Isolation and characterization of bovine mesenchymal stem cells derived from peripheral blood and endometrium.

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INTRODUCTION

Mesenchymal stem cells have the ability to migrate to damaged, inflamed and tumor tissues where they proliferate and exert their immunomodulatory properties through the release of cytokines and exosomes. Considering that pregnancy is a proinflammatory condition, we suggest that bovine endometrial mesenchymal stem cells (eMSCs) may contribute to uterine regeneration regulating embryo implantation and pregnancy in cows.

METHODS

We isolated and immortalized (using retroviral vector LXSN-16E6E7 to prevent proliferative arrest), eight eMSCs lines from the uterus of heifers at different estrous cycle stages: (i) one from early luteal phase (ii) four from late luteal phase and (iii) three from follicular phase. In addition, a total of five bovine pbMSCs were isolated from male calves, and maintained during more than twenty passages: two control cell lines (cpbMSC) and three cell lines obtained after mobilization from bone marrow by granulocyte colony-stimulating factor (G-CSF) treatment (bmpbMSC). eMSCs and pbMSCs were characterized to comply the following MSCs criteria: (i) plastic-adhesion and (ii) expression of mesenchymal CD44 and embryonic Pou5F1 markers and lack of CD34 and MHC-II. For a deeper characterization, we also analyzed (iv) Cytokeratin and Vimentin expression by Immunocytochemistry and Flow Cytometry (v) proliferation rate and (v) their ability to migrate in vitro by agarose spot assay.

RESULTS

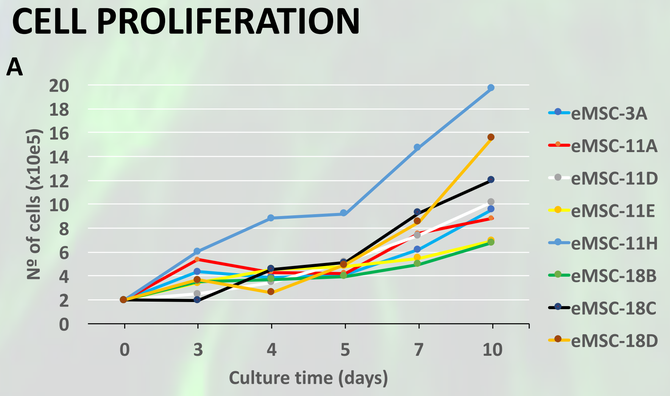
All lines expressed vimentin but not the epithelial marker Cytokeratin, with the exceptions of eMSC-11H from late luteal phase and eMSC-18C from follicular phase, that expressed high levels of both markers. bmpbMSC-81 and eMSC-18D were the lines had a higher growth rate with an average population-doubling time of 2.60±0.75 and 2.60±0.56 days respectively. eMSC-18C showed a significantly higher migration rate than the rest of either pbMSC or eMSC lines with 1007.89±38.26 µm/day 7; being bmpbMSC-84 significantly the most migratory pbMSC line with 606.27±97.62 µm/day.

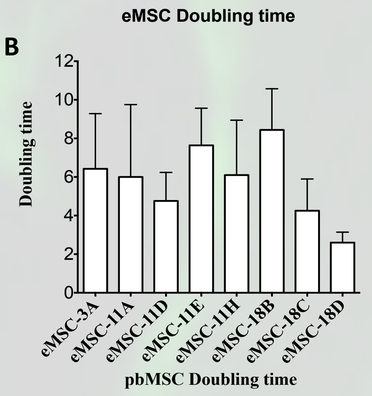
CELL LINES ESTABLISHMENT

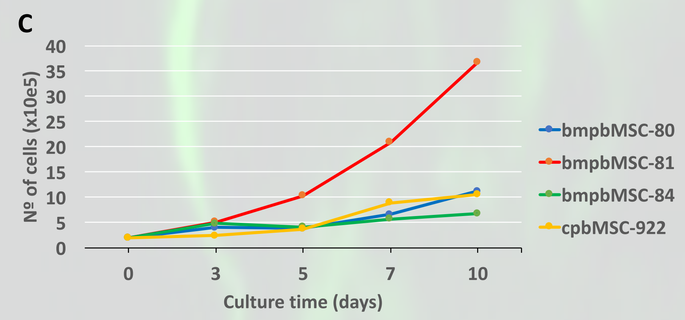
Cell lines isolated from primary cultures					
Bovine estrous cycle stages	Early luteal	Medium luteal	Late luteal	Follicular	Total
Bovine uterine stromal mesenchymal stem cells (BUSC).	3	4	6	6	19
Immortal cell lines. Retroviral vector PA-317 LXSN 16E6E7					
Endometrial Mesenchymal stem cells (eMSCs)	1	-	4	3	8
Cell lines	**3A**		**11A 11D 11E 11H**	**18B 18C 18D**	

