

## Endometrial and miometrial stem cells in the human uterus

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Valencia Spain

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## LEARNING OBJECTIVES

At the conclusion of this presentation,  
participants should be able to:

1. Understand the concept of somatic stem cells and the niche in adult tissue.
2. Describe the direct and indirect evidences of SSC in the murine and human endometrium.
3. Address the evidences of the presence of SSC in the human myometrium.

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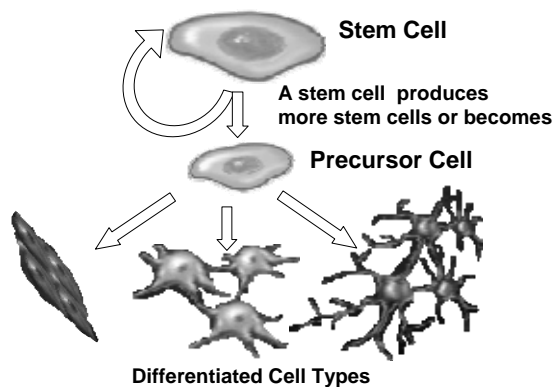
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## SOMATIC STEM CELLS (SSC)



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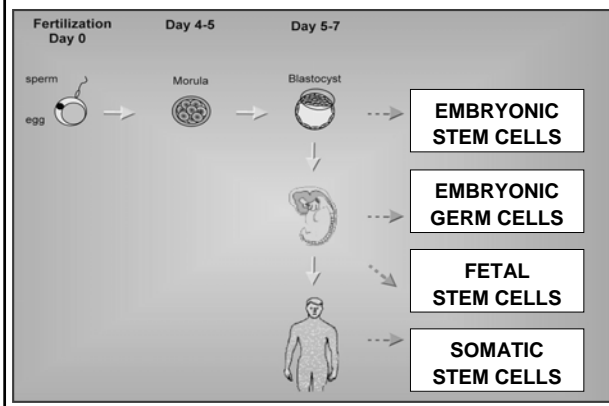
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## STEM CELLS. SOURCES AND TYPES




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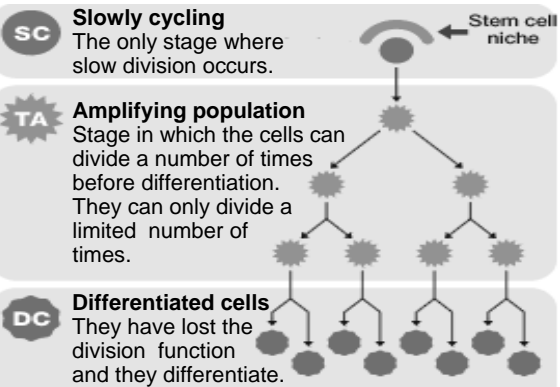
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## SSC IN ADULT TISSUES




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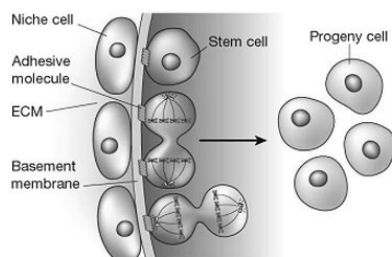
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## THE STEM CELL NICHE



The role of the niche would be the support of the SSC population in the tissue/organ

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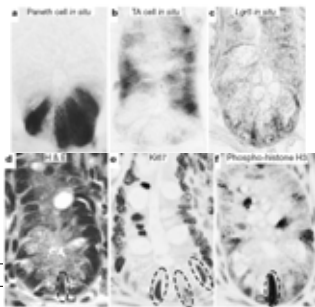
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## THE STEM CELL NICHE IN THE SMALL INTESTINE



Barker N., et al 2007. Identification of stem cells in small intestine and colon by marker specific Lgr5. Nature 449 (7093-99)

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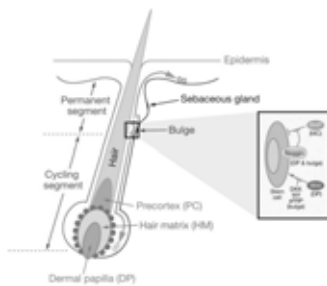
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## EPITHELIAL STEM CELL NICHES



BrdU mark demonstrates that cells labeled are more quiescent, and these cells may be the Bulge Stem Cells Side Population (*Bickenbach, 1981*).

