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Effect of antioxidant on *in vitro* maturation of bovine follicular oocytes

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Abstract
The present study was conducted to assess the effect of α-tocopherol on *in-vitro* maturation of bovine follicular oocytes. The aspirated follicular oocytes were *in-vitro* cultured in TCM-199 medium at 38.5ºC with 5% CO_2 for 24 hours in a CO_2 incubator. The medium TCM-199 supplemented with follicular fluid, oestradiol-17β, p-FSH, gentamicin, sodium pyruvate, cysteamine and fetal bovine serum was used as control. In the treatment groups, the control medium was supplemented with 100 µM and 150 µM α-tocopherol. The rates of cumulus cell expansion recorded in 100 µM, 150 µM α-tocopherol supplemented and the control group of oocytes were 74.28 ± 1.66, 73.59 ± 0.78 and 74.46 ± 2.68 per cent and polar body formation were 50.06 ± 2.40, 45.43 ± 4.20 and 44.02 ± 4.37 per cent, respectively.

Keywords: antioxidant, bovine, IVM, oocytes, TCM-199

Introduction
The success of *in-vitro* maturation necessitates both nuclear and cytoplasmic maturation in oocytes. An asynchrony between these two processes occurs when cytoplasmic maturation lags behind during the process of *in-vitro* maturation (Fulka *et al*., 1998; Smith, 2001) [4, 9]. The advancement of an oocyte from deplotene of prophase I (Germinal vesicle/ GV) to metaphase II stage along with cytoplasmic maturation are essential during *in-vitro* maturation for both fertilization and embryonic development (Smitz *et al*., 2001; Trounson *et al*., 2001; Ali *et al*., 2006) [10, 12, 1]. The generation of pro-oxidants (such as reactive oxygen species), which commonly arise following pro- and anti-oxidant imbalance, affect these developmental changes in oocytes (Guerin *et al.*, 2001; Agarwal *et al*., 2006) [5, 2]. In such a situation, antioxidants may act as a beneficial additive to the synthetic *in-vitro* maturation culture media by acting as reactive oxygen scavengers.
The present study was, therefore, undertaken to assess the effect of using α-tocopherol (vitamin E) as an antioxidant additive on *in-vitro* maturation of bovine oocytes.

Materials and Methods
The media and chemicals used to conduct the present study were procured from Sigma-Aldrich.

Collection of oocytes
Bovine ovaries were collected from local abattoirs and brought to the laboratory within 3 hours of slaughter in physiological saline (37ºC) containing Penicillin G (100IU/ml) and Streptomycin (0.125 mg/ml). Oocytes were collected by aspiration and slicing techniques and examined under a stereo-zoom microscope for grading and selection before *in-vitro* maturation. Briefly, in aspiration technique, a 10 ml disposable syringe containing aspiration medium (1 ml) fitted with an 18-gauge needle was used. The needle was introduced at the base of the follicle (3-8 mm in diameter) through the ovarian stroma and the cumulus-oocytes-complexes (COCs) were aspirated along with the follicular fluid. The aspirated content was immediately transferred to an oocyte searching Petridish containing washing medium, and examined under the stereo zoom microscope. In the slicing technique, ovaries were chopped into small pieces using a sterilized surgical blade in a Petridish containing aspiration medium. After 10-15 minutes, the content of the Petridish was examined in search of quality oocytes under a stereo-zoom microscope and the oocytes were then transferred to a small Petridish (35 mm) containing washing medium.

In-vitro maturation

The oocytes with compact cumulus cell layers and homogenous ooplasm were selected for *in-vitro* maturation (IVM) as per Hazelerger *et al.* (1993) [6]. Briefly, the oocytes were washed 4-6 times in washing medium before IVM. The IVM droplets were prepared by using 50 µl of *in-vitro* maturation medium in a 35 mm Petridish and covered with sterile mineral oil. A group of oocytes (8-12) were then transferred into IVM droplets and put in a CO_2 incubator maintained at a temperature of 38.5ºC with 5% CO_2 in humidified air for 24 hrs. Under phase contrast microscope, these *in-vitro* matured oocytes were then examined for assessment of the status of maturation based on volumetric expansion of cumulus cells and extrusion of polar body.

Media

The medium TCM-199 supplemented with follicular fluid, oestradiol-17β, p-FSH, gentamicin, sodium pyruvate, cysteamine and fetal bovine serum was used for *in-vitro* maturation of cumulus-oocyte-complexes, which used as the control group. In the treatment groups, the cumulus-oocyte-complexes were *in-vitro* cultured in two sub-groups, where the control medium was supplemented with 100 µM and 150 µM of α-tocopherol (Vitamin E).

