Irene Cervelló and Carlos Simón

Endometrial Stem Cells.

Endometrial remodelling and regeneration in reproductive disorders satellite symposium 2009. 17&18 March 2009.

ENDOMETRIAL SOMATIC STEM CELLS

ENDOMETRIAL SOMATIC STEM CELLS (ESSC):

- **INDIRECT EVIDENCE OF THE EXISTENCE OF ESSC.**
- **PUTATIVE ENDOMETRIAL STEM CELL MARKERS AND BONE MARROW IMPLICATION.**
- **DEMONSTRATION OF ESSC IN MURINE AND HUMAN ENDOMETRIUM.**

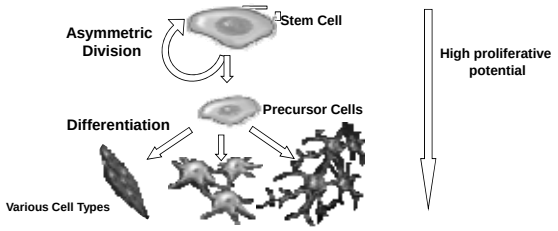
SOMATIC STEM CELLS (SSC)

STEM CELLS:
Non-specialized cells which have the capacity to divide and to differentiate into more mature cells with specialized functions.

SOMATIC STEM CELLS (SSC):
Undifferentiated cells present in a tissue/organ from an adult organism that are capable to renew itself and may differentiate to specialized cell types of the tissue from which it originated.

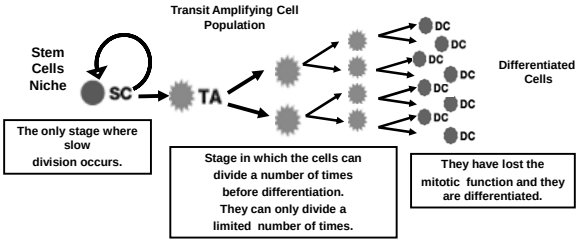
SOMATIC STEM CELLS (SSC)

BIOLOGY OF STEM CELLS



CONCEPT OF NICHE

HIERARCHY OF STEM CELLS



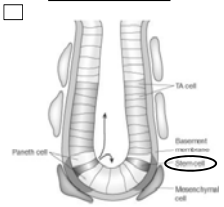
The only stage where slow division occurs.

Stage in which the cells can divide a number of times before differentiation. They can only divide a limited number of times.

They have lost the mitotic function and they are differentiated.

CONCEPT OF NICHE

THE NICHE



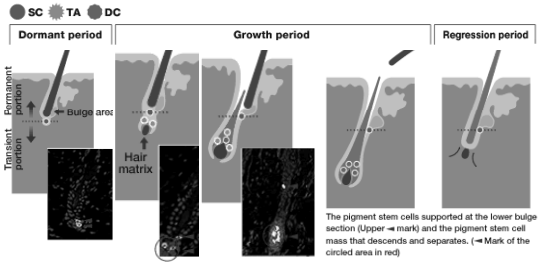
Mammalian gut crypt

"It constitutes a basic unit of tissue physiology, integrating signals that mediate the balanced response of stem cells to the needs of organisms. The interplay between stem cells and their niche creates the dynamic system necessary for sustaining tissues."

Scadden, Nature, 441 (7097), 1075-1079 (29 June 2006)

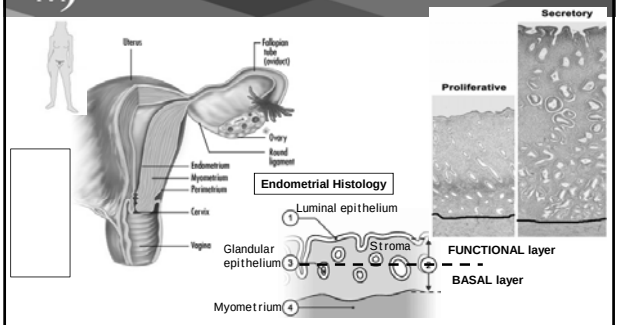
CONCEPT OF NICHE

HIERARCHY OF STEM CELLS



Nishikawa et al.,2003.

