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Effect of butafosfan in expression of genes associated oocyte quality

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Butafosfan is an organic phosphorus molecule that has been studied as a metabolic modulator. Phosphorus is fundamental for the growth, differentiation and cellular integrity, it acts in the processes of phosphorylation and dephosphorylation of proteins and cellular signals, as well as in the cycle ADP/ATP. Associated with cyanocobalamin, butafosfan had positive effects on cow folliculogenesis. In view of this, butafosfan becomes a viable alternative to improve oocyte metabolism and thus the acquisition of competence. The aim of this study was to evaluate the effect of butaphosphan addition in the maturation medium in expression of genes associated with apoptosis, cumulus cells expansion, resumption of meiosis and energy metabolism. Bovine ovaries were collected from a local slaughterhouse and transported to the laboratory in NaCl 0.9% solution with gentamicin 0.5% at 30 °C. Complex cumulus oocytes (COCs) were aspirated from follicles (3-8 mm in diameter) using a stereo and then, washed three times in washing medium (Animal Biotechnology®, Brasília, DF, Brazil). In total of 809 COCs (n = 809) were randomly assigned to groups of ± 60 COCs/group/routine as supplemented with butafosfan in IVM medium (GC: 0 mg/ml, T1: 0.05 mg/ml; 0.1 mg/ml and T3: 0.2 mg/ml butafosfan, Bayer Animal Health, São Paulo, SP, Brazil). The maturation occurred in 500 µL drops of MIV-TCM medium (Animal Biotechnology®) supplemented with 10% fetal bovine serum at 39 °C in 5% CO_2 atmosphere and at maximum humidity for 24 h. After the IVM, 15 COCs from each group were stripped through successive pipings, the rest of COCs continued in the PIVE routine for further analysis. Cumulus cells and oocytes were stored separately in microtubes containing 100 µL TRIzol (Invitrogen, Carlsbad, California, USA) at -70 °C until analysis of gene expression. In this way 3 routines were conducted. Total RNA was extracted from cumulus and oocyte cells using TRIzol and quantified on NanoVue spectrophotometer (General Electric Healthcare Limited, UK). The cDNA synthesis was performed using iScript Reverse Trascription Supermix (Bio-Rad, Hercules, California, USA) according to the manufacturer's instructions. Real-time PCR reactions were conducted on Applied Biosystems 7500 (Applied Biosystems, Foster City, USA) using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, USA). Expression of the genes: BAX and BCL2 as markers of apoptosis; AREG and EREG as genes related to the expansion of cumulus cells and resumption of meiosis; GDF9 and BMP15 as indicators of oocyte quality and GLUT1 and PFKP related to energy metabolism in oocytes. The results were analyzed using the 2-ΔΔCT method, using the H2A gene as internal control. Statistical analysis was performed in the SAS 9.0 program (SAS, Cary, NC, USA) using the General Linear Model test to determine the linear, quadratic or cubic effect of the supplementation with 0.0, 0.05, 0.1 and 0.2 mg/ml butafosfan in the maturation medium. The relative expression of the genes studied was similar between the groups in both oocytes and cumulus cells (P> 0.05). In conclusion, supplementation of the IVM medium with different doses of butafosfan does not improve oocyte quality.