ENDOMETRIAL STEM CELLS

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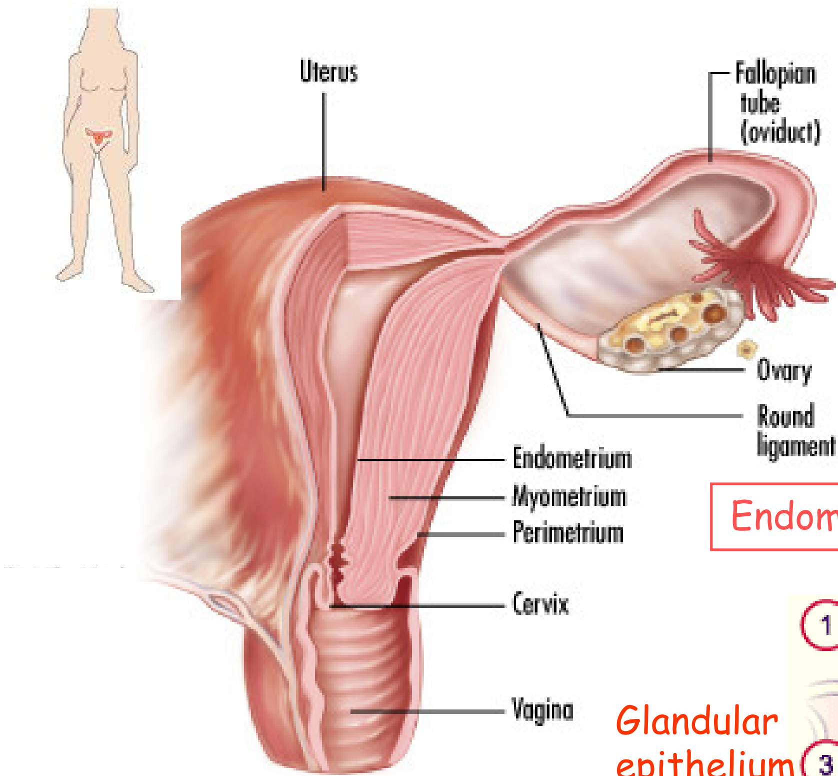
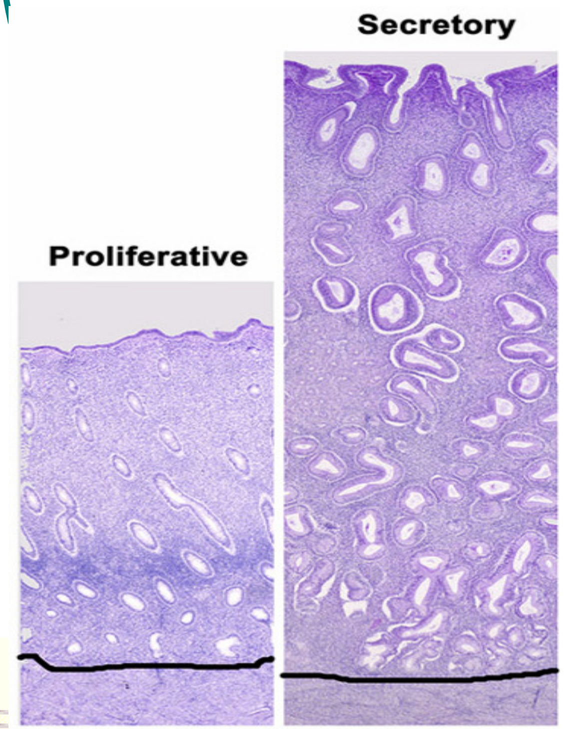
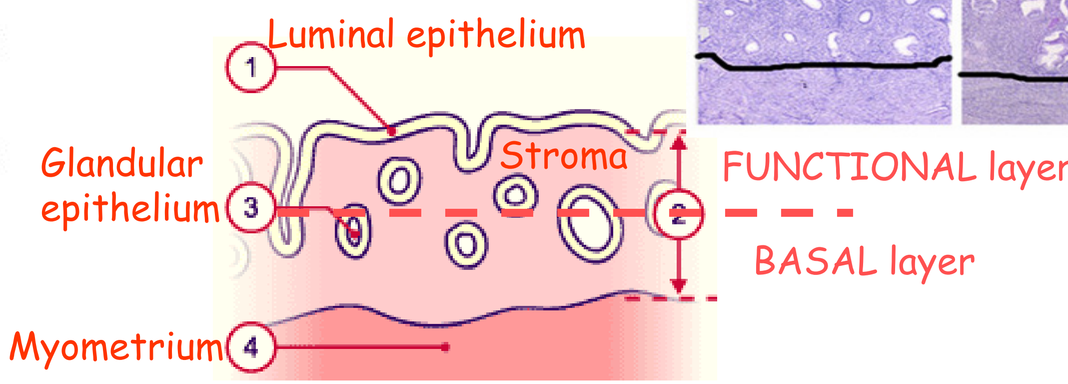
HUMAN ENDOMETRIUM





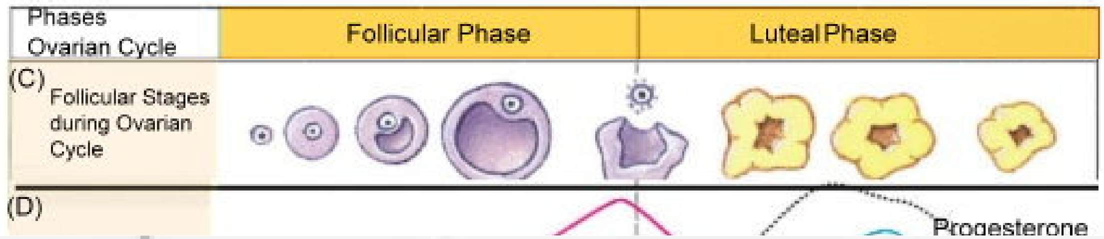
Endometrial Histology

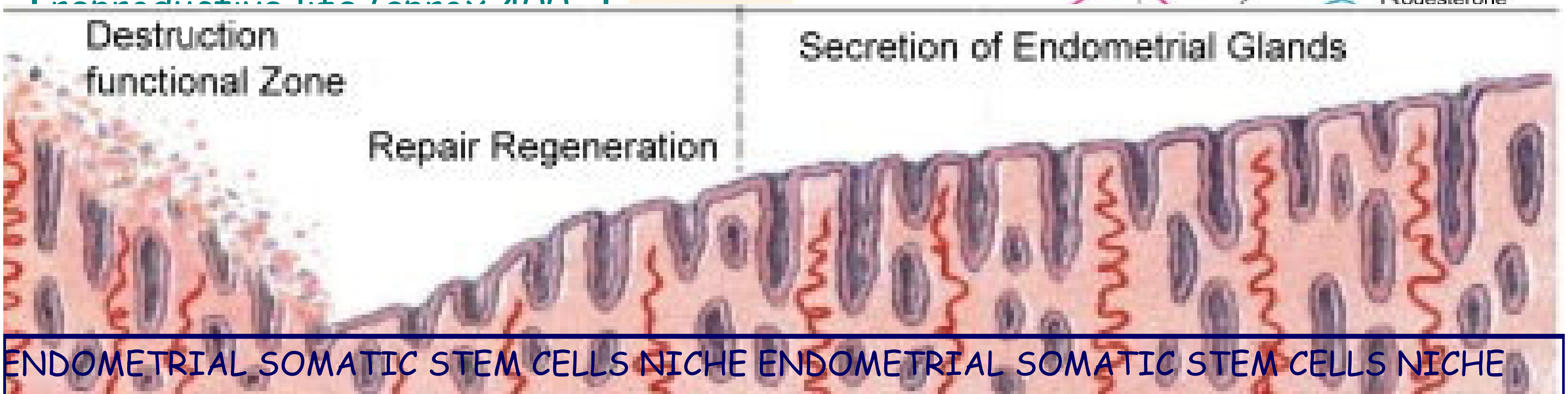


The endometrium is composed of luminal epithelium and epithelial-lined glands surrounded by a supportive stroma. It can be divided into a functional (removed layer) and basal (regenerating layer) compartments.

HUMAN ENDOMETRIUM

- Remarkable regenerative capacity during the

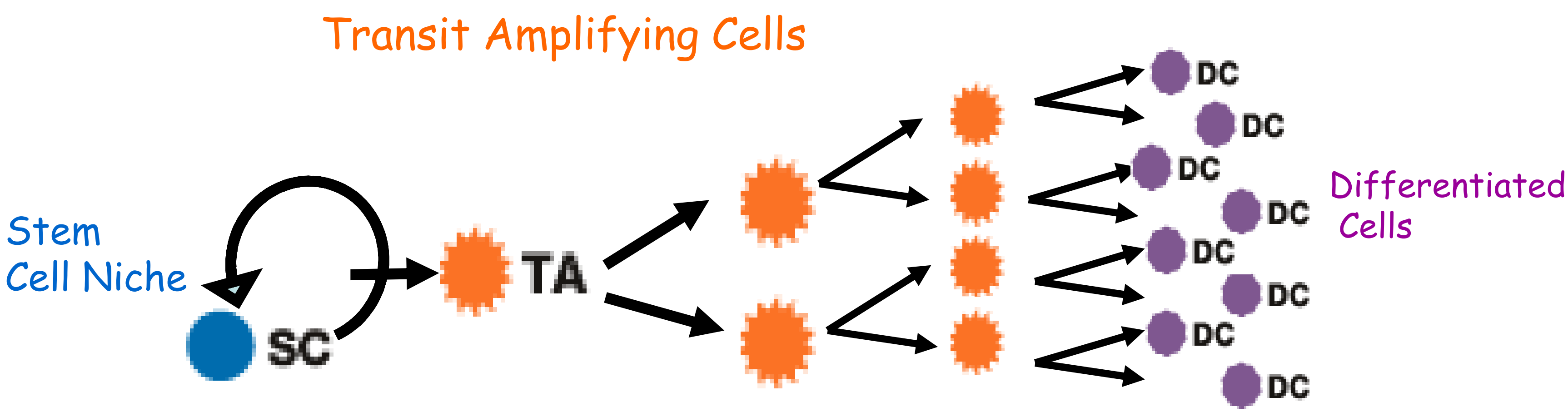




ENDOMETRIAL SOMATIC STEM CELLS NICHE ENDOMETRIAL SOMATIC STEM CELLS NICHE

CONCEPT OF NICHE

HIERARCHY OF STEM CELLS

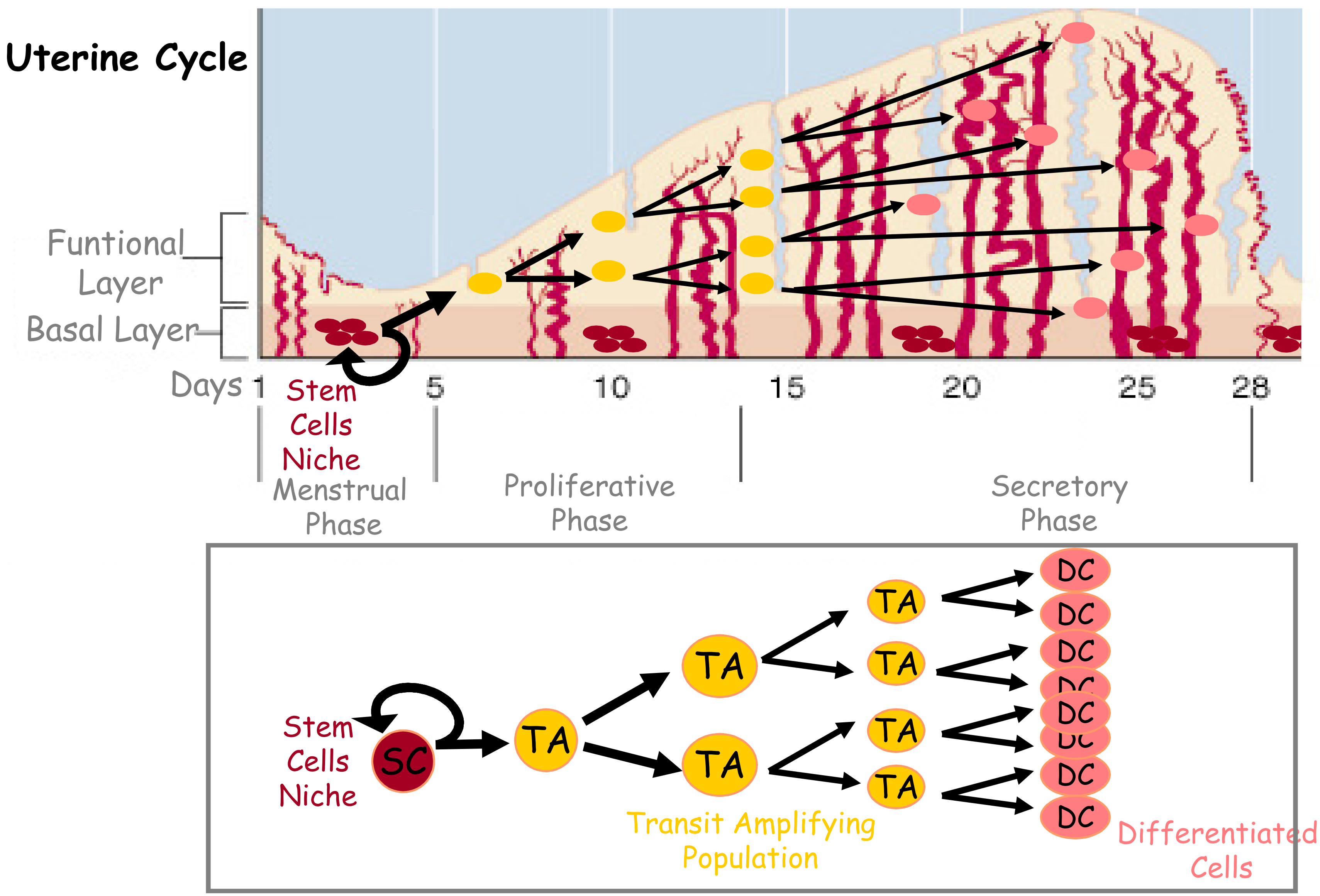


The only stage where slow division occurs.