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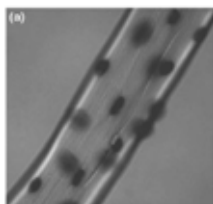
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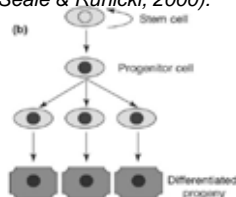
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## ADULT SKELETAL MUSCLE NICHE



Myogenic Satellite Cells reside beneath the lamina basalis of skeletal muscle and are mitotically quiescent until muscular growth or regeneration is necessary. (*Beauchamp et al., 2000; Seale & Rudnicki, 2000*).




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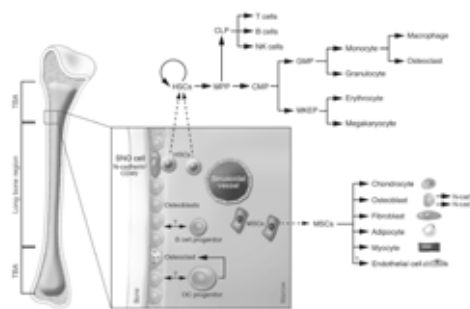
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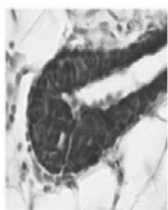
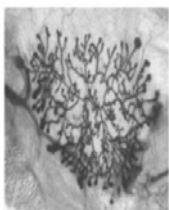
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# THE STEM CELL NICHES IN BONE



Two different stem cell niches have been identified in BM: the osteoblastic niche and the vascular niche (Wagers, 2005).

# MAMMARY GLAND STEM CELL NICHES

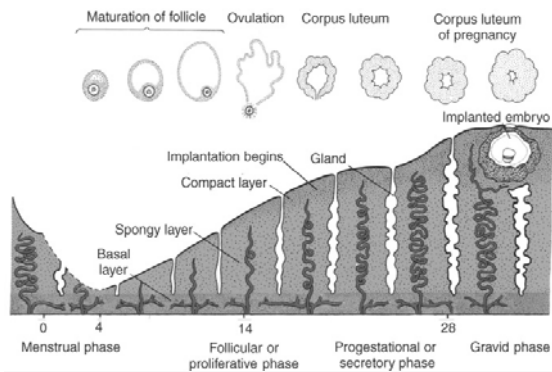


Any portion of mammary gland could reconstitute the entire mammary ductal tree on transplantation into the mammary fat pad of a mouse.


(DeOme et al., in 1959)

Shackleton et al. y Stinnig et al., both in 2006, were the first groups who isolated MaSP labelling it with CD29, CD29 and CD49f using FACS. These isolated cells are also able to extrude Hoescht 33342.

# UTERINE PHYSIOLOGY

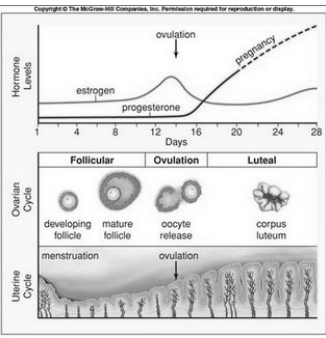


INTRODUCTION



## Human Endometrium

- Aprox. 500 menstrual cycles along the reproductive life of the women.
- Putative tissue for the existence of **Somatic Stem Cells**.
- Putative implication of endometrial SSC in pathologies like endometriosis, endometrial cancer .