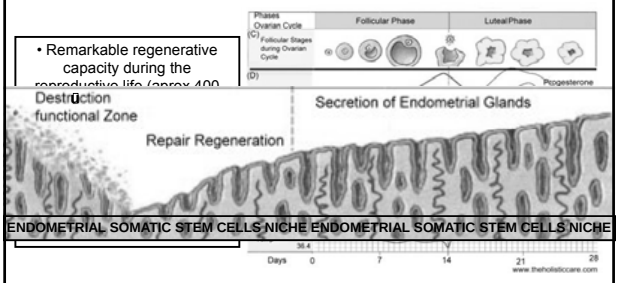
HUMAN ENDOMETRIUM



The endometrium is composed of luminal epithelium and epithelial-lined glands surrounded by a supportive stroma. It can be divided into a functional (removed layer) and basal (regenerating layer) compartments.

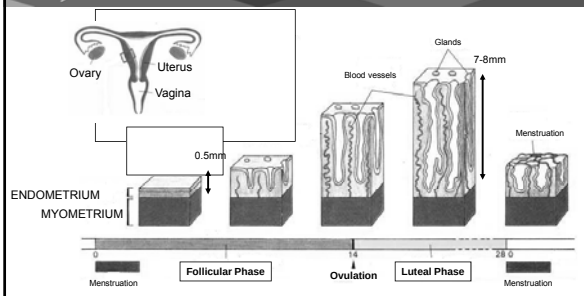
HUMAN ENDOMETRIUM

- Remarkable regenerative capacity during the reproductive life (aprox 400



ENDOMETRIAL SOMATIC STEM CELLS NICHE ENDOMETRIAL SOMATIC STEM CELLS NICHE

HYPOTHESIS: SSC IN HUMAN ENDOMETRIUM



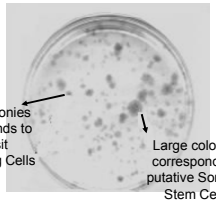
The endometrium is a highly proliferative tissue (0.5mm to 7-8 mm) in which the presence of SOMATIC STEM CELLS has been postulated from Prianishnikov 1978.

INDIRECT EVIDENCE FOR THE EXISTENCE OF ESSC

CELL-CLONING STUDIES
Capacity of forming colonies, one of the typical characteristics of stem cells.


Cell Culture

Populations of endometrial epithelial/stromal cells were seeded in triplicate at clonal density, 300-500 cells/cm2. Cultures were stained with 0.5% toluidine blue (after 15 days). Clusters, 50 cells, were counted and the colony-forming ability determined.



Clones	Clonogenicity (%)	
	Epithelial	Stromal
Large	0.08 ± 0.03	0.82 ± 0.01**
Small	0.14 ± 0.04	1.23 ± 0.18**
Total	0.22 ± 0.07*	1.25 ± 0.18*

The cloning efficiency does not vary significantly across the menstrual cycle; or between active, cycling and inactive endometrium *(Schwab et al.,Fertil Steril 2005).*

Chan et al., Biol Rep 2004.

PUTATIVE ENDOMETRIAL STEM CELL MARKERS

LACK OF SPECIFIC STEM CELLS MARKERS IN HUMAN ENDOMETRIUM

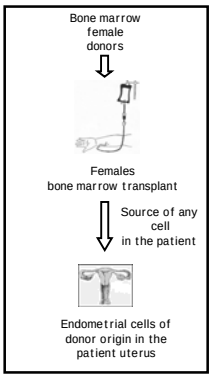
- To resolve this a combination of different markers was used in different works.
- Many of the classical stem cells markers have been examined in human endometrium to characterize the ESSC.
- Known adult stem cell markers are also expressed on mature cells (CD34 is a Hematopoietic Stem Cell and mature endothelial cell marker).
- There is a need to identify epithelial or stromal stem cells markers in human endometrium.
- To determine the exact location of the endometrial niche.

