Endometrial-peritoneal cross-talk and development of endometriosis

Christian Becker

Nuffield Dept. of Obstetrics and Gynaecology

University of Oxford

Commercial disclosure

- Astellas
- Bayer Healthcare
- Roche
- TEVA Pharmaceutical
- ValiRx

Outline

- Background
- Endometriosis
- Peritoneum
- Stem/progenitor cells
- Mesothelial damage
- Conclusions

Endometriosis

	Number of studies	Number of patients	Number with disease	% with disease (range)	% with Stage I-II disease (range)
Pelvic pain	15	2,400	688	24.5 (4.5 – 62.0)	69.9 (61.0 – 100)
Infertility	32	14,971	2,812	19.6 (2.1 – 78.0)	65.6 (16.3 – 95.0)
Sterilisation	13	10,634	499	4.1 (0.7 – 43.0)	91.7 (20.0 – 100)

Eskenazi B, Warner ML, Obstet Gynecol Clin North Am 1997

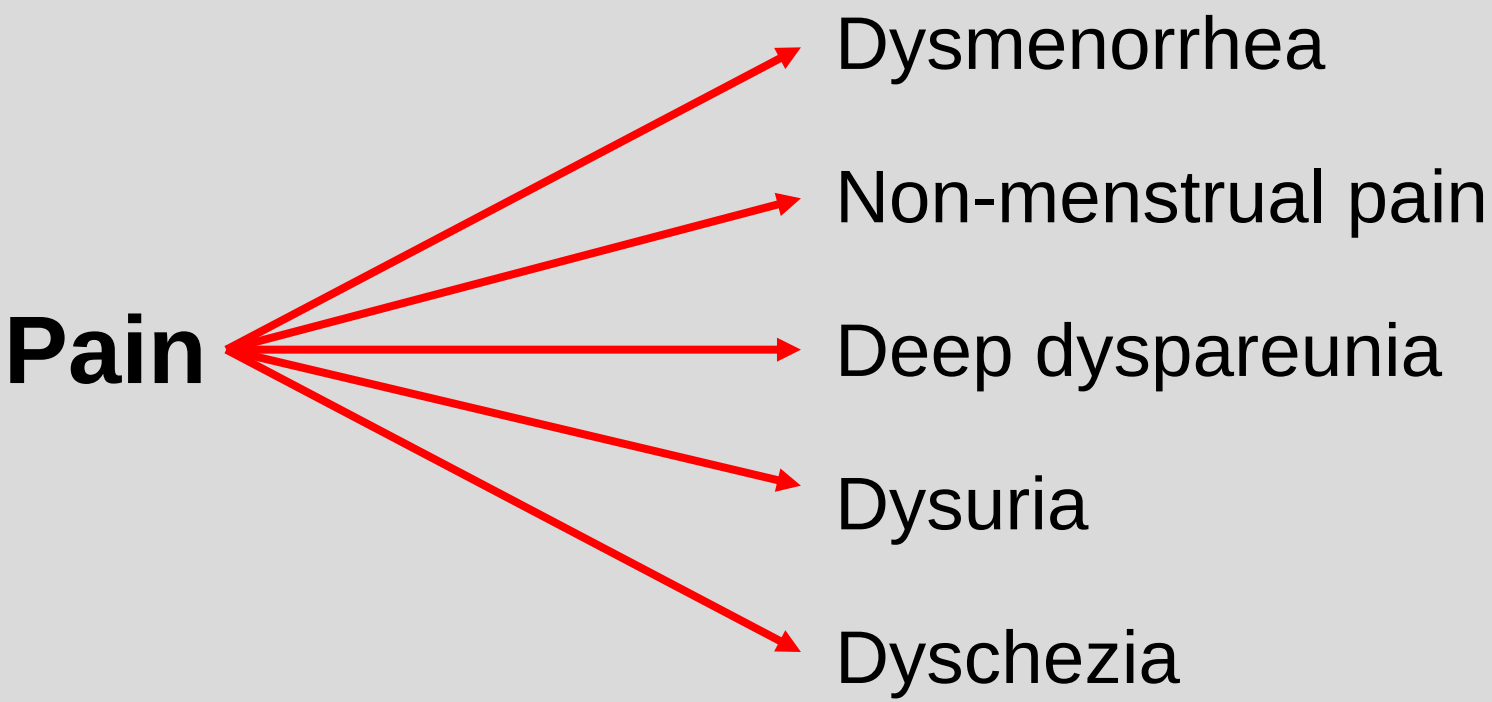
Why care?

Patients

Society

Patients

Infertility



Delay in diagnosis (years)

	Symptom onset	Surgical diagnosis	Delay
Brazil [1]	20.5 *	33.0 *	7.4 *
USA [2]	18.9	28.7	9.8
Australia	17.1	29.6	12.5
UK [3]	22.0	30.5	8.5
USA	19.2	30.9	11.7
UK [4]	24.4	32.6	8.0
GER/AUT [5]	21.2	32.0	10.4

* median values
otherwise all means

[1] Arruda *et al., Hum Reprod* 2003
[2] Sinaii *et al., Hum Reprod* 2002
[3] Treloar *et al.* , *Fertil Steril* 2002
[4] Hadfield *et al.* , *Hum Reprod* 1996
[5] Hudelist *et al., Hum Reprod* 2012

Society

RESULTS (n=905)

Average annual cost = €9,579 (95% CI €8,559-€10,599)

- Average cost of direct healthcare costs = €3,113
  - surgery (29%)
  - monitoring tests (19%)
  - hospitalisation (18%)
  - medication (10%)

- Average cost of productivity loss = €6,298

Cost of loss of productivity is twice that of healthcare costs!

