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Development of bovine embryos *in vitro* in co-culture with mesenchymal stem cells and murine embryonic fibroblasts

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Mouse embryonic fibroblasts (MEFs) have been widely used as a feeder layer to support embryonic stem cells due to their capacity to release growth factors. Mesenchymal stem cells (MSCs) also release bioactive factors which support cell growth. This study aims to investigate the effect of co-culture of MSC from rat bone marrow or MEF as a feeder layer for *in vitro* production of bovine embryos. Oocytes from slaughterhouse were collected and matured (TCM 199 medium in incubator with a temperature of 38°C, 5% CO2 concentration and 95% relative humidity) in control condition (CTRL) or in co-culture with previously inactivated MSC or MEF with 10ug/mL of mitomycin C (Sigma-Aldrich). Fertilization was performed in CTRL condition for all groups, and the embryos were cultured from fourth day in CTRL, or in co-culture with inactivate MSC or MEF, thus the following groups were performed in IVM/IVF: (CTRL/CTRL) - maturation and embryonic culture in CTRL condition; (CTRL/MSC) - maturation in CTRL condition and embryonic culture with MSC; (CTRL/MEF) - maturation in CTRL and embryonic culture with MEF; (MSC/CTRL) - maturation with MSC and embryonic culture in CTRL condition; (MSC/MSC) - maturation and embryonic culture with MSC; (MEF/CTRL) - maturation with MEF and embryonic culture in CTRL condition and (MEF/MEF) - maturation and embryonic culture with MEF. Cell inactivation was performed using mitomycin C. The data was analyzed by chi-square test for oocytes and Kruskal-Wallis nonparametric with Dunn's post-test for embryos. No significant difference in oocytes metaphase II and apoptosis rates and in embryo cleavage rate at 4th day after the beginning of the in vitro culture was found among the oocytes matured in CTRL, MSC or MEF conditions. The rates of blastocyst formation, expanded, hatched and the total of blastocysts did not differ among experimental groups ($P > 0.05$) at 7th day of embryo development. At 8th day of embryo culture we observed a difference ($P < 0.05$) in hatched blastocyst rate which was higher in the CTRL/CTRL group (14.3±1.9%) when compared to MSC/MSC group (3.6±1.4%), however, the proportion of blastocyst, expanded and total blastocysts were not different ($P > 0.05$) among the groups. The number of cells in the inner cell mass, trophoblast cells, apoptotic cells and total cells were similar ($P > 0.05$) in the embryos cultivated at all experimental groups. We conclude that the co-culture in IVM or IVC with MSC or MEF did not affect the bovine embryos development.