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

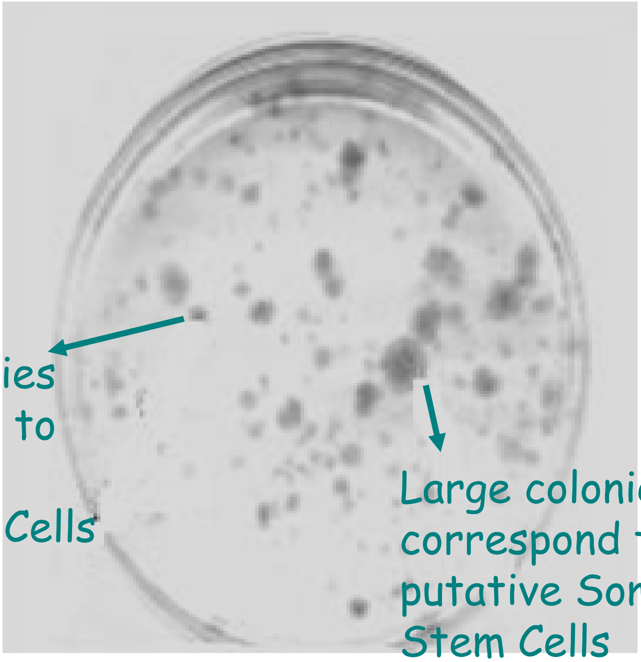
They have lost the mitotic function and they are differentiated.

Endometrial Stem Cell Niche: Hypothesis



INDIRECT EVIDENCE FOR THE EXISTENCE OF ESSC

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



Small colonies correspond to Transit Amplifying Cells

Large colonies correspond to putative Somatic Stem Cells

Clones	Clonogenicity (%)	
	Epithelial	Stromal
Large	0.08 ± 0.03	0.02 ± 0.01**
Small	0.14 ± 0.04	1.23 ± 0.18**
Total	0.22 ± 0.07*	1.25 ± 0.18*

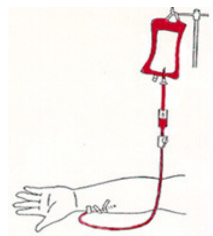
The cloning efficiency does not vary significantly across the menstrual cycle; or between cycling and inactive endometrium

(Schwab et al.,Fertil Steril 2005).

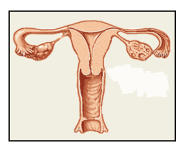
Chan et al., Biol Rep 2004.

IMPLICATION OF BONE MARROW

Bone marrow
female
donors



Females
bone marrow transplant



Endometrial cells of
donor origin in the
mismatched recipient endometrium

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:
- 0.25% at 24 months,
- 4% at 35 months,
-10.5% at 129 months,
-50% at 147 months.

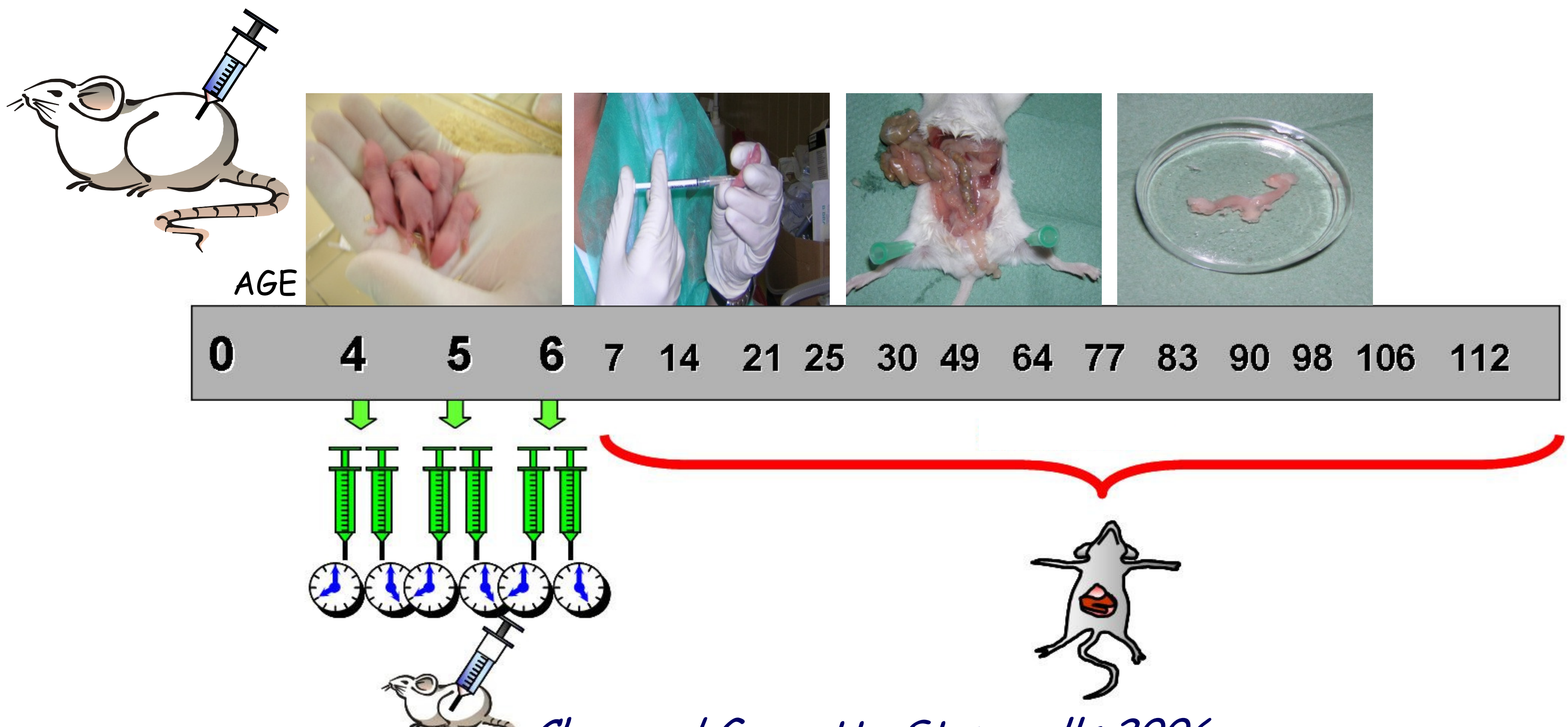
• First study suggesting hematopoietic origin in the repopulation and regeneration of the endometrial tissue after bone marrow transplantation.

Taylor., JAMA 2004.

ESSC IN MURINE ENDOMETRIUM

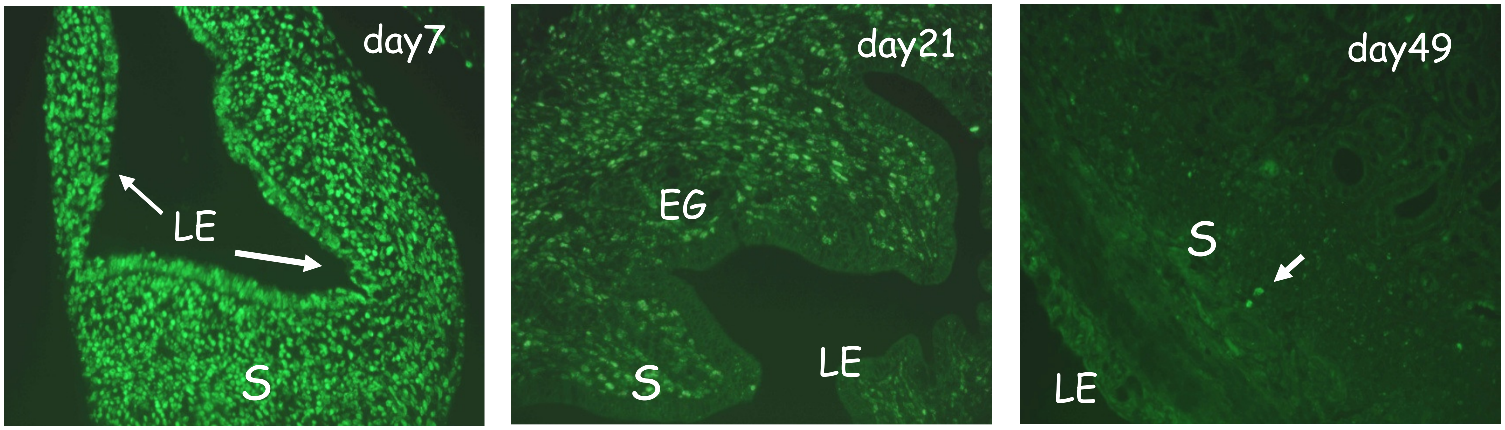
METHOD OF 5-BROME-2-DEOXYURIDINE (BrdU)

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

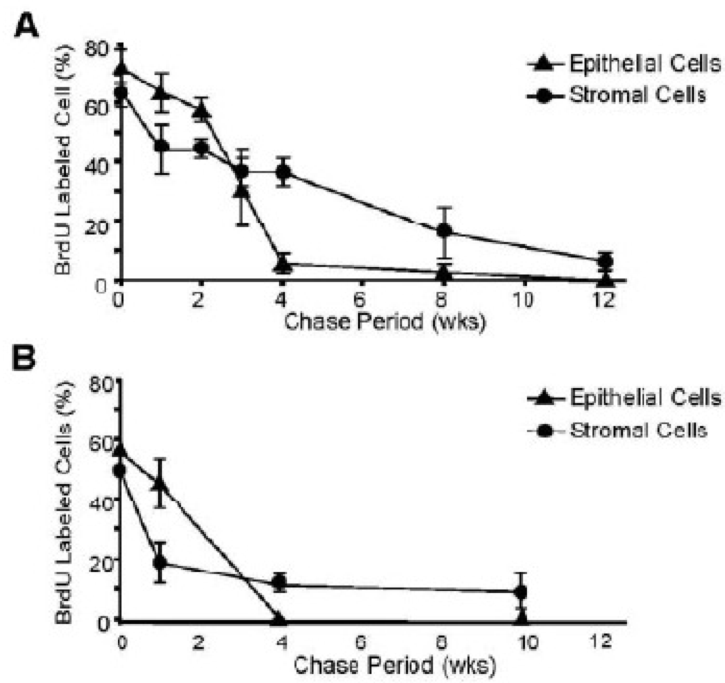


The BrdU retained cells in mice endometrium decreases with age.

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.



PUTATIVE ENDOMETRIAL STEM CELL MARKERS

	Stem Cell Marker	Endometrial localization	Reference
POU5F1	Embryonic stem cell	In humans, it co-localise 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.

• Existence of SSC in murine endometrium was demonstrated by BrdU method.

(Chan and Gargett., Stem cells, 2006; Cervelló et al., Human Rep., 2007)

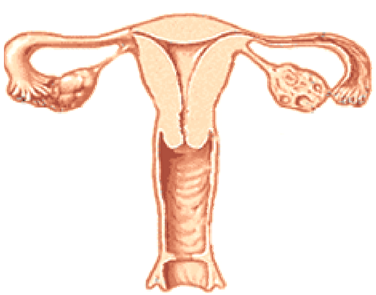
• Recent studies demonstrate that human endometrium contains a rare MSC-like population .

(Chan et al., Biol Rep., 2004; Schwab and Gargett,. Human Rep, 2007; Wolff et al., Reprod.Sci., 2007.; Gargett et al., Biol Rep.,2009)

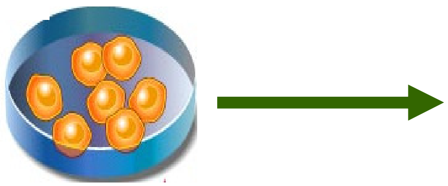
• In the menstrual blood the existence of a stem cell-like population has been demonstrated.

