Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU AND IVF

Chromatin compaction and transcriptional activity in oocytes recovered from early antral follicles and cultured in vitro with Trichostatin A

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Trichostatin A (TSA) promotes histone hyperacetylation and has been used during in vitro culture of oocytes in order to increase their transcriptional activity. In order to establish a culture system capable of increasing the accumulation of transcripts, aiming to improve the acquisition of oocyte competence, oocytes retrieved from antral follicles <2 mm in diameter were pre-matured (PM) in vitro with the meiotic blocker C-type natriuretic peptide (NPPC) associated to TSA. The follicles present in the cortical portion of the ovary were measured with a ruler attached to the stereoscope and those with a diameter <2 mm were ruptured for recovery of COCs, which were PM for 24h in 100 µL microdrops of TCM-199 with 0.2 mM pyruvate, 25 mM sodium bicarbonate, 75 µg/mL amikacin, 0.3% BSA, 1x10-4UI/mL rFSH, 100 nM NPPC and different concentrations of TSA (0 nM; 2.5 nM; 5.0 nM and 10.0 nM). Next, the oocytes were IVM (TCM199 with 1x10-1 UI/mL rFSH, 100 UI/mL hCG and 10% FBS) for 24h. Immature oocytes were also evaluated immediately after removal from the follicle (group 0h). At the end of each moment, the COCs were stripped from cumulus cells and their diameter was measured. To assess chromatin compaction, the oocytes were stained with Hoechst 33342; immature oocytes (germinal vesicle - GV) were classified from GV0 to GV3 (from least to most compact chromatin) and oocytes with contracted metaphase chromosomes and a polar body were classified as metaphase II (MII). The global transcription activity was assessed after staining with Click-it RNA Imaging Kit. The images were evaluated in an epifluorescence microscope to determine the arbitrary units of fluorescence (AUF). Data were analyzed by analysis of variance (ANOVA) followed by Tukey's test ($P<0.05$). At the end of the PM, there was no difference between the groups regarding the oocyte diameter (101.9 to 104.3 µm; $P>0.05$), but there was an increase ($P<0.05$) in the diameter of the oocytes at the end of IVM (106.9 to 109.1 µm, regardless of the presence of TSA) compared to immature oocytes (0h: 102.6 µm). At the end of PM and IVM, most oocytes were still under meiosis blockage and chromatin compaction was evenly distributed in the GV phases ($P>0.05$). The rate of oocytes that reached MII ranged from 11.8% to 18.0%, with no difference between groups ($P>0.05$). The fact that the oocytes were unable to complete their growth may explain why they did not acquire meiotic competence. The transcriptional activity of oocytes in the GV0 phase at the end of PM was higher ($P<0.05$) in TSA10 group (32.4±1.7 AUF) compared to TSA5 (22.1±2.3 AUF), TSA2.5 (16.8±1.2 AUF) and control (20.3±2.9 AUF). We conclude that TSA at 10.0 nM was efficient to promote the increase in transcriptional activity when associated with NPPC during the PM of oocytes recovered from follicles <2 mm, however, these oocytes were not able to complete the growth and to acquire the meiotic competence.

Acknowledgements: CAPES and FAPESP (#19/11174-6).