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EFFECTS OF TRICHOSTATIN A, A CHROMATIN-MODIFYING AGENT, ON MEIOTIC PROGRESSION OF BOVINE OOCYTES AND EMBRYONIC DEVELOPMENT

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One important aspect that has been the subject of recent research is the role of epigenetics in oocyte competence acquisition. Numerous precisely timed and coordinated events must occur in nucleus and cytoplasm in order to achieve full meiotic and developmental competence by the oocyte. Histone acetylation is one of those epigenetic events that occurs in oocyte nucleus. Considering that trichostatin A (TSA), an inhibitor of histone deacetylases (HDACs), causes global hyperacetylation of histones, the aim of this study was to evaluate the possible benefits of optimized acetylation levels caused by this agent on bovine oocyte competence and on subsequent embryonic development. For this purpose, oocytes from ovaries of slaughtered animals were *in vitro* matured (IVM) in TCM 199 supplemented with 10% FBS and hormones, and treated with 0 (control), 5, 10, 15, 50 or 100 nM TSA for 28 h. In the first experiment, samples were collected at 20, 24 and 28 h IVM, and oocytes were stripped from *cumulus* cells with 2% hyaluronidase, stained with 10 µg/mL Hoechst 33342 for 15 min and evaluated regarding meiotic progression. In the second experiment, after 24 h IVM the oocytes were *in vitro* fertilized in TALP-IVF medium for approximately 20 h, and the presumptive zygotes were cultured in SOFaa supplemented with 0.6% BSA and 2.5% FBS at 38.5° C in 5% CO_2 in air atmosphere. All results were analyzed by chi-square (χ^2) or, when appropriate, by Fisher's exact test, in SAS v.8.2. In relation to the first experiment, after 20 h IVM we observed inhibitory effect of TSA on oocyte maturation rates (metaphase II with extrusion of the 1st polar body) at all concentrations, whereas the concentrations 5 nM (66/112 - 58.9%), 10 nM (64/100 - 64.0%) and 15 nM (61/99 - 61.6%) differed from the control (68/75 - 90.7%), 50 nM (36/93 - 38.7%) and 100 nM (36 / 82 to 43.9%) groups. Control group was superior to all other treatments, while no significant difference was observed between 50 and 100 nM TSA. No differences were detected in the subsequent evaluations (24 h and 28 h IVM), with maturation rates ranging from 62.2% to 91.5%. In the second experiment, results were similar ($p > 0.05$) among all groups regarding cleavage rates (76.9% to 89.1%), despite the lower numerical values presented by the 50 nM (277/360 - 76.9%) and 100 nM (209/268 - 78.0%) groups. However, blastocyst development was superior in the group treated with 5 nM TSA (118/279 - 42.3%) when compared to the other groups (control: 108/334 - 32.3%; 10 nM: 76/242 - 31.4%; 15 nM: 96/300 - 32.0%; 50 nM 80/301 - 26.6%, 100 nM: 50/212 - 23.6%). Damaging effects of TSA on development were observed when high concentrations were used (50 and 100 nM). We conclude that TSA slows meiotic progression in low concentrations but allows maturation rates similar to those obtained for the control group after 24 h IVM. This effect was shown to be beneficial to the embryo development in the concentration of 5 nM TSA. Further experiments to evaluate the effect of TSA on embryo quality are required, to better characterize the potential benefits caused by this agent during oocyte maturation.
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