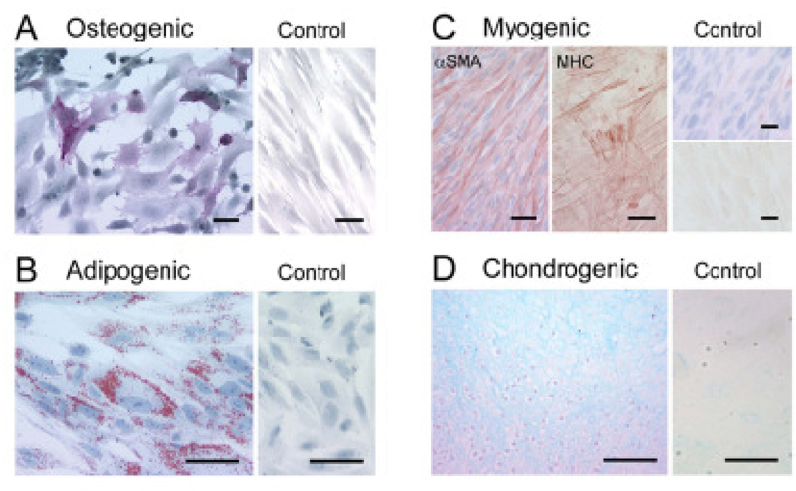
(Meng et al., J Transl Med, 2007)

• SSC have differentiated into mesoderm-derived lineages *in vitro.*



Stromal cells

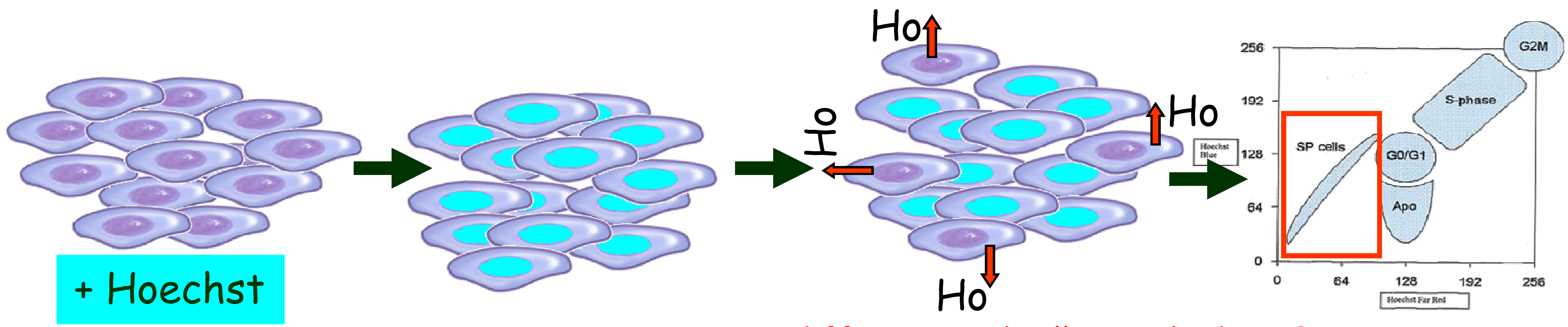




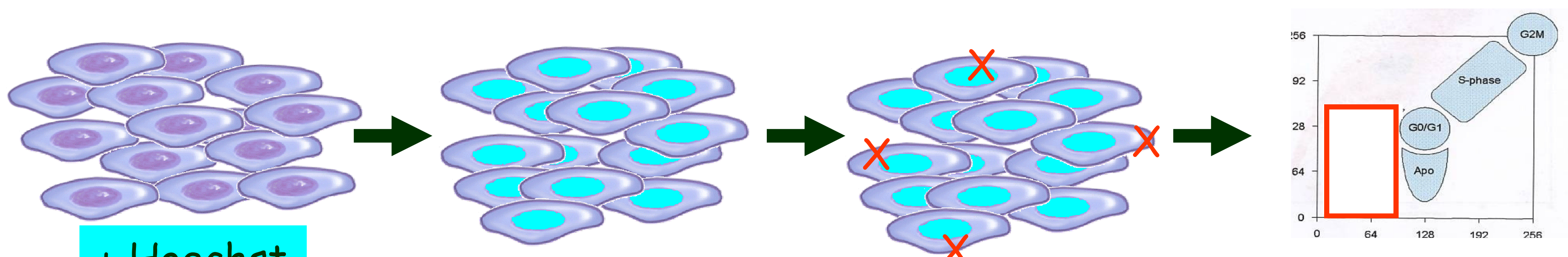
Schwab and Gargett,. Human Rep, 2007
Wolff et al., Reproductive Sciences, 2007.

ESSC IN HUMAN ENDOMETRIUM

Side Population: Hoechst Method and Cell Sorting



Undifferentiated cells enriched in ABC or MDR transporters can exclude hoechst dye



ALL THE CELLS STAINED

SIDE POPULATION METHOD

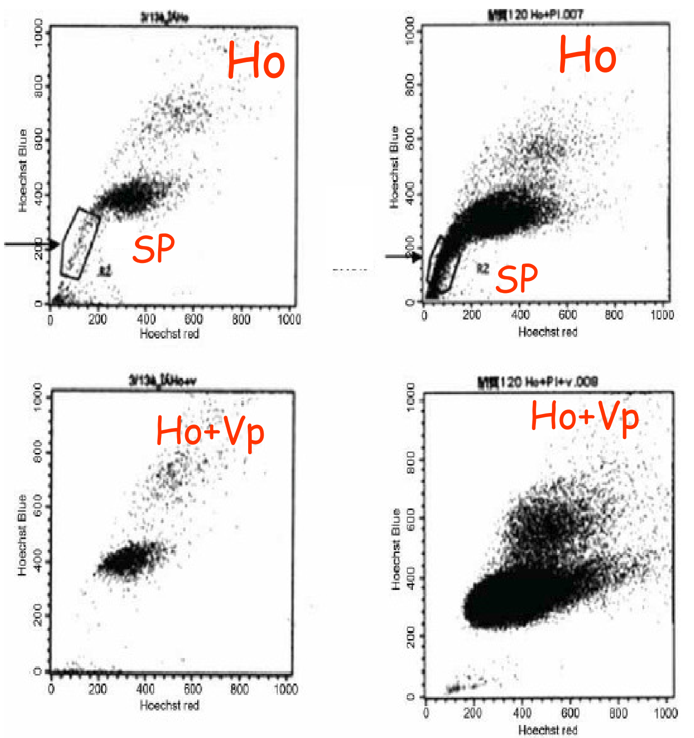
• Side Population (SP) method was described for SSC isolation in bone marrow based on the ability to efflux Hoechst33342-fluorescence dye.
(Goodell et al., J Exp Med. , 1996)

• This property is present in cells enriched in ABC transporters and has been documented in the detection of SSC in human myometrium, lung and dental pulp.
(Ono et al., PNAS, 2007; Martin et al., Cytotherapy, 2008; Iohara et al., Stem Cells, 2008)

• It has also been proposed recently in the human endometrium although not functionally demonstrated yet.
(Kato et al., Human Rep., 2007; Tsuji et al.,Fertil Steril., 2008)

ESSC IN HUMAN ENDOMETRIUM

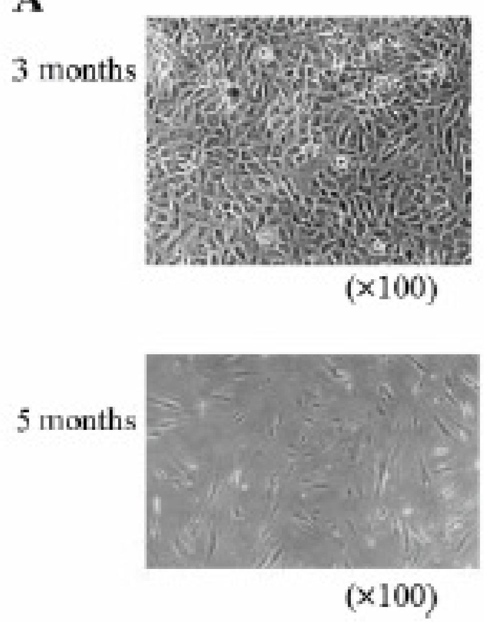
SP IN HUMAN ENDOMETRIUM
after cell culture

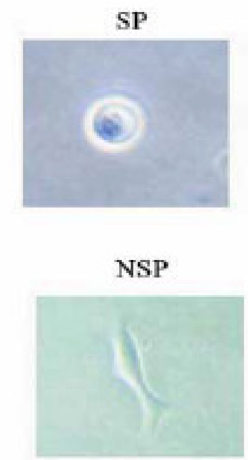


Glandular fraction Stromal fraction

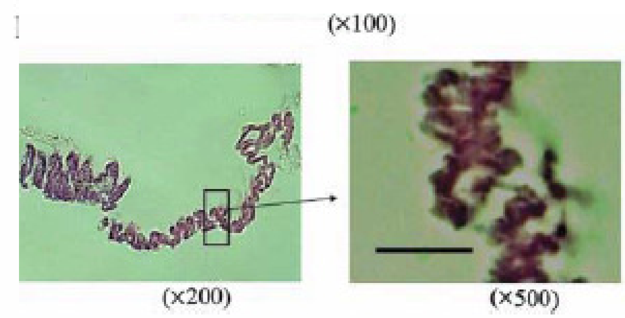
SP CELLS IN CULTURE

SP STROMAL fraction
5 months in culture





SP GLANDULAR fraction
5 months in culture

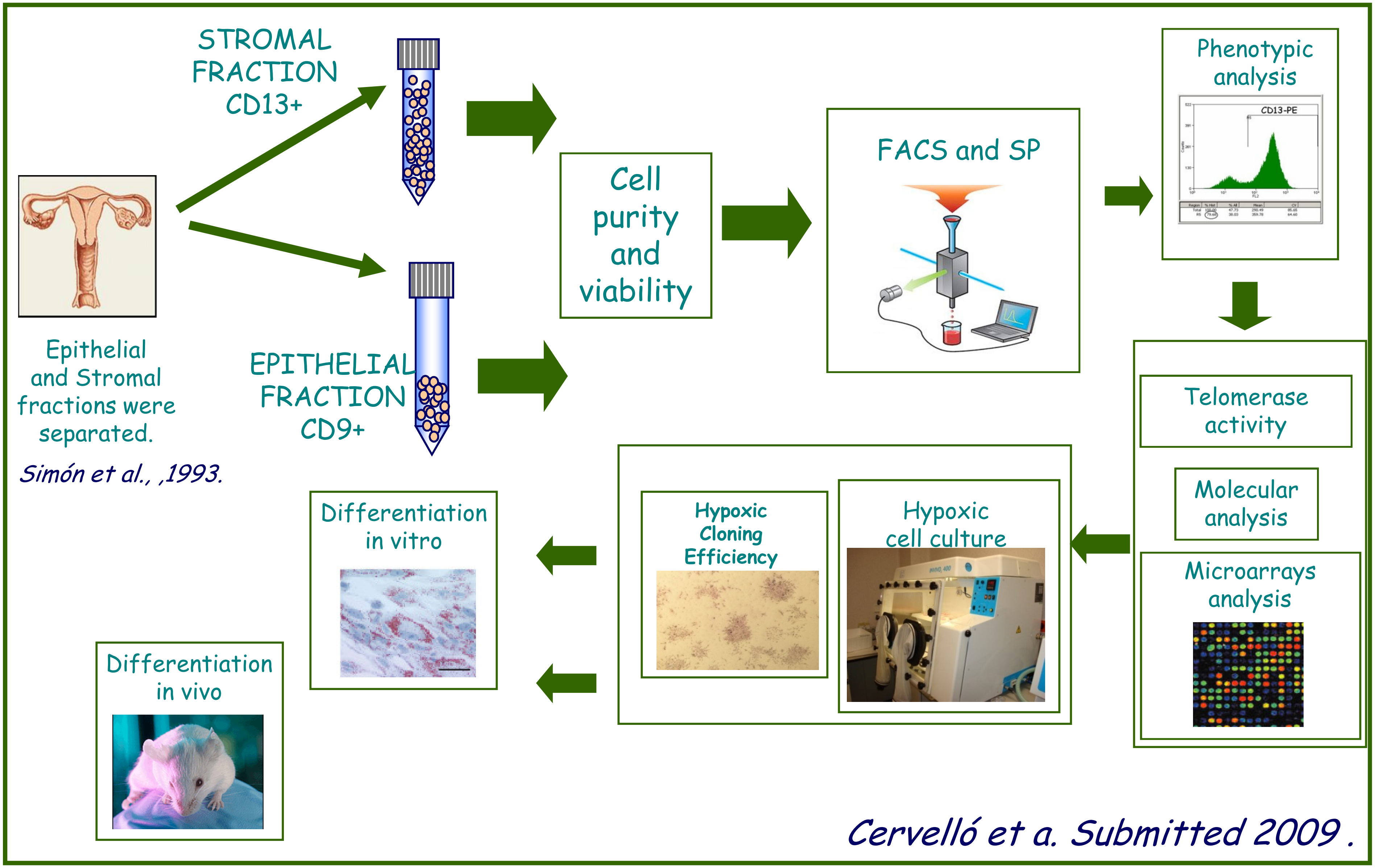


Kato et al., Human Reprod 2007.

