Adult stem cells in human endometrium

Stanimir Kyurkchiev

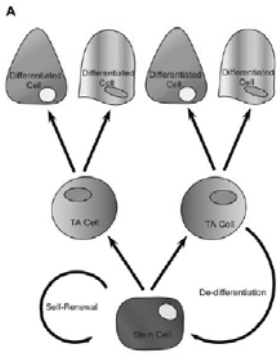
Institute of Biology and Immunology of Reproduction,
Bulgarian Academy of Sciences

Introduction to stem cells

Basic characteristics of the stem cells:

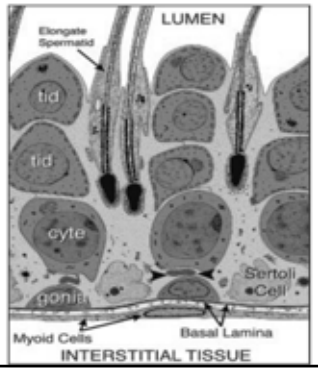
- Self-renewal – symmetric division
- Capacity to differentiate – asymmetric division
- Clonogenicity

Stem cell hierarchy

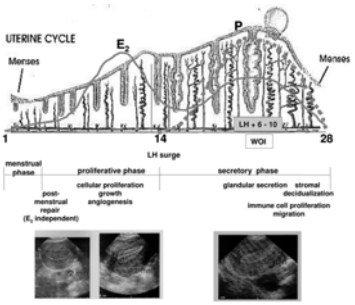


Stem cell ‘niche’ in testis

Ralph L. Brinster, SCIENCE 21 JUNE 2002 VOL 296



Human endometrium



It has been estimated that if the doubling time of endometrial cells taken place from 5th to 20th day of the menstrual cycle is maintained at the same level for 1 year, the mass of the endometrium would be 1 ton. These changes are due to cell proliferation and differentiation which are under strict hormonal control.

Stem cells in endometrium

BASIC SCIENCE EVIDENCE:

- Prianishnikov VA (1978) On the concept of stem cell and a model of functional-morphological structure of the endometrium. Contraception, 18,213–223;
- Padykula HA (1991) Regeneration of the primate uterus: the role of stem cells. Ann N Y Acad Sci 622,47–56.

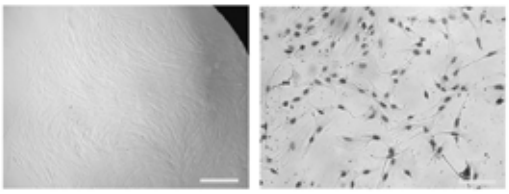
CLINICAL EVIDENCE:

- Abbott JA and Garry R (2002) The surgical management of menorrhagia. Hum Reprod Update 8,68–78;
- Wood C and Rogers PAW (1993) Pregnancy after planned partial endometrial resection. Aust NZ J Obstet Gynaecol 33,316–318.

PRESENCE OF STEM CELLS IN ENDOMETRIUM

- Human endometrium contains clonogenic cells
- Chan et al, 2004;
- Dimitrov et al., 2007
- Human endometrium contains “side-population” cells
- Kato et al., 2007
- Human endometrium contains “label-retaining cells”
- Cervello et al, 2007;
- Chan and Gargett, 2006

R.Dimitrov, T.Timeva, D.Kyurkchiev, M.Stamenova, A.Shterev, P.Kostova, V. Zlatkov, I.Kehayov, S.Kyurkchiev. Characterization of clonogenic stromal cells isolated from human endometrium. Reproduction, 2008, 135 551–558



Morphology of cultured human stromal endometrial cells.
A - confluent swirled layer of fibroblast-like cells is formed about 14 days of culture (Scale bar = 400μm); B - after Giemsa staining of sub-confluent cells the fibroblast-like morphology of the cells is clearly seen (scale bar = 100μm);

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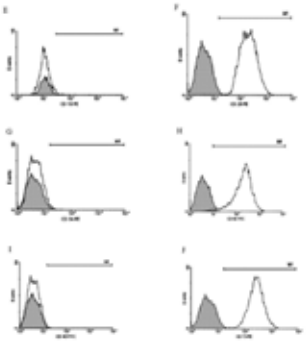

Flow cytometry analysis of cultured endometrial stromal cells. The shaded area is the isotype control. The cell cultures are negatively stained for CD45, CD14, CD19, CD 56/16, CD34, CD3 and HLA-DR. Endometrial stromal cells are positively stained with anti-CD90, anti-CD73 monoclonal antibodies and anti-CD29 antibody. These data is representative for all 6 samples collected from patients. Markers analyzed are as follows: E – CD19, F – CD29, G – CD34, H – CD90, I – CD45, J – CD73.

