Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

Structural changes of in vitro matured buffalo and bovine oocytes following cryopreservation

Bianca Gasparrini, Serena Di Francesco, Marcello Rubessa, Salvatore Velotto, Evelina Mariotti & Marina De Blasi

To cite this article: Bianca Gasparrini, Serena Di Francesco, Marcello Rubessa, Salvatore Velotto, Evelina Mariotti & Marina De Blasi (2009) Structural changes of in vitro matured buffalo and bovine oocytes following cryopreservation, Italian Journal of Animal Science, 8:sup2, 90-92, DOI: 10.4081/ijas.2009.s2.90

To link to this article: http://dx.doi.org/10.4081/ijas.2009.s2.90

Structural changes of *in vitro* matured buffalo and bovine oocytes following cryopreservation

Bianca Gasparrini[1], Serena Di Francesco[1], Marcello Rubessa[1], Salvatore Velotto[2], Evelina Mariotti[1], Marina De Blasi[1]

[1]Dipartimento di Scienze Zootecniche ed Ispezione degli Alimenti, Università di Napoli "Federico II", Italy

[2]Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università di Napoli "Federico II", Italy

Corresponding author: Bianca Gasparrini. Dipartimento di Scienze Zootecniche ed Ispezione degli Alimenti, Facoltà di Medicina Veterinaria, Università di Napoli "Federico II". Via F. Delpino 1, 80137 Napoli, Italy - Tel. +39 081 2536494/461 - Fax: +39 081 292981 - Email: bgasparr@unina.it

ABSTRACT - The aim of this work was to evaluate chromatin and spindle organization of buffalo and bovine *in vitro* matured oocytes after vitrification/warming by Cryotop and after their exposure to cryoprotectants (CP). *In vitro* matured oocytes were vitrified/warmed and exposed to the vitrification/warming solutions containing ethylene glycol (EG), dimethyl sulfoxide (DMSO) and sucrose as CP. Two hours after warming, oocytes were fixed and immunostained for microtubules and nuclei and examined by fluorescence microscopy. Data were analyzed by Chi Square test. A higher percentage of Telophase II stage oocytes was found in the toxicity (26 and 34% in bovine and buffalo) and the vitrification groups (13 and 7% in bovine and buffalo) compared to the control, indicating occurrence of activation. An increased percentage of oocytes with abnormal spindle and chromosome organization was found in oocytes exposed to CP (24 and 13% in bovine; 32 and 30% in buffalo respectively) and in those vitrified (26 and 31% in bovine; 26 and 29% in buffalo respectively) compared to the control (0 in bovine and 2.5 % in buffalo).

Key words: Ultrastructure, Oocyte vitrification, Buffalo, Bovine.

Introduction - The progress in oocyte cryopreservation would improve domestic animal breeding by genetic selection programs. However, despite the enormous progress in embryo cryopreservation, oocyte cryopreservation still remains a demanding task in the majority of mammalian species. In particular, buffalo oocytes are particularly sensitive to chilling injuries, because of their high intracytoplasmic lipid content (Gasparrini *et al*., 2006). One of the most successful ultrarapid vitrification techniques is the Cryotop vitrification (CTV) that has resulted in excellent survival and developmental rates with human and bovine Metaphase II (MII) oocytes (Kuwayama *et al.*, 2005). This method has also been successfully used for cryopreservation of ovine (Succu *et al.*, 2008), pig (Fujihira *et al.*, 2005), and buffalo oocytes (Gasparrini *et al.*, 2007). However, despite the improvement in survival and cleavage rates, development into blastocysts is still poor, probably because of ultrastructural and physiological changes that occur as a consequence of vitrification. It is known that vitrification causes several ultrastructural (Hyttel *et al.*, 2000) and structural alterations, including damages of the meiotic spindle apparatus in the oocyte of several species (Rho *et al,* 2002; Rojas *et al*., 2004; Succu *et al.,* 2008). The aim of this work was to evaluate chromosome and spindle organization of buffalo and bovine *in vitro* matured oocytes after vitrification/warming by Cryotop and after their exposure to cryoprotectants (CP).

