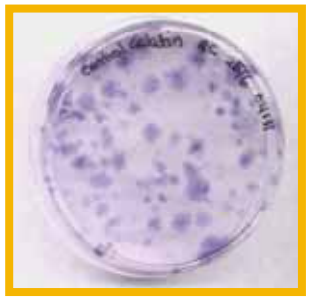
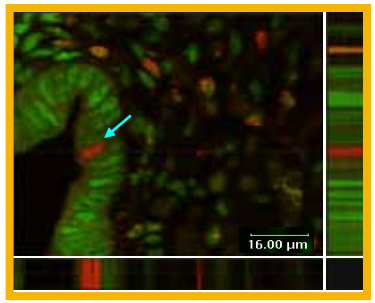
Evidence for Endometrial Stem/Progenitor cells





Caroline E Gargett

The Ritchie Centre
Monash Institute of Medical Research
Monash University Department Obstetrics and Gynaecology

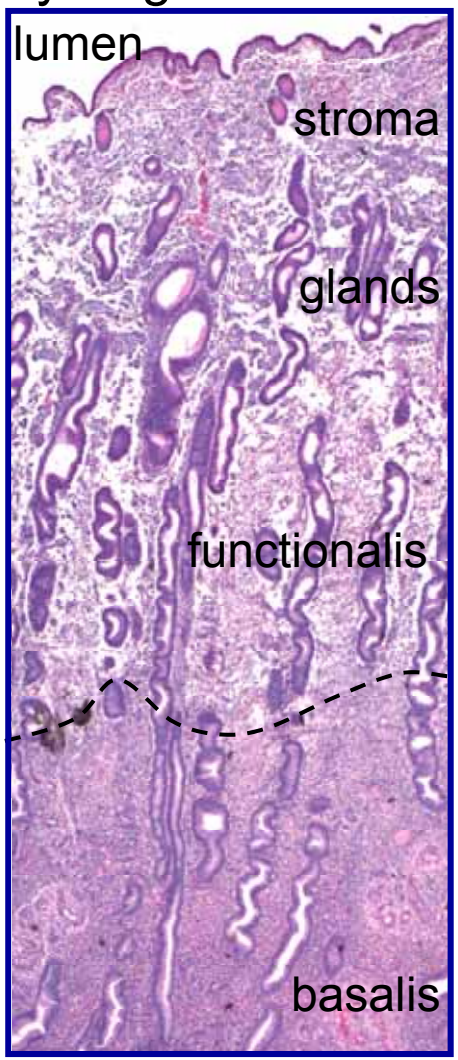
MONASH University

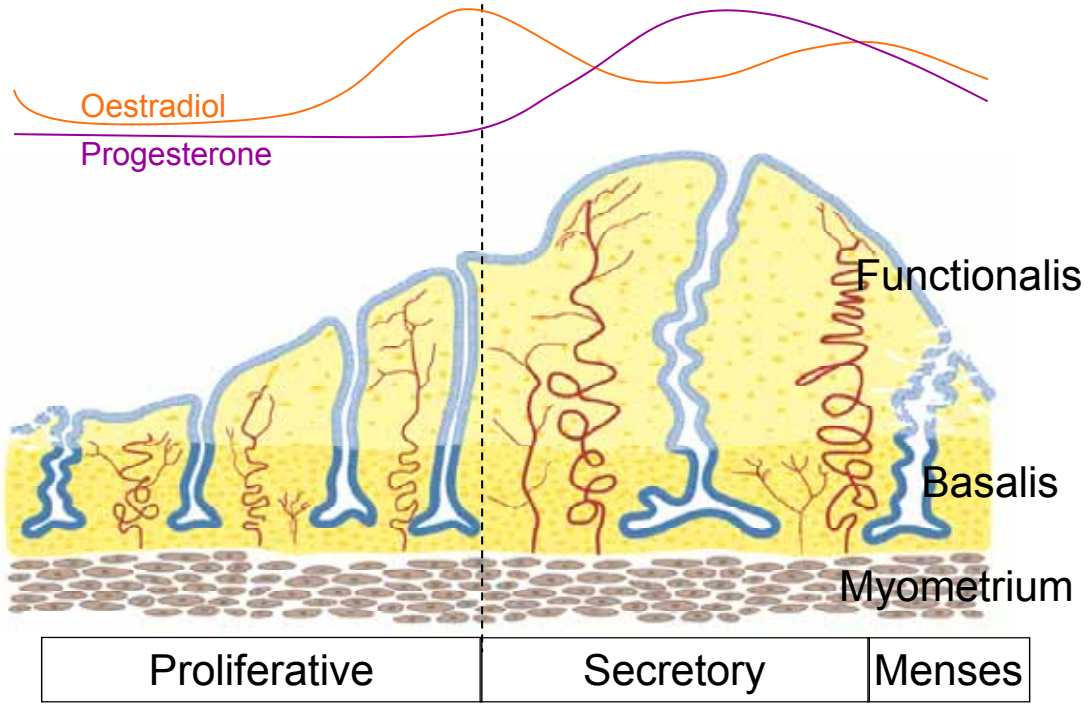
Presentation Plan

- Endometrial regeneration, the stem cell hypothesis and gynaecological disease
- Evidence for Stem/progenitor cell activity in human and mouse endometrium
- Markers of endometrial stem/progenitor cells
- Origin of endometrial stem/progenitor cells
- Endometrial cancer and evidence for cancer stem-like cells
- Endometrial Stem/progenitor cells in endometriosis

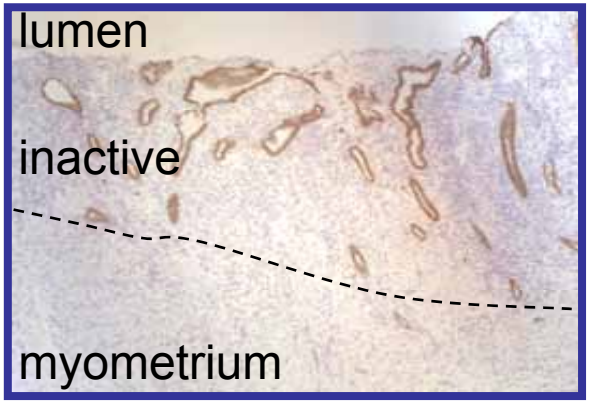
Regenerative Capacity of Human Endometrium

Cycling endometrium





- Menstrual cycle
- Parturition
- Resection
- Postmenopausal

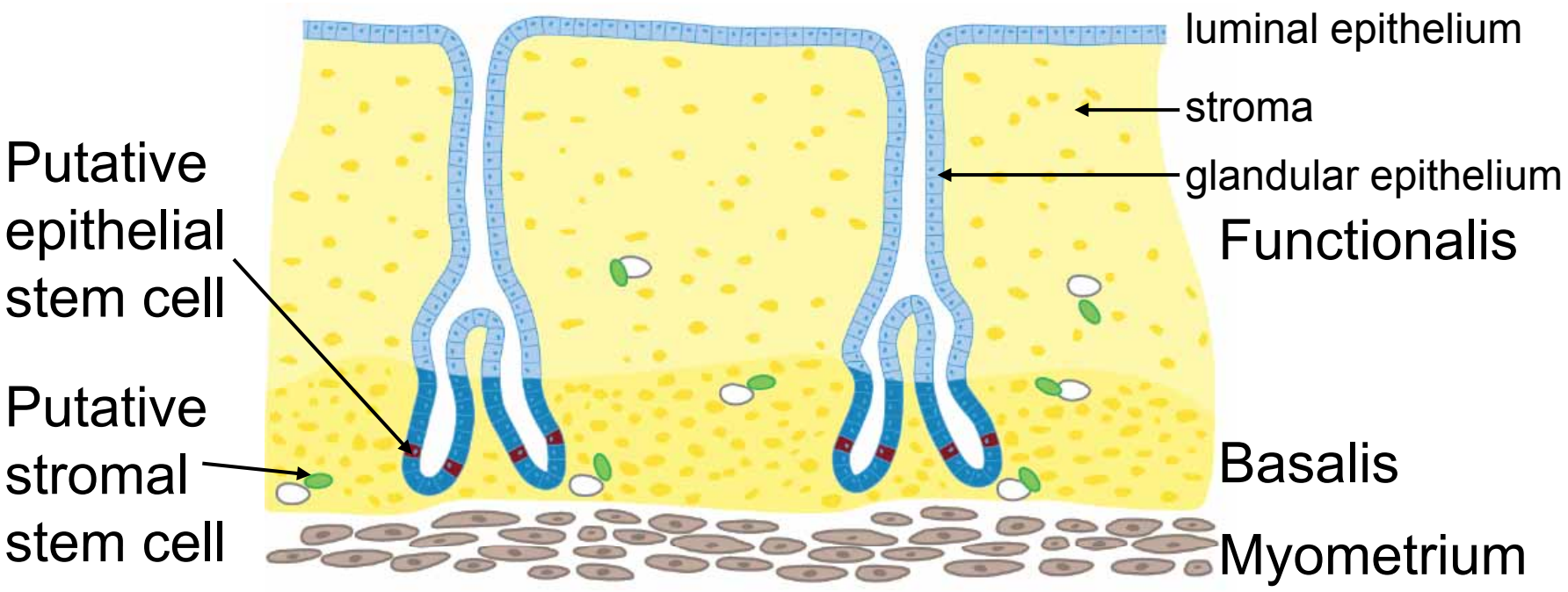


Postmenopausal endometrium

Gargett et al 2008

Hypothesis

The endometrial basalis contains a small population of epithelial stem cells and stromal stem cells

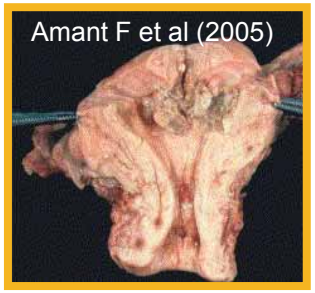


Gargett, 2007

Endometrial Stem Cell Disorders

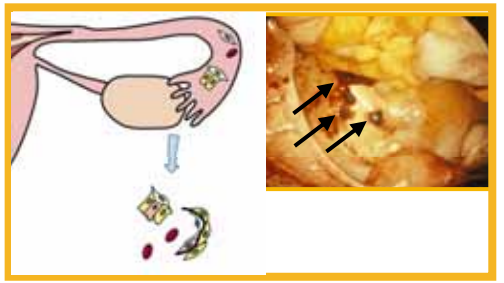
Endometrial Cancer

Mutated stem/progenitor cell → tumor
responsible for progression, metastasis, recurrence



Endometriosis

Normal stem/progenitor cell shed into peritoneal cavity → ectopic implant



Adenomyosis

Normal stem/progenitor cells, abnormal niche, inappropriate differentiation → ectopic growth, SMC hyperplasia

