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OVINE BONE MARROW-DERIVED MULTIPOTENT MESENCHYMAL STEM CELLS: ISOLATION AND CRYOPRESERVATION

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Sheeps are often used as the preclinical large animal models when the therapeutic potential of multipotent mesenchymal stem cells (MMSCs) is tested. Optimization of the Ficoll density gradient extraction method harvested the cell culture with characteristics similar to the MMSCs. Protocols using specialized tube SepMate-15 were demonstrated to be more advantageous then classical Ficoll method. The harvested cells had a strong adhesion to the cul-

ture plastic, a fibroblast-like morphology, as well as the induction capability to the *in vitro* formation of the cells of fatty, bone and cartilaginous tissues. During the adipogenic differentiation on day 14, the adipocytes with lipid vesicles were formed, as detected by a specific dye Oil red O staining. On the day 14 of cultivation, the osteogenic medium revealed specific activity of the endogenous alkaline phosphatase. The staining of MMSCs by von Kossa found the presence of insoluble calcium salts in the intercellular space. The chondrogenic differentiation was detected after 14 days, accompanied by the appearance of multilayered aggregates with a large amount of the matrix which were visual of the isogenic groups similar to hyaline cartilage lacunae. After 21 days, the cells formed dense microgranules, which detected production of the glycosaminoglycan matrix. The comparative analysis of the three cryoprotective media characterized their effects on the cell viability. The derived cell culture was deposited in the Specialized Collection of somatic cell cultures of farm and commercial animals at the K.I. Skryabin and Ya.R. Kovalenko VIEV and can be used in preclinical studies.

Keywords: multipotent mesenchymal stem cells, bone marrow, ovine, isolation, cultivation, differentiation, cryopreservation