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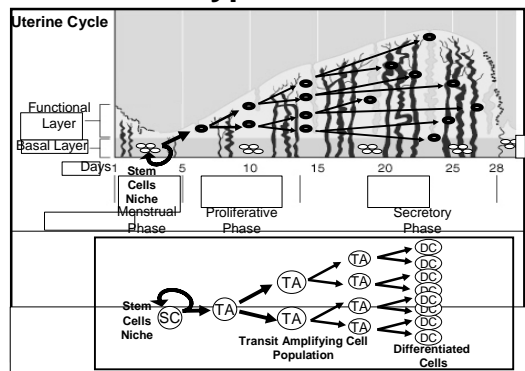
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## ENDOMETRIAL STEM CELL NICHE: Hypothesis



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## MATERIALS and METHODS:

Based en 2 models and 2 experimental techniques:

- Murine Model:

Use of 5-Bromo-2'-desoxyuridine (BrdU)


Localization of the label retaining cells by immunohistochemistry.



- Human Model:

Use of vital dye Hoechst 33342

Identification of the Side Population y Isolation with flow cytometry



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## SSC IN MURINE ENDOMETRIUM

### IDENTIFICATION:



- Using the label retention method (LRC) with the DNA marker BrdU.

- Complemented with the detection of typical markers of undifferentiation at the protein and molecular levels.

*Cervelló et al., Human Rep 2007*

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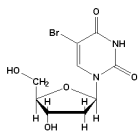
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## BrdU INCORPORATION METHOD

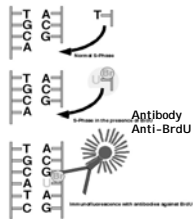


5-Bromodeoxyuridine (BrdUrd)

### 5-Bromo-2'-deoxyuridine

BrdU is a thymidine analogue that is incorporated to the genomic DNA during the replication phase of mitotic cycle.

After labelling, BrdU signal is progressively LOSS in each division. Labelled Retaining Cells (LRC) mean quiescence one of the main characteristic of the SSC.



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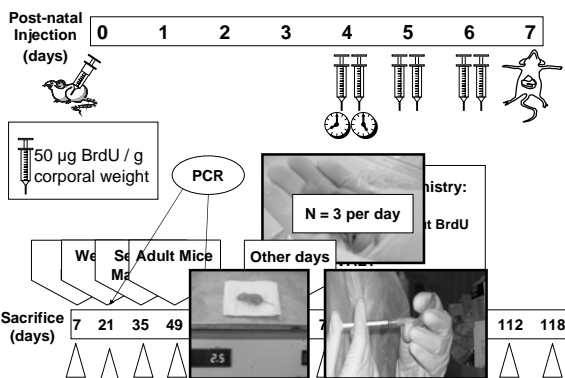
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## EXPERIMENTAL DESIGN



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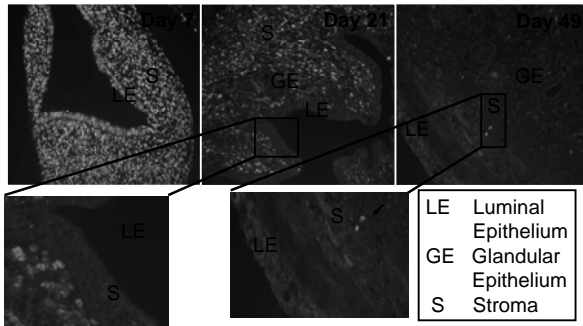
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## RESULTS

- Localization of BrdU retaining cells in murine endometrium



*Cervelló et al., Human Rep 2007*

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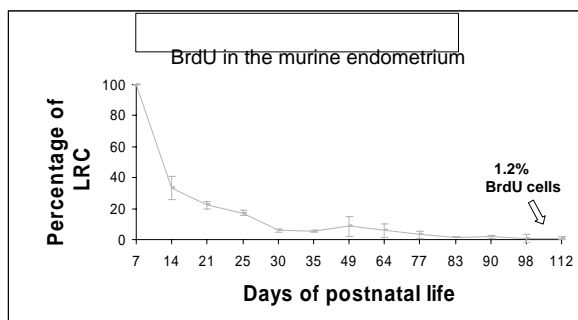
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## RESULTS

