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Human amniotic fluid stem cells from third trimester cultured under hypoxia and endothelial differentiation: *In vitro* and *in vivo* study

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Human amniotic fluid stem (hAFS) cells represent an attractive cell source in regenerative medicine since their ability to differentiate in different cell types. In particular, in this work we aimed at further investigating this potential both *in vitro* and in vivo, in relation to gestation trimester and culture oxygen condition. Human amniotic fluids were collected during routine amniocentesis (2nd trimester) and eligible caesarean sections (3rd trimester), after informed consent. Fresh cells were plated, later adherent cells were positively selected for CD117 and resulting hAFS cells were further expanded both in normoxia (20% O_2) and hypoxia (5% O_2). Human AFS cells from both gestation trimesters cultured in normoxia and hypoxia did not shown significant differences in surface marker expression and cell cycle stages (flow cytometry analysis), but the proliferation rate was significantly higher at 5% O_2 for both 2nd and 3rd trimester. Independently from trimester and oxygen pressure, hAFS cells displayed a clear endothelial capacity both *in vitro*, and *in vivo*, after Matrigel plug assay and local injection in a mouse model of acute ischemia, where hAFS cells were able to rescue the blood flow. Interestingly, hAFS cells expressed even before endothelial induction the ETS factors ETV2, FLI1 and ERG1, which represent three master regulators of the endothelial molecular program. The endothelial dysfunction represent one of the major health problem since reduced vasodilation start a protrombic state associated with diabetes, renal and heart failure. Here we reported the clear endothelial regenerative potential of hAFS cells, in particular regarding cells obtained from the 3rd trimester of gestation and cultured at 5% O , conditions that could ensure an easier recovery and a faster expansion before therapeutic application.

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