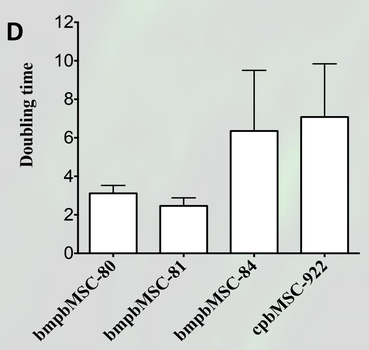
Granulocyte colony-stimulating factor: Bone marrow source tissue.	**Control group**
Bone marrow peripheral blood mesenchymal stem cells (bmpbMSC)	Peripheral blood mesenchymal stem cells (cpbMSC)
L80, L81, L84	**L922, L923**

CELL PROLIFERATION



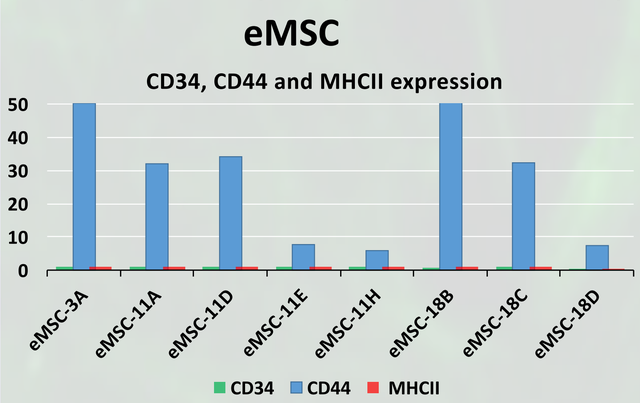


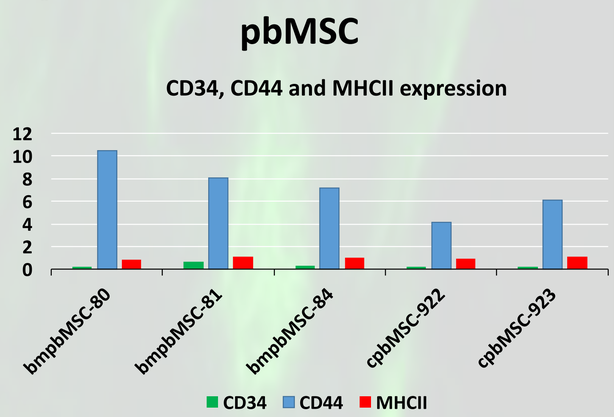


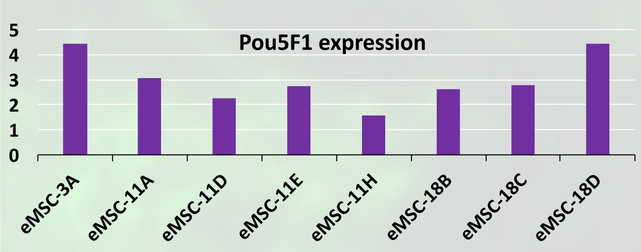


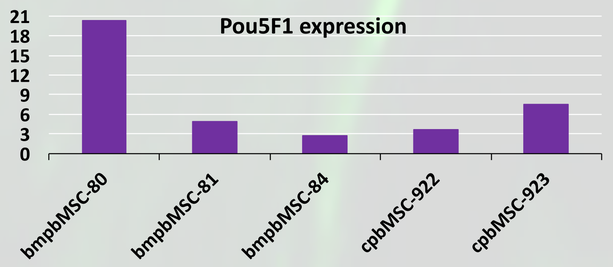
FLOW CYTOMETRY ANALYSIS

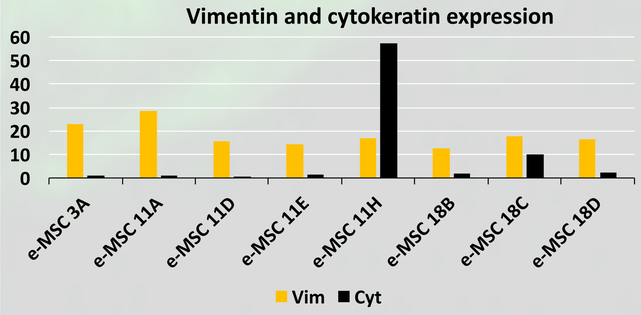
Flow cytometer results: FL1 protein expression median value divided by FL1 negative control median value.