- Localization of BrdU retaining cells in murine endometrium



*Cervelló et al., Human Rep 2007*

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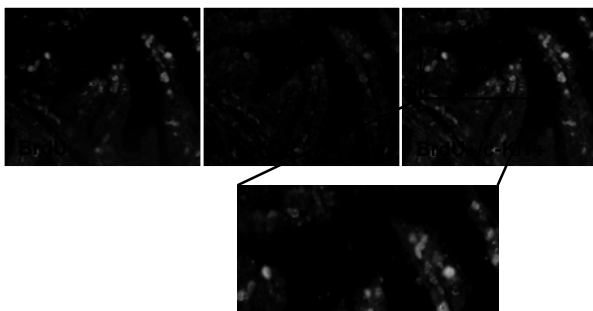
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## RESULTS

- Co-localization BrdU/c-KIT in the murine endometrium (day21)



*Cervelló et al., Human Rep 2007*

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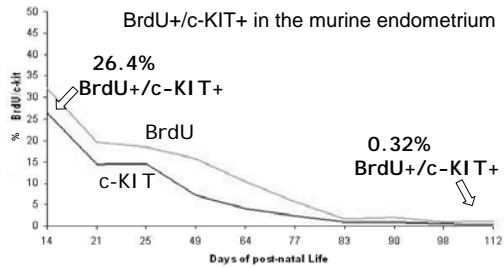
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## RESULTS

- Co-localization BrdU/c-KIT in the murine endometrium



*Cervelló et al., Human Rep 2007*

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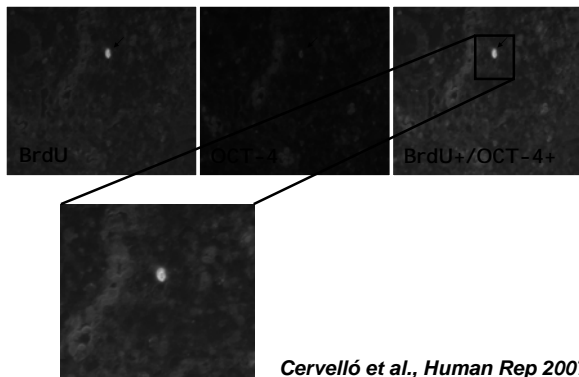
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## RESULTS

- Co-localization BrdU / OCT-4 in murine endometrium (day 49)




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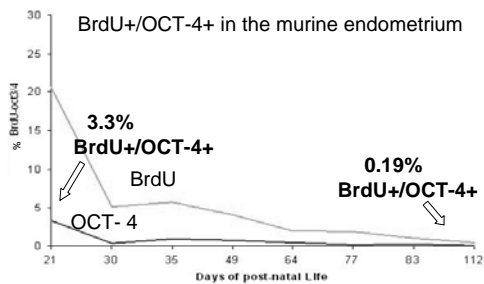
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## RESULTS

- Co-localization BrdU / OCT-4 in murine endometrium



*Cervelló et al., Human Rep 2007*

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## RESULTS

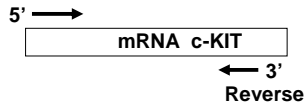
- PCR c-KIT



VAL1	Human stem cell line
U21	Uterus day 21
O21	Ovary day 21
U50	Uterus day 50
O50	Ovary day 50
C-	Water

Mouse c-kit mRNA : Fragment amplified 389 pb

Forward



Reverse

*Cervelló et al., Human Rep 2007*

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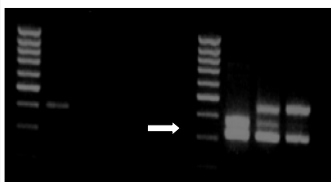
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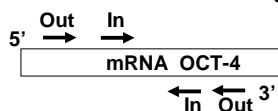
## RESULTS