WORLD ENDOMETRIOSIS RESEARCH FOUNDATION

Simoens S et al., Hum Reprod 2012

Definition

The presence of endometrial LIKE tissue

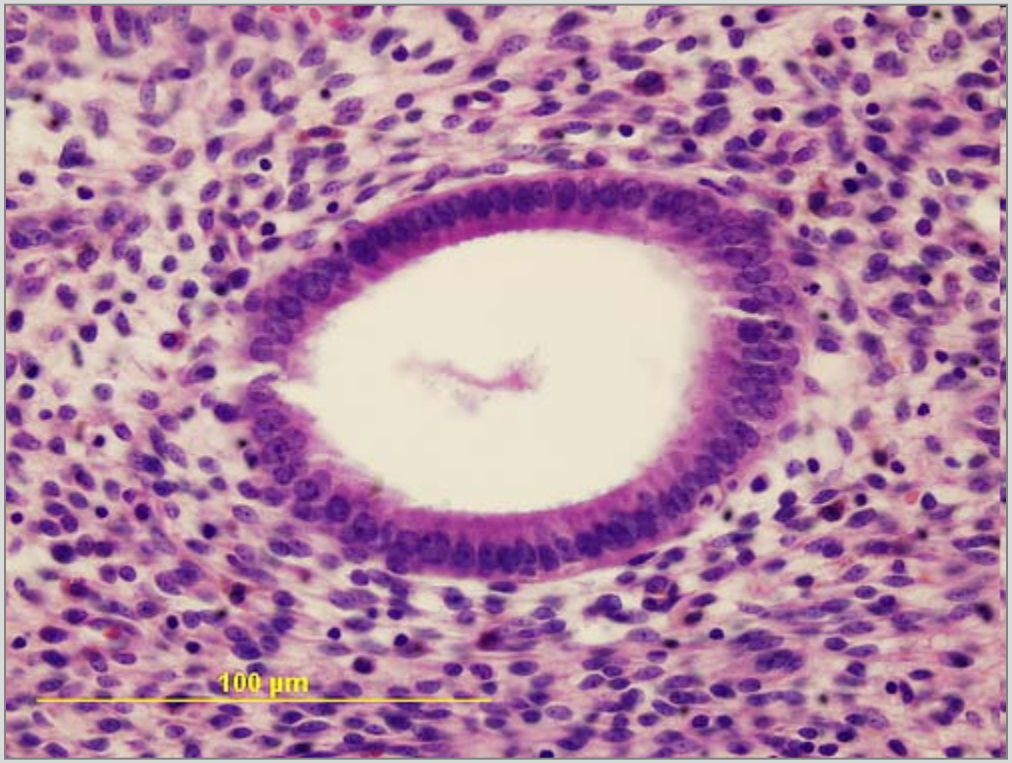
in sites outside the uterine cavity

Kennedy SH et al., Hum Reprod 2005

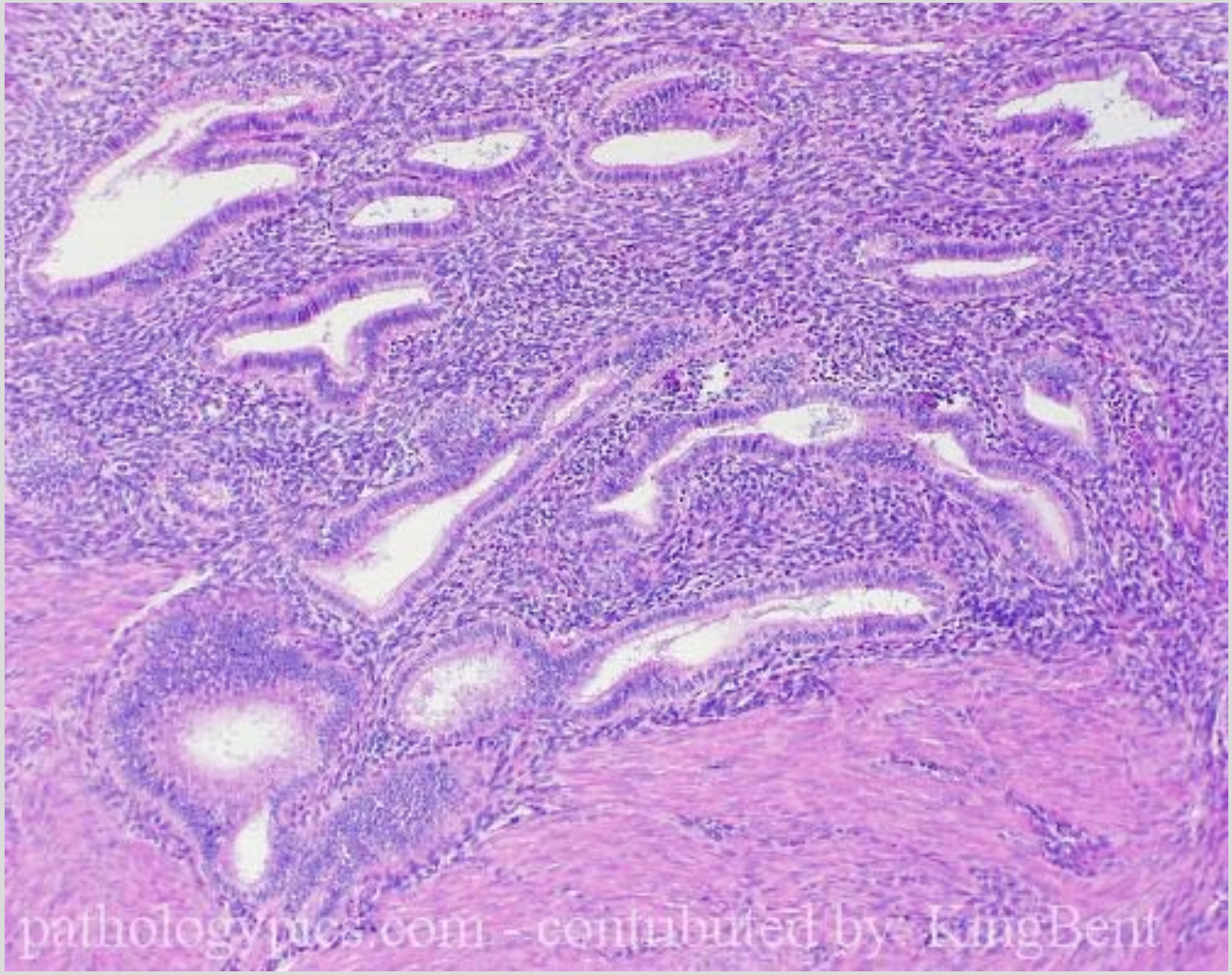
Histology

Glandular epithelial cells

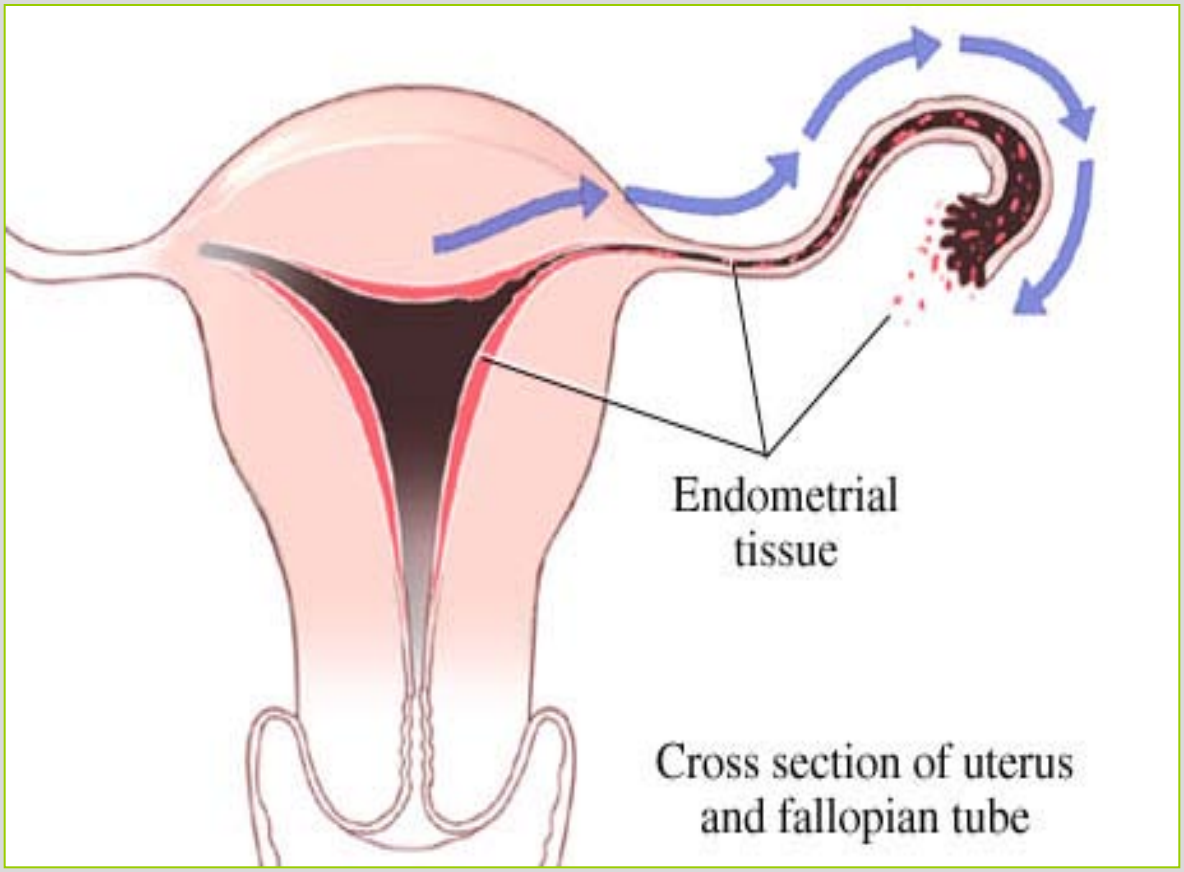
Stromal cells



Endometrium



Sampson theory? - 1927



Supporting Sampson (1)