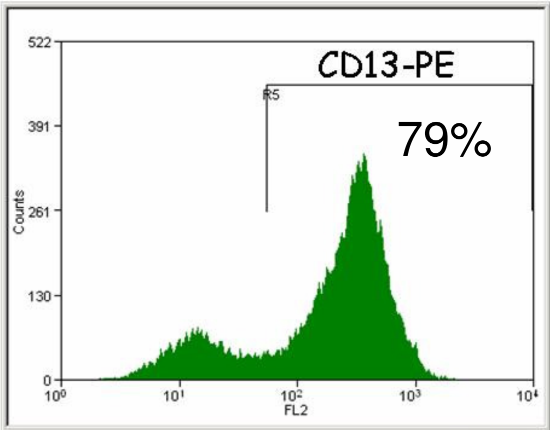
HYPOTHESIS

COULD THE SP REPRESENT THE SOMATIC STEM CELL POPULATION IN THE HUMAN ENDOMETRIUM?

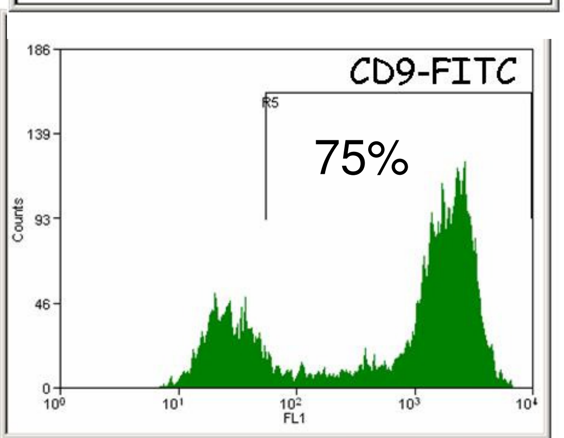
STUDY DESIGN



EPITHELIAL AND STROMAL CELL PURITY

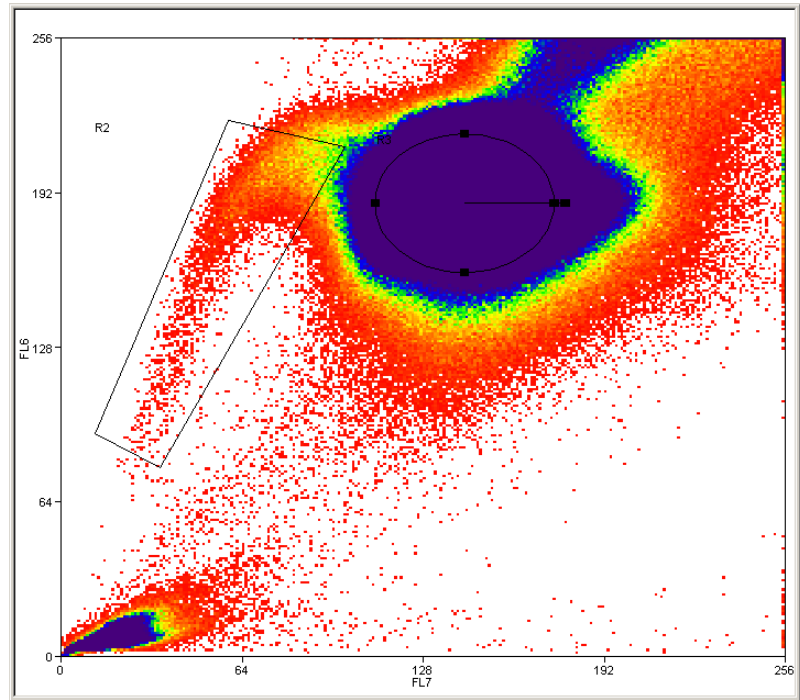


Region	% Hist	% All	Mean	CV
Total	100.00	47.73	290.49	85.65
R5	79.68	38.03	359.78	64.60



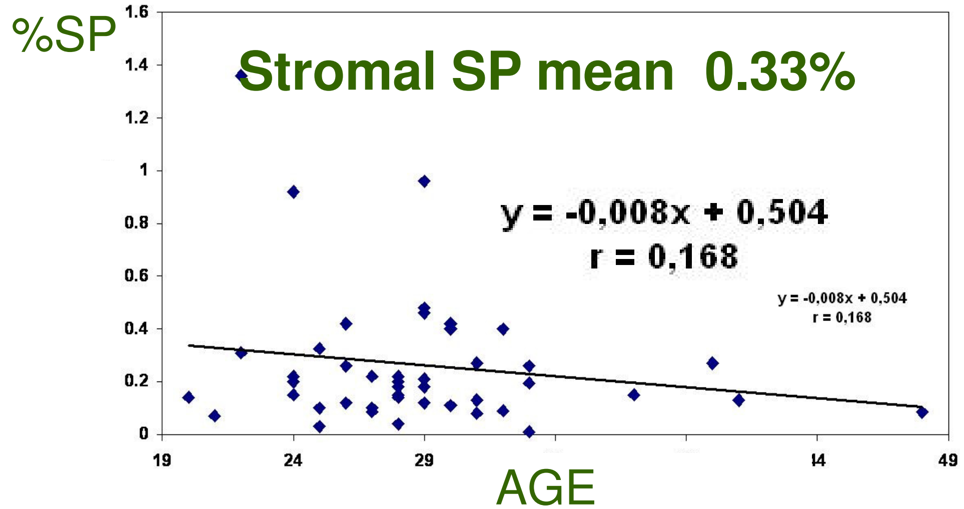
Region	% Hist	% All	Mean	CV
Total	100.00	8.12	1420.45	87.85
R5	75.89	6.16	1863.25	59.73

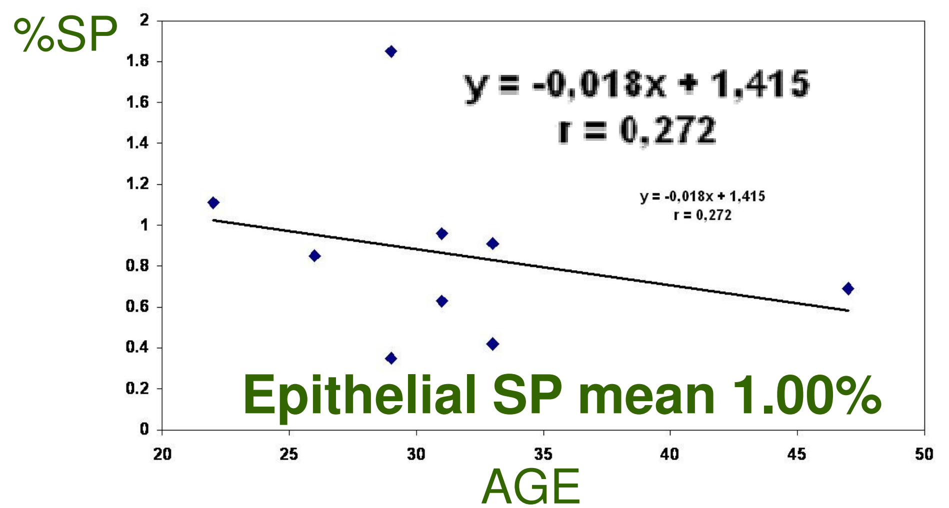
SP IN HUMAN ENDOMETRIUM



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

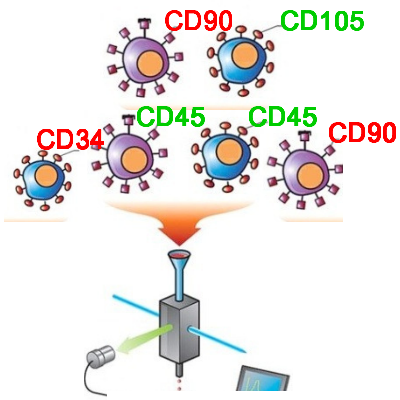
% SP during Reproductive Life





PHENOTYPIC ANALYSIS

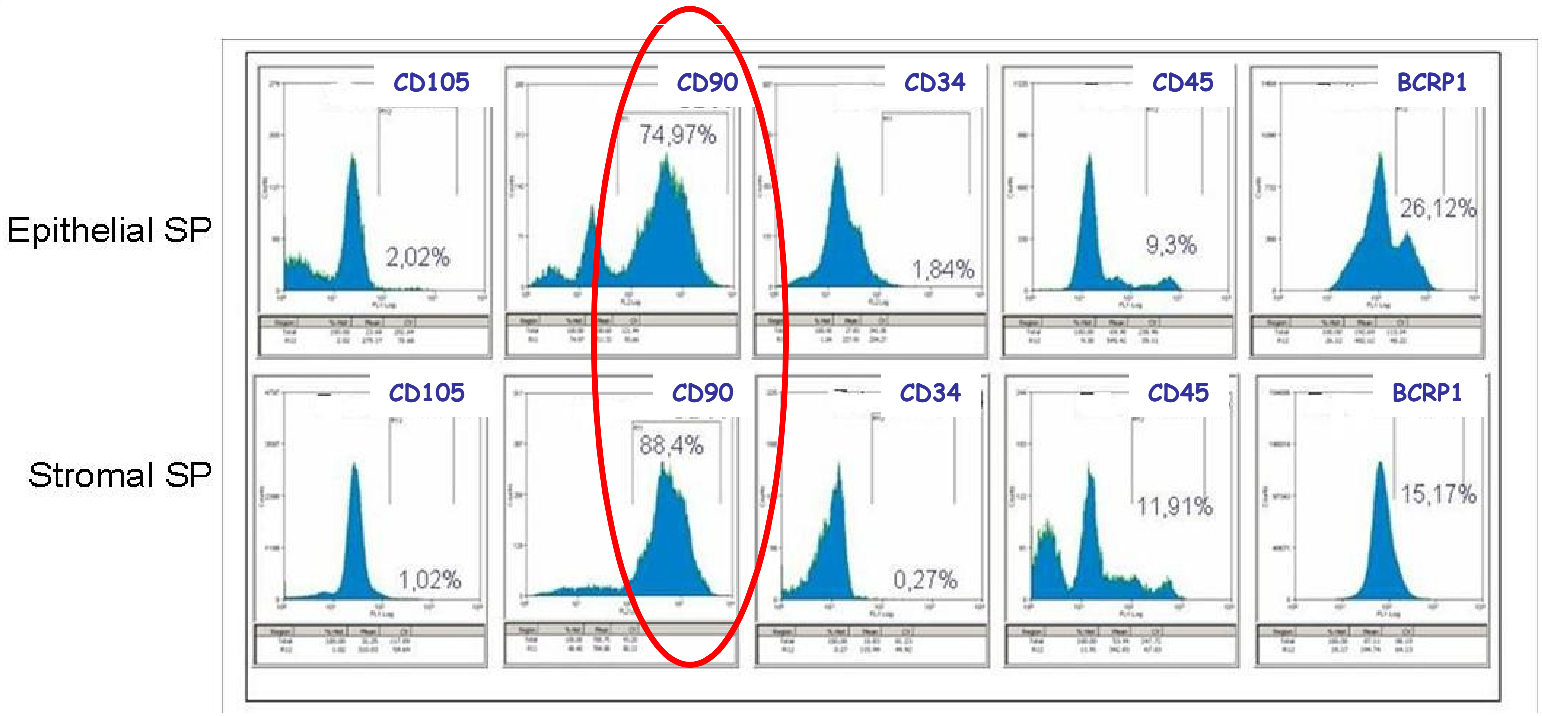
✓ Phenotypic analysis of Side Population cells:



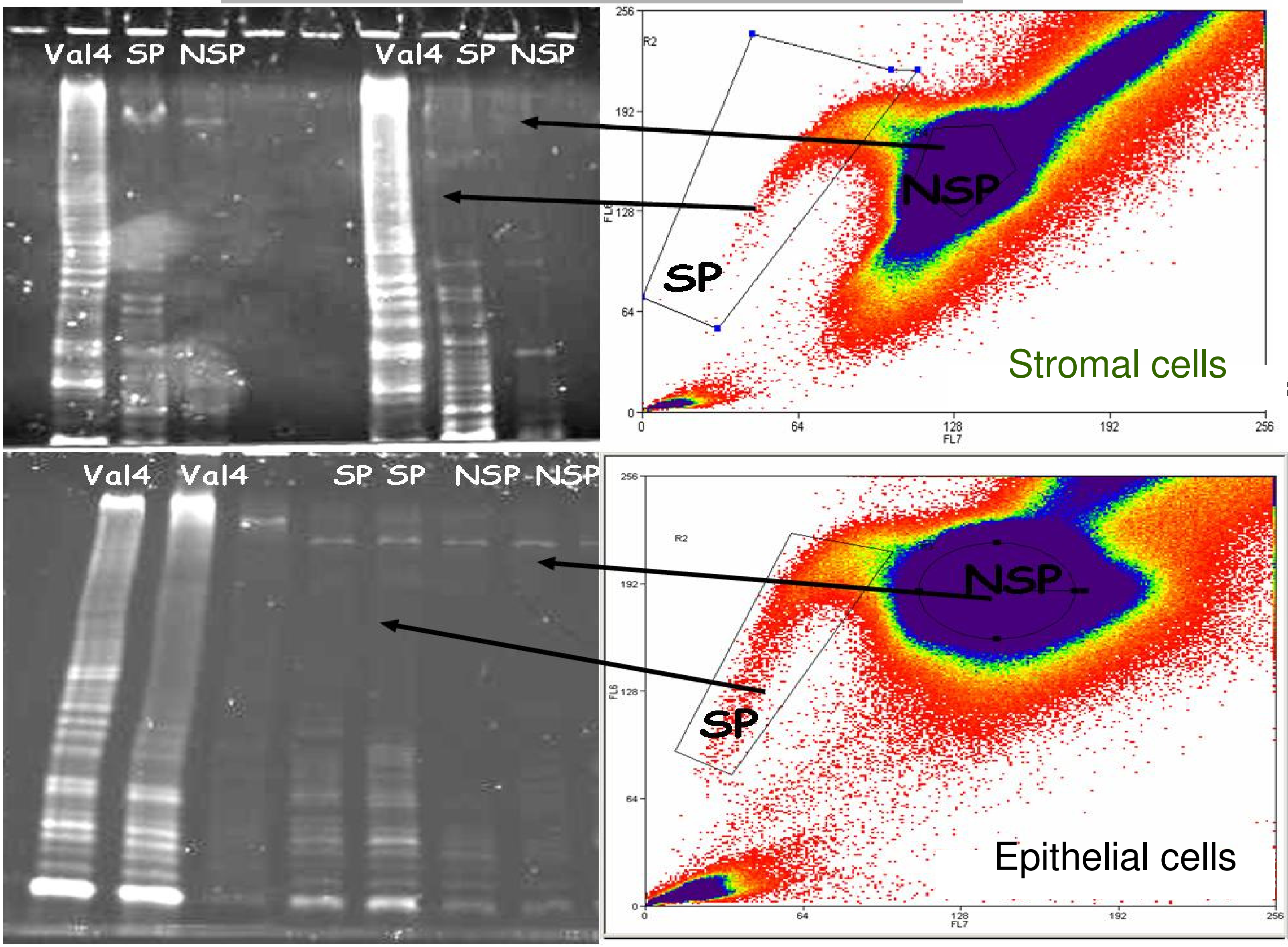
The SP cells were labeled with:

- markers associated with Hematopoietic progenitor cells like CD45-FITC and CD34-PE.

- Mesenchymal stem cells markers like CD90-PE and CD105-FITC.

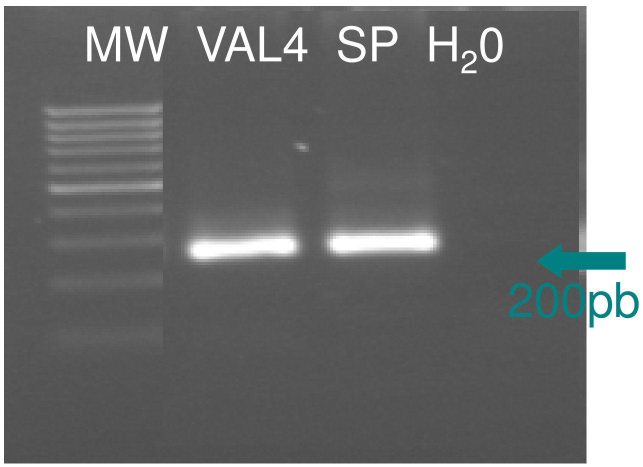


TELOMERASE ACTIVITY

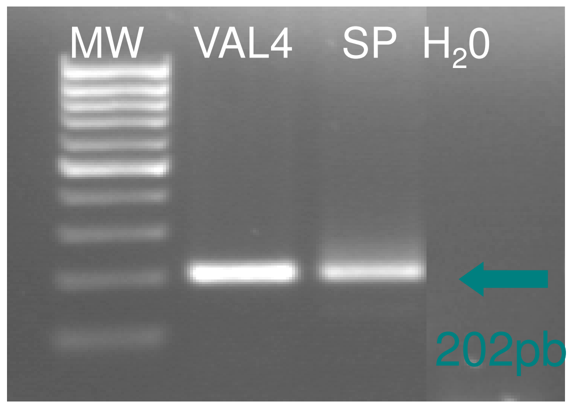


SP MOLECULAR ANALYSIS

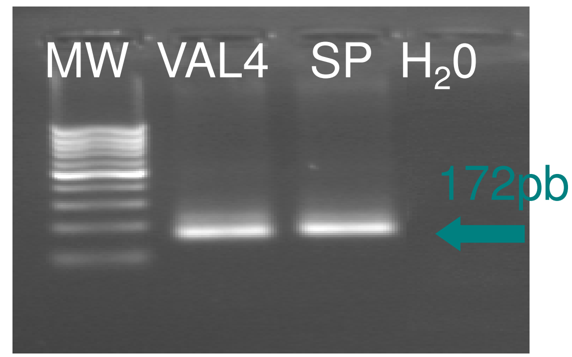
OCT-4



c-Kit

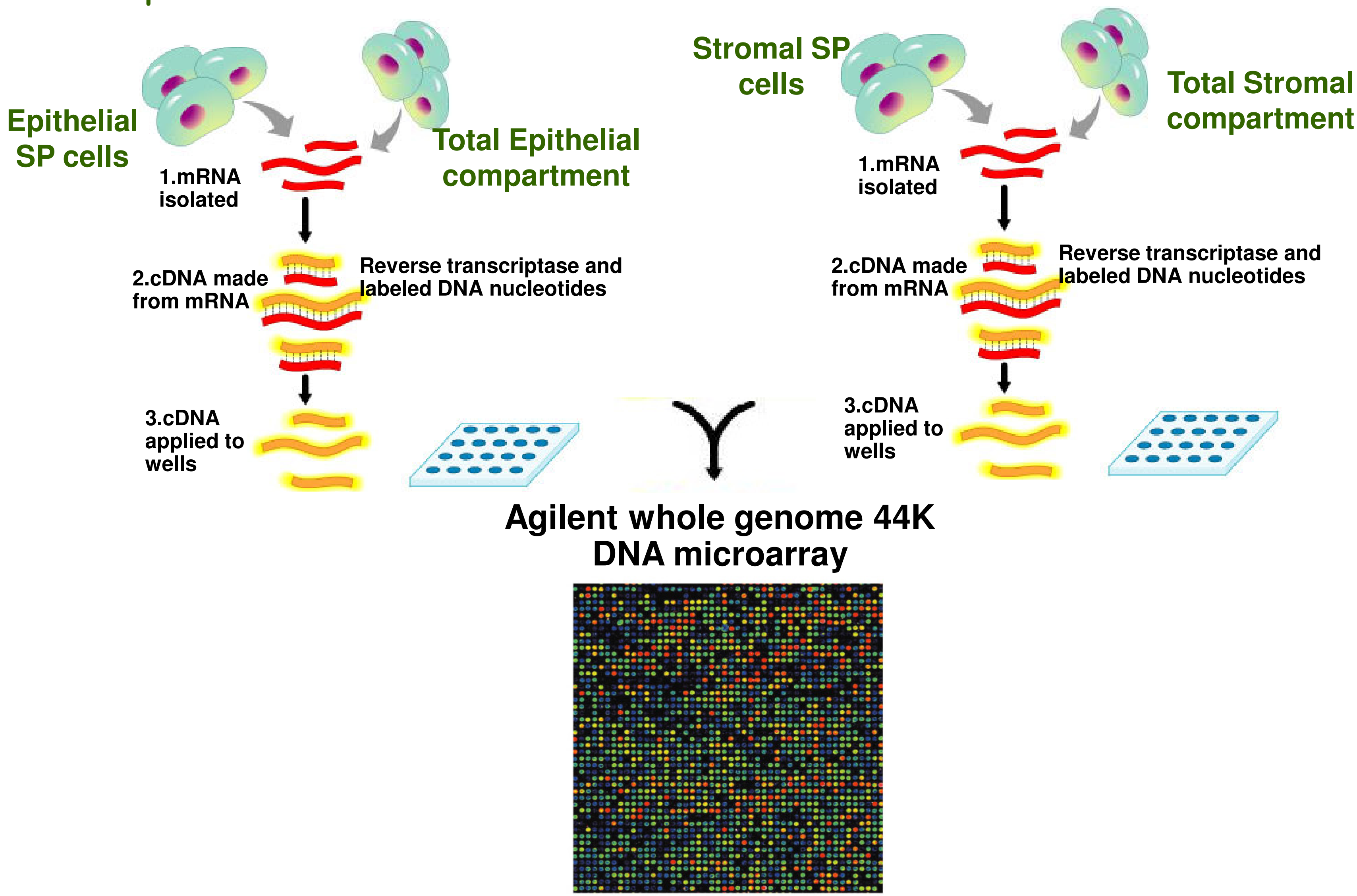


BCRP1



MICROARRAYS ANALYSIS

✓ Microarrays analysis of Epithelial and Stromal Side Population versus total compartments:



MICROARRAYS ANALYSIS

TOP TEN MOLECULES SP stromal cells

Molecules	Exp. Value
MMP3	↑6.532
RND3	↑6.412
SERPINB2	↑6.275
SLC4A1	↑6.026
ANGPTL4	↑5.820
INHBA	↑5.674
IER3	↑5.578
KRT34	↑5.502
GDF15	↑5.367
ADM	↑5.324

Molecules	Exp. Value
VWF	↓-4.331
SCGB1D2	↓-4.321
SERPINA5*	↓-4.185
ASRGL1*	↓-3.589
SOX17	↓-3.567
SCGB2A1	↓-3.169
HGD	↓-3.105
ACSL5	↓-3.105
TPD52L1	↓-3.085
ST6GALNAC1	↓-3.083

SP stromal cells vs Stromal cells:
121 genes up-regulated
74 genes down-regulated

Molecular and cellular Functions:
-Cell signalling and interaction
-Cellular growth and proliferation
-Cellular movement
-Cell death
-Cell cycle

TOP TEN MOLECULES SP epithelial cells

Molecules	Exp. Value
IL1B	↑19.823
CXCL1	↑19.069
HSPA6	↑16.887
TUBA4A	↑13.661
CCL4	↑13.402
POLR2J2	↑13.321
CACNG5	↑13.281
GDF15	↑12.487
CD69	↑11.971
RGS1	↑11.604

Molecules	Exp. Value
FOXE1	↓-12.402
PCSK1N	↓-11.151
POU3F3	↓-9.126
NEUROG1	↓-8.531
SYNPO	↓-7.969
CACNA1E	↓-7.794
CRHR2	↓-7.654
IER5*	↓-7.654
NEUROG3	↓-7.593
SYN1	↓-7.002

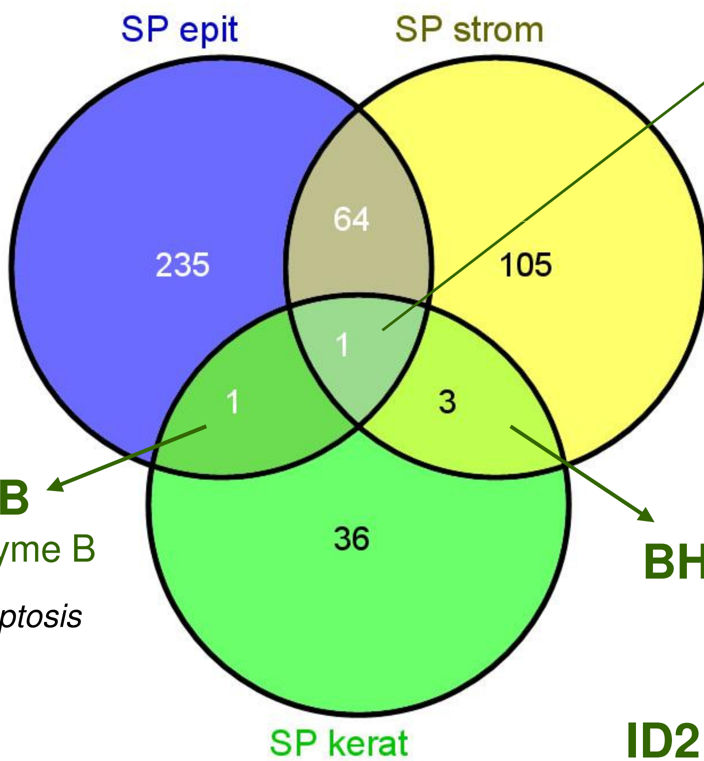
SP epit cells vs Epithelial cells:
196 genes up-regulated
116 genes down-regulated

Molecular and cellular Functions:
-Cell signalling and interaction
-Cellular growth and proliferation
-Cellular movement
-Cell death

MICROARRAYS ANALYSIS

✓Microarrays comparison with keratynocites SP:

Larderet et al., Keratynocites. Stem cells. 2006.



DUSP5
Dual specific protein phosphatase 5
Displays phosphatase activity.

