MULTILINEAGE DIFFERENTIATION OF EQUINE ADIPOSE TISSUE DERIVED PROGENITOR CELLS AFTER CRYOPRESERVATION.

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Introduction: In horses, stem cell therapies are a promising tool to the treatment of many injuries, which are common consequences of athletic endeavor, resulting in high morbidity and often compromising the performance. The aim of this study was to isolate progenitor cell from equine adipose tissue (eAT-PC), to evaluate their proliferative potential and to evaluate their capacity towards osteogenic, chondrogenic, adipogenic and neurogenic differentiation before and after cryopreservation.

Isolation and Therapeutic Potential

Morphology of eAT-PC.

Figure 1. Morphology of eAT-PC: (A) Representative image of eAT-PC just after isolation. (B) Monolayer of rapidly expanding fibroblast-like cells before cryopreservation, at early passage (P2). (C) Monolayer of rapidly expanding fibroblast-like cells after cryopreservation at late passage (P20). Phase Contrast. Scale Bars: A=20µm; B, C=50µm.

Proliferating Potential Rate of eAT-PC

Figure 3. Representative graphic showing proliferating potential of eAT-PC cells during 63 days (23 passages) was built up using calculations based on Neubauer-counting chamber. After thawing, significant decline of eAT-PC proliferation was observed at day 30 (P30), while those, which were not submitted to cryopreservation, presented it later at day 39 (P15). Each black bar (l) in all points of the graphic represent the standard deviation.

Osteogenic Differentiation

Figure 4. Representative image of adipogenic differentiation induced in eAT-PC before (A-C) and after (D-F) cryopreservation. Oil Red O staining showed accumulation of lipid-filled droplets (arrows) within the differentiated eAT-PC cultured in adipogenic medium during 4 (A) and 7 (B) days. Inset in (B) shows details of Oil Red O staining. (C) Control culture, 7 days after growing in basal culture medium. No Oil Red O staining has been observed. (D-E) Same as in (A,B) observed after cryopreservation. (F) Same as in (C) after thawing. A-F = 20µm

Chondrogenic Differentiation

Figure 5. Representative image of chondrogenic differentiation induced with TGFβ1 (A-D). Positive Toluidine blue staining observed in pellet culture of eAT-PC before (A) and after (B) cryopreservation. Positive immunostaining (red) with anti-chondroitin sulphate proteoglycan (aggrecan) (C) and anti-collagen Type II (D) antibodies (both after cryopreservation). Insets in C and D, monolayer culture of eAT-PC used as a control did not present any fluorescent signal. Nuclei stained with DAPI (blue). C, D and Insets =10µm.

Neuronal Differentiation

Figure 6. Representative image of Neuronal Differentiation (A-D). (A-B) Experimental culture four days after induction (C-D): Immunocytochemistry by confocal microscopy analysis with specific antibody against β-tubulin III-FITC. We do not observe the expression of β-tubulin III in undifferentiated cells (D). Nuclei stained by DAPI in blue. A-D = 20µm

Conclusion: Our data strongly suggest that the cells successfully isolated and expanded in vitro from equine adipose tissue are a promising candidate for cell therapy, which can be used to the treatment of several in equine, at least those, which need mesodermal derivates for the repairing process. We also demonstrated that eAT-PC showed high proliferative rate and capacity to differentiate into derivatives of mesoderm and ectoderm. These characteristics of eAT-PC can be maintained even after cryopreservation. These findings will also provide the significant biological, technical and procedural knowledge for further studies towards technological advance in stem cells applications on horse health.

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