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Efficiency in production equine induced pluripotency cells (IPS) using fibroblasts and umbilical cord mesenchymal stem cells

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Introduction: An alternative to creating banks of pluripotent stem cells involves reprogramming the somatic cell nucleus. This induced reprogramming results from the action of different molecules, including transcription factors and other proteins. Recently, several groups have reported the induction of pluripotency in human fibroblasts through the transduction with viral vectors expressing *OCT4, C-MYC, KLF4* and *SOX2* genes. The cells called induced pluripotent stem cells (iPS) have a morphology characteristic of embryonic stem cells, express pluripotent markers and are capable to differentiate in tissues from the three germ layers *in vitro* and *in vivo*. Thus, the generation of iPS cells from the reprograming of different cell types by specific factors, could represent an alternative to obtaining a bank of stem cells with pluripotent characteristics.

Objective: Equine fibroblasts and umbilical cord cells (EUCC) were genetically modified using lentiviral vectors containing STEMCCA excitable polycistronic human cDNA of OCT4, SOX2, c-MYC and KLF4 (EF1a-hSTEMCCA) in order to transform unipotent and multipotent cells in pluripotent cells.

Materials and Methods: EUCC and fibroblasts were plated at a concentration of $5x10^3$ cells per well of 9.6 cm^2 and cultured in DMEM high glucose with 20% FBS, 1% penicillin/streptomycin and 1.2% amphotericin. When cultures reached approximately 60% confluence they were transducted in a medium containing 50 mL of viral concentrate plus 8 ηg/ml polybrene (hexadimethrine bromide, Sigma). The medium was renewed after 14 hours of incubation. Five days post-transduction, the cells were transferred into MEFs. During reprogramming period cell were cultured in a specific medium for iPS. Between days 6 and 15 post-transduction we evaluate the time to the beginning of the appearance of colonies, colony morphology and number of colonies (greater than 1 mm diameter) formed per well.

Results: In fibroblasts cultures the colonies began to form on day 7 and in EUCC´ cultures on day 6. Both cultures developed thin colonies, with well-defined edges, formed by small cells of uniform size and hexagonal appearance, with evident nucleus containing one or two nucleoli. In each well 18 colonies were formed, on average, independently of the cell type. The cultures remained viable and colonies multiply in the course of 15 days.

Conclusion: Lentiviral vectors were efficient to deliver the transcription factors to cells, formed colonies showed morphological characteristics similar to embryonic stem cells between 6 and 7 days post-transduction. Future experiments will be conducted for immuno genotypic characterization of these cells.

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