Asherman's Syndrome, Ablation

Damage/loss of normal stem/progenitor cells

Inadequate endometrium for IVF

Diminished activity of normal stem/progenitor cells

Gargett ,2007 13: 87-101

Adult Stem Cells - Properties

There are no specific markers for adult stem cells

Rare cells in tissues

Undifferentiated

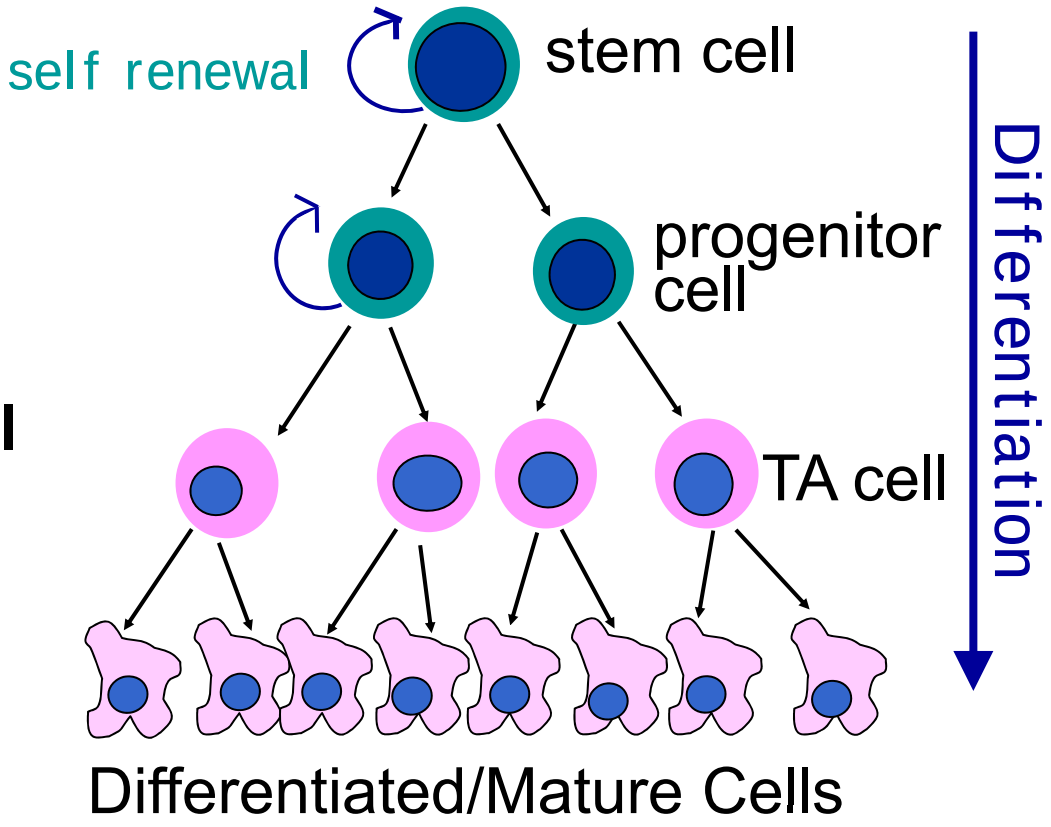
Self renew

Differentiation capacity

High proliferative potential

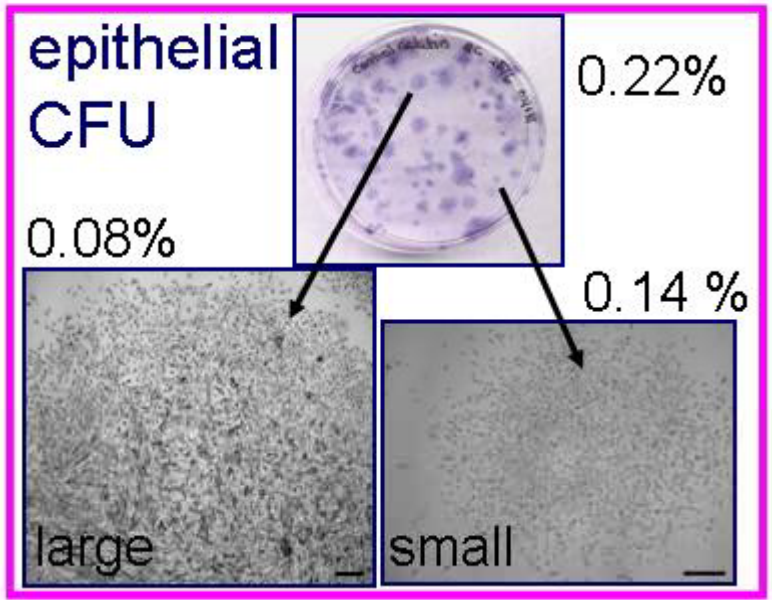
Quiescent

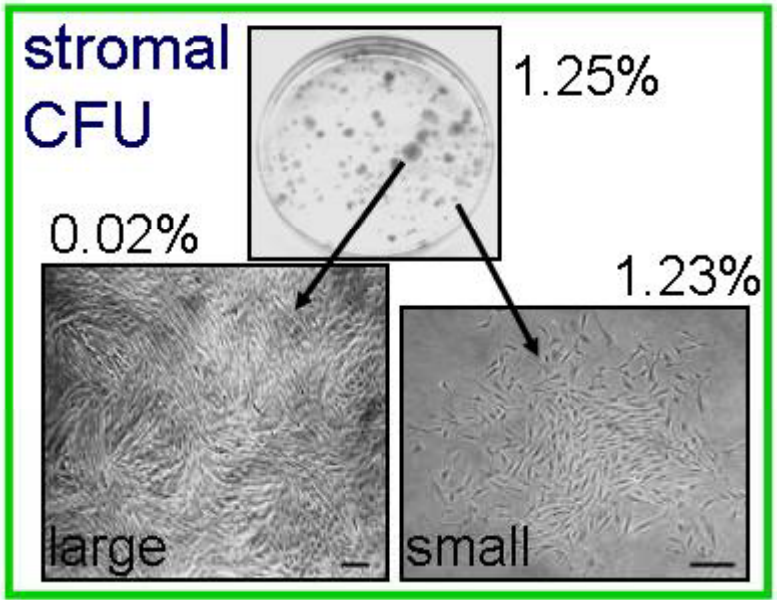
Clonogenic *in vitro* **(CFU)**



Chan et al 2004

CFU Activity of Human Endometrial Cells



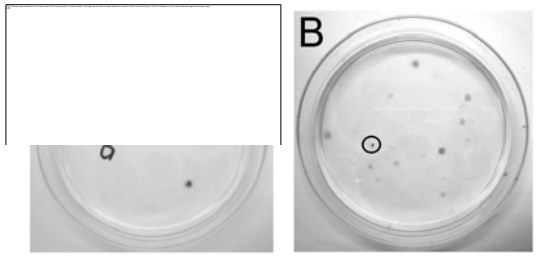


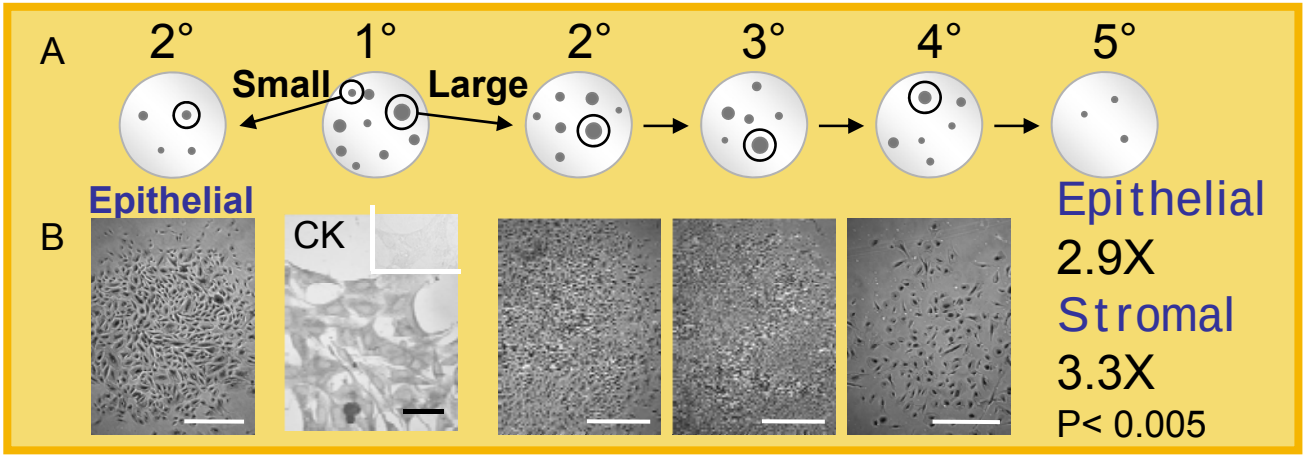
- CFU are present in inactive endometrium
- CFU activity similar for proliferative and secretory stages

Chan R et al (2004), Schwab et al (2005)

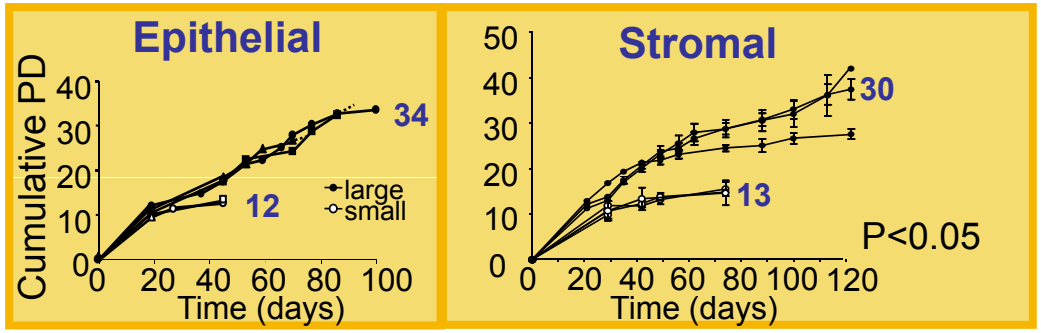
Stem/Progenitor Cell Activity in Normal Human Endometrium (CFU)