PUTATIVE ENDOMETRIAL STEM CELL MARKERS

	Stem Cell Marker	Endometrial localization	Reference
POU5F1	Embryonic stem cell	In humans, it colocalise with Vimentin and Cytokeratin. In murine populations, co-localization of BrdU- retaining cells.	Matthai *et al.*,2006 Cervelló *et al.*,2007
CD90	Cultured Mesenchymal stem cell	In humans, it differentiates the expression in the basalis and functionalis stroma.	Schwab and Gargett, 2008
CD146	Endothelial cell, perivascular cell and Mesenchymal stem cell	In humans, it co-expresses with PDGF-Rβ.	Schwab and Gargett, 2007,2008
c-Kit	Hematopoietic stem cell and mast stem cells	In humans, mainly in the stroma. In murine samples, co-localization of BrdU- retaining cells.	Cho *et al.*,2004 Cervelló *et al.*,2007 Goodell *et al.*,2008
CD34	Hematopoietic stem cell and endothelial cells	In humans, mainly in the stroma.	Cho *et al.*,2004
STRO-1	Mesenchymal Stem cells	In humans, is located on the perivascular regions of the endometrium	Schwab et al., 2008.

Cervelló et al., Expert Reviews 2009.

IMPLICATION OF BONE MARROW



Taylor., JAMA 2004.

IMPLICATION OF BONE MARROW

• Presence of donor-derived cells were detected in endometrial biopsy. A significant chimerism were confirmed in the cell composition of the glands of four patients who had undergone a bone marrow transplant.

• Percentage of donor- derived cells in endometrium increased with time and represented:
- 50% at transplantation time of 147 months, 10.5% at 129 months, 4% at 35 months and 0.25% at 24 months.

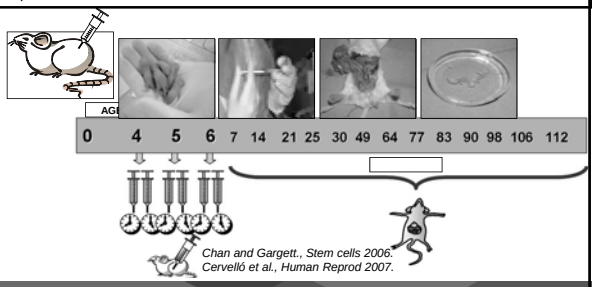
• First study suggesting hematopoietic origin in the repopulation and regeneration of the endometrial tissue after bone marrow transplantation.

Taylor., JAMA 2004.

ESSC IN MURINE ENDOMETRIUM

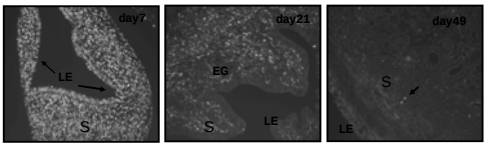
METHOD OF 5-BROME-2-DEOXYURIDINE (BrdU)

BrdU is incorporated into genomic DNA during the replication phase of the mitotic cycle labelling new cells. Identification of label retaining cells (LRC) in epithelial and stromal compartment of murine endometrium.



Chan and Gargett., Stem cells 2006.
Cervelló et al., Human Reprod 2007.

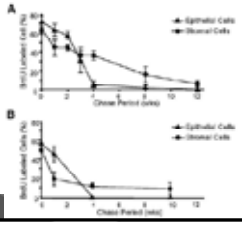
ESSC IN MURINE ENDOMETRIUM



The BrdU retained cells in mice endometrium decreases with age.

Cervelló et al., Human Reprod 2007.

After labelling, the BrdU signal is progressively decreased in each division. The retention of the labelling in some populations means no division or very low rate of division, one of the main characteristic of somatic/progenitor stem cells.



Chan and Gargett., Stem Cells 2006.

ESSC IN MURINE ENDOMETRIUM

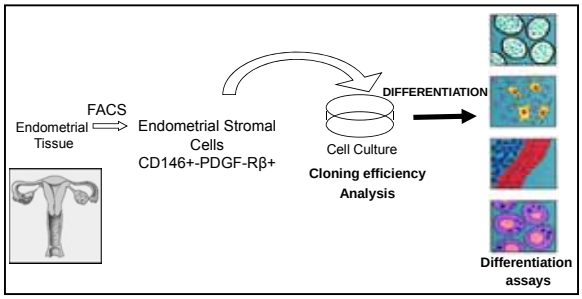
CONCLUSIONS OF MURINE MODEL

- ✓ Presence of LRC in the murine endometrium.
- ✓ Signal disappears in epithelium around 3-4 weeks of mice life.
- ✓ In the lower stromal compartment LRC remains constant during adulthood.
- ✓ LRC co-localized with estrogen receptor factor suggesting their capacity to hormonal response in endometrial remodelling.
- ✓ LRC co-localized with undifferentiated stem cells markers: c-Kit and OCT-4; in the lower part of the stroma, indicating features of SSC of these cells.
- ✓ Stem-like cells may be responsible of remodelling regeneration in murine endometrium.

