately after vitrification. Therefore, the aim of this study was to compare the effects of taxol treatment before and after vitrification on bovine immature oocytes.

Materials and Methods

Materials

Tissue culture medium (TCM)-199, bovine serum albumin (BSA), orcein, phenol red, FSH, LH, distilled water and HEPES were from Sigma-Aldrich (St. Luis, USA). Ethylene glycol (EG), Di methyl sulfoxide (DMSO) were purchased from Merck, Germany. Fetal Calf Serum (FCS) was provided from a local slaughterhouse and was exposed to 56°C for 30 min. Taxol (Ebetaxel®, Paclitaxel Ebewe, EBEWE pharma, Austria) was used in different media with 1 µmol concentration in the respective experimental groups. The OPS was produced with warming and pulling a 0.25 ml French straw (IMV technologies, France) on a hotplate and cutting it at the narrowest point with a sharp surgical blade.

Media preparation

Oocyte collection medium (OCM) was modified -TCM-199 (25 mM HEPES buffered) with 10% FCS. Oocyte maturation medium (OMM) was OCM with FSH (10 µg/ml), LH (10 µg/ml) and estradiol (1 µg/ml). Vitrification procedure was composed of 4 vitrification, warming and dilution media; V1 (OCM + 10% EG + 10% DMSO), V2 (OCM + 20% EG + 20% DMSO + 0.5 M sucrose (Sisco Research Laboratories, Mumbai, India)), a warming medium (OCM + 0.25 M sucrose) and a dilution medium (OCM + 0.15 M sucrose).

Oocyte collection and *in vitro* maturation

Bovine ovaries were collected from a local slaughterhouse and transported to the lab within 1 h in a flask, (35-37°C), containing physiologic saline with 105 IU/l penicillin and 1 g/l streptomycin. The cumulus oocyte complexes (COCs) were aspirated from follicles (2-8 mm) using an 18 gauge needle that was attached to a 5 ml disposable sterile syringe. The COCs were qualified and grade A COCs (oocytes with homogenous cytoplasm and more than 4 compacted layers of cumulus cells) were selected. The COCs were cultured in the OMM in an atmosphere with a temperature of 39°C, 5% CO_2 and 95% humidity for 24 h.

Vitrification and warming procedures

The immature COCs were vitrified by placing them in V1 for 5 min then they were transferred in V2 (for 30 sec), loaded in OPS tips (in 1-2 µL) and plunged in liquid nitrogen (LN_2). Oocytes were kept in LN_2, for at least 48 h. Warming was undertaken by placing the OPS tips in warming medium for 5 min then transferring them to dilution medium (for 5 min) prior to subjecting to OMM. All of the vitrification and warming procedures were undertaken on a stereomicroscope (Blue light®, USA) that was equipped with heat stage and temperature was set at 39°C (temperature of medium drops should be 37-39°C).

Experimental design

The COCs were assigned to six experimental groups (the duration of incubation in taxol and the concentration of taxol were based on Morató *et al*. (2008a, b)); the first group was (n=100) the COCs which were subjected to IVM. The second group was the COCs (n=51) that were exposed to vitrification and warming solutions and then subjected to IVM. The third group was the COCs which were incubated in OMM containing taxol (n=51; 1 µM) for 20 min then transferred to OMM. The fourth group was the COCs (n=50) that were vitrified, warmed and transferred to OMM. The fifth group (n=57) was the COCs which were incubated in OMM containing taxol (1 µM for 20 min), vitrified (V1 and V2 solutions were supplemented with 1 µM taxol), warmed and transferred to OMM. The last group was the COCs (n=58) that were vitrified and warmed, then incubated in OMM containing taxol (1 µM for 20) and finally were transferred to OMM. After 24 h of incubation in an

atmosphere with a temperature of 38.5°C, 95% humidity and 5% CO_2, the COCs were denuded and fixed in 1/3 aceto-ethanol (v/v) for 24 h and nuclear maturation was analysed using conventional aceto-orcein stain (Gordon, 2001).