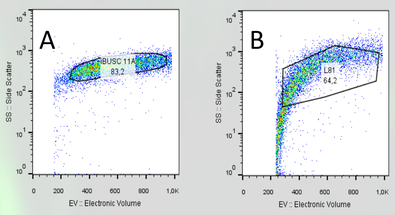






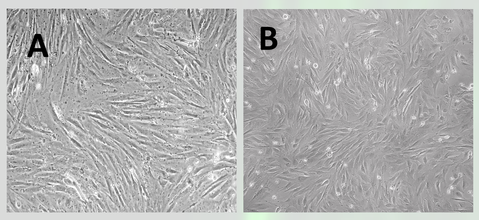






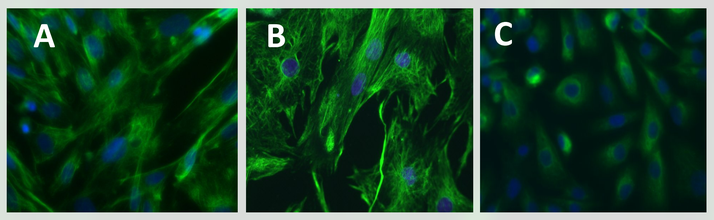
Scatterplot of eMSC population (A) and pbMSC population (B)

LIGHT MICROSCOPY



Representative images of eMSC (A: eMSC-11A) and pbMSC (B: bmpbMSC-84) showing fibroblast-like adherent cells with homogeneous morphology (100x).

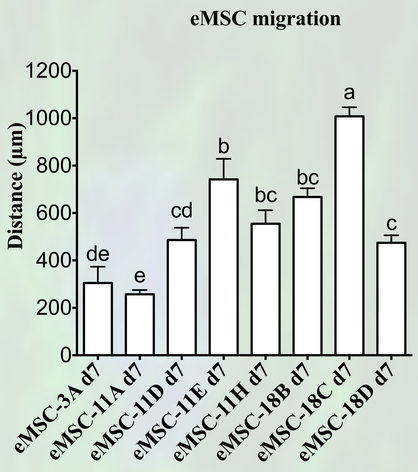
IMMUNOCYTOCHEMICAL ANALYSIS

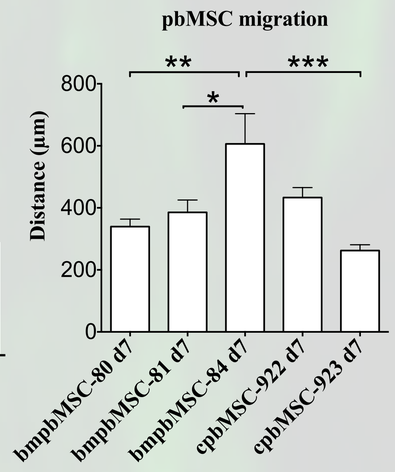


Representative images of eMSC (A: eMSC-18D) and pbMSC (B: cpbMSC-922) showing vimentin expression and eMSC-11H (C) positive for cytokeratin expression (200x).

CELL MIGRATION

Image of mitomycin-C treated bovine MSC migrating under an agarose spot. Cells remain attached while crawling under spots. Agarose is apparent in the lower left side of the image.





CONCLUSIONS

In the present work, the isolation of bovine pbMSC is shown for the first time. Bovine pbMSC and eMSC show characteristics of human MSCs including the high proliferative potency. One of the most remarkable but least understood findings it is the migration ability they may use to reach the uterus from bone marrow and peripheral blood. Subsequent analysis of communication between pbMSC or eMSC and the bovine embryo, through inflammatory or implantation cytokines and through extracellular vesicles, will shed light on the functions of MSC during the implantation process.

FUNDING

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