A252 Embryology, developmental biology, and physiology of reproduction

Effect of *in vitro* co-culture of buffalo embryos with bovine cumulus cells on the potential for early embryonic development

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In vitro embryo production (IVEP) in buffaloes is a promising technique for the multiplication of genetic material from maternal origin, however, embryo production rates are still lower than those observed in cattle, probably due to the lower quality of buffalo oocytes (fragility of the zona pellucida and the cumulus cells). Thus, cell co-cultures are widely used during IVC of buffalo embryos, especially with cumulus cells from the IVM stage. The present study aimed to evaluate the possible benefits provided by the co-culture of buffalos embryos with bovine cumulus cells newly obtained in the IVM, believing in the contribution of these cells to the production of growth factors that, in turn, stimulate the initial embryonic development. After IVM for 22 h in TCM199 medium supplemented with 10% FBS, hormones, sodium pyruvate and antioxidants, buffalo oocytes were fertilized in Talp-IVF medium supplemented with 0.6% BSA for 24 h. Then the development culture was performed in modified SOF medium supplemented with 2.5% FBS and 6 mg/mL BSA, and the structures maintained at 38.5 °C and 5% CO_2 atmosphere in air during 7 days, when the blastocyst production rate was evaluated. Three replicates of IVEP were performed, totalling approximately 100 oocytes per group. Analyses were performed in the program GraphPad Prism 7, and the proportions of blastocysts were evaluated by Fisher's exact test. Although there was a higher average blastocyst production in the group co-cultured with bovine cells, there was no difference ($P>0.05$) between the groups (co-culture with buffalo cells - 17/106 - 20.0% , Co-culture with bovine cells - 22/110-16.0%). Attanasio et al. (Theriogenology, v.74, p.1504-1508, 2010) performed a study in which buffalo oocytes were cultured with bovine somatic cells after the vitrification process and observed that only those exposed to intact bovine cumulus oocytes (CCO) complexes presented the restoration of initial developmental capacity. It is possible that co-cultivation in these systems fails to mimic the association between oocytes and radiate corona cells provided by gap junctions. Thus, it is concluded that the use of buffalo embryo co-culture with bovine cumulus cells originated from IVM does not provide preimplantation development increments, and we recommend that the strategy of co-culture of buffalo embryos with intact bovine CCOs also be investigated.
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