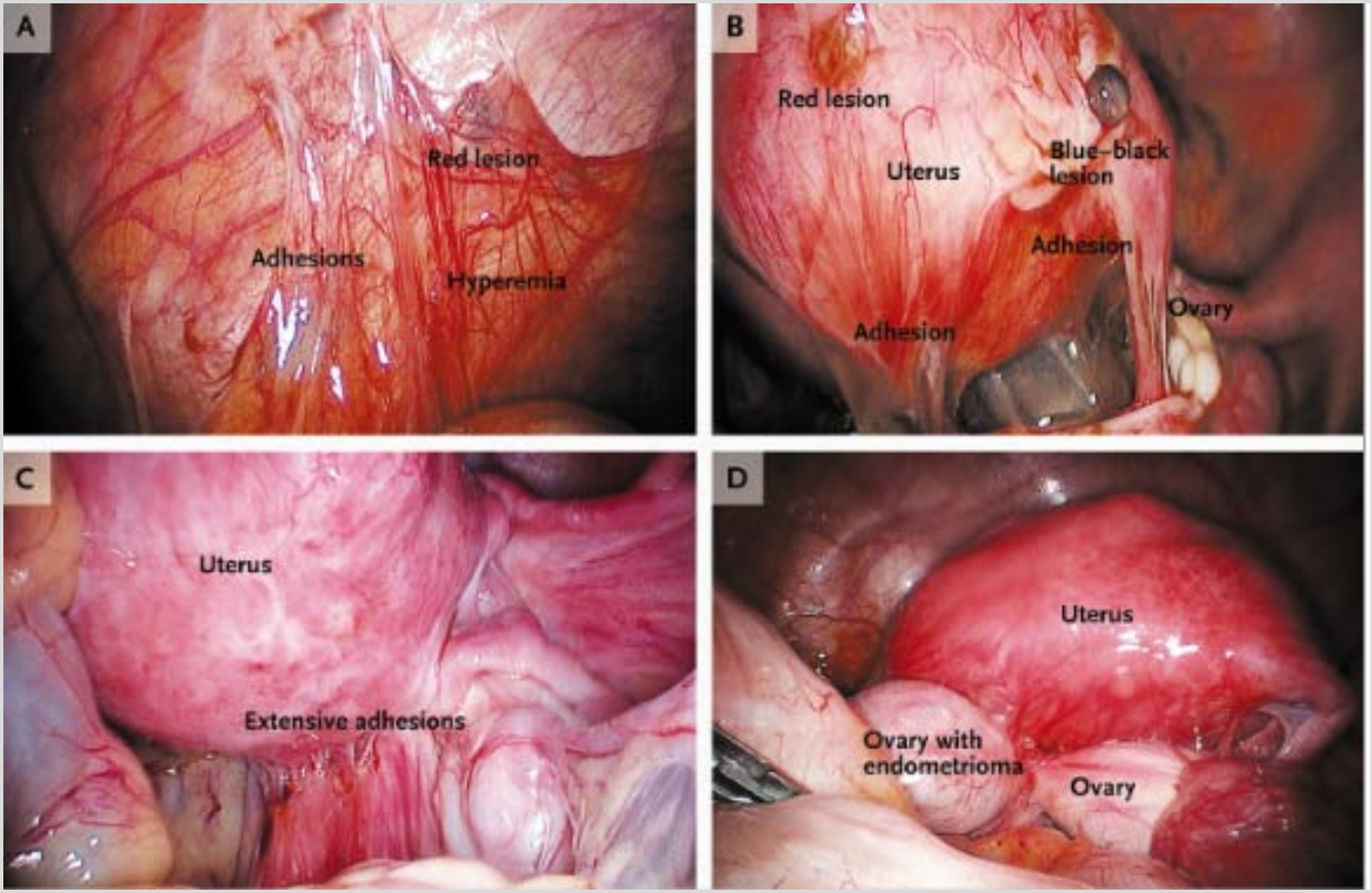
Higher incidence:

- short menstrual cycles
- increased duration of bleeding
- decreased parity
- obstructed outflow (Müllerian anomalies)

Supporting Sampson (2)

- The anatomical distribution of the disease is predominantly in dependent areas of the pelvis
- 90% of women (with patent tubes) have blood in peritoneal fluid at time of menstruation
- **Problem** – although most women have retrograde menstruation most do not have endometriosis

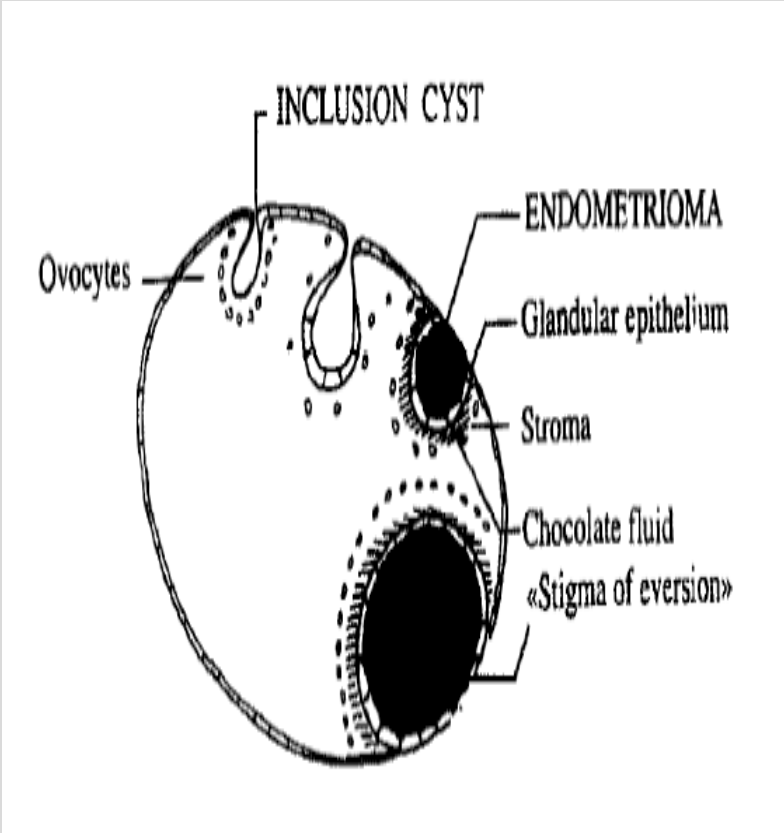
Clinical presentation



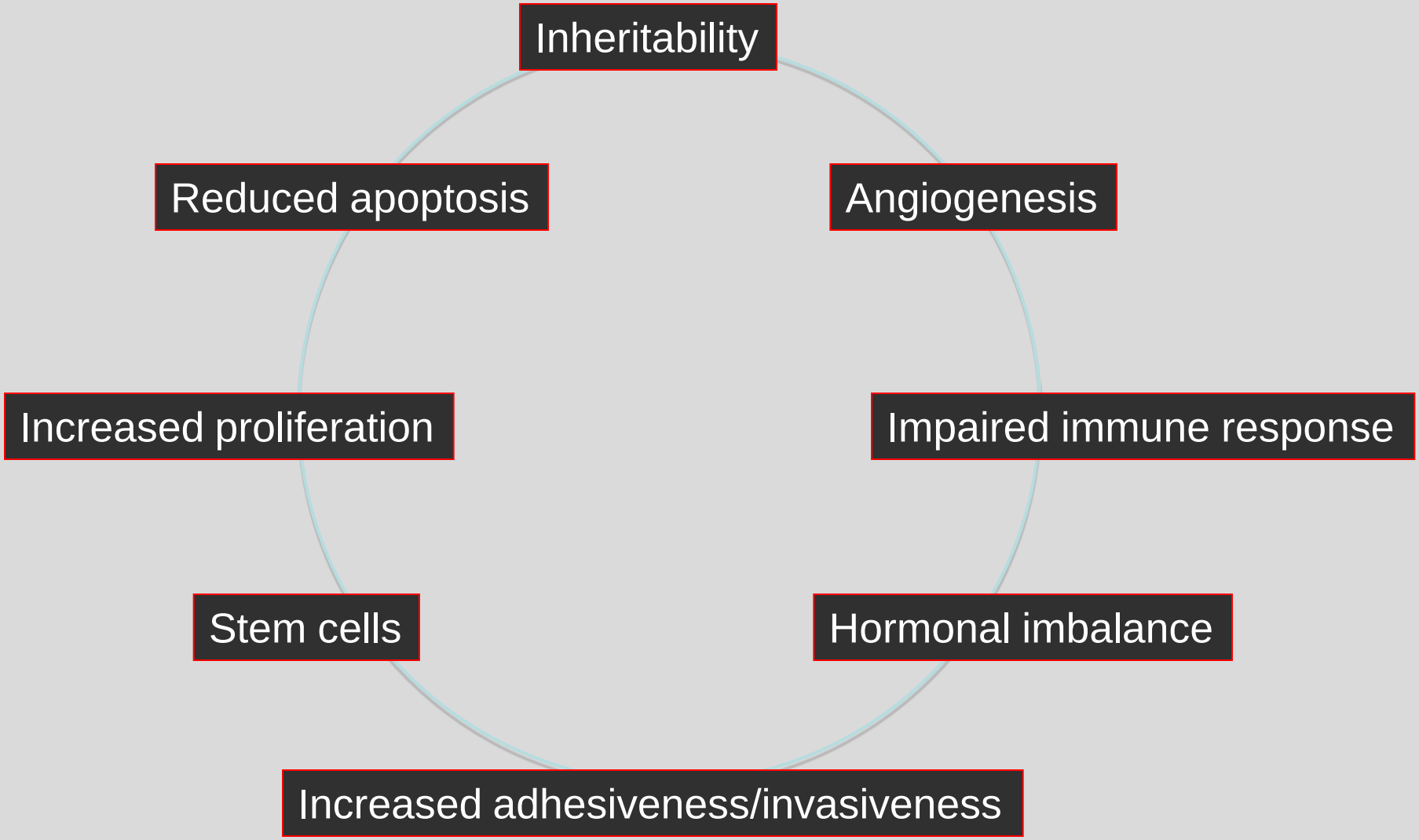
Giudice LC, NEJM 2010

Coelomic metaplasia

- Starts with invagination of mesothelium
- Mesothelial inclusions
- Metaplastic process



Possible mechanisms



Disease progression

Always progressive – self curing?

