

V.D. Patianna, B. Okere, P. Bruzzi, B. Predieri, L. Iughetti
 Department of Maternal-Infant and Adult Medical and Surgical Sciences, Modena, Italy

BACKGROUND

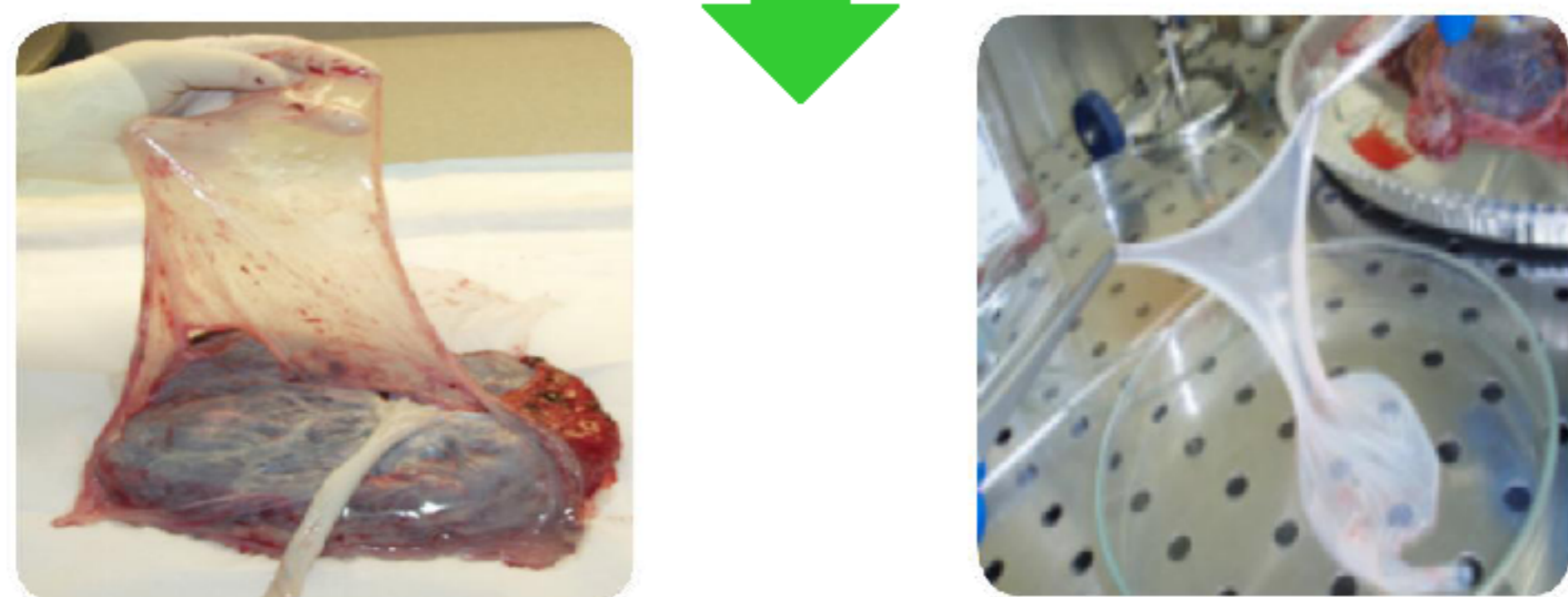
- ✓ Placenta tissue hold great promise as a source of cells for regenerative medicine due to its plasticity and easy availability.
- ✓ Amniotic Mesenchymal Stem Cells (AMSC) represent a potentially unlimited source of functional pancreatic endocrine lineage cells, used to replenish the islet mass in diabetic patients.

OBJECTIVE AND HYPOTHESES

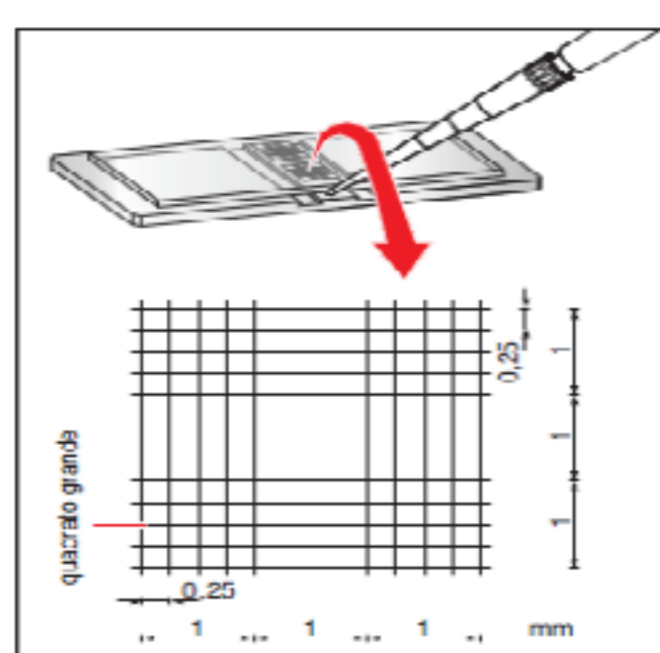
The aim of our study is to culture AMSC in serum-free condition preserving their phenotypic traits. These cultures could differentiate into pancreatic lineage on exposure to lineage-specific cocktails of growth factors.