Statistical analysis

The percentage of matured oocytes within each replicate of groups subjected to arcsin transformation was then analysed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS (1996). The least square means (LSMean) were compared with Tukey's multiple comparison test. The level of significance was set at $P<0.05$.

Results

Table 1 shows the mean percentage of the oocytes that reached MII stage (Fig. 1a) in different groups. The results show that vitrification has a considerable detrimental effect on maturation (Fig. 1b; the oocyte arrested at condense chromatin stage) of bovine immature oocyte compared to the control ($P<0.05$). Oocyte maturation rates were not different between the COCs which were exposed to the taxol or vitrification solutions (without vitrification) and the control group ($P>0.05$). Oocyte maturation rates were apparently higher in the taxol treated (before or after vitrification) vitrified COCs than the non-treated vitrified COCs ($P>0.05$). However, oocyte maturation rates in the taxol treated vitrified COCs were significantly less than the control COCs ($P<0.05$).

Table 1: Effects of taxol treatment (1 µM) on vitrification of bovine immature oocytes (LSMean±SEM) using OPS

Groups	No. oocytes cultured	Maturation rate (%)
Control*	100	84.8±8.4[a]
VS**	51	83.4±8.4[a]
Taxol†	51	78.7±6.3[a]
Vit††	50	20.1±7.7[b]
Tax-vit‡	57	30.9±9.4[b]
Vit-Tax‡‡	58	32±10.9[b]

*The oocytes were subjected to IVM. **The oocytes were exposed to the vitrification and warming solutions, then subjected to IVM. †The oocytes were exposed to taxol for 20 min then subjected to IVM. ††The oocytes were vitrified, warmed and subjected to IVM. ‡ The oocytes were exposed to taxol (for 20 min), vitrified, thawed and subjected to IVM. ‡‡The oocytes were vitrified, warmed, incubated in OMM supplemented with taxol for 20 min then transferred to IVM. [ab]values with different superscript within the column significantly differ ($P<0.05$). Each group was replicated ten times

Discussion

The results of the present study showed that pre and post vitrification taxol treatment did not improve the vitrification of bovine germinal vesicle (GV) stage oocyte. These results are in agreement with the previous studies which reported high sensitivity of immature bovine (Rojas *et al*., 2004; Diez *et al*., 2005), murine (Suo *et al*., 2009), porcine (Rojas *et al*., 2004; Fujihira *et al*., 2005) and human (Kyono *et al*., 2002) oocytes to vitrification. Rojas *et al*. (2004) showed that porcine GV stage (12 h incubation in OMM) oocytes were very sensitive against vitrification; they had used OPS for their

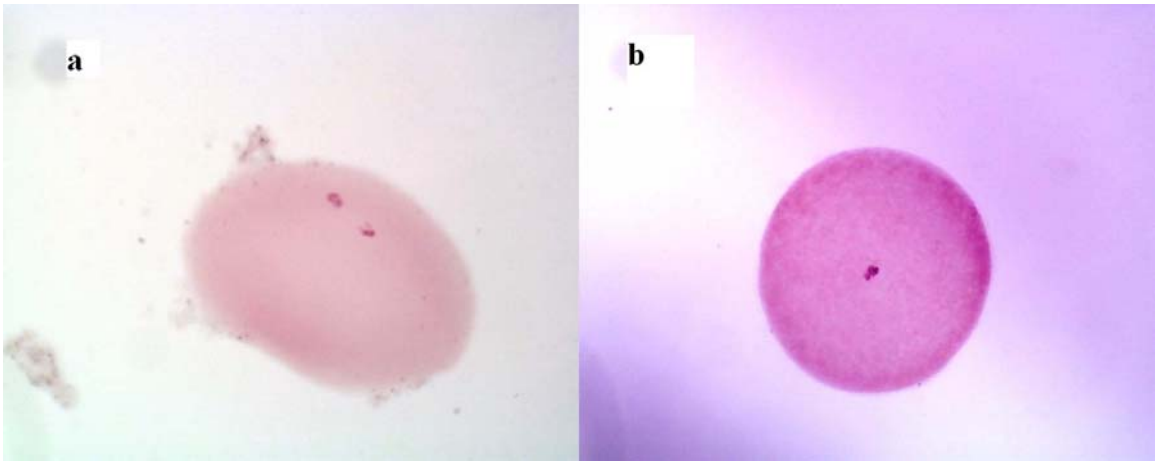

Fig. 1: Bovine matured (a) immature (condensed chromatin, b) oocytes, (aceto-orecein stain, ×40)