Self renewal



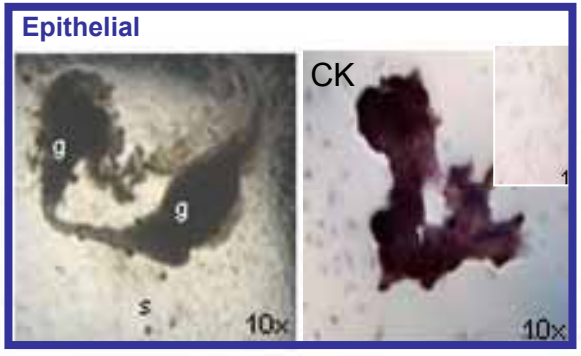


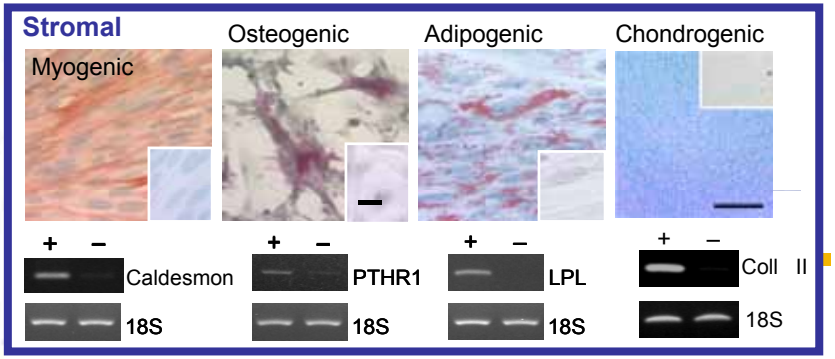
High proliferative potential



Differentiation

Gargett et al 2009





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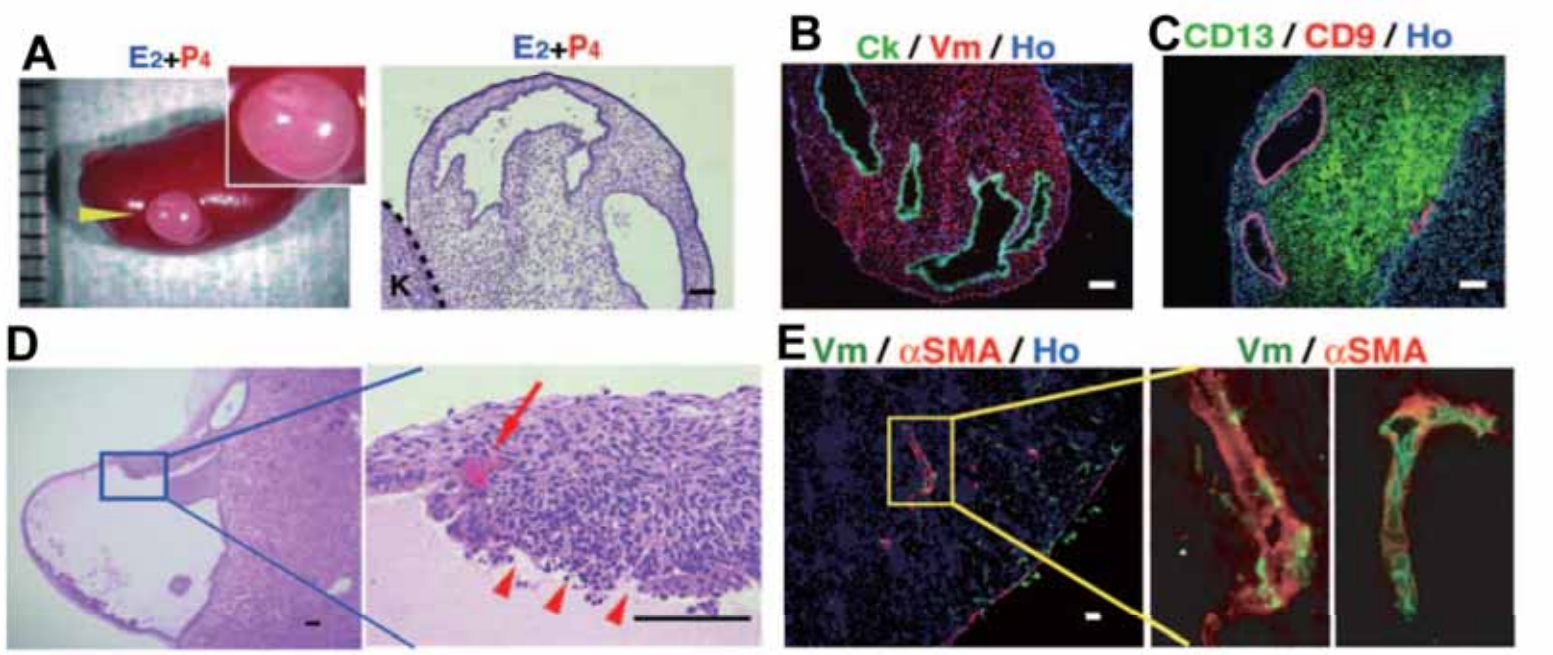
Human Endometrial Stem/Progenitor Cell Activity in vivo

Reconstruction of human endometrial like tissue from transplanted human endometrial cells



Hormone-dependent functional changes of reconstructed endometrium

Masuda H. et.al. 2007

Side Population (SP) Cells in Human Endometrium

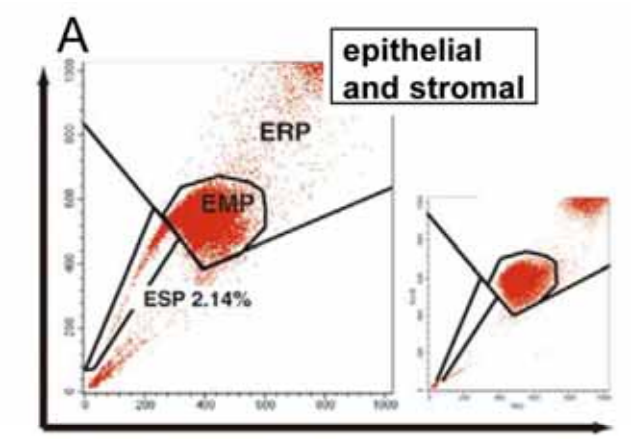
- Endometrial SP cells are heterogeneous
  - Endothelial, Epithelial, Stromal cells

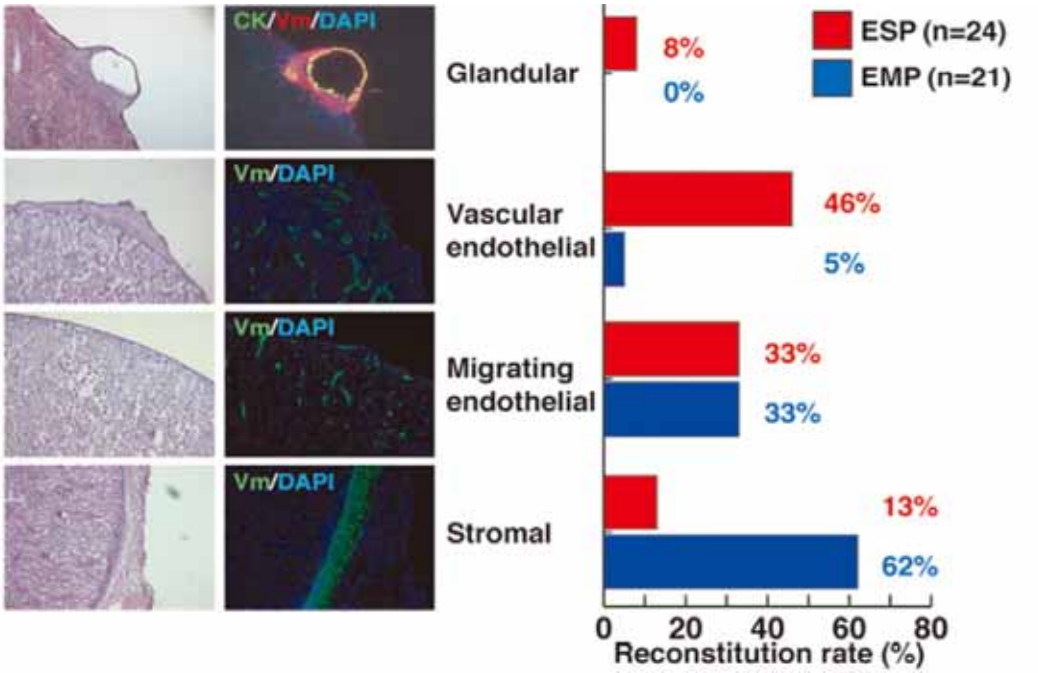
Kato K et al 2007
Tsuji S et al 2008

- Express ABCG2/Brcp1, telomerase, OCT-4, c-KIT

Cervello I et al, 2010

- Clonogenic
- Differentiate
- Reconstruct endometrial tissue components

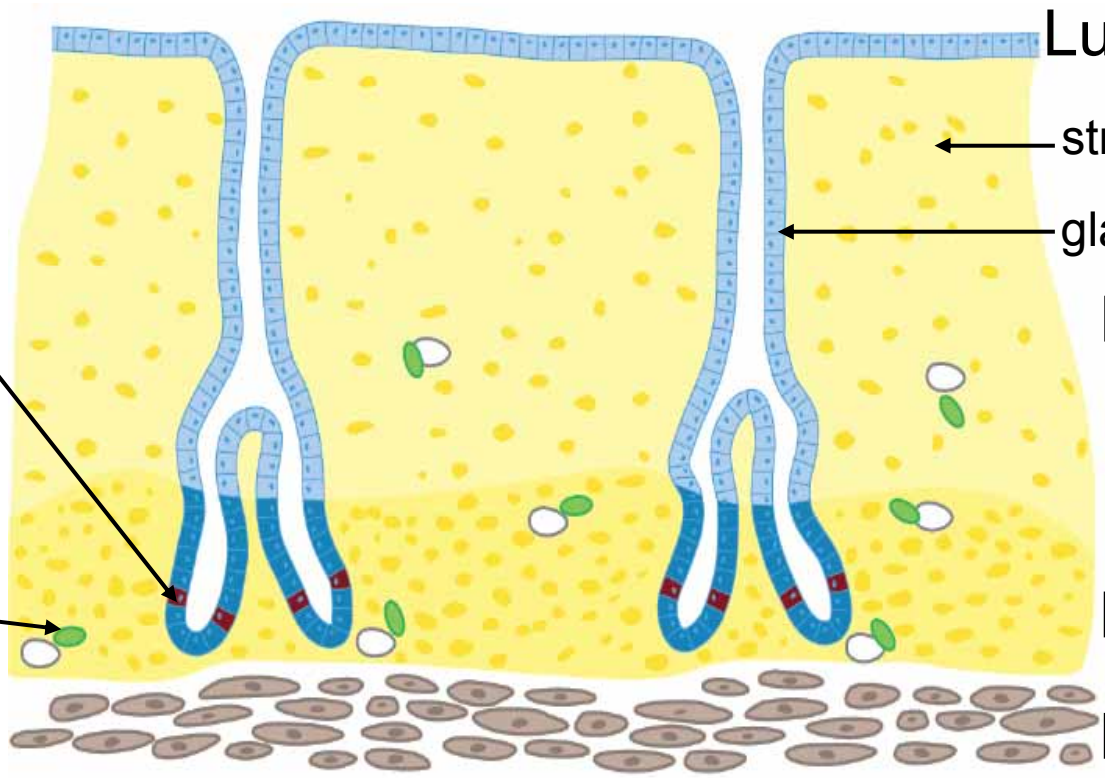




H Masuda et al, 2010

Where are Endometrial Stem/Progenitor Cells Located?