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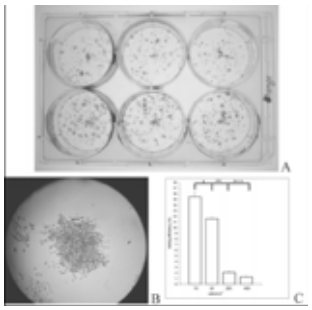

Clonogenicity of human endometrial cells cultured in vitro. A - Wells of 6 –well plates seeded with 400 cells/cm^2. Separate colonies originating from single cells are seen. B - Separate colonies consisting of fibroblast-like cells after Giemsa staining are seen. C - Quantitative data of cloning efficiency for different cell densities (n=6). Results shown are means ± SEM of n= 8 patient samples. Statistical significance between cloning efficiencies at different cell densities is shown (*p=0.00026; **p<0.0001; ***p=0.0003).

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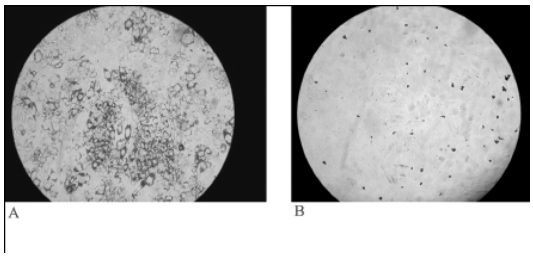
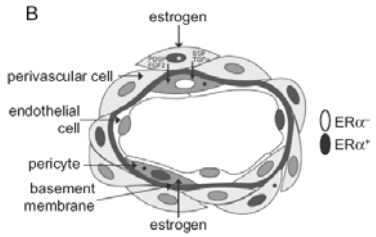


Adipogenic differentiation was demonstrated by the accumulation of neutral lipid vacuoles stainable with Oil-Red O (A cells treated with adipogenic factors; B are non-induced controls; (magnification, x 200).

Uterine stem cells: What is the evidence?
C.E.Gargett
Human Reproduction Update doi:10.1093/humupd/dml045



Mesenchymal stem niche in a perivascular location, indicating the possible pericyte and/or perivascular nature of the mesenchymal stem cells (MSC)-like cell (*), some of which express ERa (black nuclei). Surrounding ERa+ perivascular cells or endothelial cells may act as niche cells to regulate MSC-like cell proliferation through production of PDGF-BB, EGF, TGF-a or FGF2. Not all MSC-like cells respond to estrogen during endometrial regeneration. Endometrial MSC-like cells could be responsible for estrogen-induced growth of stromal tissue (perivascular MSC-like cells) and blood vessels (pericyte MSC-like cells).

Possible clinical implications

- Endometrial carcinoma – carcinoma stem cells, uncontrolled proliferation;
- Endometriosis – ectopic growth, invasiveness, monoclonality, possible transition to ovarian clear cell carcinoma and endometrioid carcinoma;
- Adenomyosis – invasion of basal endometrium of the myometrium
- Endometrial hyperplasia

Stem cells in human decidua

- R.Richards et al. Fibroblast Cells from Term Human Decidua Closely Resemble Endometrial Stromal Cells: Induction of Prolactin and Insulin-Like Growth Factor Binding Protein-1 Expression'. Biol. Reprod., 52, 609-615 (1995);
- E.Olivares, M.Montes, C.Oliver, J.Galindo, C.Ruiz. Cultured Human Decidual Stromal Cells Express B7-1 (CD80) and B7-2 (CD86) and Stimulate Allogeneic T Cells. Biol.Reprod. 57, 609-615 (1997);
- J.Garcia-Pacheco, C.Oliver, M.Kimatrai, F.Blanco, E.Olivares. Himan decidua stromal cells express CD34 and STRO-1 and are related to bone marrow stromal precursors. Mol Human Reprod, 7, 1151, 2001;
- M.Kimatrai, C.Oliver, A.Abadia-Molina, J.Garcia-Pacheco, E.Olivares. Contractile Activity of Human Decidual Stromal Cells. J.Clin.Endocrin.and Metabolism 88(2):844–849

R.Dimitrov, D.Kyurkchiev, T.Timeva, M.Yunakova, M.Stamenova, A.Shterev, S.Kyurkchiev.
First trimester human decidua contains a population of mesenchymal stem cells

Fert.Steril (submitted)

- Formation of human decidua is a specific remodelling and restructuring process strictly controlled by reproductive hormones and particularly essential for the development of successful pregnancy. Pre-decidual stromal cells are the major players in this process but their origin and characteristics have not been completely understood, yet.
- The aim of this study was to characterize pre-decidual stromal cells in reference to their phenotype, clonogenicity and differentiation potential.