- Nested-PCR OCT-4



Testis	Positive Control
U21	Uterus day 21
O21	Ovary day 21
U50	Uterus day 50
C-	Water

Mouse OCT-4 mRNA: Fragment amplified 321 pb



*Cervelló et al., Human Rep 2007*

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## CONCLUSIONS

- BrdU retained cells were co-localized with c-KIT and OCT-4 in cells located in the stromal compartment representing in the adulthood 0.32% and 0.19% of cell population, respectively.
- Molecular analysis demonstrate the presence of c-KIT and OCT-4 in the murine endometrium.
- LRC decrease with age.

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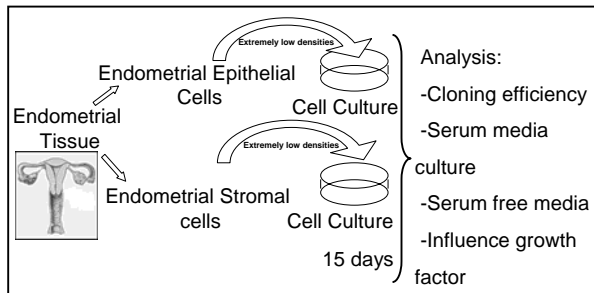
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## EVIDENCE FOR THE PRESENCE OF SSC IN HUMAN ENDOMETRIUM



Chan et al, Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod. 2004.

## INDIRECT EVIDENCES OF ENDOMETRIAL SSC

- Descriptive study that analyzes the expression of CD34+ /c-kit+ cells located in the stroma of the basal layer.

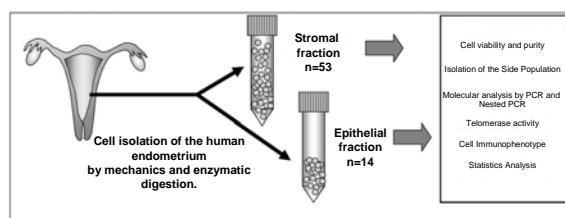
	Fetus	Reproductive Age	Pregnancy	Menopause
c-kit	-	++	+++	+
CD34	-	++	-	+

Cho et al, Fertil Steril. 2004.

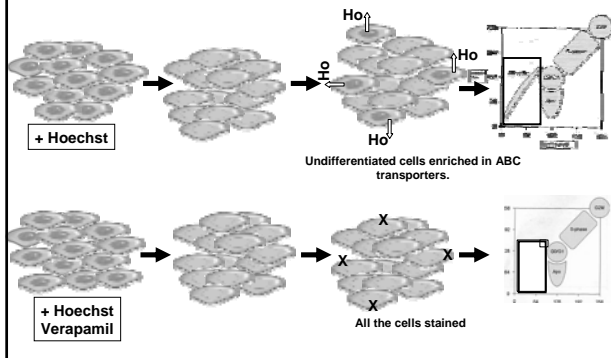
- 44% of patients (n=25) analysed showed endometrial cells expressing Oct-4 at protein level by immunohistochemistry, and at the molecular level using RT-PCR. Restricted to the stromal cell compartment.

Matthai et al, Mol Hum Reprod. 2006.

## Human Model

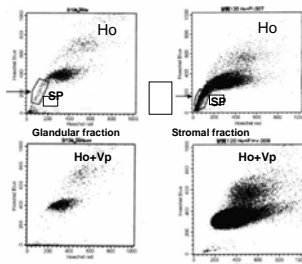


### Side Population: Hoechst Method and Cell Sorting



### INDIRECT EVIDENCES OF ENDOMETRIAL SSC

- First study describing the Side Population cells in human endometrium after IN VITRO culture.