The search for endometrial stem/progenitor cell markers



Gargett, 2007

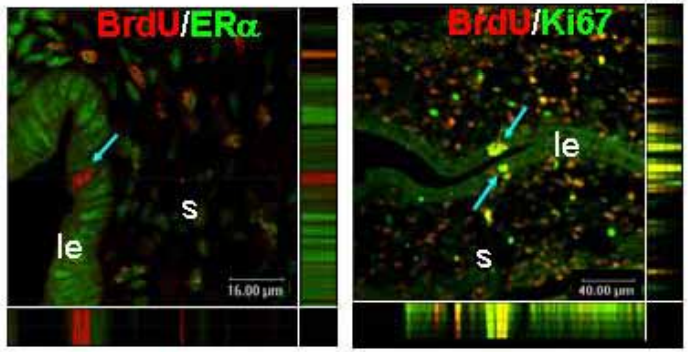
Mouse Endometrial Epithelial and Stromal Stem/Progenitor Cells - Label Retaining Cells (LRC)

Epithelial LRC

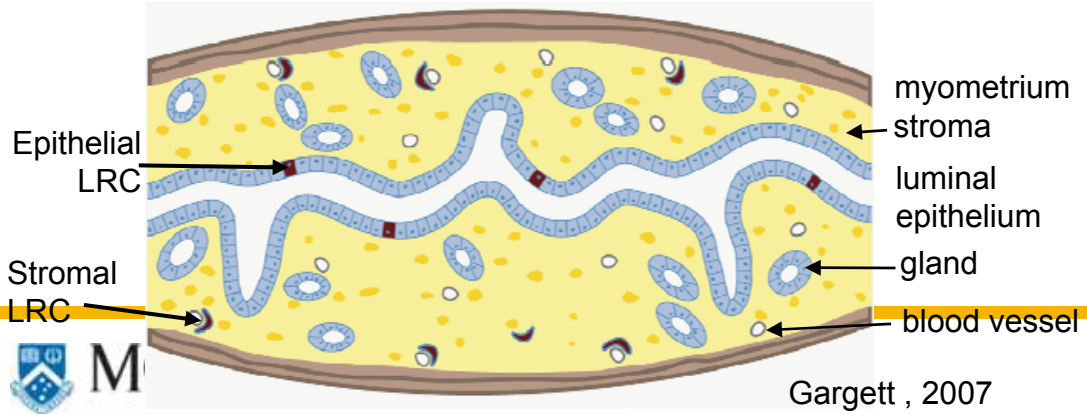
3% of epithelial cells
Luminal epithelium
ERα⁻
Proliferate in response to E

Stromal LRC

6% of stromal cells
Perivascular
Most ERα⁻, 16% ERα⁺,
Some proliferate in response to E



Chan & Gargett 2006



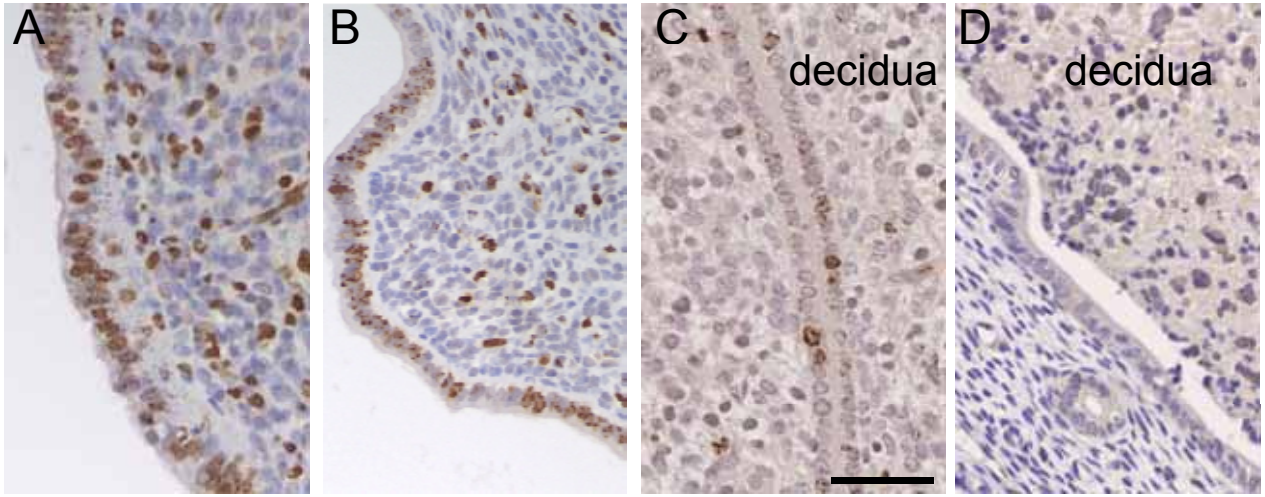
Gargett , 2007

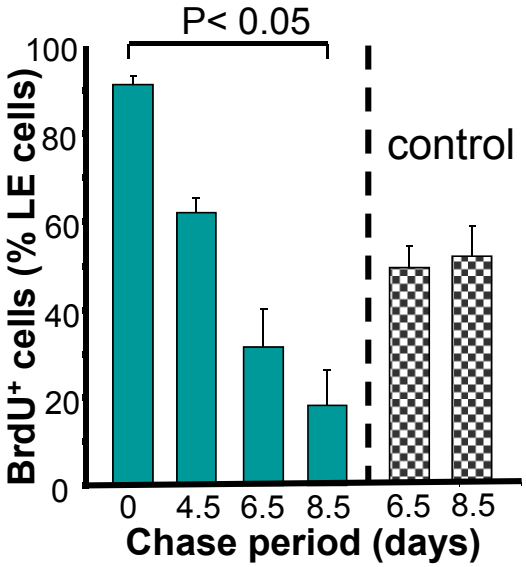
Stromal LRC

αSMA⁺, CD45⁻
Some OCT4⁺ Cervello et al 2007

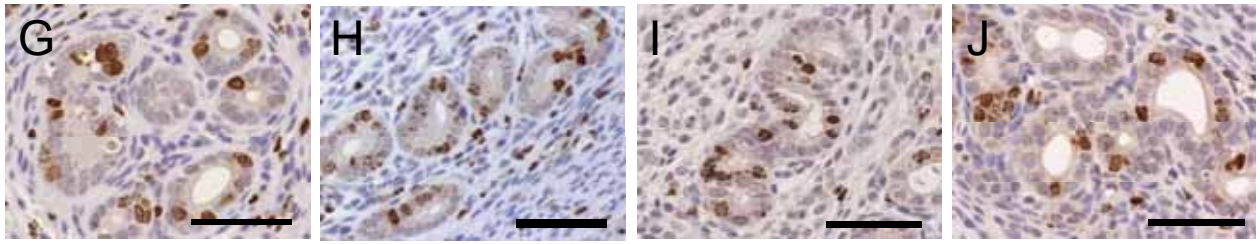
LRC in Mouse Model of Endometrial Breakdown and Repair

Luminal epithelium

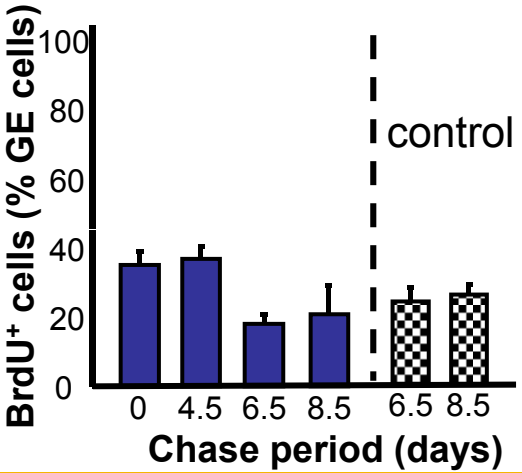




Glandular epithelium



initial labeling after E2 decidualisation repair



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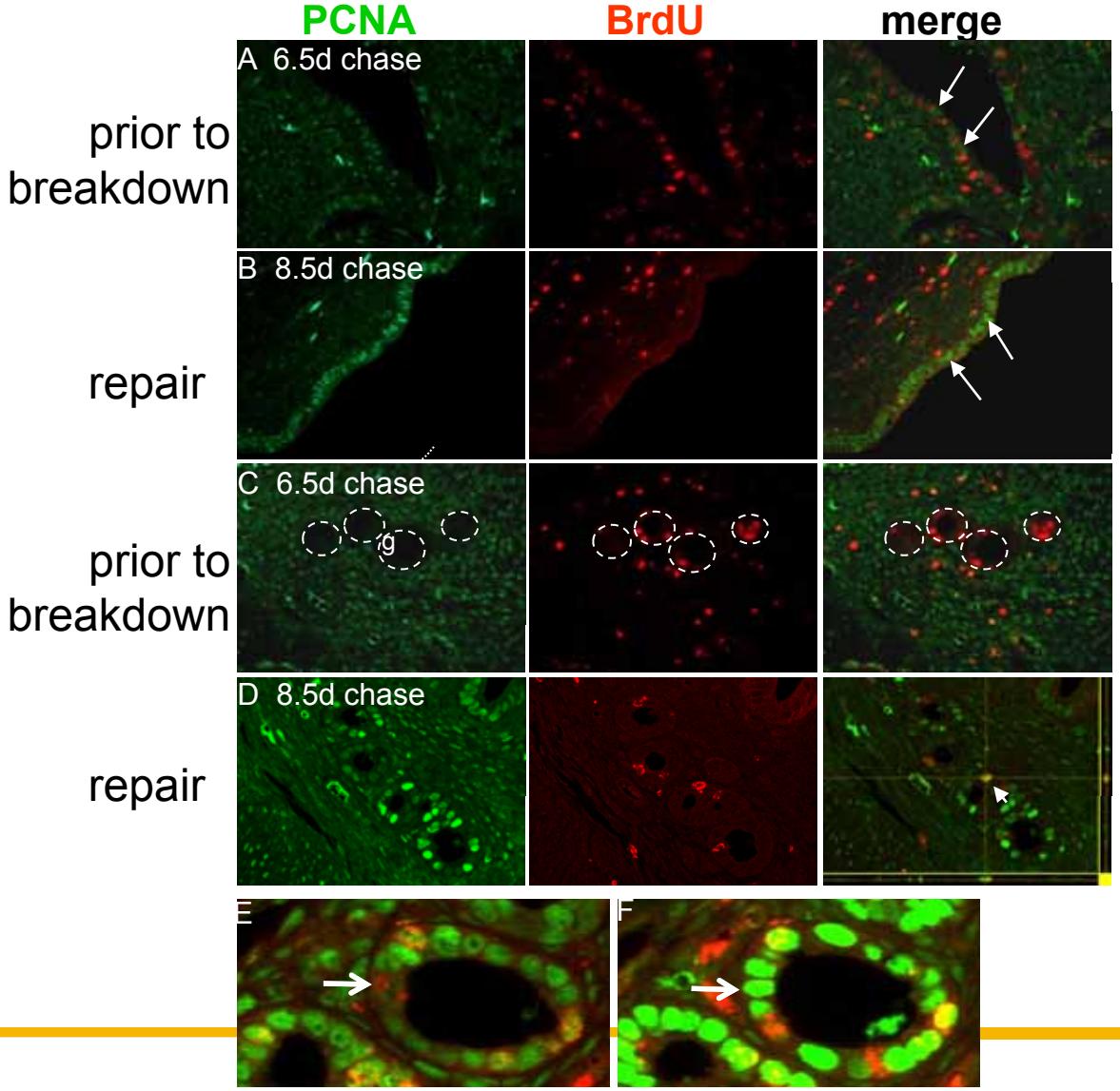
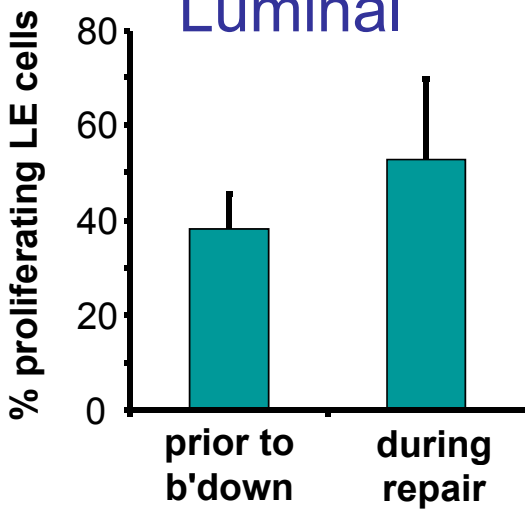
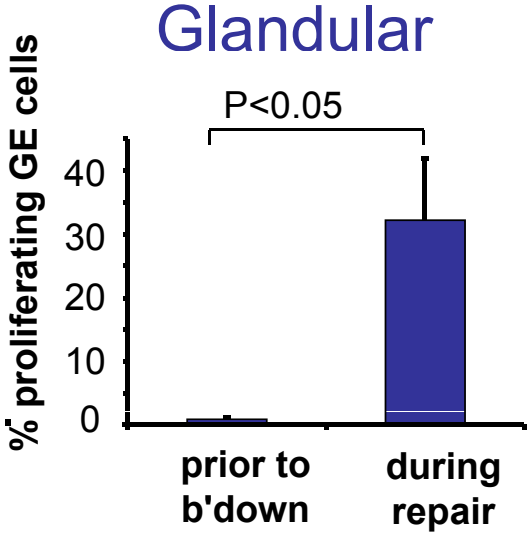
Kaitu'U-Lino et al, 2010