INSIG1 *Regulatory role during G0/G1 transition phase.*
Insuline induced gene 1 protein

GZMB
Granzyme B
Responsible for apoptosis

BHLHB2 Class B basic helix loop helix protein
Transcriptional factor

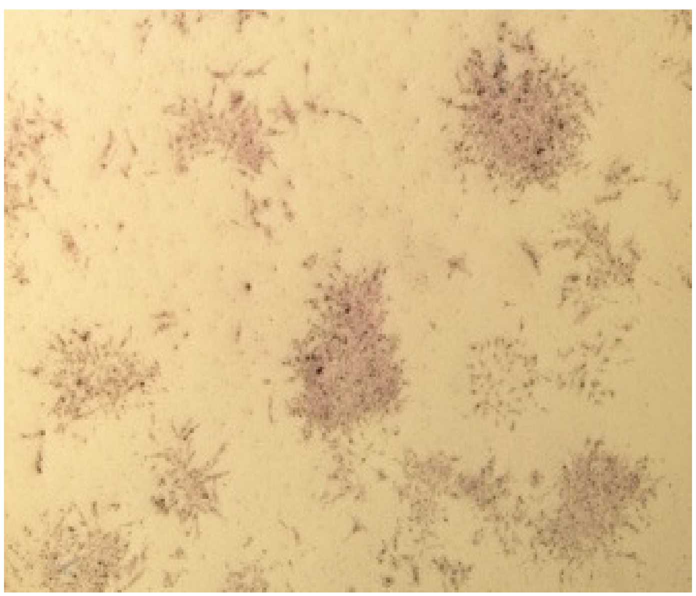
ID2 DNA binding protein inhibitor 2
Inhibitor of tissue-specific gene expression.

CLONING OF SP CELLS IN HYPOXIC CONDITION

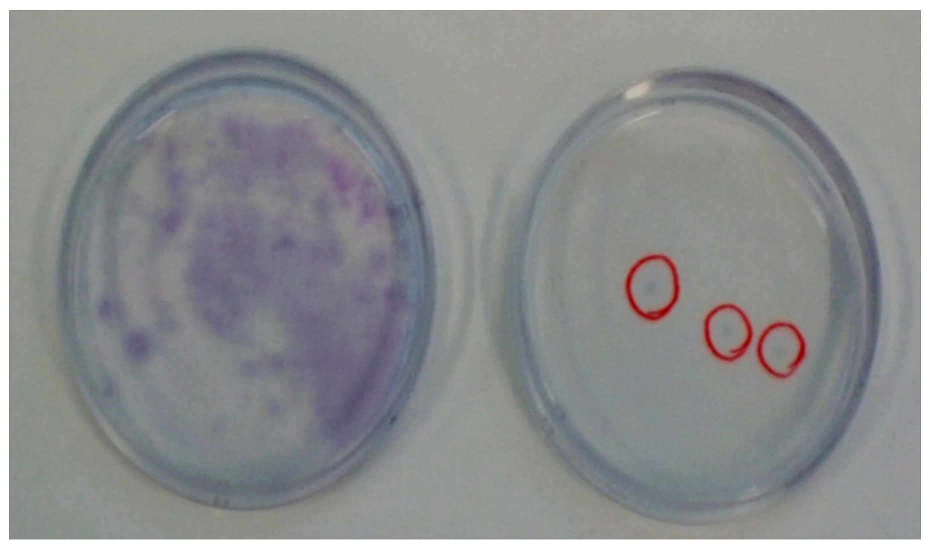
✓ Cell culture conditions: 2% O_2, 37°C, 5% CO_2, 90 % humidity:

300-500 cells/cm² were seeded into 60-mm Petri dishes coated with gelatine 0.1%, cultured in serum medium, incubated for **15 days** and stained with 0.5%Toluidine Blue for 5 min.

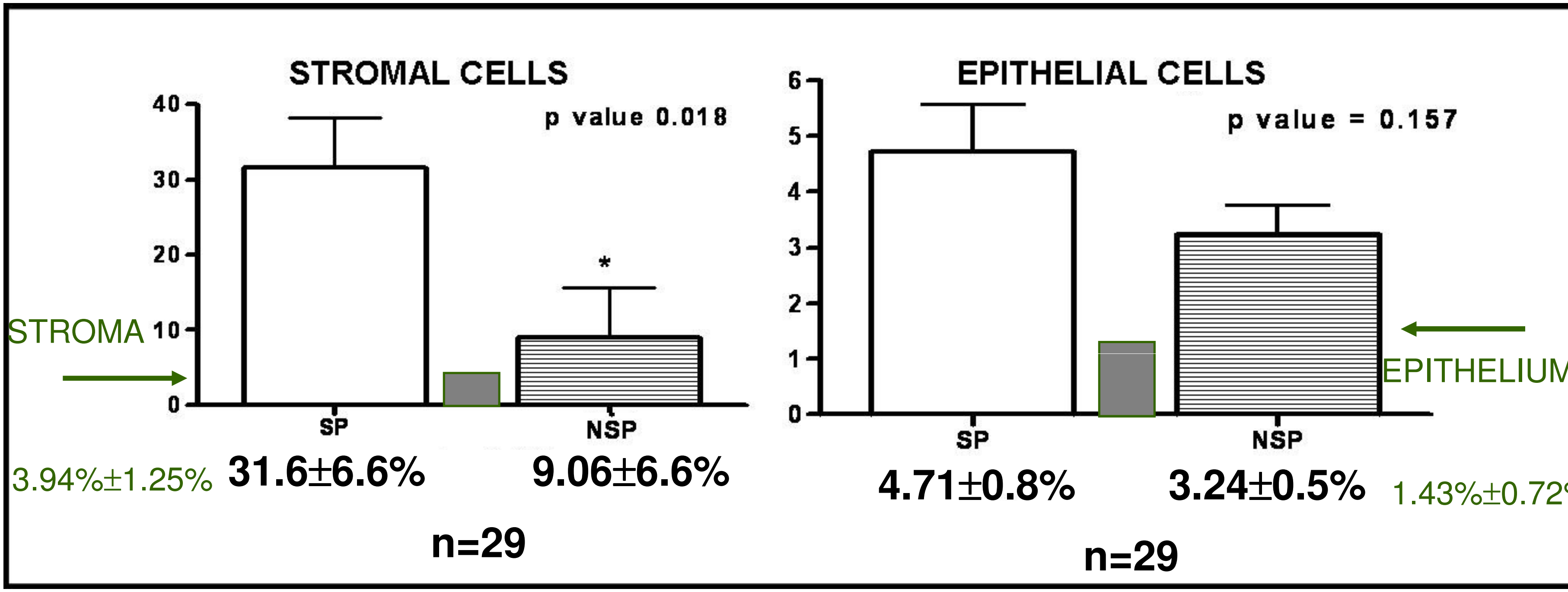
CLONING EFFICIENCY CE (%) = (Number of colonies/number of cells seeded) x 100.



SP CELLS NSP CELLS

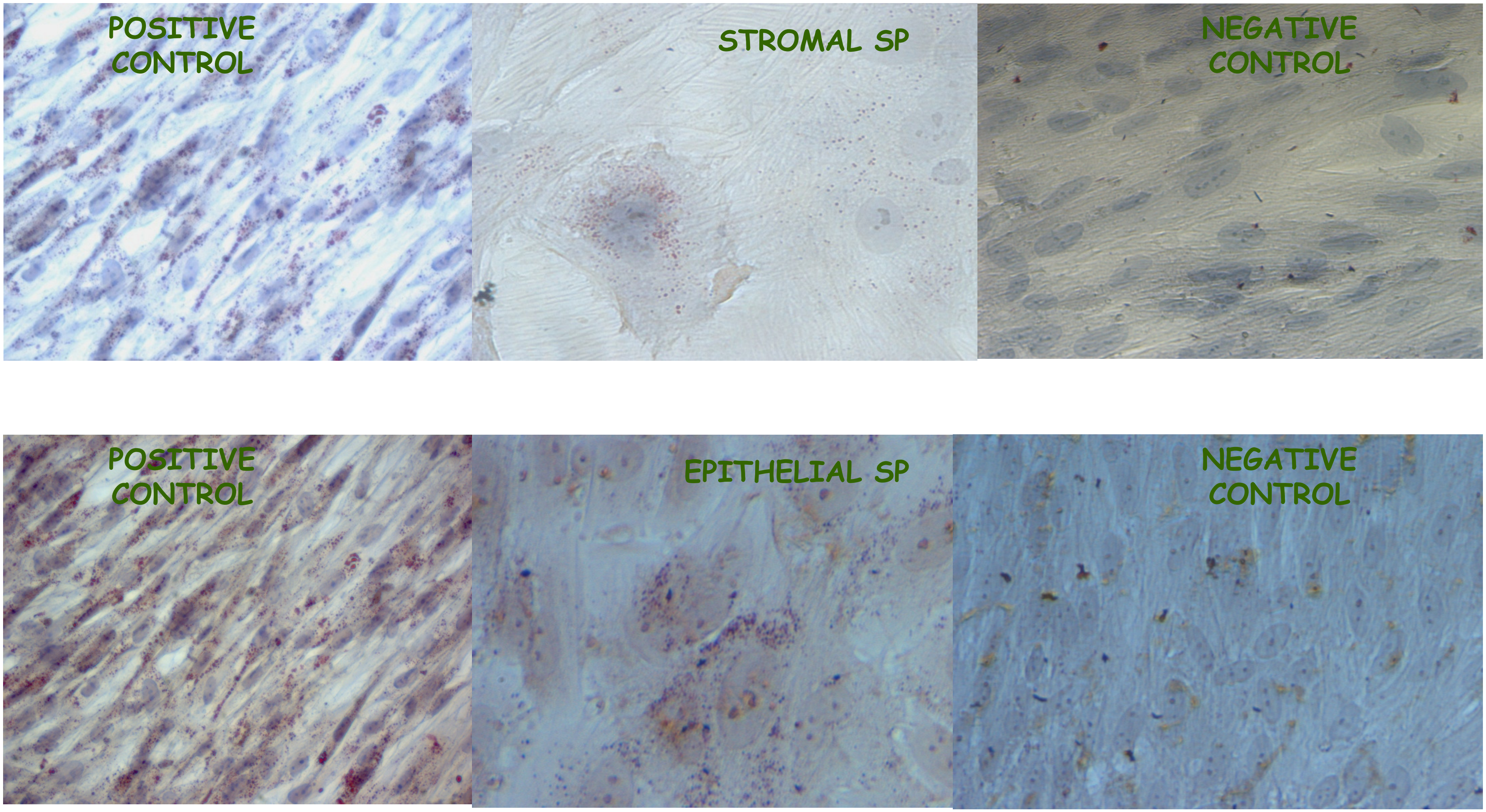


CLONING EFFICIENCY



DIFFERENTIATION IN VITRO

✓ Functional proof of concept in vitro inducing adipocyte differentiation:



IN VIVO DIFFERENTIATION

✓ Functional proof of concept in vivo using NOD-SCID female mice:

Endometrial biopsy → Mechanic and Enzymatic Digestion → SP cells → Cell Cloning Culture → Subcutaneous injection of 100.000 SP cells

+pellet E_2

↓

Tissue growth

↓

Laser Capture and PCR

Morphologycal Analysis & Demonstration of Human Origin

PCR in order to detect human housekeeping versus mouse housekeeping genes. Primers used according to *Flasza et al., Cloning and Stem Cells, 2006.*

IN VIVO DIFFERENTIATION

• mRNA human (79 pb)

CTCTCTGCTCCTCCTGTTCGACAGTCAGCCGCATCTTCTTTTGCGTCGCCAGCCGAGCCACATCGCTCAGACACCATGGGGAAGGTGAAGGTCG
GAGTCAACGGATTTGGTCGTATTGGGCGCCTGGTCACCAGGGCTGCTTTTAACTCTGGTAAAGTGGATATTGTTGCCATCAATGACCCCTTCATTG
ACCTCAACTACATGGTTTACATGTTCCAATATGATTCCACCCATGGCAAATTCCATGGCACCGTCAAGGCTGAGAACGGGAAGCTTGTCATCAATG
GAAATCCCATCACCATCTTCCAGGAGCGAGATCCCTCCAAAATCAAGTGGGGCGATGCTGGCGCTGAGTACGTCGTGGAGTCCACTGGCGTCTT
CACCACCATGGAGAAGGCTGGGGCTCATTTGCAGGGGGGAGCCAAAAGGGTCATCATCTCTGCCCCCTCTGCTGATGCCCCCATGTTCGTCATG
GGTGTGAACCATGAGAAGTATGACAACAGCCTCAAGATCATCAGCAATGCCTCCTGCACCACCAACTGCTTAGCACCCCTGGCCAAGGTCATCCA
TGACAACTTTGGTATCGTGGAAGGACTCATGACCACAGTCCATGCCATCACTGCCACCCAGAAGACTGTGGATGGCCCCTCCGGGAAACTGTGG
CGTGATGGCCGCGGGGCTCTCCAGAACATCATCCCTGCCTCTACTGGCGCTGCCAAGGCTGTGGGCAAGGTCATCCCTGAGCTGAACGGGAAG
CTCACTGGCATGGCCTTCCGTGTCCCCACTGCCAACGTGTCAGTGGTGGACCTGACCTGCCGTCTAGAAAAACCTGCCAAATATGATGACATCAA
GAAGGTGGTGAAGCAGGCGTCGGAGGGCCCCCTCAAGGGCATCCTGGGCTACACTGAGCACCAGGTGGTCTCCTCTGACTTCAACAGCGACAC
CCACTCCTCCACCTTTGACGCTGGGGCTGGCATTGCCCTCAACGACCACTTTGTCAAGCTCATTTCCTGGTATGACAACGAATTTGGCTACAGCA
ACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGAC
CCTCACTGCTGGGGAGTCCCTGCCACACTCAGTCCCCCACCACACTGAATCTCCCCTCCTCACAGTTGCCATGTAGACCCCTTGAAGAGGGGAG
GGGCCTAGGGAGCCGCACCTTGTCATGTACCATCAATAAAGTACCCTGTGCTCAACC

