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Effect of antioxidant on *in-vitro* maturation of vitrified bovine oocytes

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ABSTRACT

The present study was undertaken to investigate the effect of α-tocopherol on *in-vitro* maturation of vitrified bovine oocytes. The cumulus-oocyte-complexes (COCs) recovered from follicles (3–5 mm diameter) by aspiration and slicing techniques were equally divided into 5 (five) groups consisting 66 COCs in each. The vitrified bovine oocytes were sub-grouped into control and treatment group. The vitrified- and non-vitrified- control group of oocytes were *in-vitro* matured in TCM-199 media without supplementation of vitamin E. In three vitrified treatment groups, the oocytes were *in-vitro* cultured in TCM-199 media supplemented with vitamin E (α-tocopherol) @ 50 μM, 100 μM and 200 μM, respectively. The mean percentage of cumulus cell expansion and polar body formation in non-vitrified control and vitrified control groups were 85.18±2.57 and 65.38±1.83, and 51.67±1.94 and 30.26±0.16, respectively. The rate of cumulus cell expansion and polar body formation in the oocytes of three vitrified treatment groups media supplemented with vitamin E @ 50 μM, 100 μM and 200 μM were 56.39±3.49 and 34.62±1.83, 69.95±3.20 and 56.23±1.61, and 54.44±4.73 and 31.62±4.50% respectively. Mean percentage of both cumulus cell expansion and polar body formation in the non-vitrified control group were significantly higher than that in the vitrified control group. In the treatment group supplemented with vitamin E @ 100 μM, both cumulus cells expansion and polar body formation were significantly higher than that in media supplemented with 50 μM and 200 μM vitamin E as well as in vitrified control group.

Key words: Antioxidant, Bovine, *In-vitro* maturation, Oocytes, Vitrified

Oocytes can be cryopreserved by vitrification to preserve its fertility for a long period. In the solidification process, the oocytes are treated with cryoprotective substances and submerged in liquid nitrogen at -196°C. Despite the advances in the field of vitrification of oocytes at different stages, no procedure was found to be reported as an established technique. Different types of cell injuries, including membrane lipids peroxidation, oxidation of amino acids and nucleic acids, apoptosis and necrosis may occur due to chilling effect of vitrification. These adverse effects tend to recover to some extent after thawing and rehydration and *in-vitro* culture (Ciotti *et al*. 2009). The most susceptible damaging effect of reactive oxygen species (ROS) to oocytes (Gupta *et al.* 2010) might arouse during thawing and rehydration following vitrification. To overcome different types of cell injuries, exogenous antioxidants, such as α-tocopherol may be useful to increase antioxidant capacity of oocytes by increasing intracellular levels of reactive oxygen species scavengers. Supplementation of a reasonable level of antioxidants to the media might be effective to obtain optimal ROS detoxification during *in-vitro* oocyte maturation.

The present study, therefore, was undertaken to investigate the effect of α-tocopherol on developmental competence of vitrified immature oocytes to metaphase-II (M II) stage on *in-vitro* maturation.

MATERIALS AND METHODS

The media and chemicals used to conduct the present study were bought from Sigma-Aldrich. Bovine oocytes were collected from abattoir ovaries after bringing it to the laboratory. Ovaries were collected soon after the slaughter of the animal and carried to the laboratory in a thermos flask containing warm (37°C) normal saline solution (NSS) with antibiotic. The cumulus-oocyte-complexes (COCs) were recovered from follicles (3–5 mm diameter) by aspiration and slicing techniques. A total of 264 numbers of vitrified-rehydrated COCs were equally subdivided into four (4) groups consisting 66 numbers in each. In three sub-groups, the COCs were separately *in-vitro* cultured in TCM-

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199 medium supplemented with vitamin E @ 50, 100 and 200 µM, respectively. The fourth group of vitrified-rehydrated oocytes was *in-vitro* cultured in TCM-199 medium without vitamin E supplementation. In another group, 66 numbers of non-vitrified COCs were *in-vitro* cultured in TCM-199 medium and it was considered as the control group.

