A261E Embryology, Developmental Biology and Physiology of Reproduction

# Alpha-tocopherol affects gene expression patterns of rabbit cumulus-oocyte complexes and reduces apoptosis rate during in vitro maturation

**M. Arias-Alvarez*[1], R.M. Garcia-Garcia[1], J. Lopez-Tello[1], K. Nieto[1], P.G. Rebollar[2], A. Gutierrez-Adan[3], P.L. Lorenzo[1]**

[1]Complutense University of Madrid, Madrid, Spain; [2]Polytechnic University of Madrid, Madrid, Spain; [3]INIA, Madrid, Spain.

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Oxidative stress compromises oocyte developmental competence during *in vitro* maturation (IVM). Antioxidants such as vitamin E may avoid this imbalance. The aim of this study was to investigate the effect of a-Tocopherol (α-TocOH) on the relative mRNA abundance of genes involved in cumulus expansion (*GJA1, PTGS2*), cell cycle and viability (*AKT1*), cell cycle regulation and apoptosis (*Tp53, CASP3*) and antioxidant response (*SOD2, GPX1, CAT*) in rabbit cumulus oocyte complexes (COCs) *in vitro* matured. The apoptosis index in cumulus cells (CCs) and the hydrogen peroxide ($H_2O_2$) released by the COCs in maturation media were also assessed. For these purposes, COCs from follicles ≥1mm were recovered, selected and *in vitro*-matured for 16h (38ºC, 5% $CO_2$) in a medium containing TCM-199 (Sigma, Madrid, Spain) with 0.3% bovine serum albumin (Sigma, Madrid, Spain) and 10 ng/mL Epidermal Growth Factor (EGF) (Sigma, Madrid, Spain) supplemented with 0, 100, 200 or 400 μM α-TocOH (Sigma, Madrid, Spain), named as 0E, 100E, 200E and 400E groups, respectively. After IVM, maturation media without cells was collected and stored at -32ºC and $H_2O_2$ concentrations were measured by the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Life Techonologies, NY, USA). The mRNA transcripts were quantified in 203 oocytes and their respective CCs (n = 51, n = 50, n = 50, n = 52 for 0E, 100E, 200E and 400E groups, respectively) by qRT-PCR to contrast relative levels of histone *H2AZ* and genes described above. Apoptotic index was studied in 43 COCs by TUNEL technique (Roche Diagnostics, SL, Barcelona, Spain) (n = 10, n = 10, n = 10, n = 12 for 0E, 100E, 200E and 400E groups, respectively). Data were analysed using one way ANOVA and Bonferroni test to compare means. In oocytes, *SOD2*, *CAT* and *Tp53* poly (A) mRNA contents were down regulated with 100 μM α-TocOH supplementation compared to the control group without this antioxidant ($P < 0.05$). In CCs, *CASP3* mRNA transcripts were lower in groups with intermediate concentrations of antioxidants (100E and 200E) compared to 0E and 400E groups ($P < 0.005$), in spite of the apoptosis rate was significantly reduced in all groups supplemented with α-TocOH (100E: 9.12 ± 1.81%, 200E: 10.26 ± 2.75%, 400E: 8.50 ± 2.63% vs 0E: 22.50 ± 3.40%, $P < 0.05$). However, the amount of $H_2O_2$ released by the COCs to the maturation media was similar in all the experimental groups (7.62 ± 0.60, 10.93 ± 1.23, 7.76 ± 0.00 and 7.75 ± 0.45 μM in 0E, 100E, 200E and 400E groups, respectively). This study has demonstrated that supplementation of α-TocOH in IVM medium induced significant changes in the molecular machinery of oocytes. Thus, α-TocOH reduced the apoptosis rate in CCs despite non-differences in $H_2O_2$ concentrations were found among groups. We acknowledge UCM, CM and MICINN for funding.

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