METHODS

1. Different placenta samples of segment cesarean section deliveries of full term pregnancies were collected.



2. AMSC were isolated and cultured in serum-free optimized media with Human Platelet Lysate (HPL).
3. Cell growth was analyzed by direct cell count to determine the log, lag and stationary phases.

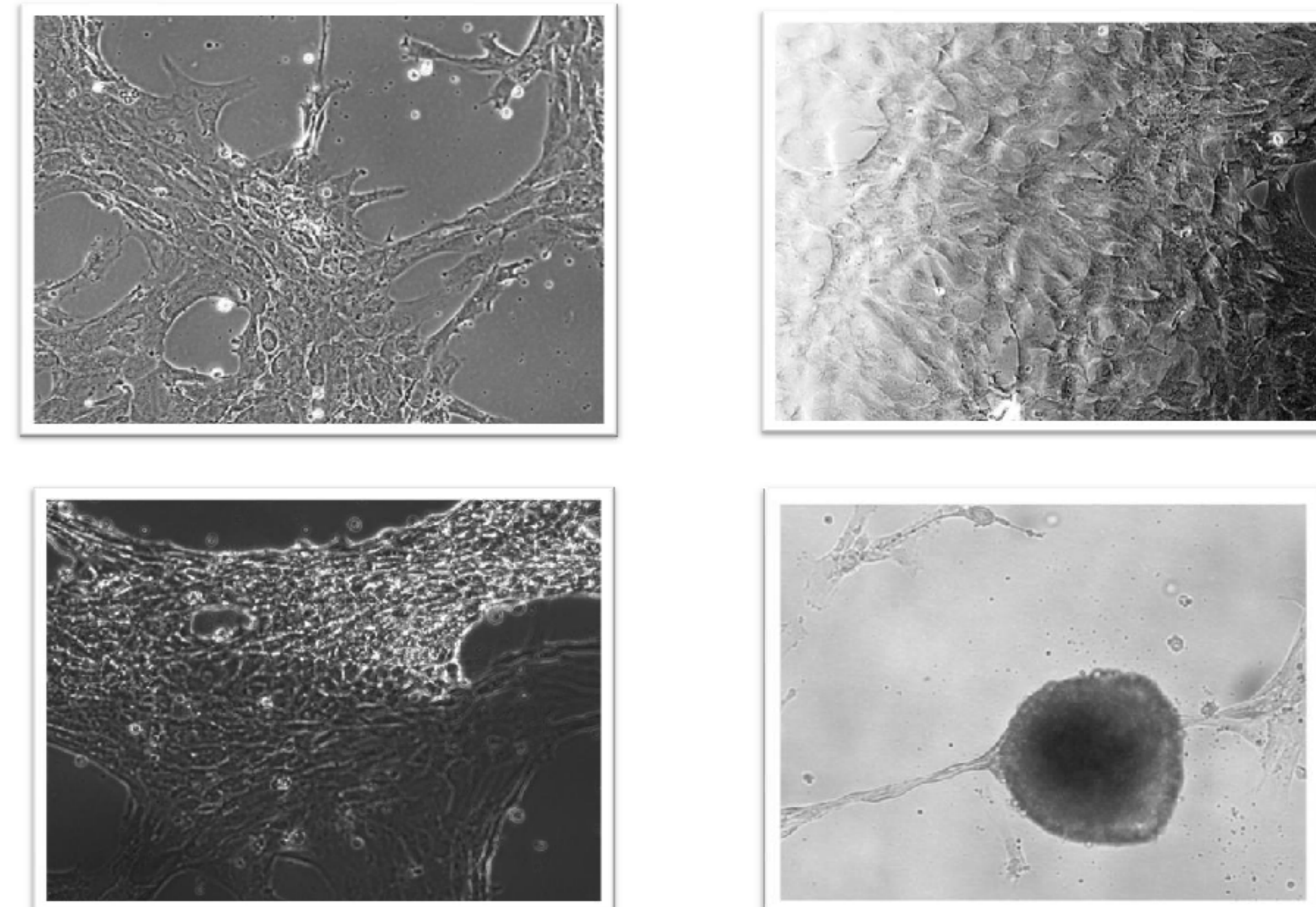


4. Differentiation was carried out in three stages and nicotinamide, taurine and retinoic acid were added to each medium.