Differential Epithelial Proliferation During Breakdown and Repair





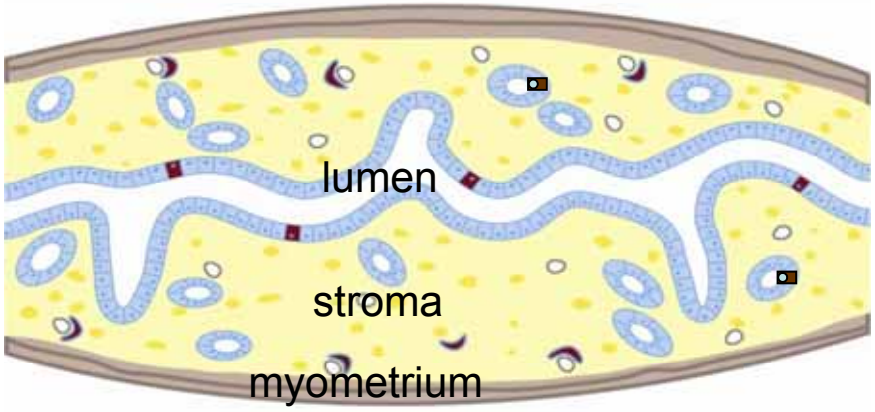


Kaitu'U-Lino et al, 2010

Location of LRC varies between Endometrial Regeneration and Repair Models

1. Endometrial growth during development
   Luminal epithelial LRC
2. Endometrial repair following breakdown
   Glandular epithelial LRC

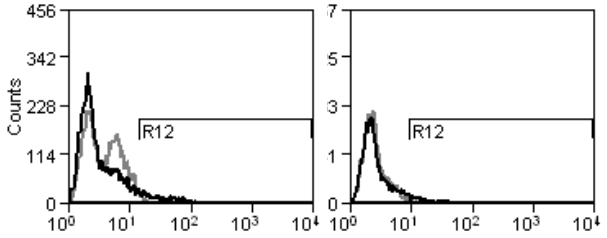
Different mechanisms involved between growth and repair following tissue damage

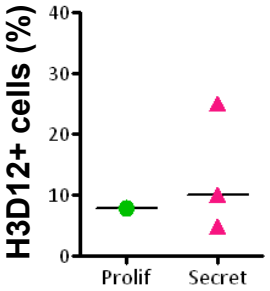


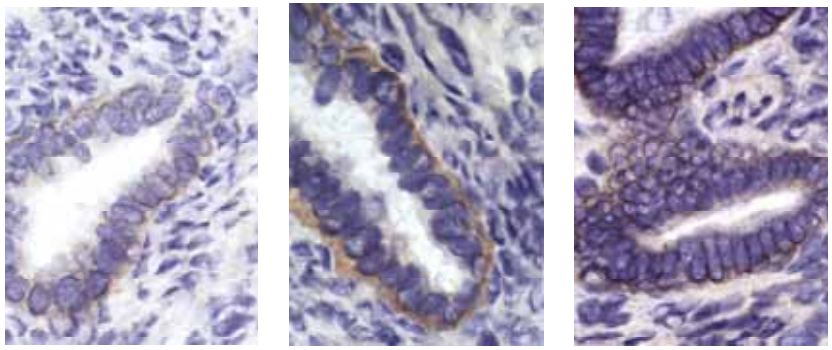
Searching for Human Endometrial Epithelial Stem/Progenitor Cell Markers

Screened with an antibody panel to 30 surface markers

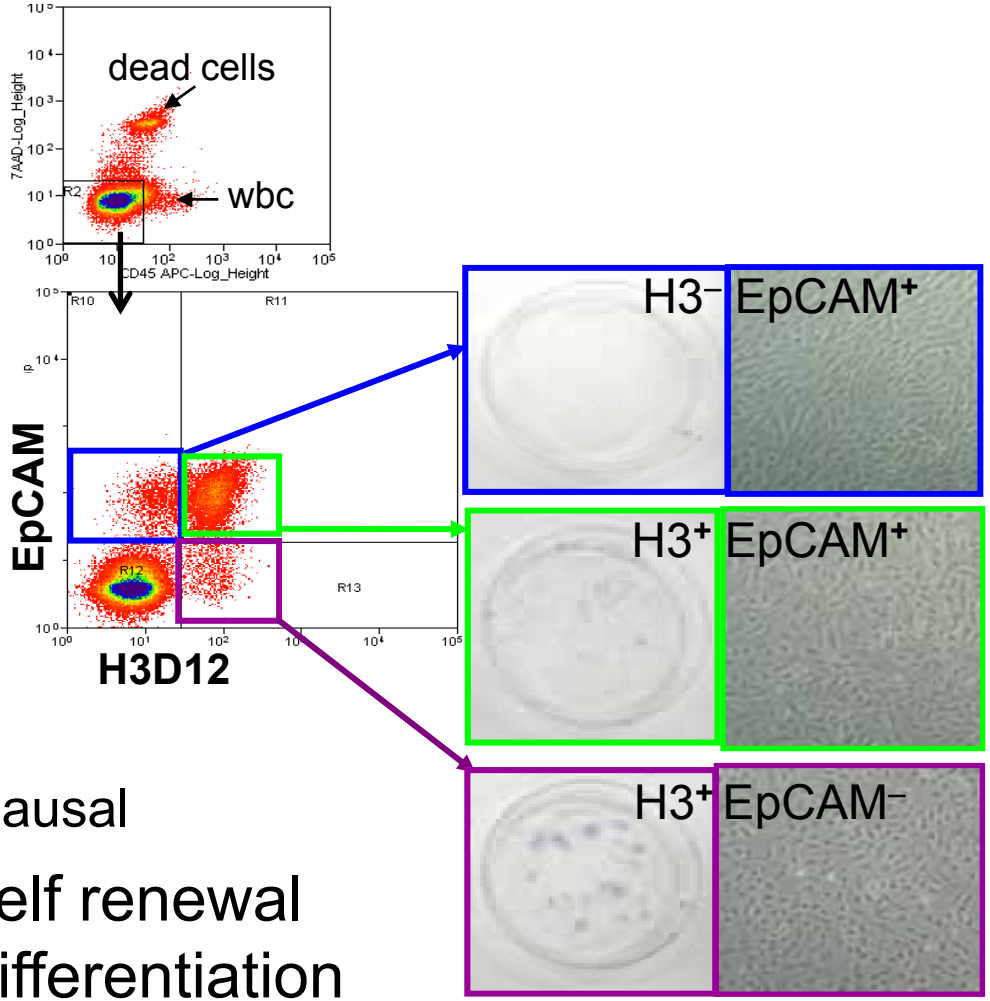
H3D12







Proliferative Secretory Post-menopausal



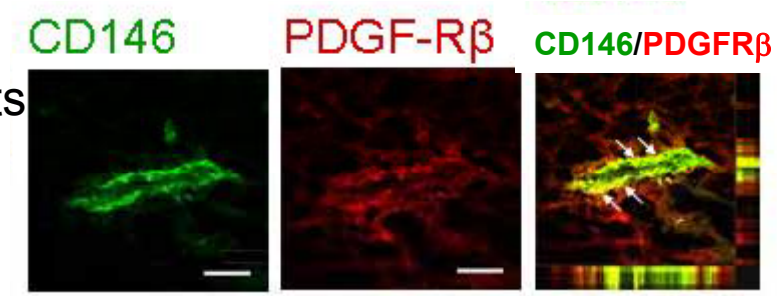
- Self renewal
- Differentiation
- In vivo reconstitution

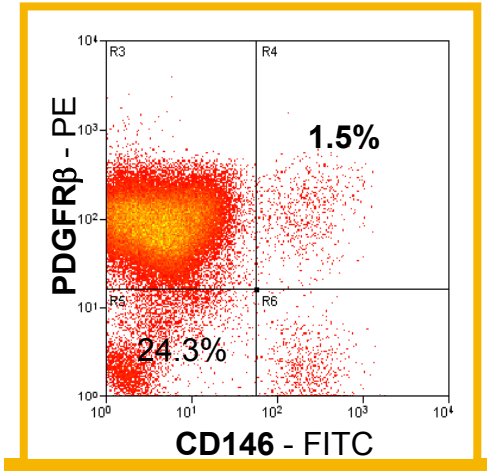
Markers to Isolate Endometrial Mesenchymal Stem Cells

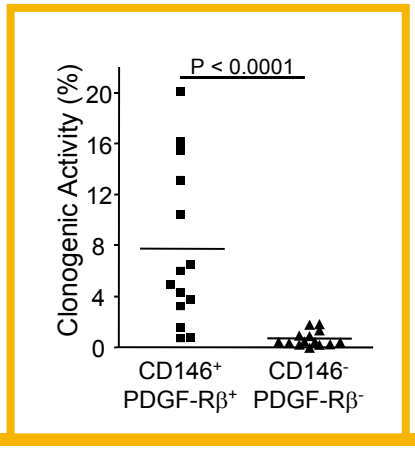
Co-expression of CD146 and PDGFRβ → 8 fold purification of MSC-like cells compared to freshly isolated stromal cells