• mRNA mice (75 pb)

AGAGACGGCCGCATCTTCTTGTGCAGTGCCAGCCTCGTCCCGTAGACAAAATGGTGAAGGTCGGTGTGAACGGATTTGGCCGTATTGGGCGCCTGGT
CACCAGGGCTGCCATTTGCAGTGGCAAAGTGGAGATTGTTGCCATCAACGACCCCTTCATTGACCTCAACTACATGGTCTACATGTTCCAGTATGACTC
CACTCACGGCAAATTCAACGGCACAGTCAAGGCCGAGAATGGGAAGCTTGTCATCAACGGGAAGCCCATCACCATCTTCCAGGAGCGAGACCCCACTA
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CTGCCACCCAGAAGACTGTGGATGGCCCCTCTGGAAAGCTGTGGCGTGATGGCCGTGGGGCTGCCCAGAACATCATCCCTGCATCCACTGGTGCTGC
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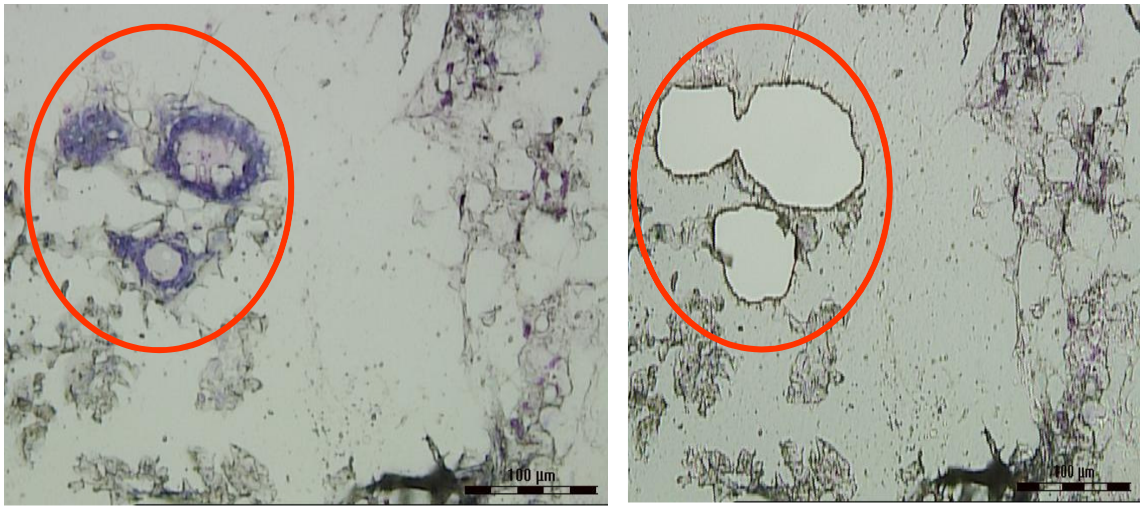
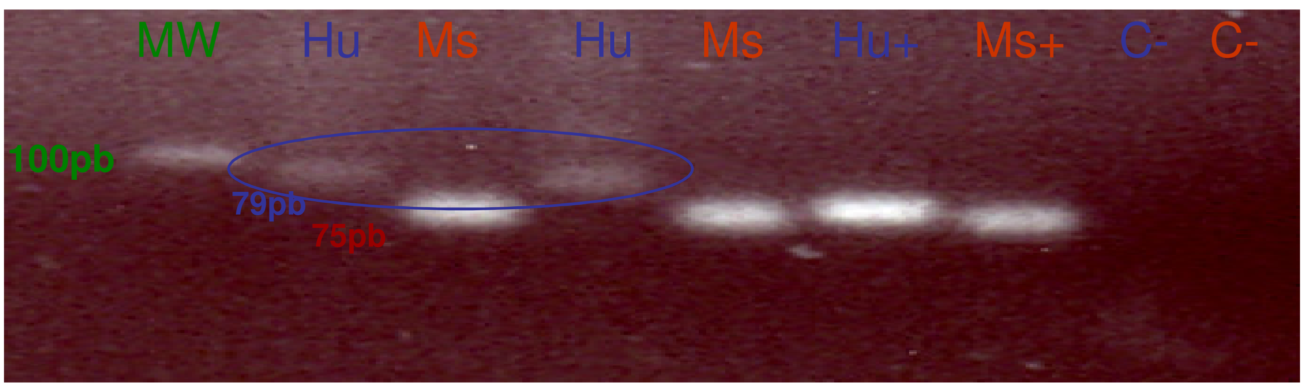
GGGCCTAGGGAGCCCTCCCTACTCTCTTGAATACCATCAATAAAGTTCGCTGCACCCACAAAAAAAAAAAAAAAAAAAAAA

Flasza et al., Cloning and Stem Cells, 2006.

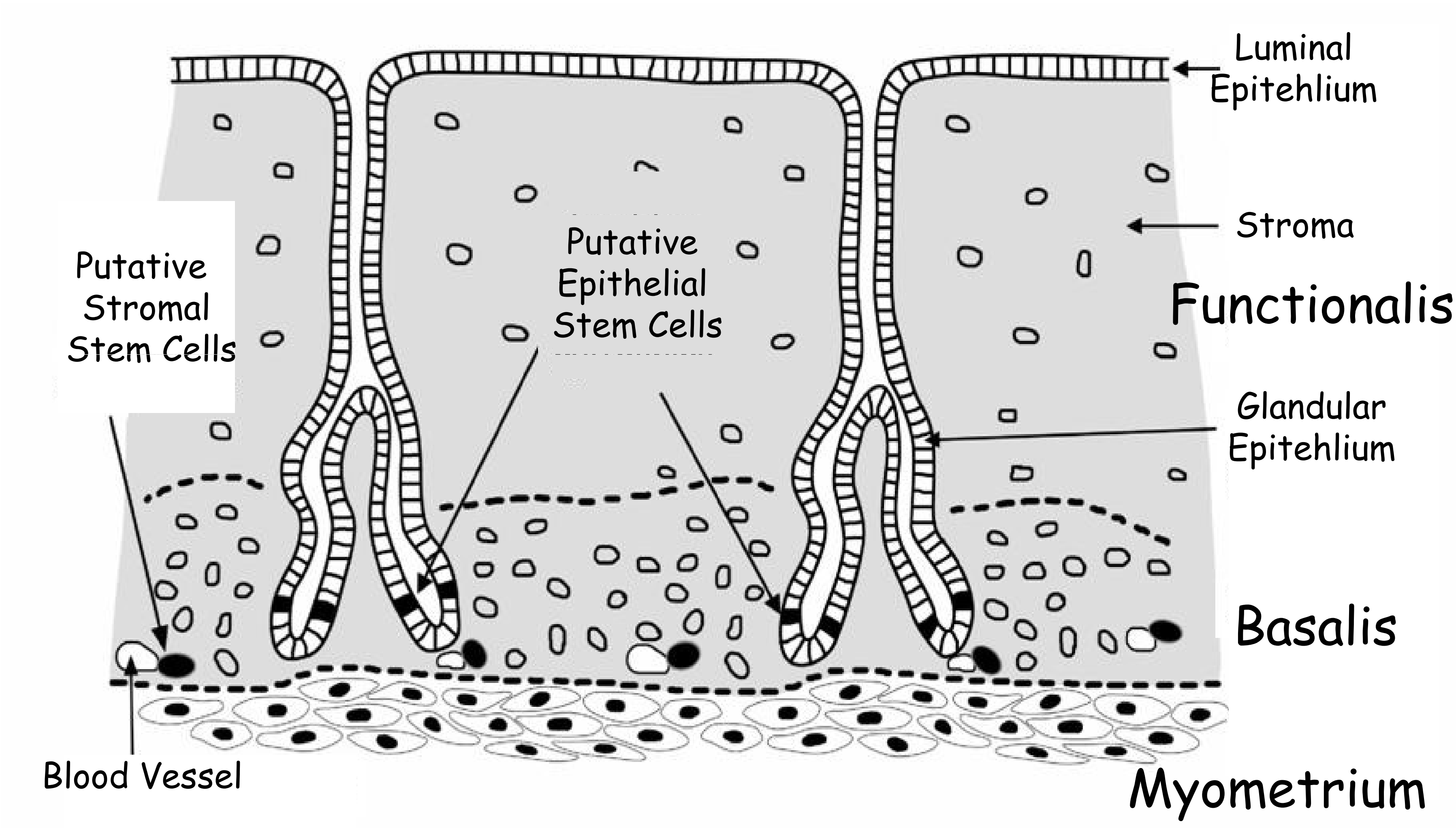
IN VIVO DIFFERENTIATION





Hu: putative human glands
Ms: mice glands.
Hu+: human endometrium
Ms+: mice endomtrium
C-: water

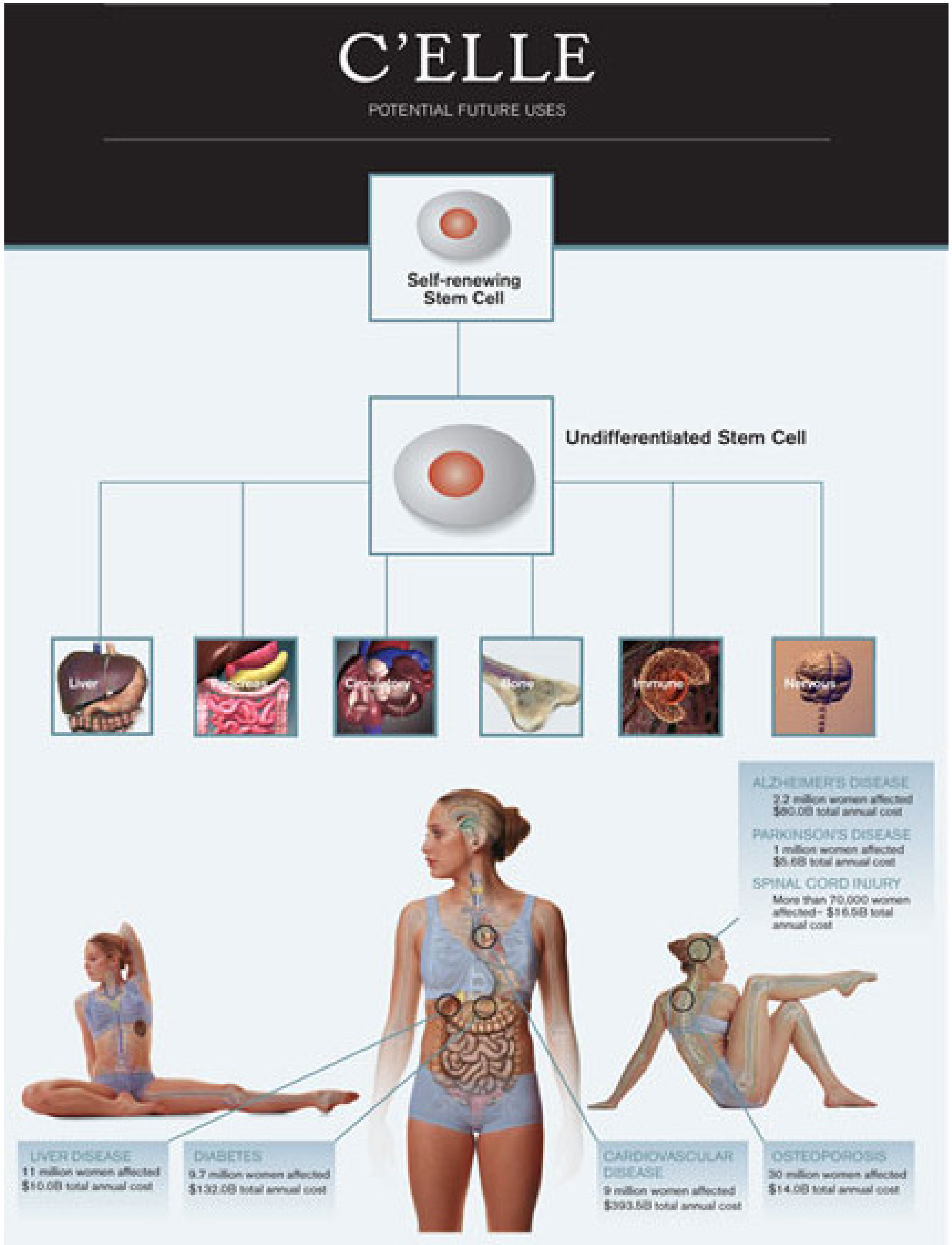
ADULT STEM CELLS IN HUMAN ENDOMETRIUM



EVERY MONTH HOLDS A MIRACLE

C'ELLE IS AVAILABLE NOW! TO START COLLECTING AND PRESERVING YOUR BODY'S PRECIOUS STEM CELLS, PLEASE CLICK THE BUTTON BELOW.