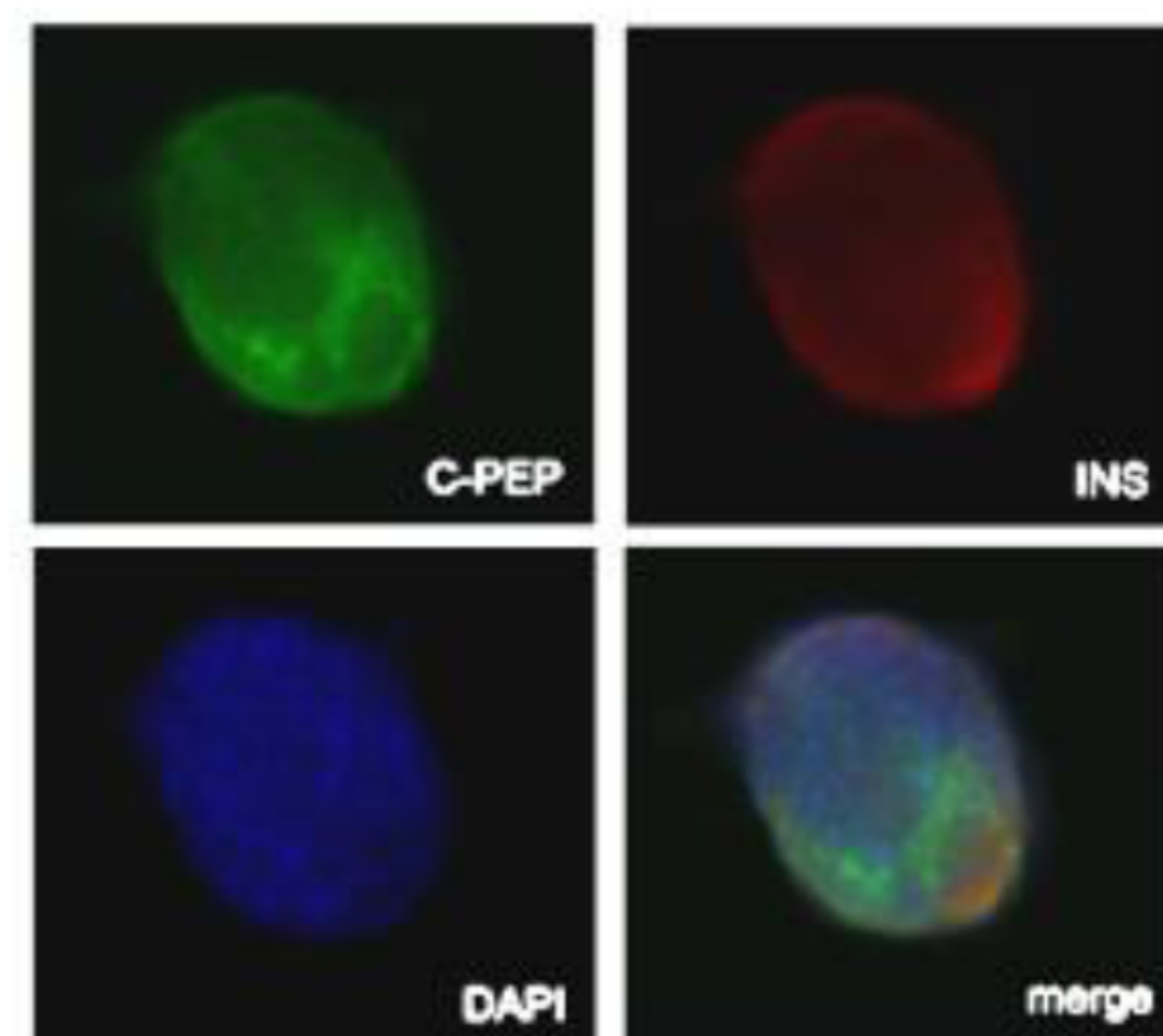
Differentiation Day	Medium composition	Cell structure
2	α MEM + 5% PLP	Adhered cells
0 (SFM-i)	α MEM + 1% BSA + 1xITS	Single cells (after trypsinization)
3 (SFM-ii)	α MEM + 1% BSA + 1xITS + 0.3 mM taurine	Loose cell clusters
7 (SFM-iii)	α MEM + 1% BSA + 1xITS + 3 mM taurine + retinoic acid + nicotinamide	Floating cell clusters
10 (SFM-iii)	α MEM + 1% BSA + 1xITS + 3 mM taurine + retinoic acid + nicotinamide	Pancreatic hormone producing ILCs

Legend: **SFM:** serum-free medium; **α MEM:** alpha minimal essential medium; **BSA:** Bovine serum albumin; **ITS:** insulin-transferrin-selenium; **ILCs:** islet-like cluster.

5. Pancreatic markers expression was assessed with fluorescence-activated cell sorting (FACS) analysis.



Isolation and expansion of AMSC



Differentiation of AMSC to pancreatic lineage

RESULTS

- ✓ Serum-free media sustained AMSC growth. Cell colonies from placental tissue began to appear after 4 days of cells isolation.
- ✓ The induction of AMSC has microscopically shown progressive cell clustering from day 2 and this led to a spheroidal structure similar to a typical Islet-Like cell formation at the end of day 10.
- ✓ Preliminary pancreatic induction assessed with FACS revealed expression of insulin and c-peptide.

CONCLUSIONS

The present study shows that placenta-derived mesenchymal cells can be isolated and expanded in medium supplemented with HPL.

Due to the easy accessibility, lack of ethical concerns and abundant availability AMSC might be an attractive, alternative source of progenitor/stem cells for basic or translational research and a reliable source of insulin producing cells in clinical applications.