Expression of OCT-4 and Nanog in mesenchymal stem cells from the perivascular portion of matrix of equine umbilical cord

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Abstract

Umbilical cord intervascular stroma is composed of a mesenchymal connective tissue know as Wharton's jelly (WJ). The WJ and umbilical cord blood are sources of primitive stem cells and can be collected, stored and used for therapeutic uses or biotechnology. The objective of this study was to evaluate the effect of the cryopreservation process at controlled freezing system on immunophenotypic profile (CD44, CD90, CD34, MHCII and Oct-4) and cromossomic stability of mesenchymal stem cells from intervascular matrix of equine umbilical cord (MSC-UCMI) using different cryopreservation medium.

Samples of umbilical cord (n=5) were collected at delivery, processed and cultured in DMEM high glucose supplemented with 20% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1.2% amphotericin B at 37.5 ºC in a humidified atmosphere, containing 5% CO_2 in air. At the end of second passage, MSCs were trypsinized and submitted to immunophenotypic analysis by flow cytometry (FC) for the markers CD44, CD90, MHCII and CD34, conjugated to fluorescein isothiocyanate and for Oct-4 conjugated with alexa fluor 488. Furthermore, MSCs-UCMI were subjected to osteogenic (n=5) and adipogenic (n=4) differentiation assay and karyotyping. MSC were cryopreserved in a controlled freezing system using Mr Frosty (-1 ºC/minute until -80 ºC, 24 hours, Nalgene®) with the following medium: Medium 1 (M1): DMEM high glucose, with 20% FBS and 10% DMSO; Medium 2 (M2) (free from FBS): DMEM high glucose, with 10% PVA and 10% DMSO; Medium 3 (M3): 90% FBS and 10% DMSO; Medium 4 (M4): 90% conditioned medium (DMEM high glucose + 20% FBS) and 10% DMSO. After three months of storage in liquid nitrogen, samples from M1, M2, M3 and M4 were thawed, analyzed on FC and cultured for karyotype and differentiation assay. Data regarding immunophenotypic analysis before and after the cryopreservation with M1, M2, M3 and M4 were analyzed and compared using the tests Kruskal-Wallis One Way analysis of variance on ranks and All Pairwise Multiple Comparison Procedures (Dunn's Method), taking $P< 0.05$ as significant. The samples were differentiated into osteogenic and adipogenic lineages. After thawing M2 group showed exceptional low viability, samples were not used for karyotype analysis and differentiation assay. Changes on immunophenotypic profile were not detected ($P>0,05$) for none of studied markers independently of cryopreservation medium. Additionally, cryopreserveded MSCs-UCMI with M1, M3 and M4 medium showed cromossomic stability after cryopreservation presenting normal karyotype (2n=64) for equine specie without aneuploidy. The

process of cryopreservation on controlled freezing system does not promote changes on immunophenotypic profile and cromossomic stability of MSC-UCMI. However, the presence of FBS seems to be important for cryosurvival of the cells.

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