

## Amniotic Fluid Stem Cells cardiomyogenic potential: a preliminary study

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The characterization of Amniotic Fluid-derived multipotent Stem Cells (AFSCs) open new paths in stem cell research. hAFSCs have characteristics intermediate between pluripotent embryonic- (ESCs) and lineage-restricted adult stem cells, and are non-tumorigenic and low immunogenic. Moreover, they are obtained without destroying human embryos, so that most of the ethical and social controversy could be prevented.

We previously observed that human AFSCs express some genes specific of ESCs and primordial germ cells. We also shown hAFSCs ability to form *in vitro* three-dimensional aggregates of cells known as embryoid bodies (EBs), that express three germ layer markers.

Recent studies reported the ability of hAFSCs to differentiate *in vitro* into adipocytes and osteocytes. Aim of our study was to analyse the cardiomyogenic potential of hAFSCs. EBs were obtained by modified hanging drops protocol from hAFSCs cultured in presence of ascorbic acid and 5-aza-2'-deoxycytidine (differentiation medium: DM). RT-PCR and Western Blotting analysis conducted on AFSCs and EBs cells evidenced the gene and protein expression of the transcription factor Nkx2.5, the earliest marker of heart precursor cells. Immunofluorescence (IF) analysis performed on EBs after 10 days in DM evidenced the cytoplasmic presence of  $\alpha$ -myosin heavy chain ( $\alpha$ -MHC) organized in parallel, oriented filaments. Microscopical analysis evidenced beating cells mainly at the periphery of the EB. In conclusion, our results evidenced that hAFSCs cultured in permissive conditions give rise to EB able to terminally differentiate in cardiomyocytes.

Keywords: hAFSCs, EBs, cardiomyocytic differentiation.