Second look (24 wks) laparoscopy (gestrinone)

Placebo group		95% CI
Disease improved	29%	9 - 50
Disease eliminated	24%	4 - 43

Thomas EJ & Cooke ID, Br Med J 1987

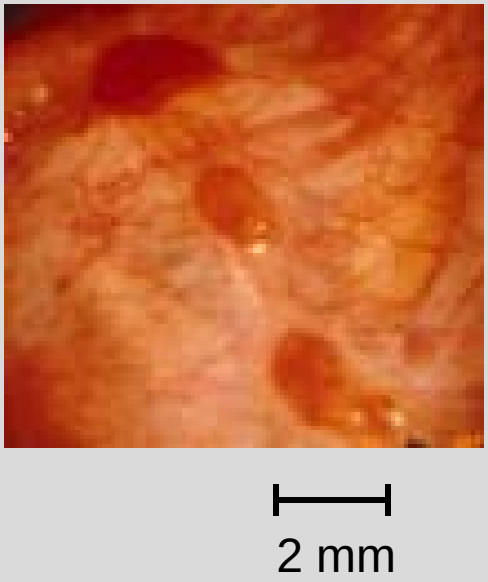
Disease progression

Second look (6 months) laparoscopy (surgery)

Diagnostic laparoscopy group	n	%
Disease progressed	8	45
Disease unchanged	6	33
Disease improved	4	22

Abbott J et al., Fertil Steril 2004

Minimal endometriosis



- All ectopic endometrium = disease?
- Only physiological variant?
- Transient phenomenon?

What is the definition of a normal pelvis?

Microscopic endometriosis

Macroscopic endometriosis	No evidence of disease	
11%	6%	Balasch et al., 1996
13%	6%	Nisolle et al., 1990

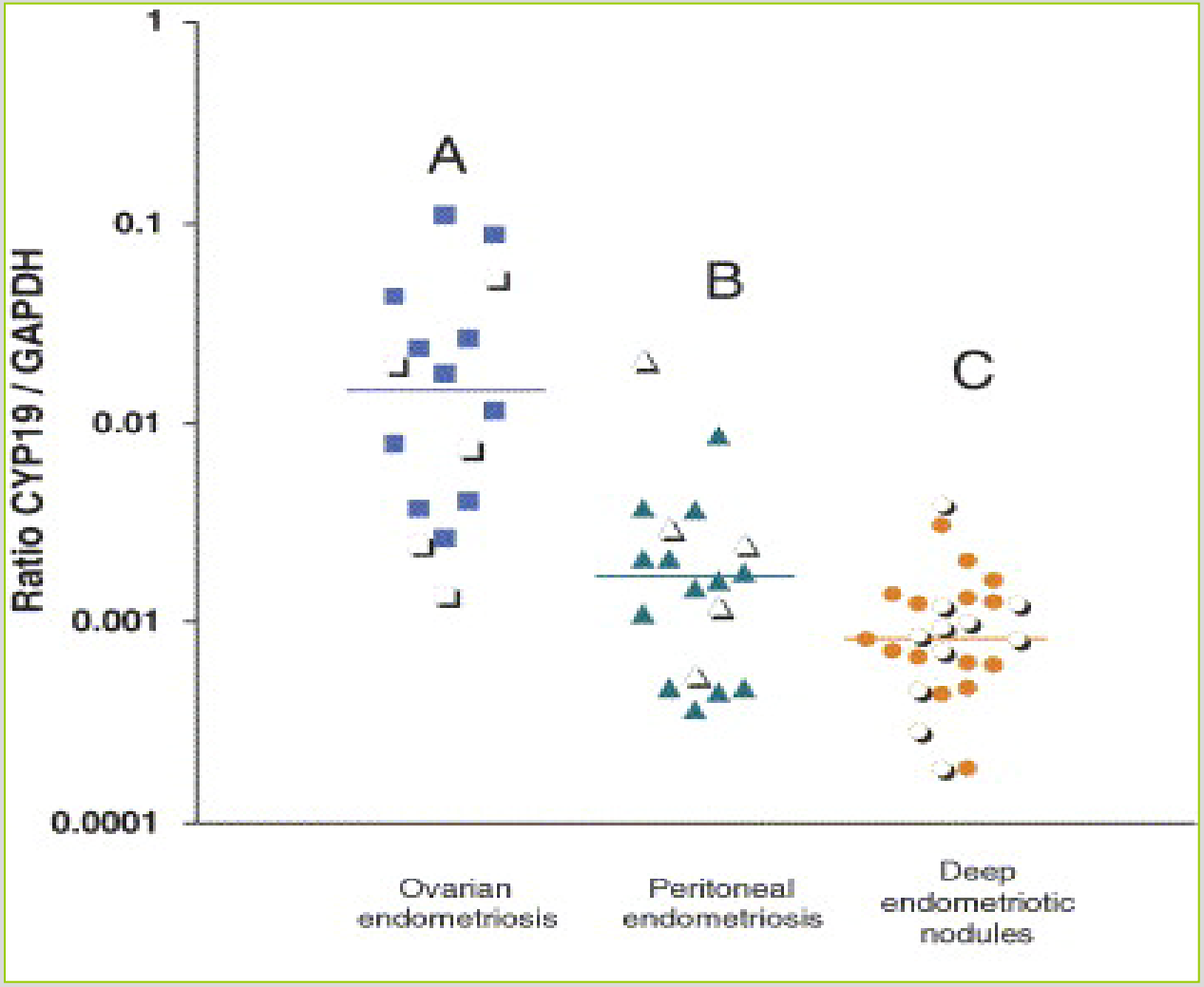
One disease?

Peritoneal endometriosis

Ovarian endometriosis

Recto-vaginal endometriosis

Distribution of aromatase in endometriosis tissue



Heilier JF et al., Fert Steril 2006

Peritoneum

Peritoneum

- *Peritonaion* = stretch around (gr.)
- Serous, semi-permeable membrane 1 - 2 m^2
- 100 ml serous transudate daily
- 80% of lymphatic drainage through subdiaphragmatic lymph vessels
- Cavity closed in males, open in females (Fallopian tubes)
- Mesothelium
- Cubical epithelium (ovaries)
- Culumnar ciliated epithelium (fimbriae)

Peritoneum

Parietal peritoneum:

- Embryologically derived from the somato-pleural layer of the lateral plate of mesoderm
- Blood supply and innervation same as overlying body wall
- Pain sensitive (somatic innervation)