C'ELLE
ColorPack


C'ELLE
POTENTIAL FUTURE USES
Self-renewing Stem Cell
Undifferentiated Stem Cell
Liver
Pancreas
Circulatory
Bone
Immune
Nervous
ALZHEIMER'S DISEASE
2.2 million women affected
$80.0B total annual cost
PARKINSON'S DISEASE
1 million women affected
$5.6B total annual cost
SPINAL CORD INJURY
More than 70,000 women affected– $16.5B total annual cost
LIVER DISEASE
11 million women affected
$10.0B total annual cost
DIABETES
9.7 million women affected
$132.0B total annual cost
CARDIOVASCULAR DISEASE
9 million women affected
$393.5B total annual cost
OSTEOPOROSIS
30 million women affected
$14.0B total annual cost

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

Masanori Ono*, Tetsuo Maruyama*†, Hirotaka Masuda*, Takashi Kajitani*, Takashi Nagashima*, Toru Arase*, Mamoru Ito‡, Kuniaki Ohta*, Hiroshi Uchida*, Hironori Asada*, Yasunori Yoshimura*, Hideyuki Okano§, and Yumi Matsuzaki†§

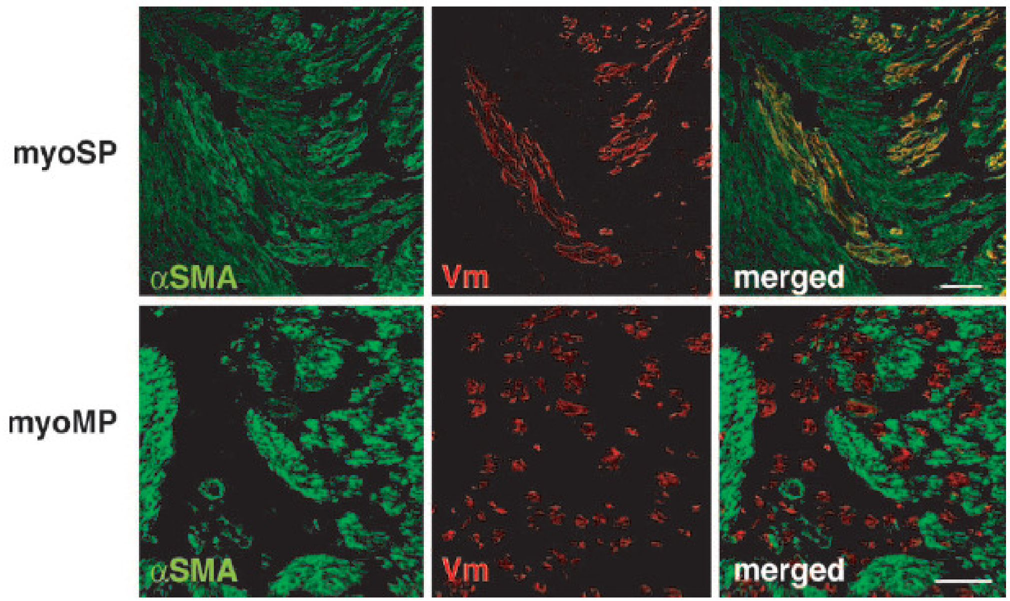
Departments of *Obstetrics and Gynecology and §Physiology, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan; and ‡Central Institute for Experimental Animals, Kawasaki, Kanagawa 216-0001, Japan

• In vivo FORMATION OF MYOMETRIUM

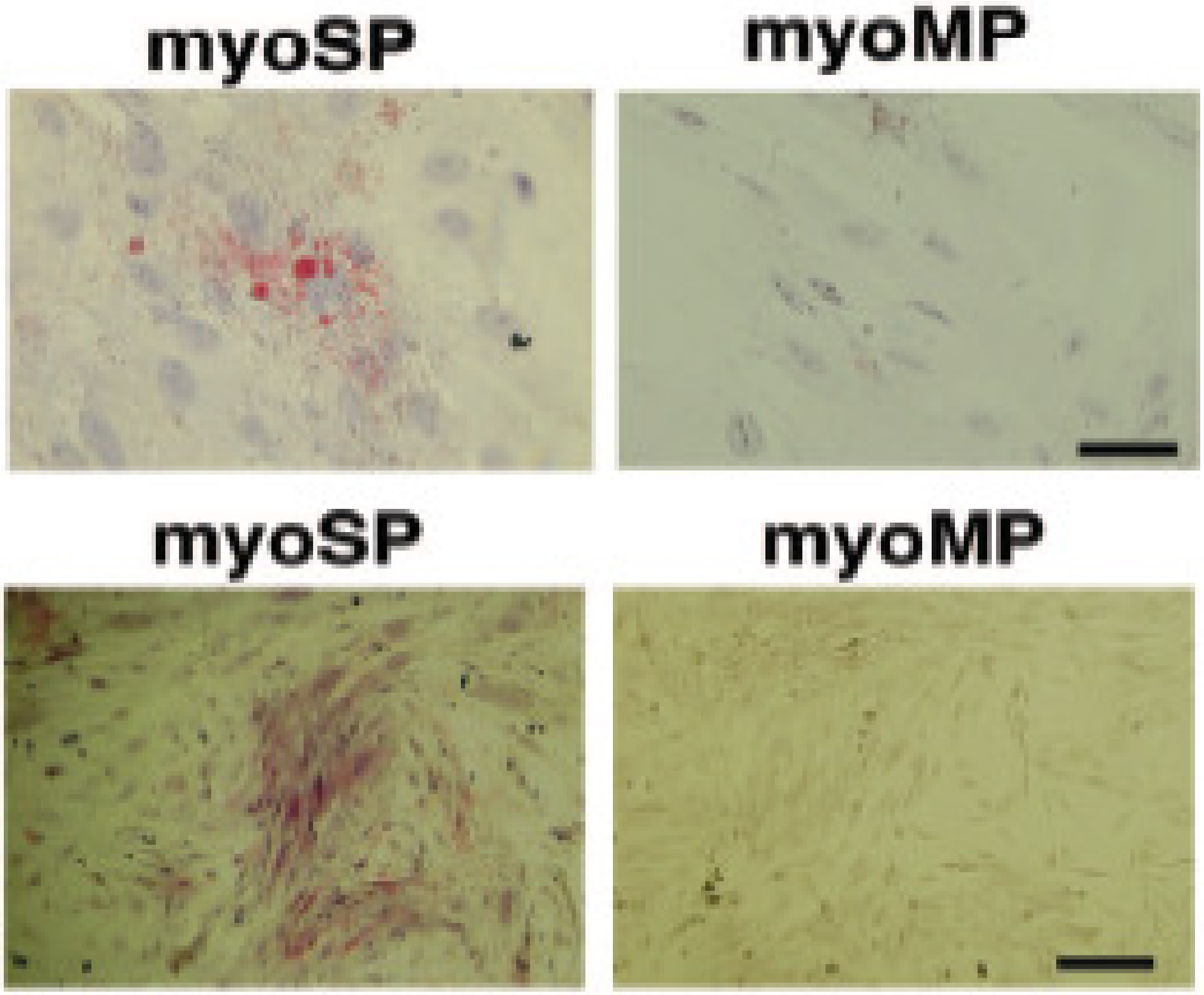
100.000 Myo SP cels por horn
100.000 Myo NSP cels por horn

+SMA hum

+Vim hum
10/16



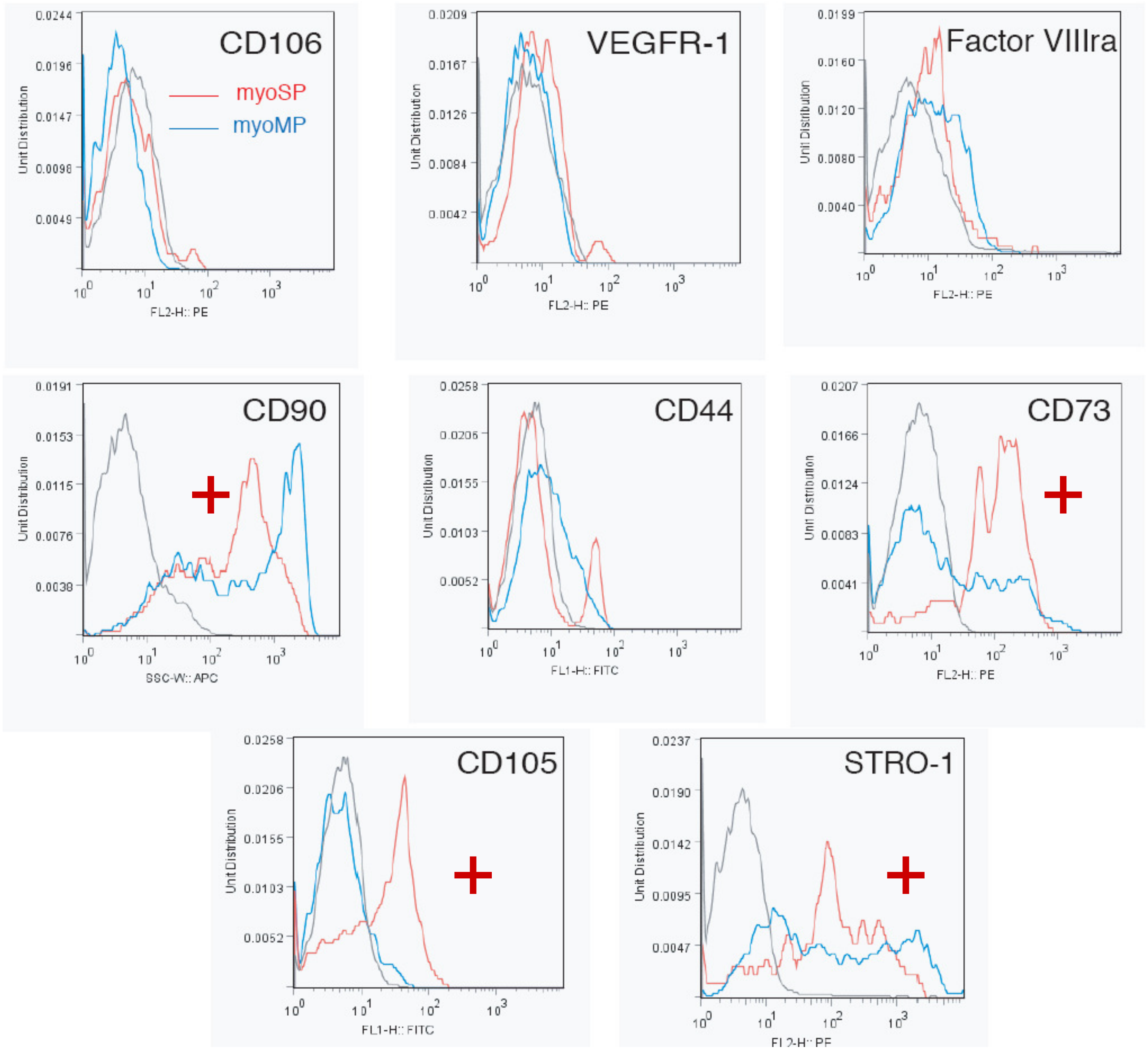
In vitro differentiation



ADIPOCITES

OSTEOCITES

PHENOTYPE


CD106
myoSP
myoMP
VEGFR-1
Factor VIIIra
CD90
CD44
CD73
CD105
STRO-1
+
+
+
+
Unit Distribution
FL2-H:: PE
SSC-W:: APC
FL1-H:: FITC

CONCLUSIONS

- SP account for 0.3% and 1% of the stromal and epithelial compartment respectively. Their percentages remain constant during reproductive life.
- Phenotype of SP suggest a mesenchymal origin.
- They display an intermediate pattern of telomerase activity, and are positive for c-Kit, Oct-4 and BCRP-1
- Wide genome analysis demonstrated a differential gene expression profile of SP compared to its endometrial fraction. A common SP signature is suggested.
- SP cells do not growth in normoxic conditions. In hypoxic conditions, SP cells display high cloning efficiency compared to NSP and total fraction.
- Stromal and epithelial SP have been differentiated in vitro to adipocytes, osteocytes and condrocytes.
- The functional proof of concept is given by the ability of SP cells to reconstruct the human endometrium in an animal model.

Claudia Gil and Aymara Mas.

fundación IVI

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

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