The expression of pluripotency genes is affected by the exposure to hypoxic conditions during the generation of equine induced pluripotent stem cells

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The objective of this study was to determine whether hypoxic (5% O_2) culture conditions could improve the efficiency of derivation and maintenance of induced pluripotent stem (iPS) cell lines from equine fetal fibroblasts (50-day fetus). A *piggyBac* system was used, as described previously (1) to induce pluripotency. Briefly, plasmids PB-TET-MKOS, PB-CAG-rtTA, PB-CAG-GFP and pCyL43 (2) were used to transfect cells using an electroporation system (Neon Transfection System, ThermoFischer Scientific). After transfection, cells were transferred to an incubator at 37°C and 5% CO_2 either in 5 % O_2 (in a chamber filled with a gas mixture of 90%N_2, 5%CO_2 and 5% O_2) or 20% O_2 (atmospheric oxygen). Cell culture media was composed of DMEM/F12 Knockout (ThermoFisher Scientific #12660-012 Waltham, USA), 20% Knockout serum replacement (ThermoFisher Scientific #10828010 Waltham, USA), 2mM GlutaMax (#35050061, ThermoFisher Scientific Waltham, USA) 0,1mM Non-essential amino acids (ThermoFisher Scientific #11140050 Waltham, USA), 0,1mM β-mercaptoetanol (SigmaAldrich # M6250 St. Louis, USA), 1% Penicilin/Streptomicyn (ThermoFisher Scientific #15140163 Waltham, USA) 1000U/ml LIF (Millipore #ESGRO Burlington, USA), 10ng/ml bFGF (Peprotech #100-18B Rocky Hill, USA), 1,5 µg/ml doxycycline (SigmaAldrich #D9891 St. Louis, USA), 3µM GSK inhibitor (StemGent #CHIR99021, Cambridge, USA), 0.5µM MEK inhibitor (StemGent #PD0325901 Cambridge, USA), 2.5µM ALK/TGF inhibitor (StemGent #A83-01 Cambridge, USA) and 1 µM Thiazovivin (StemGent #Thiazovivin Cambridge, USA). After formation of the colonies an alkaline phosphatase staining was performed (Sigma Aldrich #86R-1Kt St. Louis, USA), followed by the assessment of *OCT4*, *SOX2*, *NANOG* and *REX*-1 endogenous gene expression using equine-specific RT-PCR primers. The statistical significance between two groups was determined using the unpaired t-test (GraphPad Prism). Differences were considered significant when $P<0.05$. iPS cells were tested for embryoid body formation in adhesion-free culture conditions. Both groups cultured in 5% and 20% O_2 formed iPS-like cell colonies (24 and 29 colonies in 5% and 20% O_2, respectively, being the reprogramming efficiency 0,0232% in high oxygen and 0,0192% in low oxygen). Both colonies from 5% and 20% O_2 were positive for alkaline phosphatase staining at passage 5. Expression of endogenous *OCT4* was higher in colonies cultured in 5% O_2 (1,079 ± 0,06235, n=9) when compared to the 20% O_2 group (0,92 ± 0,03629, n=9) (P=0,0426). SOX2 expression, however, was lower in 5% O_2 (1,087 ± 0,1059, n=9) than 20% O_2 (1,76 ± 0,1249, n=9) (p=0,0008). No difference was observed for *NANOG* or *REX-1* expression. iPS colonies derived at both 5% and 20% O_2 were equally capable of forming embryoid bodies. Therefore, hypoxic culture conditions seem to have a positive effect on increasing the expression levels of *OCT4*, a key pluripotency marker. However, more studies are necessary to examine the epigenetic mechanisms by which low oxygen levels affect the expression of pluripotency genes.

1. Nagy, K. et al., 2011. Induced pluripotent stem cell lines derived from equine fibroblasts. Stem Cell Reviews and Reports, 7(3), pp.546-546.

2. Wang, W. et al., 2008. Chromosomal transposition of PiggyBac in mouse embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America, 105(27), pp.9290-9295.

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