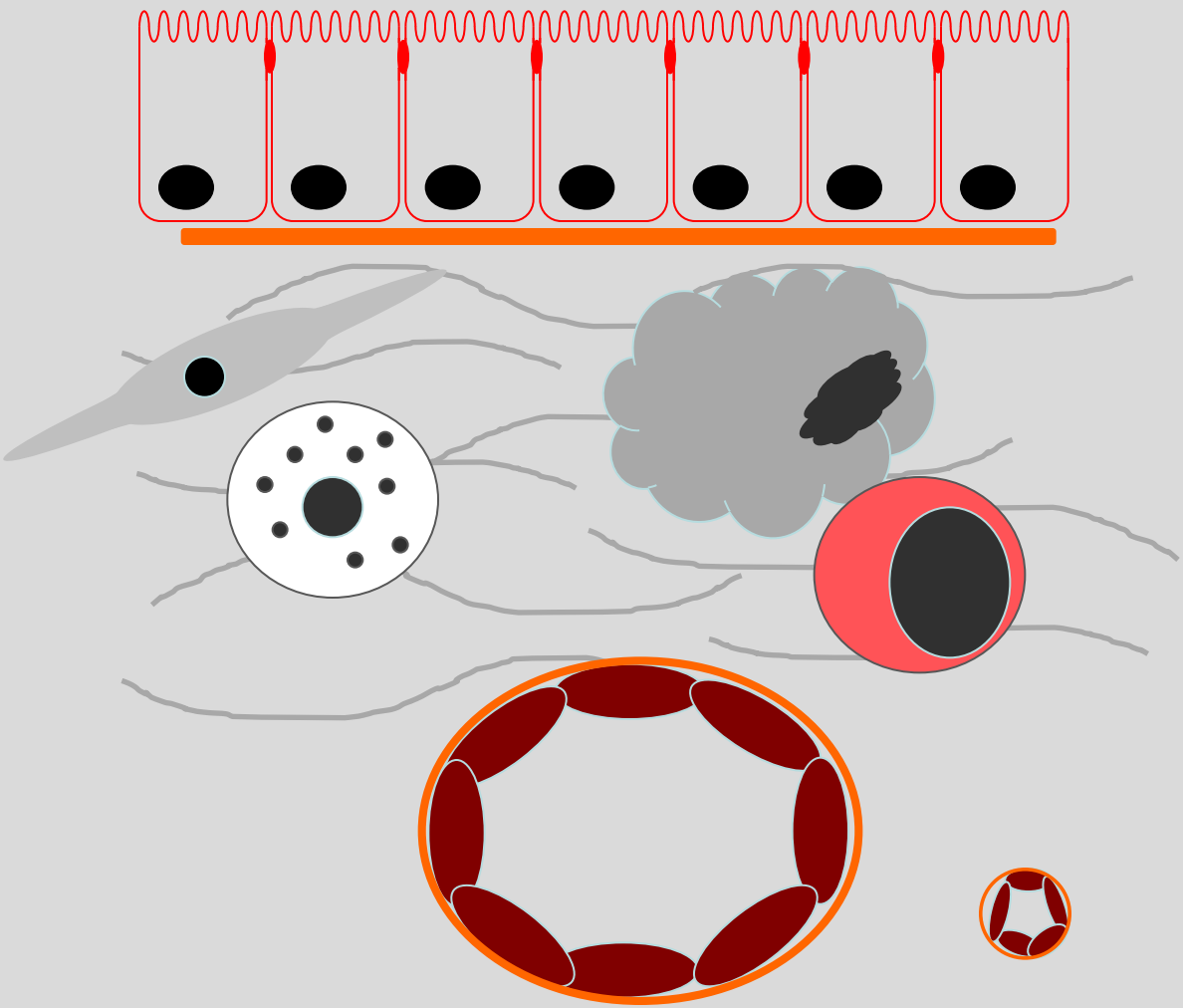
Visceral peritoneum:

- Embryologically derived from the splanchno-pleural layer of the lateral plate of mesoderm
- Blood supply and innervation same as underlying viscera
- Pain insensitive (autonomic innervation)

Peritoneal function

- Contains phagocytic cells
- Contains lymphocytes for humoral and cellular immune reaction
- Movements of viscera
- Absorption
- Storage of fat
- Transformation of mesothelial cells for wound healing

Peritoneum



Mesothelium

Basement membrane

Fibroblasts

Macrophages

Mast cells

Lymphocytes

Blood vessels

Lymph vessels

Origin of endometriotic cells

- Locally through transformation/differentiation
- Endometrium (Retrograde menstruation)
- Bone marrow
- Combination

Endometrial stem cells

Necessary growth factors:

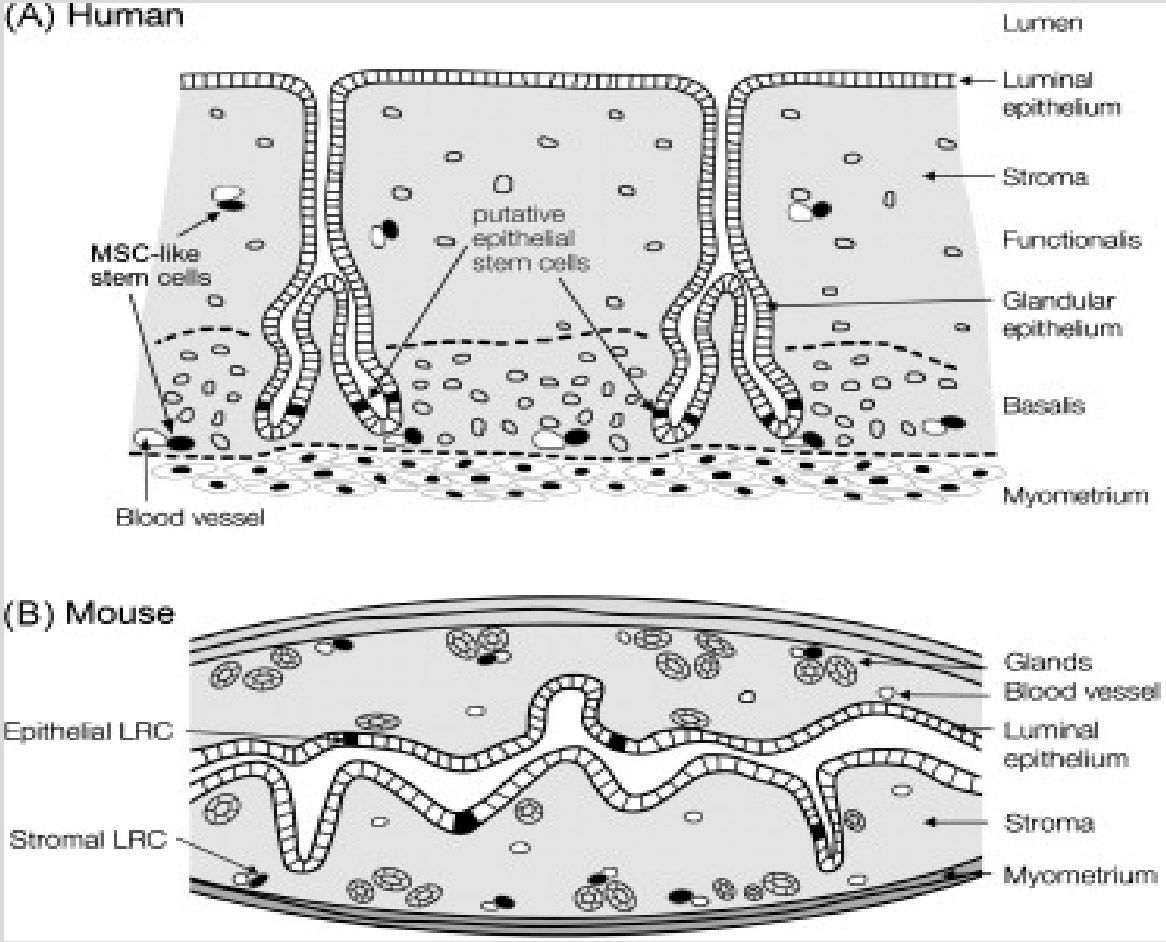
Epithelial colony-forming units:

- EGF
- TGF-α
- PDGF-BB

Stromal colony-forming units:

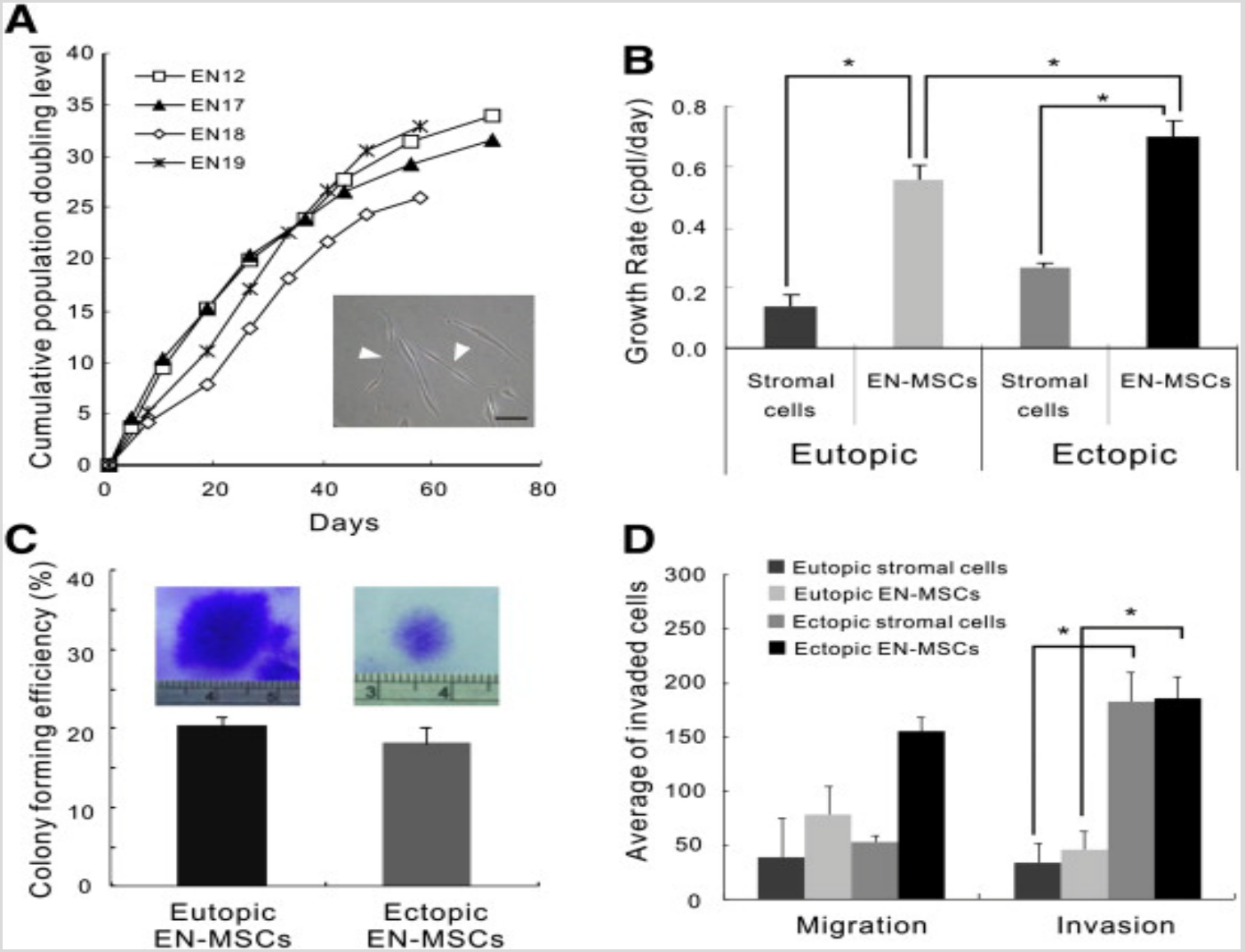
- EGF
- TGF-α
- PDGF-BB
- bFGF

Side populations



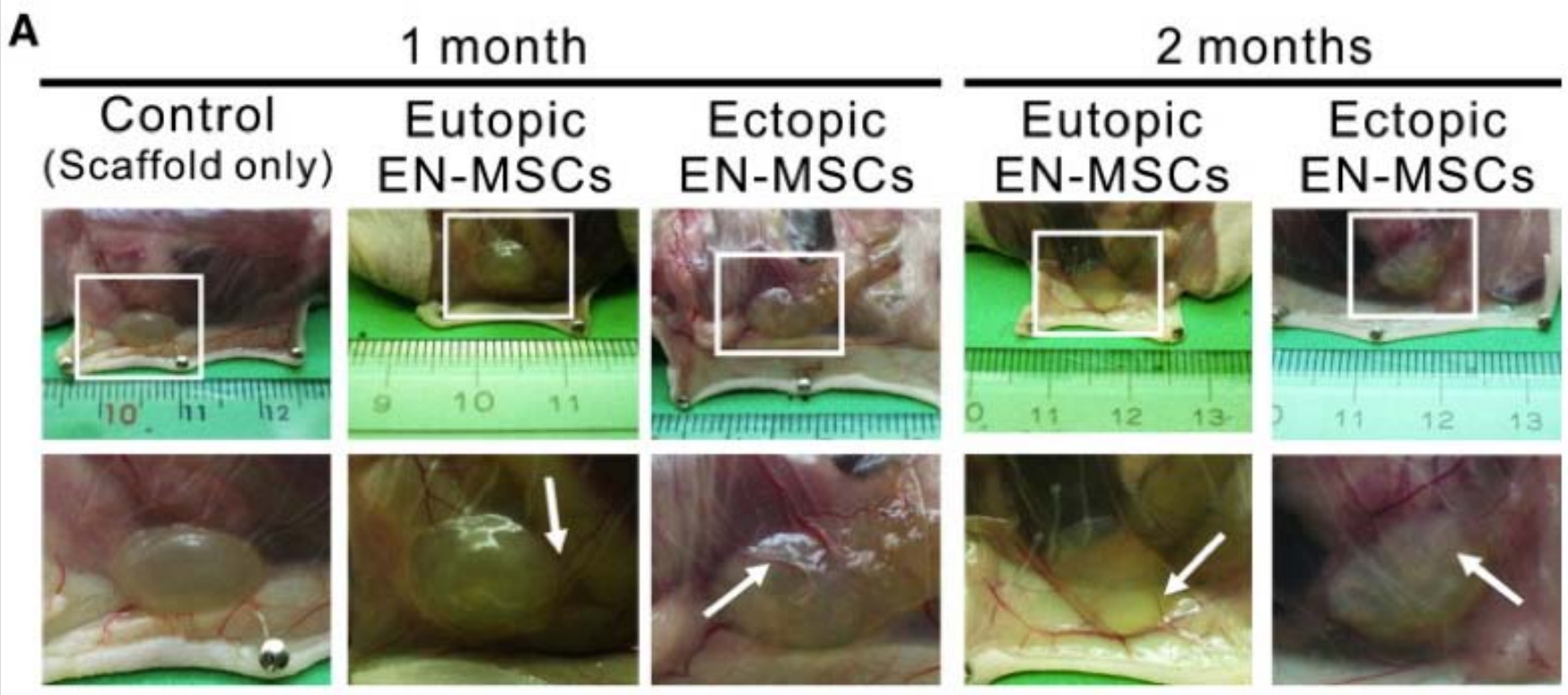
Gargett CE et al., Mol Cell Endocrinol 2008

Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells



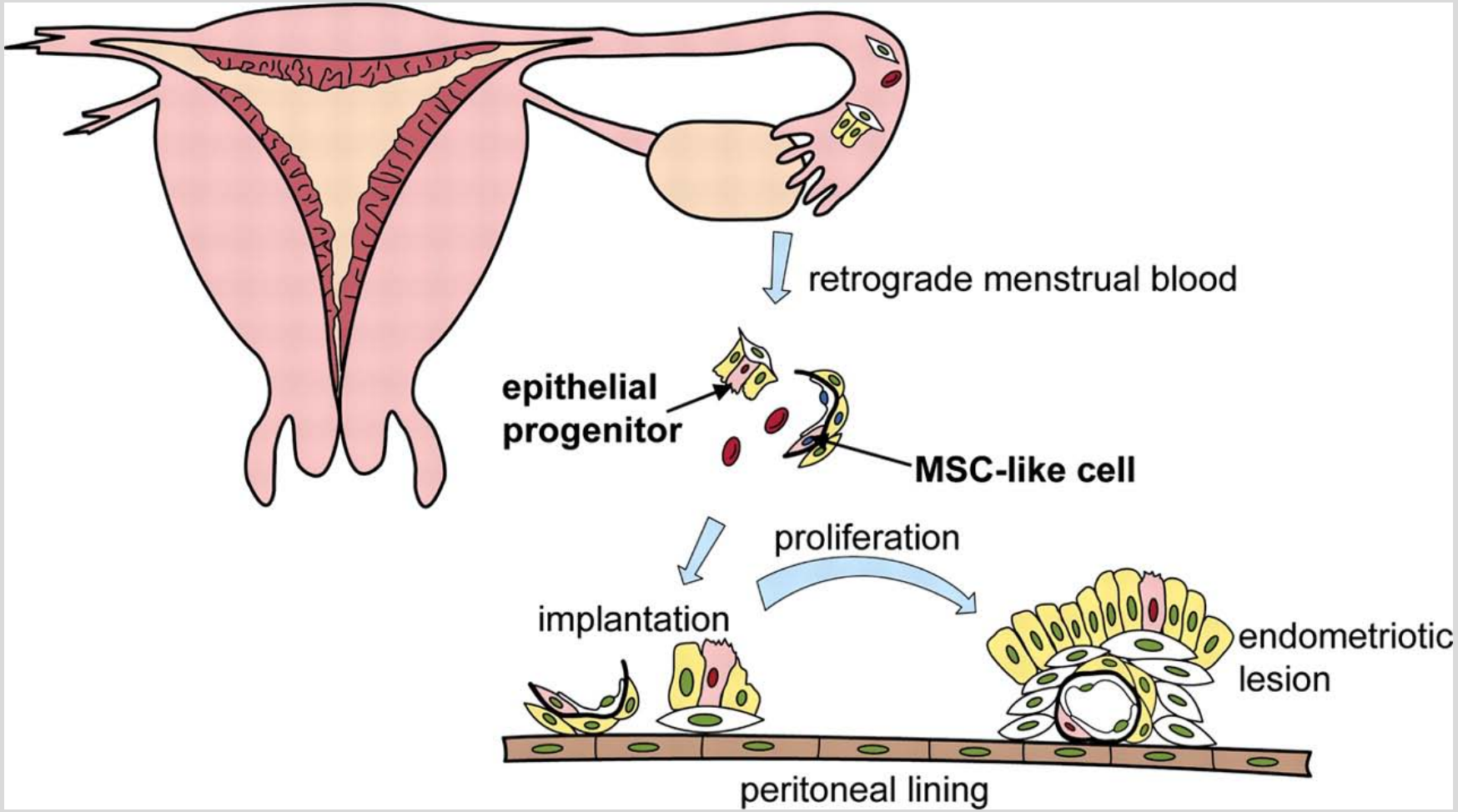
Kao AP et al., Fertil Steril 2011

Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells



Kao AP et al., Fertil Steril 2011

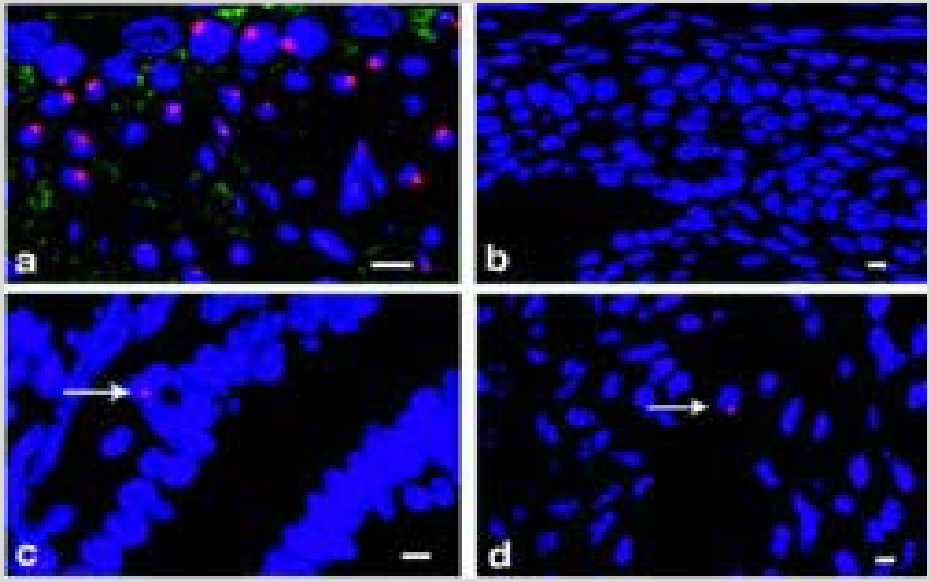
Possible role of endometrial stem/progenitor cells in the pathogenesis of endometriosis.