Material and methods - Abattoir-derived cumulus–oocyte complexes (COCs) with a compact, non-atretic cumulus and a homogeneous cytoplasm were matured *in vitro* according to our standard procedures (Gaspar-

rini *et al.*, 2006, 2008). After *in vitro* maturation (IVM), COCs were mechanically stripped of their cumulus cells by gentle pipetting in Hepes-buffered TCM 199 (H199) supplemented with 10% fetal calf serum (FCS). All media for vitrification/warming were made in a base solution consisting of H199 medium supplemented with 20% FCS, and all equilibration and dilution steps were carried out at room temperature. The denuded oocytes were vitrified by the Cryotop method, previously described (Kuwayama and Kato, 2000). Briefly, oocytes were equilibrated in 10% EG and 10% DMSO for 3 min, transferred into 20% EG and 20% DMSO in TCM 199 with 20% FCS +0.5 M sucrose, loaded on Cryotops, and plunged into liquid nitrogen within 25 s. For warming, the Cryotop strip was immersed directly into 1 ml of 1.25 M sucrose solution for 1 min, and recovered oocytes were then exposed to decreasing concentrations of sucrose (0.62 M, 0.42 M, and 0.31 M) for 30 s each. In order to test CP toxicity, COCs were exposed to the vitrification and warming solutions. In both cases, oocytes were rinsed and allocated to IVM drops for 2 h. To evaluate the status of the meiotic spindle 2 h after warming, oocytes were fixed and immunostained for microtubules using a method previously described (Messinger and Albertini, 1991), stained for nuclei with Hoechst and examined by fluorescence microscopy. Fresh *in vitro* matured oocytes were fixed and stained as control. Data were analyzed by Chi Square test.

Results and conclusions - The results of vitrification and exposure to CP (toxicity) on bovine and buffalo IVM oocytes are shown in Table 1 and 2, respectively. The most interesting finding of the present study was the occurrence of spontaneous parthenogenetic activation, indicated by the presence of oocytes in Telophase stage (TII). It was previously reported that vitrification or exposure to CP causes a transient increase in intracellular calcium concentration in mouse oocytes that is comparable to the calcium rise triggered by sperm penetration (Larman *et al*., 2006).

Recently, high rates of parthenogenetic activation were reported in pig oocytes following vitrification but not following exposure to CP (Somfai *et al*., 2006). Parthenogenetic activation also occurred in ovine oocytes exposed to CP and, to a greater extent, to vitrification (Succu *et al.*, 2008). An unexpected datum of our study was the evidence of a significantly higher percentage of spontaneously activated oocytes in the toxicity group compared to the vitrification group in both species. We speculate that the lower activation observed in the vitrification group may be referred to the slowing down of the metabolic activity subsequent to thermal shock, and hence that activation after vitrification may occur later than 2 h post-warming. To confirm this hypothesis, the same assessment needs to be carried out in oocytes fixed at later times post-warming. The other conclusion of this study is that the exposure of oocytes to CP causes damages to the meiotic spindle and chromosome organization similar to those induced by the whole vitrification pro-

Table 1. Effect of vitrification and exposure to cryoprotectants (toxicity) on bovine IVM oocytes, spindle and chromosome organization.

		Maturation rate on total number of oocytes			Spindle configuration and chromosome organization in MII oocytes				
		Non matured oocytes	TII n (%)	MII n (%)	Spindle configuration n (%)			Chromosome organization n (%)	
Groups	n				normal	abnormal	absent	normal	abnormal
control	91	12 (13)^{a}	0 (0)^{A}	79 (87)^{A}	75 (95)^{A}	0 (0)^{A}	4 (5)^{a}	79 (100)^{A}	0 (0)^{A}
toxicity	101	8 (8)^{aA}	26 (26)^{Bb}	67 (66)^{B}	49 (73)^{Ba}	16 (24)^{B}	2 (3)^{A}	58 (87)^{Ba}	9 (13)^{Ba}
vitrification	111	29 (26)^{bB}	14 (13)^{Ba}	68 (61)^{B}	38 (56)^{Bb}	18 (26)^{B}	12 (18)^{bB}	47 (69)^{Bb}	21 (31)^{Bb}

^{a,b}*Values with different superscripts within columns are significantly different, P<0.05;*
^{A,B}*Values with different superscripts within columns are significantly different, P<0.01.*

tocol. The damages to the meiotic spindle and DNA fragmentation may lead to aneuploidy incompatible with subsequent embryo development and account for the poor embryo development currently recorded. The evidence of these structural alterations provides the basis for developing new strategies to improve the efficiency of cryopreservation in bovine and buffalo species.

Table 2. Effect of vitrification and exposure to cryoprotectants (toxicity) on buffalo IVM oocytes, spindle and chromosome organization.