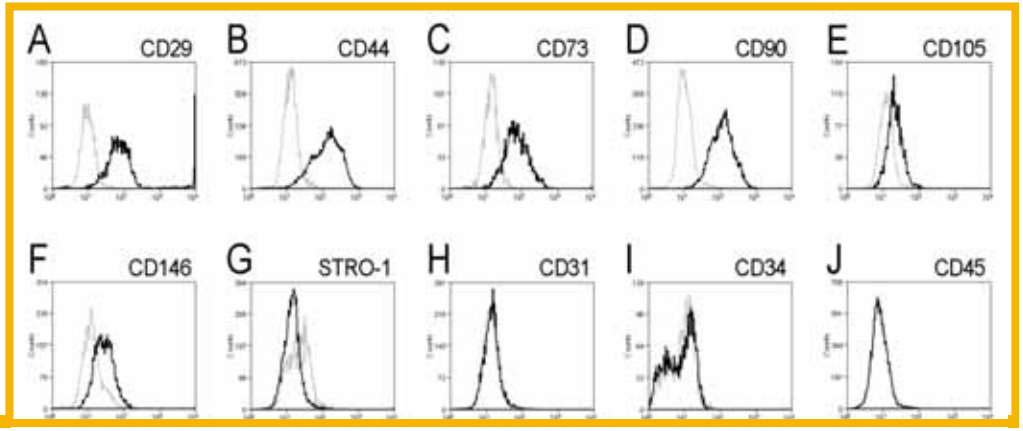
- clonogenic
- Multipotent

  adipocytes, SMCs, chondrocytes, osteoblasts
- MSC surface phenotype
- perivascular location









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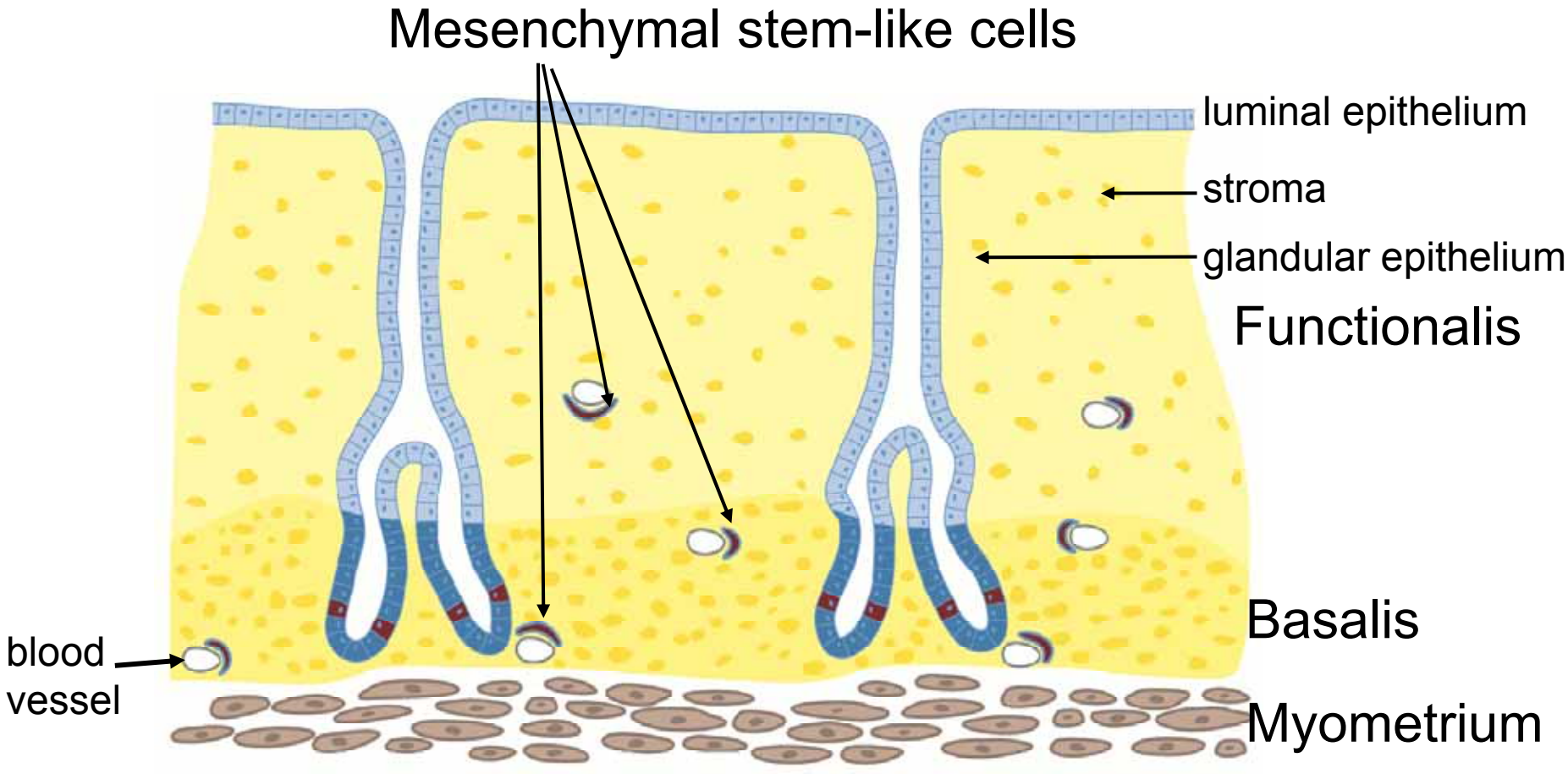
Schwab & Gargett, 2007

Are Endometrial MSC-like cells in the Basalis or Functionalis?



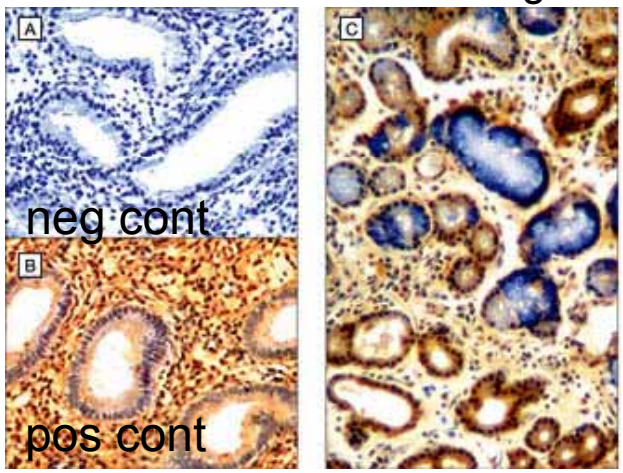
Endometrial MSC will be shed in menstrual blood

Origin of Human Uterine Stem/Progenitor Cells

- Residual Müllerian duct fetal stem cells Gargett, Hum Reprod Update, 2007
- Circulating bone marrow (stem) cells
  - HLA or gender mismatch BMT recipients with donor HLA type or male glands, stroma

Human

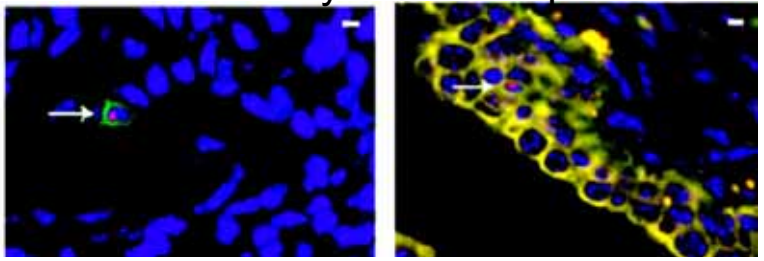
monoclonal glands



Taylor, H. S. 2004.

Mouse

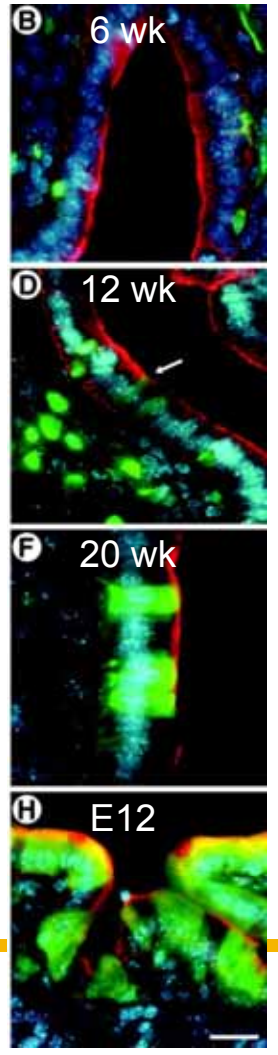
CD45⁺ Y⁺ leukocyte CK⁺Y⁺ epithelial cell



Du H & Taylor 2007

Conditional expression of EGFP in CD45 expressing cells using *CD45/Cre-Z/EG* mouse (n=1)

