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Effects of cAMP modulators during *in vitro* prematuration of bovine oocytes on gap junctional communication and embryo development potential

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In vitro oocyte maturation is fundamentally limited by the quality of the oocyte, namely by the intrinsic developmental competence of this structure. This study aimed to evaluate the effects of the addition of cAMP modulators during IVM of bovine oocytes in the SPOM system (simulated physiological oocyte maturation) by checking the status of communication between oocytes and cumulus cells through gap junctional communication (GJC), and early embryonic development from structures subjected to these modulators, seeking positive impact of this system on oocyte competence. Oocytes were *in vitro* matured in TCM 199 supplemented with 10% FBS, and subjected in treated groups to 100 µM or 150 µM forskolin and 750 µM IBMX for the first 2 h of culture. Subsequently, oocytes were transferred to base medium supplemented with 20 µM cilostamide. To evaluate the level of connection between the oocyte and the cumulus cells, it was measured the transfer of dye calcein AM for GJC, according to the protocol described by Thomas et al. (Biol. Reprod. 70, 548-556). The emission of intra-oocyte fluorescence was measured with a fluorescence microscope aid, and the images captured by camera and analyzed in ImageJ software. Oocytes from the same treatments were also submitted to IVF procedure in TALP medium and CIV in SOFaa with 6 mg/mL BSA and 2.5% FBS, evaluating early embryonic development. Analyses were performed in GraphPad INSTAT 6.01, and the fluorescence results were submitted to ANOVA and means were compared by Tukey test, and proportions of cleaved embryos and blastocysts evaluated by chi-square test (χ^2). IVM process caused a significant increase ($P < 0.05$) in the GJC fluorescence intensity in all groups, with the superior means in the groups treated with cAMP modulators. There was no difference between groups 100/750 and 150/750. In relation to preimplantation development, we found that the treatments affected negatively the cleavage rates (control - 585/677 (86.4)^a; 100/750 - 542/663 (81.7)^b; 150/750 - 558/688 (81.1)^b). When the proportion of blastocysts (D8) was calculated by the total number of oocytes, we verified no significant differences between the groups (35.2 to 37.9%). Nonetheless, when the proportions were calculated from the cleaved embryos, we observed superiority of groups 100/750 (53,3)^a and 150/750 (48,8%)^a in comparison to the control group (38.9%)^b. Thus, we conclude that the use of cAMP modulators during IVM causes inhibitory or retarding effect on the first cleavages; however, stimulatory effect in those structures which overcome the initial block phase. Moreover, cAMP modulators caused considerable increase of GJC during IVM.

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