		Maturation rate on total number of oocytes			Spindle configuration and chromosome organization in MII oocytes				
		Non matured oocytes	TII n (%)	MII n (%)	Spindle configuration n (%)			Chromosome organization n (%)	
Groups	n				normal	abnormal	absent	normal	abnormal
control	90	10 (11)	0 (0)^{aA}	80 (89)^{A}	78 (97)^{A}	2 (3)^{A}	0 (0)	78 (97)^{A}	2 (3)^{A}
toxicity	117	6 (5)	40 (34)^{B}	71 (61)^{B}	48 (68)^{B}	23 (32)^{B}	0 (0)	50 (70)^{B}	21 (30)^{B}
vitrification	114	8 (7)	8 (7)^{bA}	98 (86)^{A}	69 (70)^{B}	26 (27)^{C}	3 (3)	70 (71)^{B}	28 (29)^{B}

^{a,b}*Values with different superscripts within columns are significantly different, P<0.05;*
^{A,B,C}*Values with different superscripts within columns are significantly different, P<0.01.*

REFERENCES - Fujihira, T., Nagai, H., Fukui, Y., 2005. Relationship between equilibration times and the presence of cumulus cells, and effect of taxol treatment for vitrification of in vitro matured porcine oocytes. Cryobiology. 51:339-343. **Gasparrini,** B., Boccia, L., Marchandise, J., Di Palo, R., George, F., Donnay, I., Zicarelli, L., 2006. Enrichment of in vitro maturation medium for buffalo (Bubalus bubalis) oocytes with thiol compounds: effects of cystine on glutathione synthesis and embryo development. Theriogenology. 65:275-87. **Gasparrini,** B., Attanasio, L., De Rosa, A., Monaco, E., Di Palo, R., Campanile, G., 2007. Cryopreservation of in vitro matured buffalo (Bubalus bubalis) oocytes by minimum volumes vitrification methods. Anim. Reprod. Sci. 98:335-342. **Gasparrini,** B., Monaco, E., Boccia, L., De Rosa, A., Attanasio, L., Killian, G., 2008. Effect of osteopontin on in vitro embryo development in cattle. 34th Annual Conference of the International Embryo Transfer Society, 5-9 January 2008. Reprod. Fert. Develop. 20:180. **Hyttel,** P., Vajta, G., Callesen, H., 2000. Vitrification of bovine oocytes with the open pulled straw method: ultrastructural consequences. Mol. Reprod. Dev. 56:80-88. **Kuwayama**, M., Kato, O., 2000. All-round vitrification method for human oocytes and embryos. J. Assist. Reprod. Genet. 17:477. **Kuwayama**, M., Vajta, G., Kato, O., Leibo, S.P., 2005. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod. Biomed. 11:300-308. **Larman,** M.G., Sheehan, C.B., Gardner, D.K., 2006. Calcium free vitrification reduces cryoprotectant-induced zona pellucida hardening and increases fertilization rates in mouse oocytes. Reproduction. 131:53-61. **Messinger**, S.M., Albertini, D.F., 1991. Centrosome and microtubule dynamics during meiotic progression in the mouse oocyte. J. Cell Sci. 100:289-298. **Rho,** G.J., Kim, S., Yoo, J.G., Balasubramanian, S, Lee, H.J., Choe, S.Y., 2002. Microtubulin configuration and mitochondrial distribution after ultra-rapid cooling of bovine oocytes. Mol. Reprod. Dev. 63:464-470. **Rojas,** C., Palomo, M.J., Albarracin, J.L., Mogas, T., 2004. Vitrification of immature and in vitro matured pig oocytes: study of distribution of chromosomes, microtubules, and actin microfilaments. Cryobiology. 49:211-220. **Somfai,** T., Dinnyés, A., Sage, D., Marosán, M., Carnwath, J.W., Ozawa, M., Kikuchi, K., Niemann, H., 2006. Development to the blastocyst stage of parthenogenetically activated in vitro matured porcine oocytes after solid surface vitrification (SSV). Theriogenology. 66:415-422. **Succu**, S., Bebbere, D., Bogliolo, L., Ariu, F., Fois, S., Leoni, G.G., Berlinguer, F., Naitana, S., Ledda, S., 2008. Vitrification of in vitro matured ovine oocytes affects in vitro pre-implantation development and mRNA abundance. Mol. Reprod. Dev. 75:538-546.