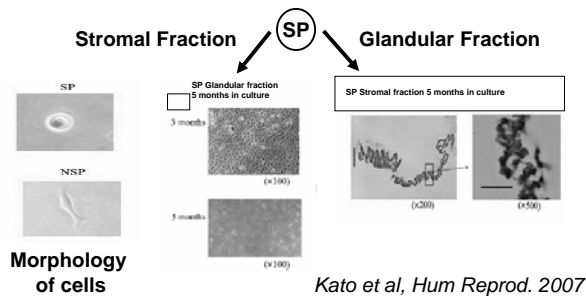


- The endometrial SP corresponds to 0.01%-3% of the stromal fraction.

*Kato et al, Hum Reprod. 2007*

### EVIDENCES OF ENDOMETRIAL SSC

- 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.



## EVIDENCES OF ENDOMETRIAL SSC

- Presence of stromal cells in the human endometrium with mesenchymal SC characteristics.

- CD146: Perivascular and endothelial cell marker to identify bone marrow cells mesenchymal stem cells.
- CD146<sup>+</sup> cells represent 3.1% of the stromal fraction.
- PDGF-Rb : Perivascular/pericyte marker. PDGF-Rb<sup>+</sup> cells represent 71.6% of the stromal cells.
- CD146<sup>+</sup>PDGF-Rb<sup>+</sup> human endometrial stromal cells (1.5% of stromal cells) produced colonies with cloning efficiency of 7.7% versus CD146<sup>-</sup>PDGF-Rb<sup>-</sup> 0.7% of cloning efficiency.

*Schwab et al, Hum Reprod. 2007*

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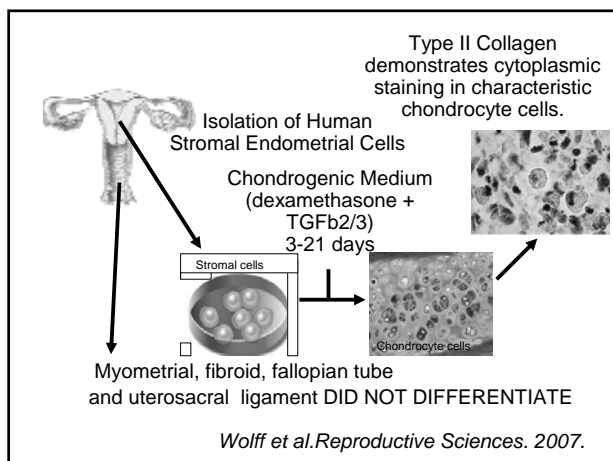
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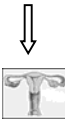
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## POSSIBLE ORIGIN OF SSC

Bone marrow female donors



Females bone marrow transplant



Endometrial cells of donor origin in the patient uterus

- Presence of donor-derived endometrial cells were detected

- First study suggesting **hematopoietic origin** in the regeneration of the endometrial tissue after bone marrow transplant.

- Percentage of donor-derived cells in endometrium were:

- 0.25% after 24 months
- 4% after 35 months
- 10.5% after 129 months
- 50% after 147 months

*Taylor. JAMA. 2004.*

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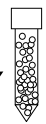
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## Isolation of Stromal and Epithelial SCs from Human Endometrium



Human endometrial biopsy and hysterectomy from healthy donors was obtained after written informed consent for this project.

Purity of the stroma and epithelial cell suspensions and viability using a Cytomics FC500 flow cytometer.



Viability of cells >60%

67% CD9+(epithelium)  
80% Vimentine+(stroma)

Isolation of Stromal and Epithelial fraction

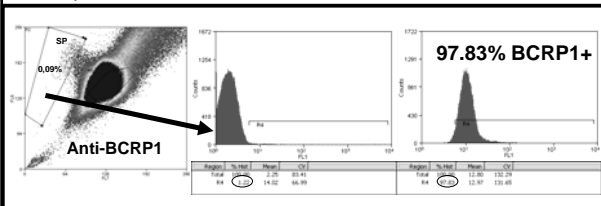
## MATERIALS and METHODS

MoFlo® high-speed sorter (Dako,CO,USA).



