De Schauwer Catharina, J Cell Sci Ther 2011, 2:4
doi: http://dx.doi.org/10.4172/2157-7013.S1.16

OMICS Group Conferences
Accelerating Scientific Discovery

International Conference & Exhibition on
Cell Science & Stem Cell Research

29 Nov - 1 Dec 2011 Philadelphia Airport Marriott, USA

Cultivation of mesenchymal stromal cells from cryopreserved mononuclear cells isolated from equine umbilical cord blood

De Schauwer Catharina[1], Evelyne Meyer[2], Gerlinde R. Van de Walle[3], Guy Wouters[4] and Ann Van Soom[1]

[1]Department of Reproduction, Obstetrics and Herd Health, Ghent University, Belgium
[2]Laboratory of Biochemistry, Ghent University, Belgium
[3]Department of Comparative Physiology and Biometrics, Ghent University, Belgium

The therapeutic potential of mesenchymal stromal cells (MSC) for cellular therapy has generated an increasing interest in this type of research. In human, as well as in veterinary medicine, considerable research has been performed on the cryopreservation of expanded MSC, but little information is available on the possibility to cryopreserve the original mononuclear cell fraction. The present study describes a protocol to successfully expand equine MSC after cryopreserving the mononuclear cell fraction of umbilical cord blood (UCB). To this end, the mononuclear cell fraction was isolated from 5 UCB samples using a Percoll gradient and cryopreserved in standard 1.8ml cryotubes at a concentration of 1-2×10^6 cells per ml cold freezing solution. Cells were kept frozen for a minimum of three weeks before thawing. Frozen cryotubes were thawed au bain marie at 37°C. Cell viability after thawing varied around 98%. Approximately 4 × 10^6 cells were seeded in a T25 culture flask in culture medium containing low-glucose DMEM, 30% FCS, 10^{-7} M low dexamethazone, 50 µg/mL gentamycine, 10 µl/ml antibiotic antimycotic solution, 250 ng/mL fungizone and 2 mM ultraglutamine. In 4 out of 5 samples, adherent spindle-shaped cell colonies occurred within 9.8 ± 3.2 days and 80% confluency was reached after 16.3 ± 2.2 days of incubation at 38.5°C and 5% CO_2. After three passages, undifferentiated MSC were immunophenotyped using multi-color flow cytometry. In conclusion, equine MSC can be cultured successfully after cryopreservation of the isolated mononuclear cell fraction, an approach that is time- as well as cost-efficient.