Sertoli cell-mediated differentiation of bovine fetal mesenchymal stem cells into germ cell lineage using an in vitro co-culture system

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In vitro gamete derivation based on differentiation of germ cells (GC) from stem cells has emerged as a potential new strategy for the treatment of male infertility. This technology also has potential applications in animal reproduction as an alternative method for dissemination of elite animal genetics, production of transgenic animals, and conservation of endangered species. Mesenchymal stem cells (MSC) are multipotent progenitor cells defined by their ability to differentiate into mesodermal lineages. Under the effect of selected bioactive factors, MSC upregulate expression of pluripotent and GC specific-markers revealing their potential for GC differentiation. In addition to the effect of trophic factors, cell-to-cell interaction with Sertoli cells (SC) may be required to guide the sequential differentiation of MSC into GC. Thus, the aim of the present study was to investigate the effect of coculture with SC on the potential for in vitro GC differentiation of bovine fetal MSC (bfMSC) derived from bone marrow (BM-MSC) and adipose tissue (AT-MSC). bfMSC were isolated from male bovine fetuses and SC were collected from adult bull testes. The effect of SC interaction with BM-MSC or AT-MSC was analyzed on the expression of pluripotent factors OCT4 and NANOG, GC genes FRAGILLIS, STELLA and VASA and male GC markers DAZL, PIWIL2, STRA8 and SCP3 at Day 14 of coculture. Flow cytometry analyses detected that the majority (95,5% ± 2.5; $P < 0.05$)

of the isolated population of SC cultures were positive for SC-specific marker WT1. Levels of mRNA of WT1 in BM-MSC and AT-MSC were lower ($P < 0.05$) compared to SC; whereas, WT1 expression was not detected in bovine fetal fibroblasts (FB). Cocultures of BM-MSC and AT-MSC with SC had higher ($P < 0.05$) OCT4 mRNA levels compared to monocultures of BM-MSC, AT-MSC and SC. Moreover, cocultures of BM-MSC with SC had higher ($P < 0.05$) proportion of cells positive for Oct4 and Nanog compared to monocultures of BM-MSC and SC. Levels of mRNA of DAZL, PIWIL2 and SCP3 were upregulated in cocultures of AT-MSC with SC compared to monocultures of AT-MSC and SC. Accordingly, the proportion of cells positive for Dazl were higher ($P < 0.05$) in cocultures of AT-MSC with SC compared to monocultures of AT-MSC and SC. Changes in gene expression profiles during coculture of SC with AT-MSC suggest that cell-to-cell interaction or bioactive factors provided by SC may induce progression of AT-MSC into early stages of GC differentiation.