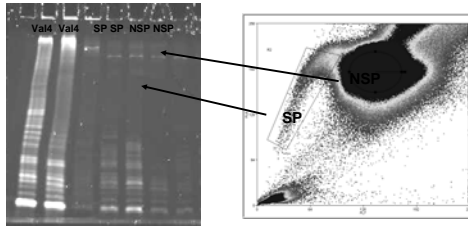
## Expression of typical ABC transporter: BCRP1

- 97.83% of cells isolated and corresponding with SP phenotype express BCRP1, typical marker of ABC transporter.



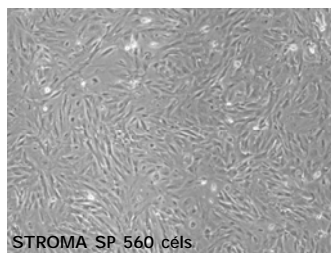
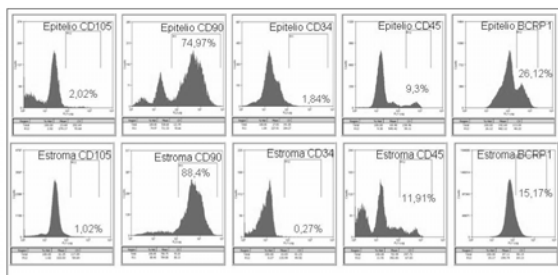
SP + Immuno anti-BCRP1

## Telomerase Activity

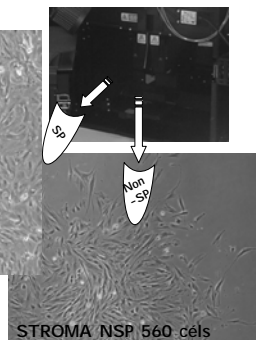


SP cells from epithelium have an intermediate profile of Telomerase Activity between human Embryonic Stem Cells line, VAL4, and differentiated cells from human endometrium NSP.

## PHENOTYPE

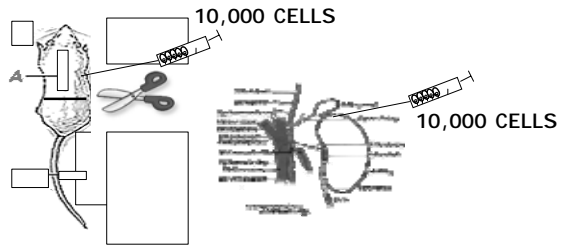


X 436

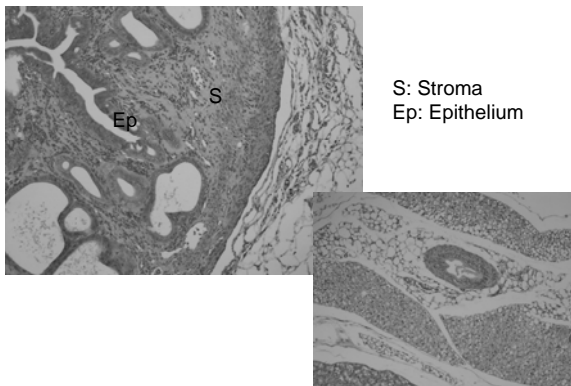


### ANIMAL MODEL:

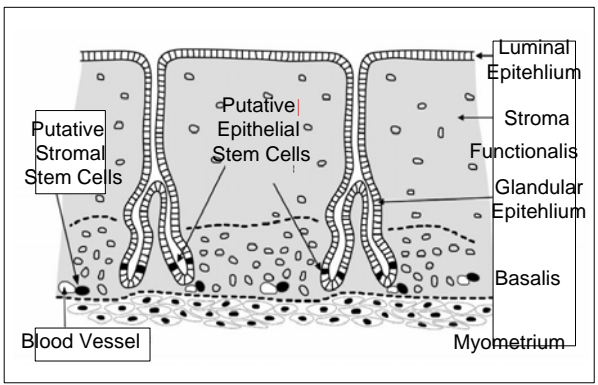
SCID MOUSE OVARECTOMIZED + E<sub>2</sub> PELLET SUPPLY (60d)