THESE INITIAL FINDINGS ARE A PREREQUISITE FOR FUTURE STUDIES TO INVESTIGATE THE ROLE OF SSC IN HUMAN ENDOMETRIUM

ESSC IN HUMAN ENDOMETRIUM

CELL-CLONING AND DIFFERENTIATION



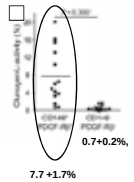
Schwab and Gargett., Human Reprod. 2007.

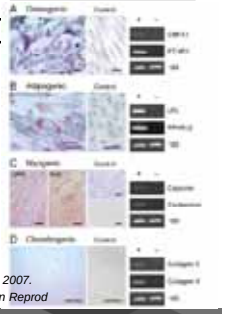
ESSC IN HUMAN ENDOMETRIUM

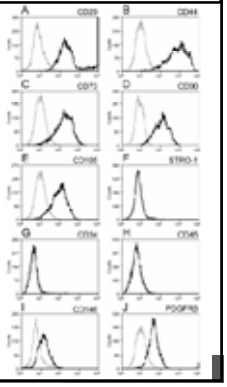
PHENOTYPING OF CD146/PDGF-Rβ+

IN VITRO DIFFERENTIATION

CLONING EFFICIENCIES

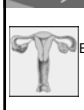






Wolff et al.,Reprod Scien 2007.
Schwab and Gargett.,Human Reprod 2007.

ESSC IN HUMAN ENDOMETRIUM



Endometrial Tissue

CD146+ PDGF-Rβ+

DIFFERENTIATION

Cloning Culture

PHENOTYPE

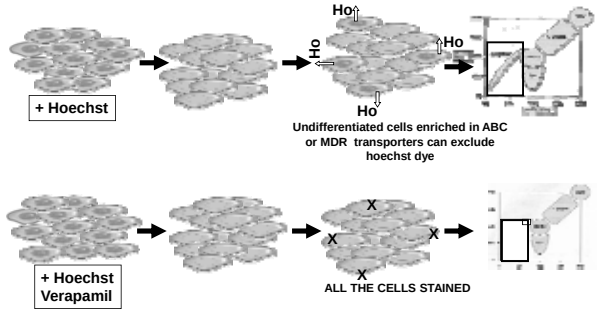
CONCLUSIONS

- ✓ Isolation of mesenchymal stem-like cells in human stromal endometrium using two perivascular cell markers: CD146 and PDGF-Rβ+.
- ✓ High cloning efficiency of the cells isolated in comparison with stromal cells and negative cells.
- ✓ Ability of these cells to differentiate in mesenchymal lineage tissues *in vitro*.
- ✓ The mesenchymal cellular phenotype may be responsible for its cyclical growth, and may provide an available source for tissue engineering applications.

Schwab and Gargett., Human Reprod 2007.

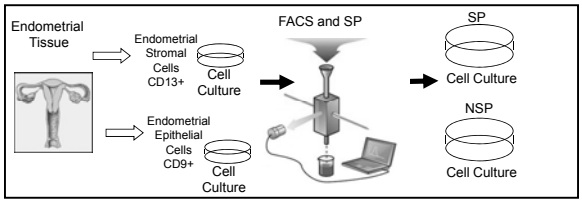
ESSC IN HUMAN ENDOMETRIUM

Side Population: Hoechst Method and Cell Sorting



ESSC IN HUMAN ENDOMETRIUM

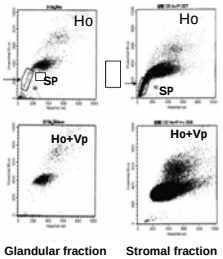
SIDE POPULATION (SP) IN HUMAN ENDOMETRIUM



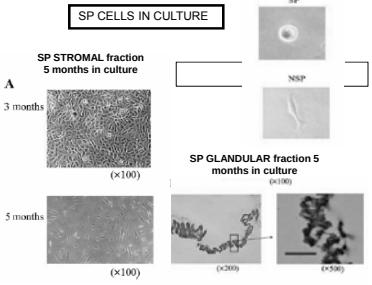
Kato et al., Human Reprod 2007.