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- Human deciduas (10 samples) were collected from pregnant women undergoing an elective surgical termination after a signed informed consent.
- The samples were used to produce single cell suspension and cultured in vitro.
- The cells were induced to differentiate as decidual cells secreting prolactin, osteogenic, adipogenic and endothelial-like cells.
- The phenotype of the cells was analyzed using flow cytometry, Western blot, immunofluorescence, reverse transcriptase polymerase chain reaction (RT-PCR).

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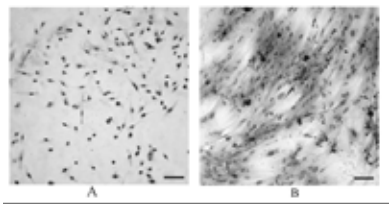
MORPHOLOGY



Light microscopy of morphology of in vitro cultured decidual stromal cells. A- individual decidual stromal cells with elongated spindle-like shape. B - homogenous monolayer of decidual stromal cells. Giemsa staining. Scale bar = 200μm

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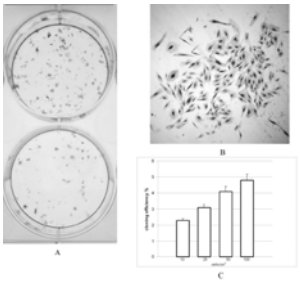
CLONOGENICITY



Colony formation unit –fibroblast (CFU-F) test. Separate colonies were generated from single cells and stained with Crystal violet (panel A, upper well - 100 cell/cm2, lower well-50 cell/cm^2), digital photograph.. B – a single colony, Scale bar = 200μm. C – Quantitative data of cloning efficiency for different cell densities (n=8). Results shown are means ± SEM of n = samples from 8 patients.

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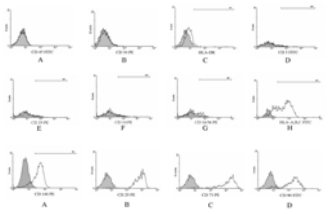
PHENOTYPE



Flow cytometry analysis of cultured DSCs. The cells were completely negative after staining with antibodies against haematopoeitic cell markers such as CD45 (panel A), CD34 (panel B), HLA-DR (panel C) was detected in 1.6% - 6.3% of the cells analyzed, CD3 (panel D), CD19 (panel E), CD14 (panel F), CD16/56 (panel G). The cells were positive for CD 146 (panel H), CD29 (panel J), CD73 (panel K) and CD90 (panel L) and CD146 (panel G) markers.

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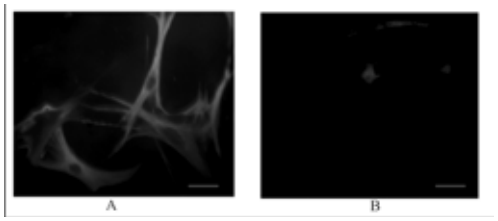
PHENOTYPE



Indirect immunofluorescence of preDSCs for vimentin. A – vimentin positive staining is located in the cytoplasm of the cells; B – negative control. Scale bar = 20μm.

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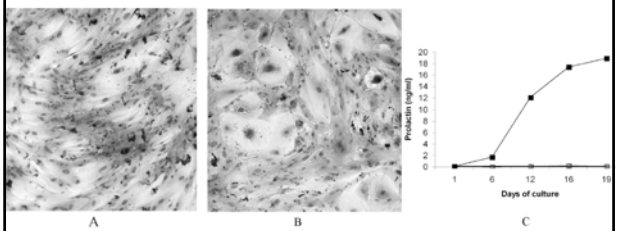
DECIDUALIZATION