Follicular fluid

The aspirated follicular fluid from visible follicles (3-6 mm) was centrifuged at 37ºC, at 10000 rpm for 30 minutes. The collected supernatant was then heat inactivated using standard protocol and stored at -20ºC for future use.

Statistical Analysis

Data obtained in the present experiment were analysed statistically by SAS enterprise 5.1.

Results and Discussion

The results obtained in respect of the effect of α-tocopherol (vitamin E) on *in-vitro* maturation of bovine oocytes are presented in Fig 1. In the present study, the rates of incidence of cumulus cells expansion were found to be 74.28 ± 1.66 and 73.59 ± 0.78 per cent, in medium supplemented with α-tocopherol @100 µM and 150 µM, respectively. In the control medium, incidence of cumulus cells expansion was recorded as 74.46 ± 2.68 per cent. The rates of incidence of polar body formation following *in-vitro* maturation were found to be 50.06 ± 2.40 and 45.43 ± 4.20 per cent and 44.02 ± 4.37 per cent in medium containing 100 µM, 150 µM and 0 µM of α-tocopherol, respectively. Both the rates of cumulus cells expansion and polar body formation differed non-significantly between the groups in the present study. Thiyagarajan and Valivittan (2009) [11] and Natarajan *et al.* (2009) [7], however, reported a significantly ($p< 0.01$) enhanced rate of *in-vitro* maturation of bovine follicular oocytes in 100 µM and 150 µM α-tocopherol (Vitamin E) supplemented medium. The discrepancy in the findings in the different studies might be due to variation in concentration of vitamin E, breeds of animals or status of the ovary and oocytes used for *in-vitro* maturation. Carlson *et al.* (1993) [3] reported that the α-tocopherol and its derivatives reduce oxidative damage by acting as a sink to the spare electrons due to its antioxidant effect. Primarily it protects polyunsaturated fatty acids in membranes. In alteration, peroxidation of these membrane lipids can lead to structural damage, affecting the function and permeability of membranes, eventually resulting in irreversible injury and death of the cells (Olson and Seidel, 2000) [8].

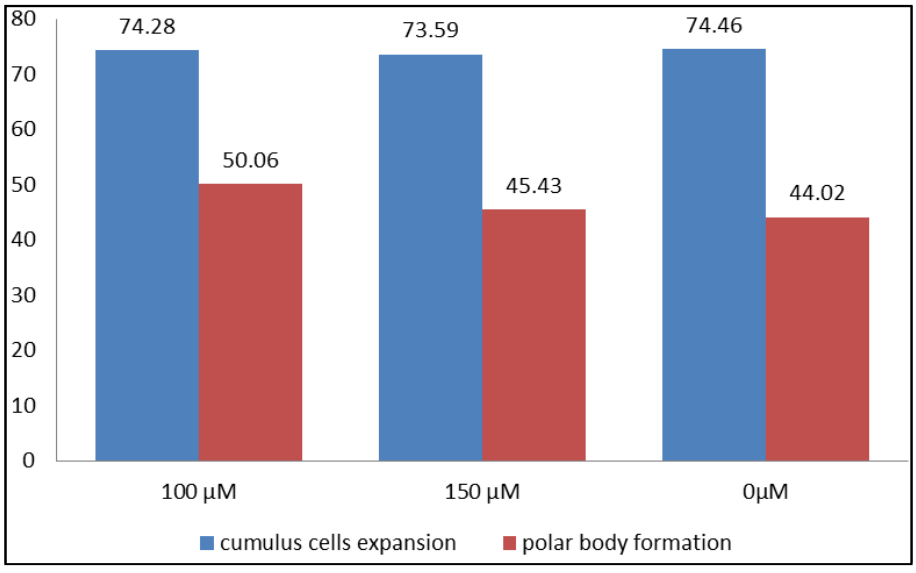

Fig 1: *In-vitro* maturation of bovine oocytes using media supplemented with vitamin E @ 100 µM, 150 µM and control medium in respect of cumulus cell expansion and polar body formation.

Conclusion

From the present study, it could be concluded that α-tocopherol (Vitamin E) may be a beneficial antioxidant which can be used as a source of reactive oxygen scavengers in TCM-199 for *in-vitro* maturation of bovine follicular oocytes. It was found that TCM-199 medium supplemented with α-tocopherol @ 100 µM has a better effect on *in-vitro* maturation of bovine follicular oocytes compared to the dose of 150 µM.

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