### Presence of endometrium in SCID mice



### SOMATIC STEM CELLS IN HUMAN ENDOMETRIUM



PNAS

## Side population in human uterine myometrium displays phenotypic and functional characteristics of myometrial stem cells

Masanori Ono<sup>a</sup>, Tetsuo Maruyama<sup>a,1</sup>, Hirotaka Masuda<sup>a</sup>, Takashi Kajitani<sup>a</sup>, Takashi Nagashima<sup>a</sup>, Toru Arase<sup>a</sup>, Munehito Ito<sup>a</sup>, Kazuki Ohta<sup>a</sup>, Hiroshi Uchida<sup>a</sup>, Hiromori Asada<sup>a</sup>, Yasunori Yoshimura<sup>a</sup>, Hideyuki Okano<sup>b</sup>, and Yumi Matsuzaki<sup>a</sup>

10760-10765 | PNAS | November 20, 2007 | vol. 104 | no. 47

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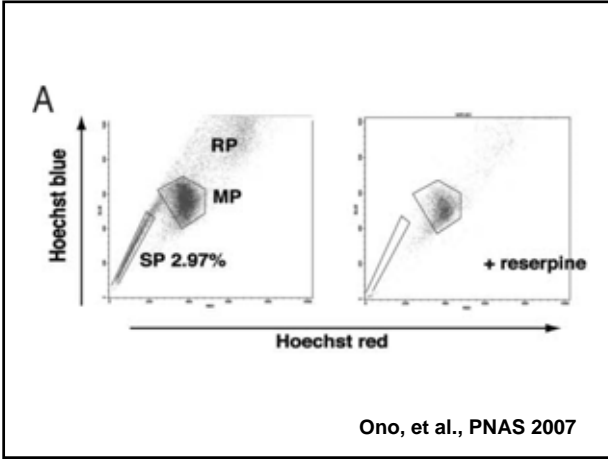
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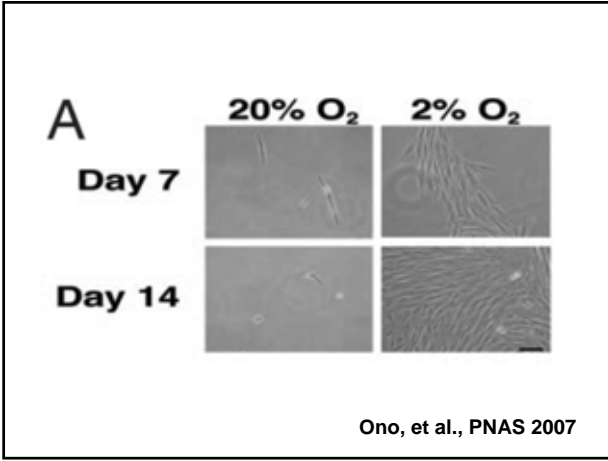
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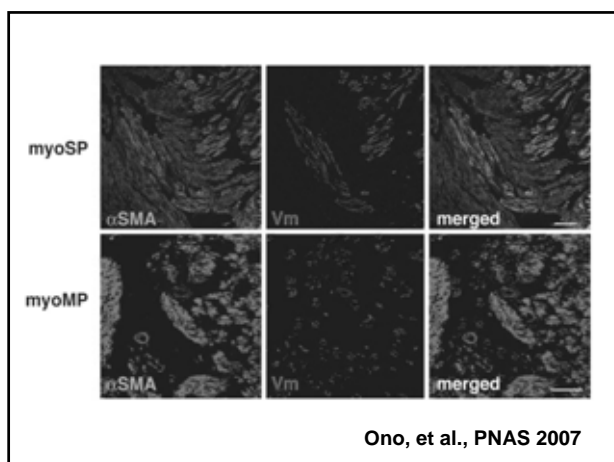
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Dr. Simón

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