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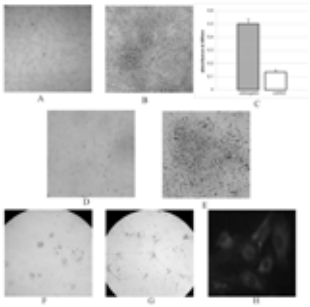
DIFFERENTIATION



Pre-decidual stromal cells can be induced to differentiate as osteogenic cells as demonstrated by von Kossa staining (A-control cells; B-osteogenic cells) and increasing the alkaline phosphatase activity (C); or adipogenic cells stained with Oil Red (D-control cells; E- adipogenic cells); or endothelial-like cells forming tube-like structures when grown in Matrigel (F- control cells; G - endothelial-like cells) which are expressing Von Willebrand factor (H).

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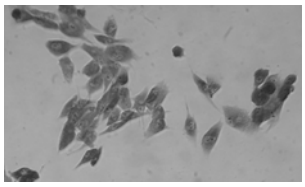
- Pre-decidual stromal cells in human decidua have the characteristics of adult stem cells in reference to their clonogenicity and multipotent differentiation potential.
- It can be speculated that these cels are involved in the processes of remodelling the endometrium.
- Where do these cells come from?

Where do the stem cells come from?

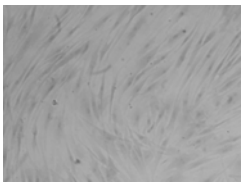
- Remnant fetal stem cells - C.E.Gargett(2006)
- M.Mints, M.Jansson, B.Sadeghi, M.Westgren, M.Uzunel, M.Hassan, J.Palmblad. Endometrial endothelial cells are derived from donor stem cells in a bone morrow transplant recipient. Human Reproduction pp. 1–5, 2007;
- H.Taylor. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. JAMA, 292, 81, 2004

Decidualization of bone marrow mesenchymal stem cells?

MORPHOLOGY



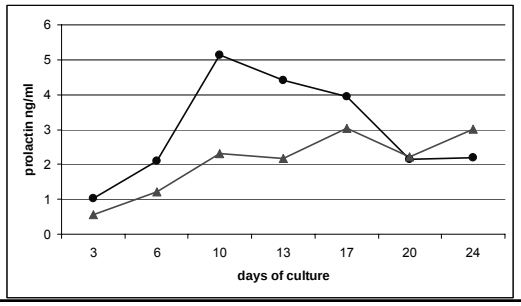
BM-MSC cultured in the presence of estradiol, progesterone and cAMP



Control BM-MSC

Decidualization of bone marrow mesenchymal stem cells?

Prolactin secretion



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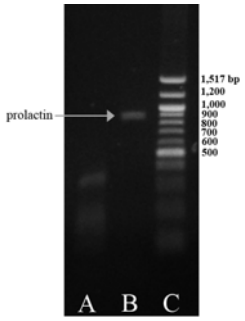
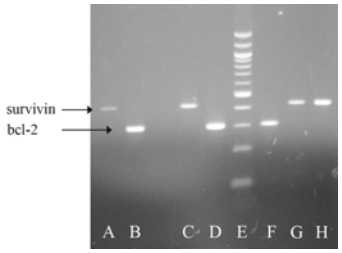


Decidualization of bone marrow-derived mesenchymal stem cells. RT-PCR to check the transcription of prolactin gene. Agarose gel electrophoresis of products after RT-PCR of RNA isolated from: A. control BM-MSCs; B. BM-MSCs cultured in the presence of estradiol, progesterone cAMP; C. DNA ladder for molecular mass markers.

Decidualization of bone marrow mesenchymal stem cells?



Expression of anti-apoptotic genes by decidualized BM-MSCs. Agarose gel electrophoresis of RT-PCR products. Lanes A,B – control mesenchymal stem cells; C,D, - BM-MSCs cultured in the presence of estradiol, progesterone and cAMP; E – molecular mass markers; F,G – adipose tissue derived mesenchymal stem cells cultured in the presence of estradiol, progesterone and cAMP; H – control adipose mesenchymal stem cells.

Conclusions

- There is strong evidence that population/s of endometrial stromal stem cells are residing in human endometrium. The functions of these cells are not defined, yet but it can be speculated that the endometrial stromal stem cells are active players in the monthly decidual reaction in humans.
- Another speculation based on the data presented is that the endometrial stromal stem cells originate from bone marrow derived stem cells recruited to the endometrium under the stringent control of reproductive hormones.