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THE EFFECT OF HYPOXIA ON HUMAN AMNIOTIC FLUID STEM CELL CULTURE: DEVELOPING A GMP-GRADE STEM CELL SOURCE FOR THE TREATMENT OF NEC

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Aim of the Study: We aim to evaluate the effect of hypoxia on human Amniotic Fluid Stem Cells (hAFSC) grown under GMP conditions as a high-yield source for cell therapy towards the treatment of necrotizing enterocolitis. Amniotic fluid stem cell niche exhibit low oxygen concentration within the amniotic sac (~3.5% - 6% O_2), while similar conditions exist in vivo with the pO_2 values of inflamed intestine in NEC. hAFSCs represent a treatment modality with promising therapeutic potential due to their proliferation and differentiation capacities as well as their ability to release protective factors (paracrine effects) and could thus be administered through enteral delivery to mobilize areas of injury in the mucosa of the gastrointestinal tract and increase enteric function.

Methods: We compared the characteristics of 2nd trimester (Gestational Age, GA :15-27weeks (w)) hAFSC samples cultured in standard Chang C media and in xeno-free Chang D media that complies with biobanking regulations, both in hypoxic (5% O_2) and normoxic (21% O_2) conditions, in terms of growth potential, generation doubling time, cell cycle analysis, viability rates, and determine the influence in cell behaviour and morphology.

Main Results: The generation doubling time was significantly shorter in hypoxia ([C:36±5.8 hrs vs. D:36± 6.1 hrs] in normoxia versus [C:29±5.1 hrs vs. D:20± 1.4 hrs]in hypoxia) allowing us to yield higher numbers of cells at an earlier passage. hAFSC in 5% O_2 showed a significant increase of the cell population arrested in S/G2/M cycle in comparison to the normoxic ($p=0.01$). hAFSCs in hypoxia showed increased chondrogenic and osteogenic capacity with upregulated NOTCH target genes.

Conclusion: In this study we have observed that the hypoxic conditions significantly increase the hAFSC cell yield in numbers compatible for clinical translation, and enhance the phenotypical and functional characteristics of hAFSCs rendering them useful for regenerative therapies in vivo.