

## Amniotic fluid mesenchymal stem cells isolation, characterization and cryopreservation.

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### ABSTRACT

Amniotic fluid draws great interest as a source of mesenchymal stem cells (AF-MSCs) for regenerative medicine. Herein we present a study aimed to characterize AF-MSCs. We analysed the chromosome stability after *in vitro* culture on 7 native AF samples. Afterwards on 10 morphologically homogeneous clones we evaluated i) the correlation between cell morphology and multipotent potential by investigating their time of doubling, expression of MSC markers and differentiation potential; ii) the microsatellite instability. Finally, we tested the yield of cell growth after cryopreservation on 40 native AF samples. Karyotype and microsatellite instabilities were excluded until the 5<sup>th</sup> and 18<sup>th</sup> culture passages, respectively. The 10 homogeneous clones displayed the following morphologies: 2 were fibroblastoid (F), 6 of amniotic fluid-type (AF) and 2 were epithelioid (E). The two F clones and 2/6 AF ones demonstrated the immunophenotype, the cell growth profile and the differential potential typical of the MSCs. Finally, the cryopreservation did not adversely affect the viability and cell growth of AF cells. In conclusion, our findings indicate that: i) in addition to F-type also the AF-type cells may display a MSCs profile; ii) the integrity of the genome is preserved during *in vitro* cell culture: this is an important parameter for a therapeutic use of AF-MSCs; iii) cryopreservation of native AF cells do not jeopardize the cell viability. The storage of native samples without laboratory procedure/manipulation (i.e.: the culture on growth media with unknown composition) is the advisable solution to provide AF-MSCs source for future applicative therapeutic protocols on humans.