Bratincsak, A. et al. 2007



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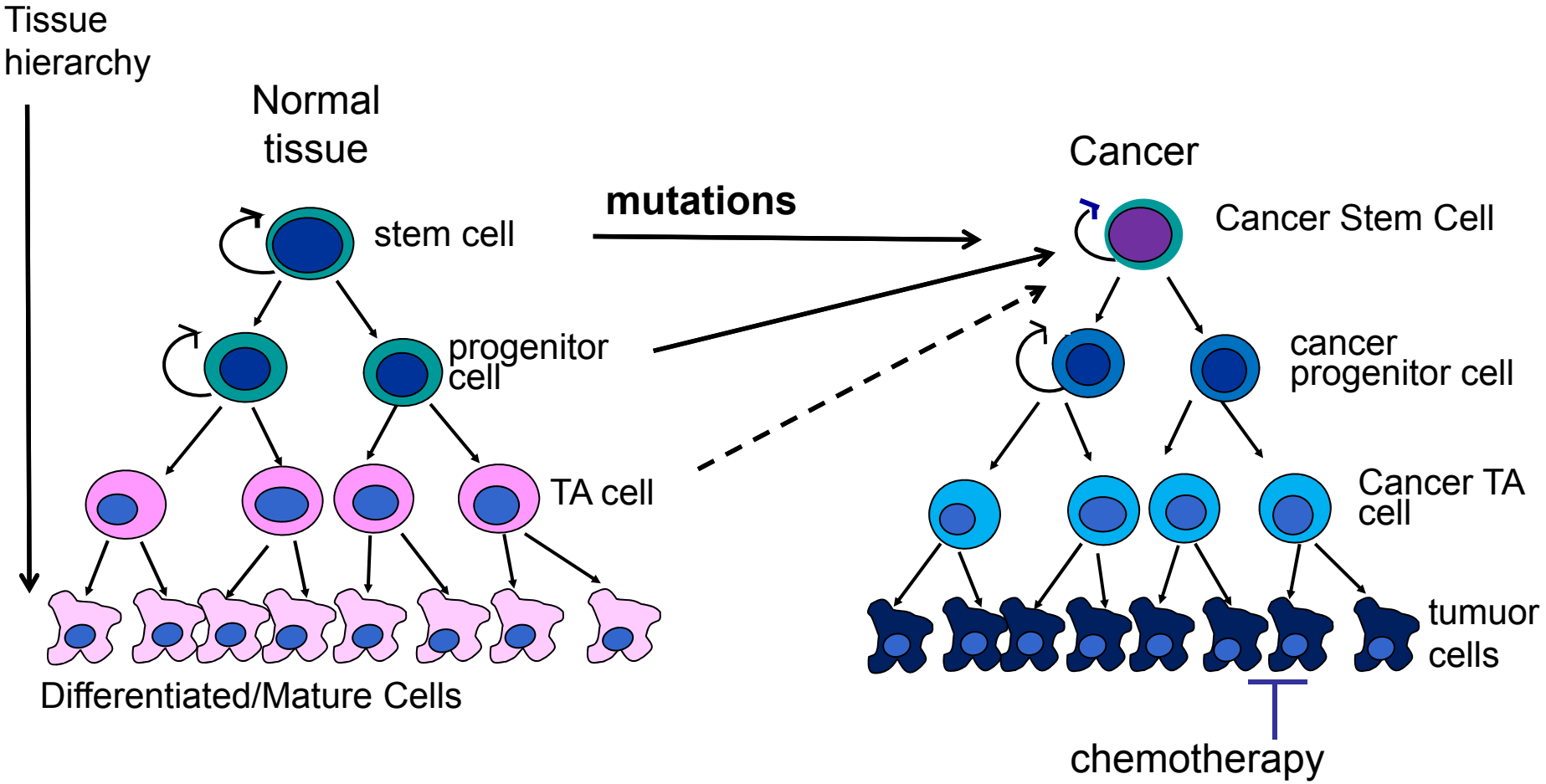
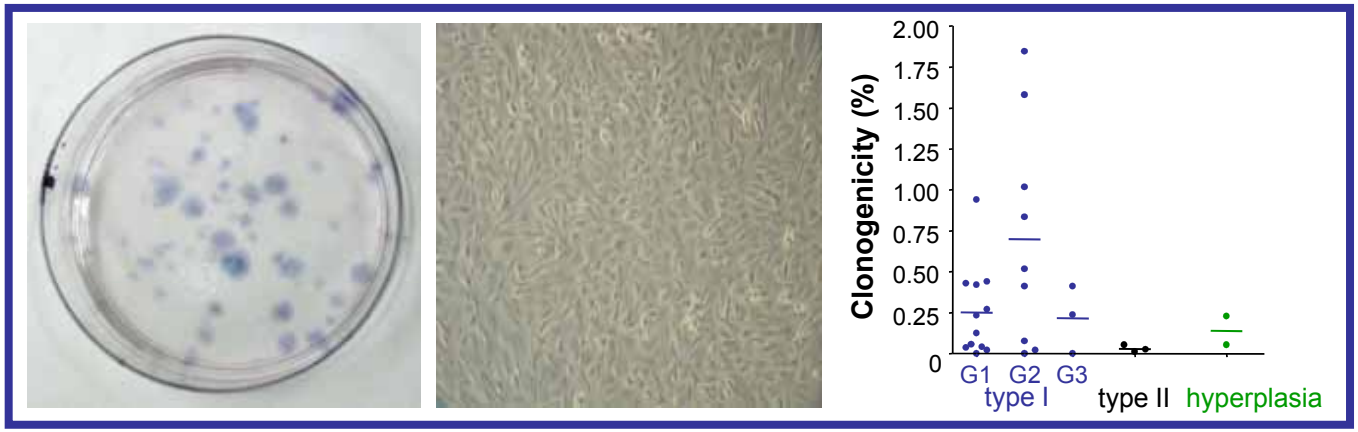
Cancer Stem Cells

Initiation, progression, metastasis, drug resistance, recurrence



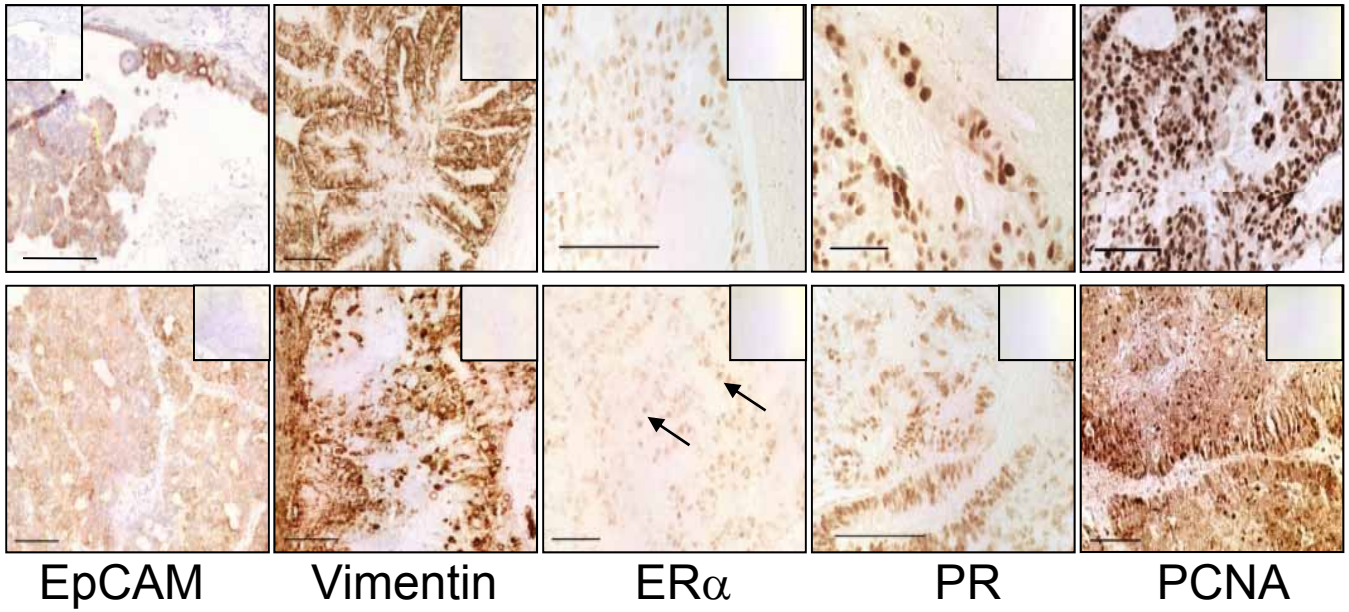
Cancer Stem Cells in Endometrial Carcinoma

- Clonogenic
- Tumorigenic
- Differentiate in vivo



Transplant 125,000 endometrial cancer cells

Parent tumour Type 1 Grade 2



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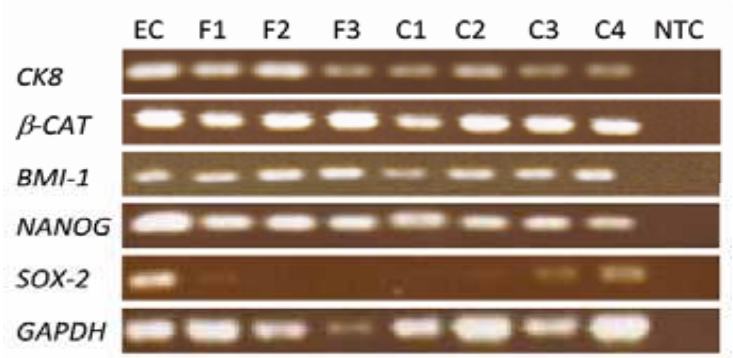
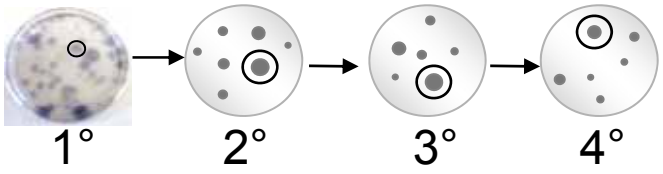
Hubbard et al, Cancer Res 2009

Cancer Stem Cells in Endometrial Carcinoma

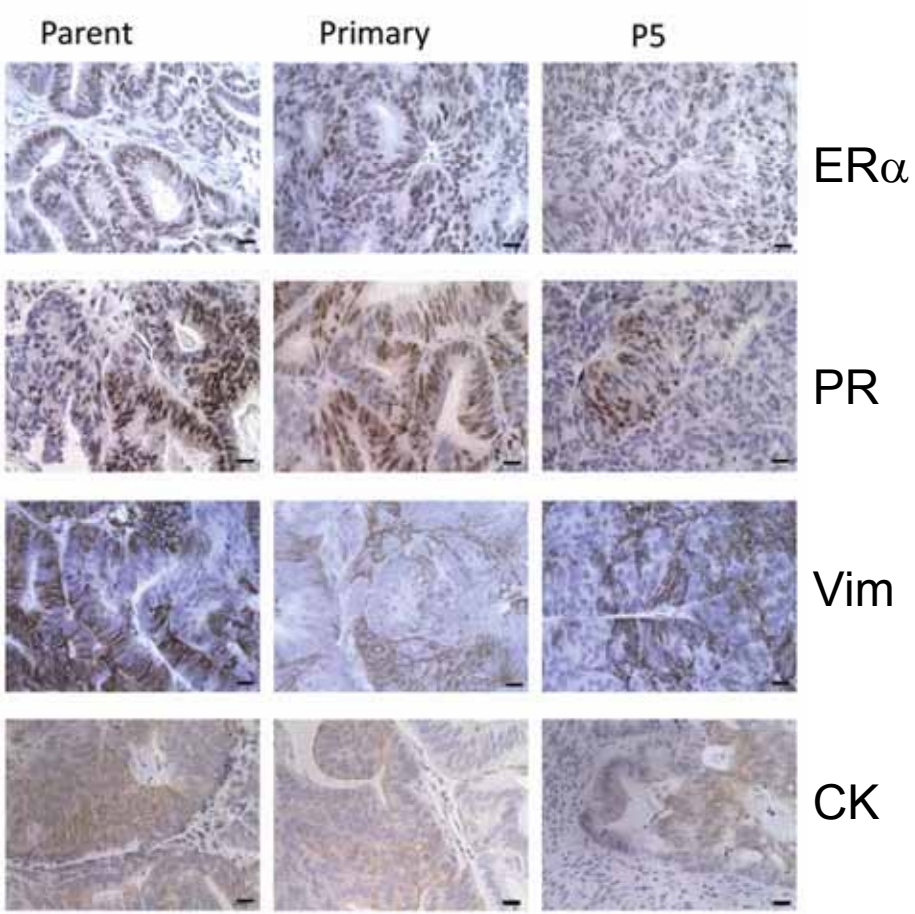
- Self renewal in vitro





Sample	Subclonings (M, range)	N
Hyperplasia	2.5 (2,3)	2
Grade 1	3 (1-5)	11
Grade 2	3 (3-4)	5
Grade 3	3.5 (3,4)	2
Type II	4 (1-4)	3