experiment. Albarracín *et al*. (2005) showed that bovine GVBD stage (after 6 h of incubation in OMM) was more sensitive than the MII stage oocyte against vitrification damages. They found that vitrification adversely affected the developmental capacity of the germinal vesicle breakdown (GVBD) stage calf oocyte and there was no embryo developed to blastocyst in the respected groups. Diez *et al*. (2005) reported that no GV stage arrested bovine vitrified oocytes developed to blastocyst, while the MII stage vitrified oocytes were able to develop to blastocyst. They explained their finding with morphological alterations that may affect the developmental competence of GV stage vitrified bovine oocytes. Fujihira *et al*. (2005) showed that taxol treatment has no positive effect on vitrification of *in vitro* matured porcine oocytes.

The results of the present study are in contrast with the previous findings that reported the beneficial effects of taxol on mature (Shi *et al*., 2006; Morató *et al*., 2008a, b, c; Zhang *et al*., 2009) oocyte vitrification. Pre-vitrification taxol treatment of MII stage porcine oocytes reduced damages and improved the development of vitrified porcine oocytes (Shi *et al*., 2006; Zhang *et al*., 2009). Morató *et al*. (2008c) treated bovine MII stage oocytes with 1 µM taxol before and during vitrification and reported no adverse changes in the cytoplasm or metaphase spindles in adult bovine matured oocytes, while stabilizing the metaphase and spindle morphology. They also showed the protective effects of taxol on developmental competence of matured bovine oocytes (Morató *et al*., 2008a). The glass capillary micropipettes were very effective in bovine oocytes vitrification (Babaei *et al*., 2006).

Effects of donor species (Hurtt *et al*., 2000; Rojas *et al*., 2004) and its sexual maturity (Albarracín *et al*., 2005), oocyte meiotic stages (Rojas *et al*., 2004; Cao *et al*., 2009; Jee *et al*., 2009) and morphologic conditions (Comizzoli *et al*., 2008) on oocyte vitrification were investigated previously. The reason for the poor maturation rate in the present study may confirm the very high sensitivity of bovine immature oocytes to vitrification or may return to the fact that we used grade A COCs that contained several layers of cumulus cells (>4 layers). The adverse effect of the presence of cumulus cell layers on oocyte vitrification was demonstrated previously (Bogliolo *et al*., 2007; Comizzoli *et al*., 2008; Suo *et al*., 2009). The results of the present study are in line with the previous studies which reported the influence of the presence of cumulus cells on mouse (Suo *et al*., 2009), sheep (Bogliolo *et al*., 2007) and cat (Comizzoli *et al*., 2008) GV stage oocytes. Removing cumulus cells, Comizzoli *et al*. (2008) improved vitrification of GV stage feline oocytes. Suo *et al*. (2009) compared the effects of the presence of cumulus cells on vitrification of GV and GVBD mouse oocytes and demonstrated that oocytes at the GVBD stage retain better cumulus membrane integrity and developmental ability during vitrification than those at GV stage. Bogliolo *et al*. (2007) provided evidence that the removal of cumulus cells before vitrification enhances survival and meiotic competence of immature ovine oocyte. However, Zhang *et al*. (2009) showed no beneficial effect of cumulus cells on ovine matured oocyte vitrification.

In conclusion, exposure to taxol or vitrification solutions had no detrimental effects on the meiotic progression of the bovine immature oocytes. Pre and post vitrification taxol treatment of bovine GV stage oocytes did not improve the oocyte maturation rate significantly.

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