ESSC IN HUMAN ENDOMETRIUM

SP IN HUMAN ENDOMETRIUM after cell culture

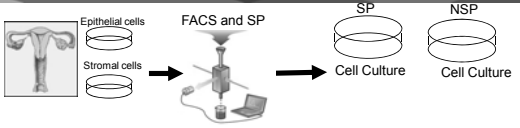


SP CELLS IN CULTURE



Kato et al., Human Reprod 2007.

ESSC IN HUMAN ENDOMETRIUM

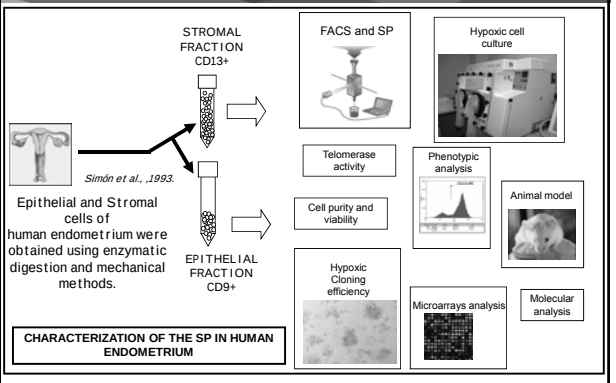


CONCLUSIONS

- ✓ First study describing the presence of Side Population cells in human endometrium after *IN VITRO* culture. The stromal and glandular fraction of endometrium was cultured during 24-48 h before Hoechst analysis.
- ✓ The SP percentage in all endometrial cells corresponds to 0.01% up to 5.1% (the higher percentage stromal fraction just after menstruation 3.91%).
- ✓ SP cells isolated from the glandular fraction can give rise to glands and stromal fraction to stromal-like compartment *in vitro* after 5 months in culture.
- ✓ These results suggest that SP cells isolated from human endometrium exhibit characteristics of self-renewal and differentiation into one cell type (glandular/stromal cells).

Kato et al., Human Reprod 2007.

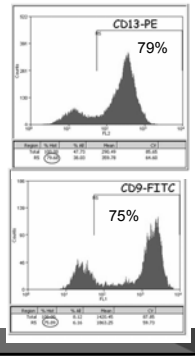
ESSC IN HUMAN ENDOMETRIUM



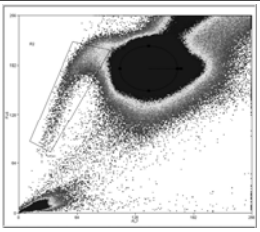
Cervelló et al., 2009 submitted.

ESSC IN HUMAN ENDOMETRIUM

EPITHELIAL AND STROMAL CELL PURITY



SP IN HUMAN ENDOMETRIUM

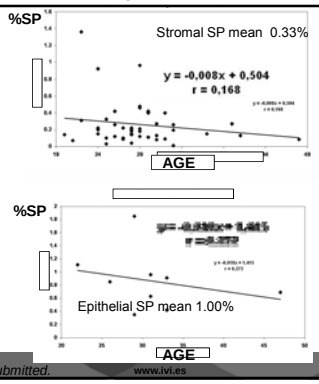


Epithelial SP 1.0 ± 0.8% (n=60)
Stromal SP 0.3 ± 0.3% (n=90)

Cervelló et al., 2009 submitted.