- Self renewal in vivo

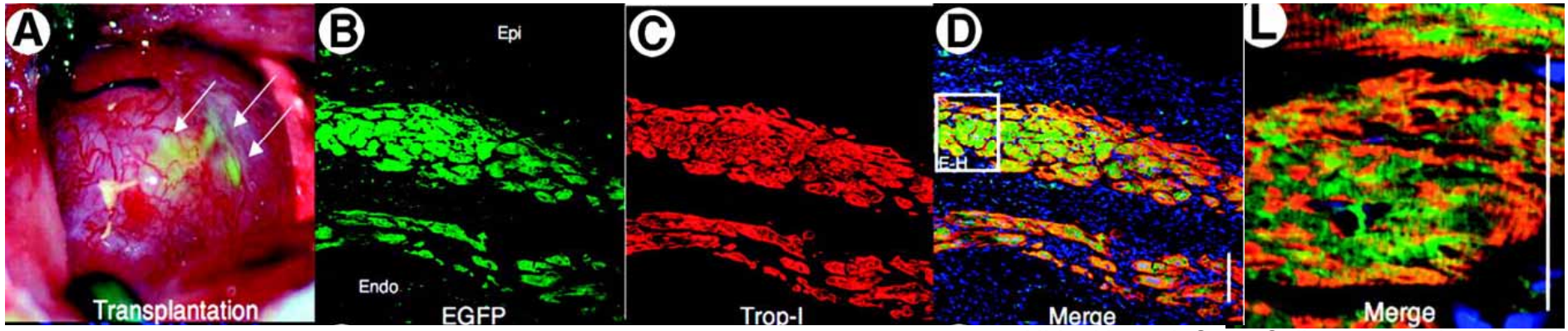


Hubbard et al, Cancer Research 2009

Stem/Progenitor Cells in Menstrual Blood

Cultured cells from menstrual blood contains MSC-like cells

- MSC cell surface phenotype
- Express OCT4, c-KIT, SSEA4 (pluripotency markers), telomerase
- Differentiate **in vitro**: cardiomyocytes, neural, hepatic, lung cells
- Differentiate **in vivo:** cardiomyocyts, skeletal muscle



Hida, N. et al. Stem Cells 2008;26:1695-1704

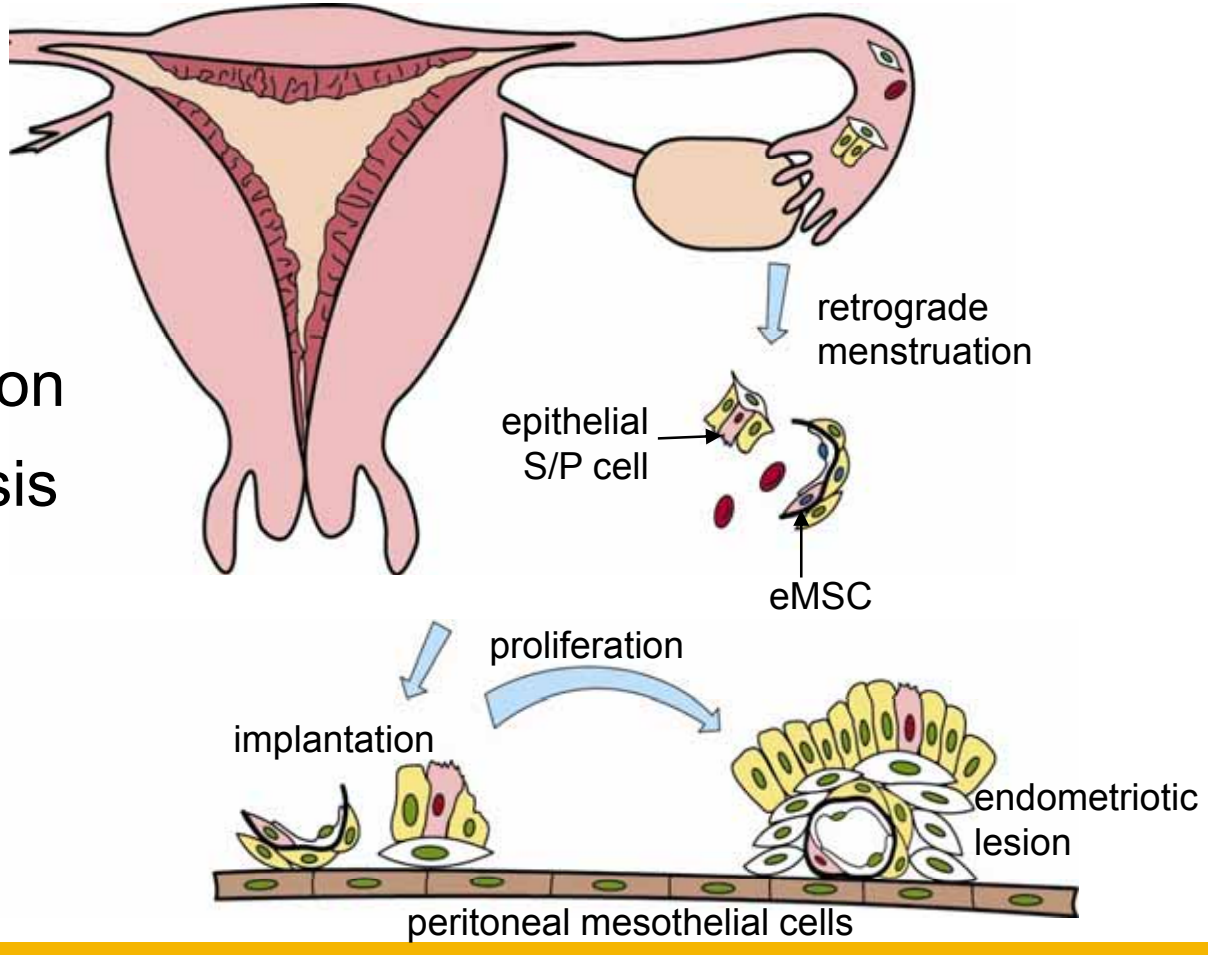
Suggested as a source of cells for regenerative medicine

Cui et al 2007; Meng et al 2007; Patel et al, 2008

Endometrial stem/progenitor cells in Endometriosis

Endometrial stem/progenitor cells may

- be shed in menstrual blood
- gain access to the pelvic cavity by retrograde menstruation
- Establish endometriosis lesions in susceptible women



Gargett & Masuda 2010

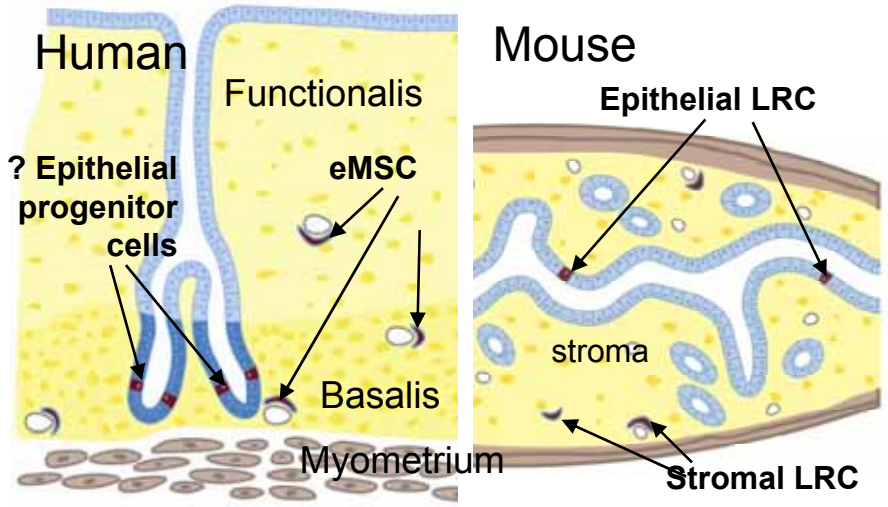
Summary
Human and Mouse Endometrium contains

Epithelial progenitor cells

- Clonogenic
- self-renew, high proliferative potential, differentiate
- SP cells
- Label retaining cells, ERα^{-}
- Markers unknown

Endometrial MSC-like cells

- Clonogenic
- self-renew, multipotent
- high proliferative potential
- Perivascular
- Purified in CD146^{+}PDGFRβ^{+} stromal fraction
- Shed during menstruation
- Proposed to use in tissue engineering applications



Gargett and Masuda (2010)

Functional identity and differentiation capacity of human endometrial stem/progenitor cells

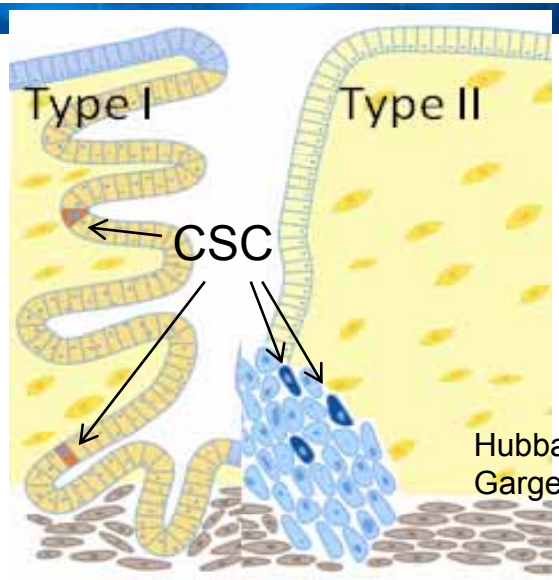
Gargett & Masuda, 2010

Stem/Progenitor Cell Type	Possible Commitment		
	epithelial cells	stromal cells	other cells
Clonogenic epithelial cells (CFU)	+		
Clonogenic stromal cells (CFU)		+	adipocytes, osteocytes, SMC, chondrocytes
$CD146^{+}PDGF\text{-}R\beta^{+}$ stromal cells		+	adipocytes, osteocytes, SMC, chondrocytes
Endometrial tissue reconstituting cells	+	+	Endothelial cells (EC)
Cultured endometrial stromal cells		+	chondrocytes, dopaminergic neurons
SP cells	+	+	endothelial cells, SMC
Bone-marrow derived cells	+	+	
Menstrual blood cells		+	cardiomyocytes, myocytes, adipocytes, osteocytes, SMC chondrocytes, neural cells
Endothelial progenitor cells (bone marrow derived)		?	EC, perivascular cells
Mouse LRC	+	+	perivascular cells

Endometrial Stem/Progenitor Cells: Clinical relevance

CSC in Endometrial Cancer

- Clonogenic, Tumour initiating cells
- Self renew in vitro and in vivo
- Differentiate in vivo
- Derived from epithelial progenitor cells or their progeny
- CD133 may be a marker (Rutella et al 2009)
- Involved in progression, metastasis, recurrence, resistance
- Target for novel treatment



Hubbard & Gargett 2010

Normal Endometrial Stem/progenitor Cells may have roles in

- Endometriosis lesion development
- Adenomyosis
- Generating adequate endometrium for IVF after biopsy/injury

Barash, Fertil Steril 2003

Potential endometrial stem/progenitor cell therapy

- MSC for autologous cell-based therapy for pelvic organ prolapse

Unresolved questions in Endometrial Stem/Progenitor Cell Research

1. Markers to identify endometrial epithelial progenitors
2. Relationship between cultured endometrial stromal cells and endometrial MSC ($CD146^{+}PDGFR\beta^{+}$)?
3. What is the relationship between
   - clonogenic cells
   - SP cells
   - Tissue reconstituting cells
   - LRC?
4. How many endometrial stem/progenitor cell types are there? One or more?
   ie epithelial, stromal (MSC), endothelial progenitor cells?
5. Endogenous &/or bone marrow origin of endometrial stem/progenitor cells?

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