Proceedings of the 28th Annual Meeting of the Brazilian Embryo Technology Society (SBTE), August 14 to 17th, 2014, Natal, RN, Brazil. Abstracts.

A183 Embriology, Biology of Development and Physiology of Reproduction

Optmization of in vitro development of mouse embryos using mesenchymal stem cells as feeder layers

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Keywords: co-culture, embryonic development, mesenchymal stem-cells.

Despite the advances in assisted reproduction techniques, the poor quality and failures in in vitro embryo development remain as a drawback resulting in low pregnancy rate. Bone marrow mesenchymal cells (MSCs) have emerged as a novel therapeutic option due to their unique properties of releasing bioactive factors and supporting others cell growth. In addition, murine embryonic fibroblasts (MEFs) have been widely used as a feeder layer to support embryonic stem cells due to their release of growth factors. In the present study we have compared the role of MSCs and MEFs in supporting C57Black6 mouse early embryo development. MSCs and MEFs were isolated from mice and cultured in DMEM-F12 with 10% fetal bovine serum up to the third passage. All the embryos were obtained in approximately 42 hours (2nd day) after mating and were randomly distributed in the following groups: CTRL - cultured in control culture medium; iMSC - co-cultured with MSCs inactivated to arrest proliferation; and iMEF - co-cultured with MEFs inactivated to arrest proliferation. Inactivation was performed using mitomycin C. Embryo development was evaluated daily for 5 days (7th day after mating). Immunocitochemistry, diameter and total cell number of blastocysts were measured at the 3rd day after culture. The statistical analysis was performed by non-parametric Kruskal-Wallis test with Dunns post-test and $p<0.05$ was considered as statistically significant. We observed at 2nd day after mating (day of embryo acquisition) the proportion of following development stages: 87.0% at 2-cell, 6.5% at 3-cell, 3.7% at 4-cell and 2.8% at 5-8-cell. After the 3rd day in culture, the embryos co-cultured with iMSC or iMEF showed a greater development when compared with the CTRL group. On the 5th day in culture the rate of hatched blastocysts in iMSC (84.1±5.8%) and iMEF (90.3±4.2%) groups were higher than CTRL group (49.2±8.8%). We did not observe any difference in the proliferation or apoptosis among the groups, however, the blastocysts co-cultured with iMSC presented a significant higher number of inner cell mass (26.1±1.6%) and a lower number of trophoblast cells (73.9±1.6%) when compared to the CTRL group (20.4±1.5% and 79.6±1.5%, respectively). The iMSC and iMEF groups presented a higher cell number (70.9±2.5 and 74.5±2.7, respectively) and diameter (133.7±2.53 and 139±2.3 µm, respectively) when compared to the CTRL group (cell number: 60.3±2.14; and diameter: 123.8±2 µm). In summary, our data suggests that co-culture with inactivated MSCs or MEFs greatly supports and improves the early embryonic development in vitro.

Supported by: FAPEMIG, FAPERJ, CNPq, CAPES and DECIT/MS.