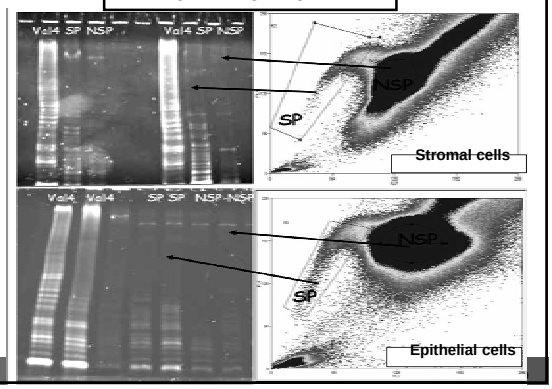
ESSC IN HUMAN ENDOMETRIUM

% SP during reproductive life



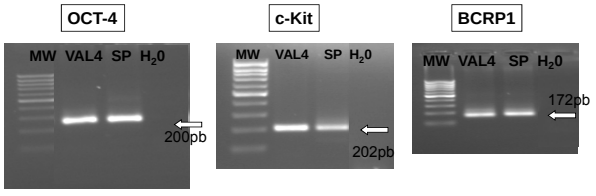
ESSC IN HUMAN ENDOMETRIUM

TELOMERASE ACTIVITY



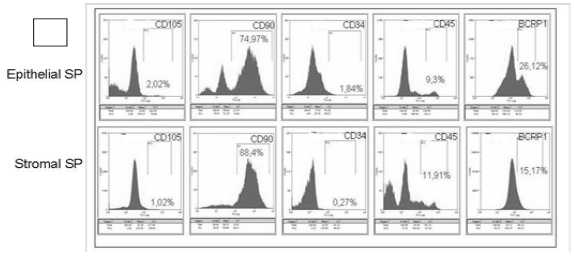
ESSC IN HUMAN ENDOMETRIUM

SP MOLECULAR ANALYSIS



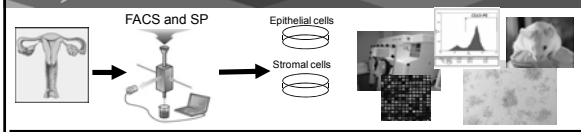
ESSC IN HUMAN ENDOMETRIUM

PHENOTYPE OF ENDOMETRIAL SP



Cervelló et al., 2009 submitted.

ESSC IN HUMAN ENDOMETRIUM



CONCLUSIONS

- ✓ **Presence of Side Population cells in fresh endometrial stromal and epithelial compartments.**
- ✓ **The SP cells in human endometrium correspond to 1.0 ± 0.8% for epithelial compartment and 0.3 ± 0.3% stromal compartment.**
- ✓ **SP from both fractions expressed C-KIT and OCT-4; and ABC-transporters BCRP1.**
- ✓ **Epithelial and stromal SP cells have a characteristic immortality pattern of telomerase.**
- ✓ **Phenotypic analysis demonstrates the putative mesenchymal origin of the SP:CD90+.**
- ✓ **Differences in the cloning efficiencies among E/S compartments of SP/NSP were appreciated in hypoxic conditions.**
- ✓ **With this work we demonstrate that Ho method could be a tool to isolate SSC in the human endometrium.**

Cervelló et al., 2009 submitted.

LIMITATIONS OF SSC

LIMITATIONS OF SOMATIC STEM CELLS

1. Found in only low quantities in adult tissues.
2. Difficult to isolate and purify.
3. Difficult to expand in culture.
4. Difficult to generate differentiated cells.
5. Not yet clear that fully functional differentiated cells types can be obtained.
6. No specific markers.

CONCLUSIONS

CONCLUSIONS:

- Published work in recent years not only confirm the existence of a SSC population in human endometrium, BUT also suggest the implication of this population in endometrial cell regeneration and associated pathologies.
- The identification of a common marker to recognize SSC in the human endometrium is desirable.
- The detection of SSC in human endometrium is an exciting outcome in reproductive and regenerative medicine.
- These results could be a strategic key in the gynecological area to provide new insights into endometrial regeneration.

IMPLICATION IN ENDOMETRIAL PATHOLOGIES

Several gynaecological conditions are associated with atypical endometrial proliferation, and it is possible that endometrial stem cells may play a role in their pathophysiology.

- Endometriosis is defined as the growth of the endometrium outside the uterine cavity. It is a very common gynaecological disorder affecting approximately 15% of women. Clinical manifestations include abnormal menstrual bleeding, pelvic pain and infertility.
- Adenomyosis is a medical condition affecting 1% of women and involves the presence of ectopic endometrium tissue within the myometrium.
- Endometrial cancer starts in the cells that line the uterus and belong to the group of cancers called carcinomas. Most endometrial carcinomas are cancers of the cells that form glands in the endometrium (American Cancer Society).
- Endometrial hyperplasia is generally considered a precursor to endometrial cancer; it is associated with a proliferation of glands of irregular size due to an excess of epithelium growth producing an increase in the glands/stroma ratio.

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