The TCM-199 + follicular fluid + oestradiol 17β + p-FSH + follicular fluid + gentamicin + sodium pyruvate + cysteamine + FBS was used for *in-vitro* maturation of bovine oocytes. *In-vitro* maturation was carried out in an environment of 5% CO_2 in a humidified environment at 38.5°C for 24 h in a CO_2 incubator.

The data obtained from the study were analyzed statistically using SAS enterprise Guide 4.3.

RESULTS AND DISCUSSION

Effects of antioxidant on *in-vitro* maturation of vitrified bovine oocytes are presented in Table 1. The mean percentage of cumulus cell expansion and polar body formation in non-vitrified control and vitrified control groups were 85.18±2.57 and 65.38±1.83, and 51.67±1.94 and 30.26±0.16, respectively. DMRT (Duncan's Multiple Range Test) indicated that the mean percentage of both cumulus cells expansion and polar body formation in the non-vitrified (control) group was significantly higher (P<0.01) than that in the vitrified control (without antioxidant) group of bovine follicular oocytes. The variation found in the present study might be due to vitrification (Moawad *et al.* 2012) and exposure of oocytes to the toxic effects of cryoprotectants. The actin filaments, junctures between cumulus cells and zonapellucida, become disorganized leading to alterations in the secondary structure of the zonapellucida proteins as well as the carbohydrate residues (Bogliolo *et al.* 2012). The mixture of cryoprotectants used in the present study might have reduced its individual toxicity as well as probable major alterations in ultra-structure of bovine oocytes (Cocchia *et al.* 2010, Purohit *et al.* 2012, Dutta *et al.* 2013).

The rate of cumulus cells expansion and polar body formation in media supplemented with vitamin E @ 50 µM, 100 µM and 200 µM were 56.39±3.49 and 34.62±1.83, 69.95±3.20 and 56.23±1.61, and 54.44±4.73 and 31.62±4.50% respectively. DMRT indicated that the mean percentage of both cumulus cells expansion and polar body formation in the group supplemented with vitamin E @ 100 µM was significantly higher (P<0.01) than that in 50 µM and 200 µM vitamin E supplemented and vitrified control groups.

Higher concentration of antioxidants may lead to formation of pro-oxidants which is toxic for the maturation of the oocytes. Vitamin E @ 100 µM might have a positive effect during *in-vitro* maturation of the bovine follicular oocytes (Thiyagarajan *et al.* 2009). Being a lipid soluble antioxidant, Vitamin E (α-tocopherol) reacts with free radicals and reactive oxygen species. Therefore, it reduces oxidative damage by acting as a sink to the spare electrons (Thiyagarajan *et al.* 2009 and Natarajan *et al.* 2010) thus leading to limited DNA fragmentation and improved developmental ability (Kitagawa *et al.* 2004). However, Natarajan *et al.* (2010) reported a better result in media supplemented with 200 µM α-tocopherol. The discrepancy in various studies might be due to techniques of vitrification and thawing, species, status of the ovary and the oocytes used for *in-vitro* maturation.

In conclusion, the results of the present study reveal that vitrification has control over the *in-vitro* maturation of bovine follicular oocytes. TCM-199 medium supplemented with Vitamin E @100 µM has a desirable effect on the *in-vitro* maturation of vitrified bovine follicular oocytes.

Table 1. Per cent cumulus cells expansion and polar body formation of vitrified and non-vitrified Oocytes in vitamin E supplemented (50, 100 and 200 µM) treatment and control media

Groups	Vitamin E		Per cent (Mean±SE) oocyte Maturation (N= 66)	
			Cumulus Cells Expansion	Polar Body Formation
Treatment		50 µM	56.39^{c}±3.49	34.62^{c}±1.83
		100 µM	69.95^{b}±3.20	56.23^{b}±1.61
		200 µM	54.44^{c}±4.73	31.62^{c}±4.50
Control	Vitrified	0 µM	51.67^{c} ±1.94	30.26^{c} ±0.16
	Non-Vitrified	0 µM	85.18^{a}±2.57	65.38^{a}±1.83

*Mean with different superscripts in a column differ significance (P<0.01)

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