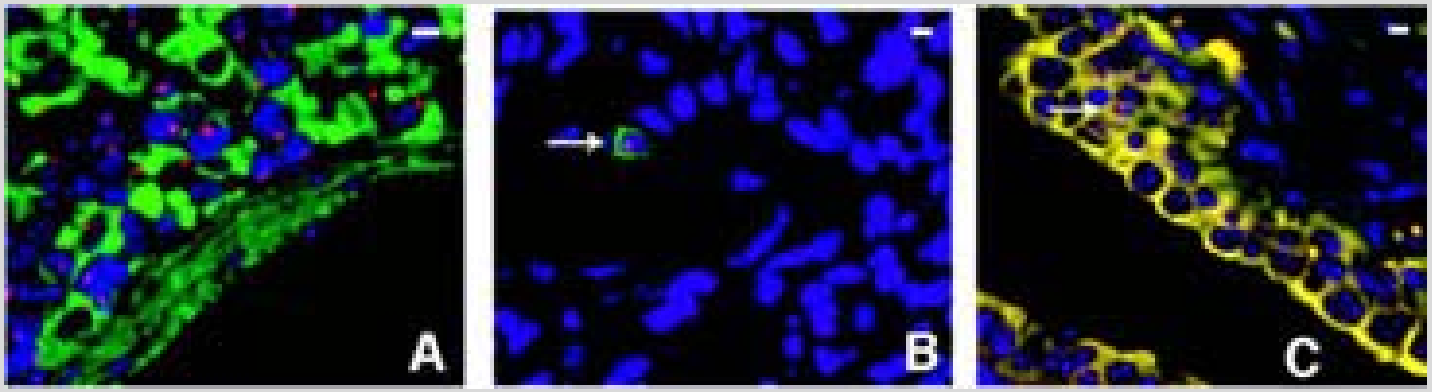


Gargett CE , Masuda H, Mol. Hum. Reprod 2010

Bone marrow origin

Male-to-female BM transplantation model

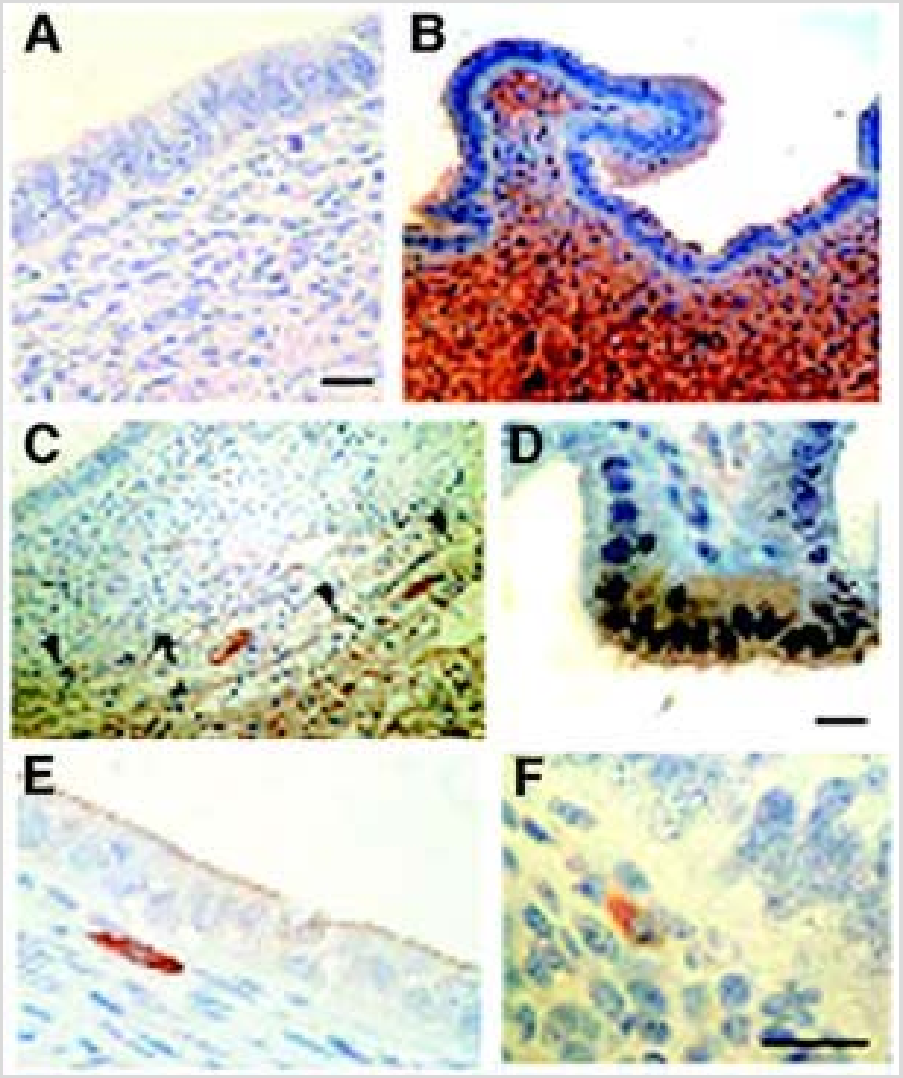




Red = Y chromosome
Green = CD45
Blue = Nuclei
Yellow = Cytokeratin

Du H and Taylor HS., Stem Cells 2007

Bone marrow origin

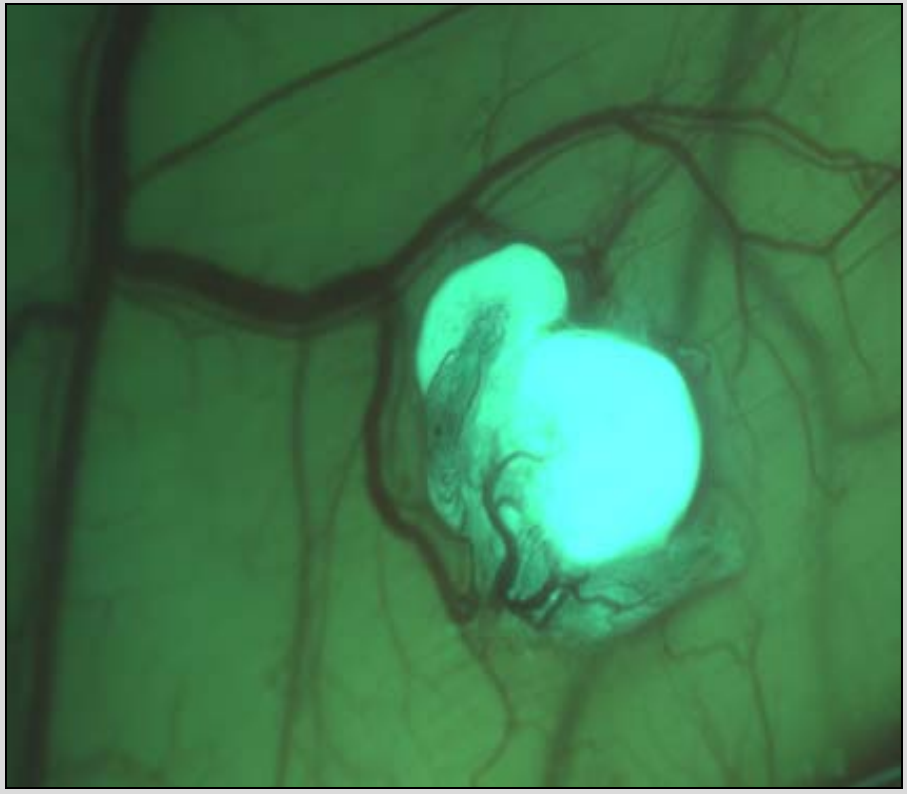


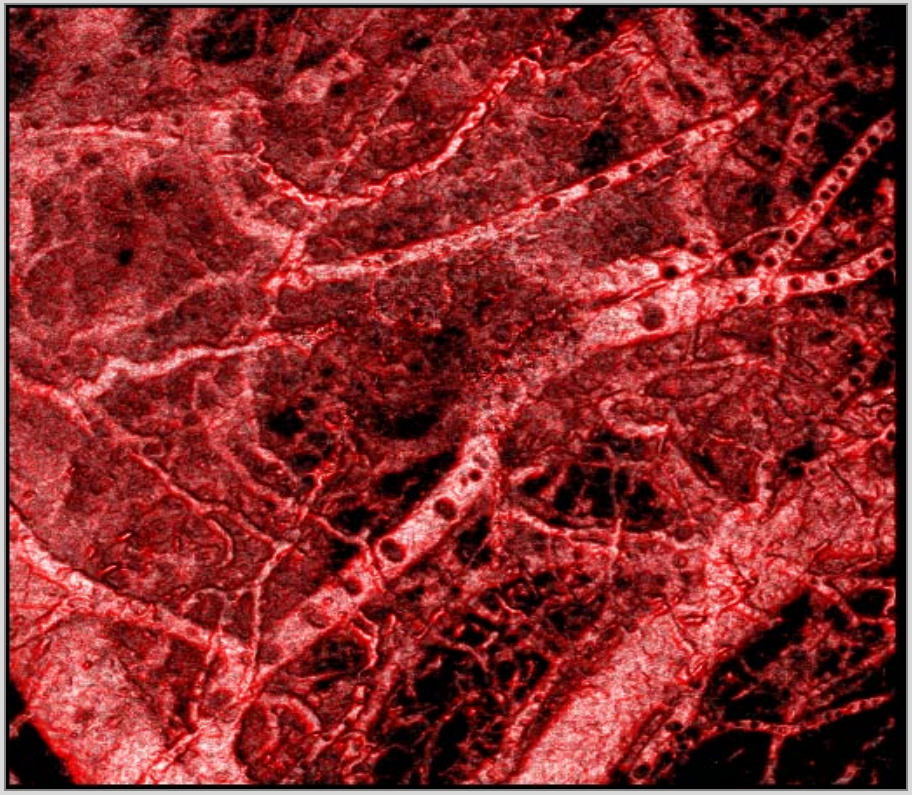
- LacZ endometriosis transplantation model
- Endometriosis auto-transplantation

➡ Incorporation of BM-derived stem cells into endometriosis-like lesions

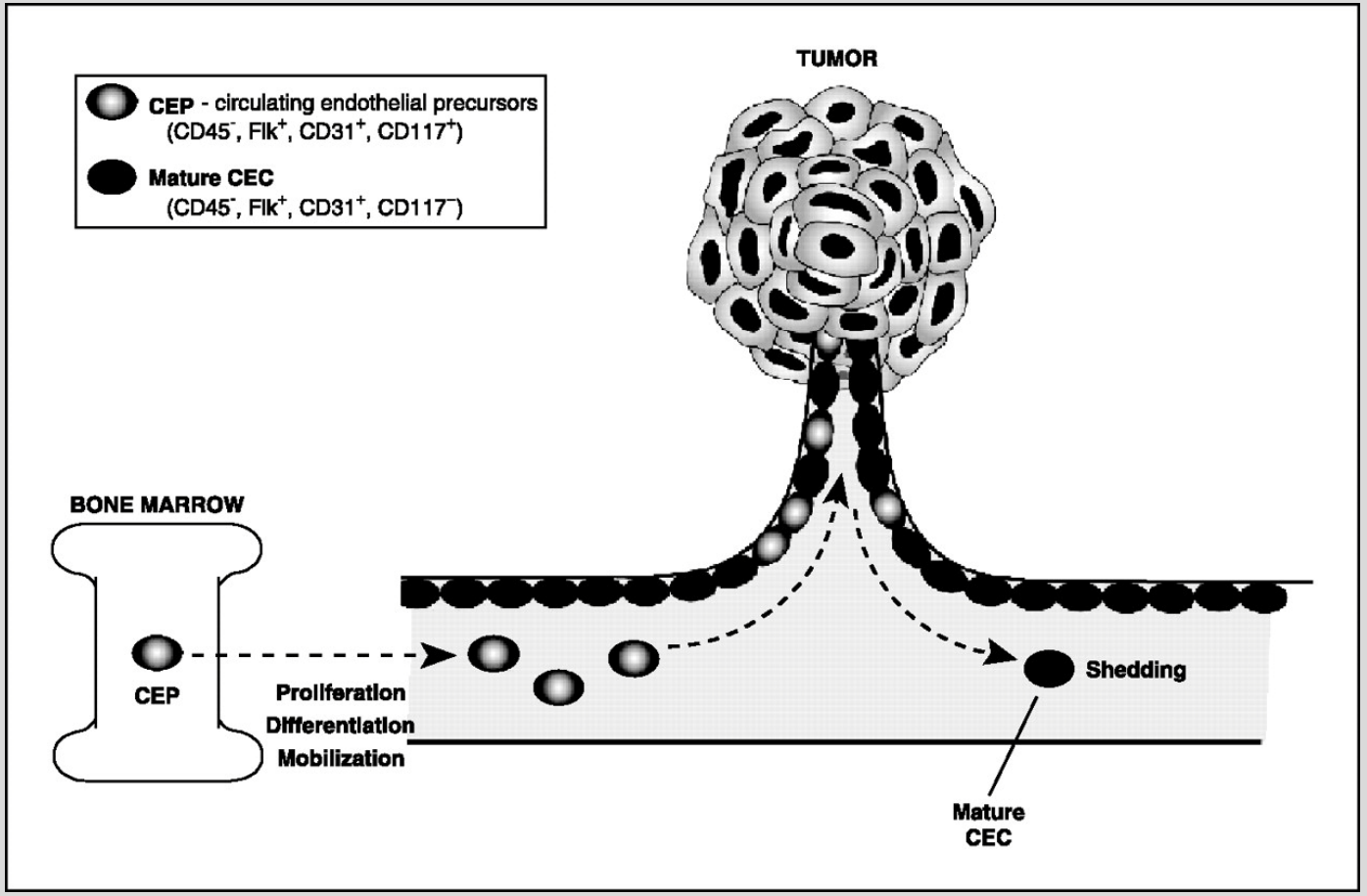
Du H and Taylor HS., Stem Cells 2007

Angiogenesis





Endothelial progenitor cells

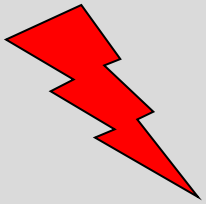


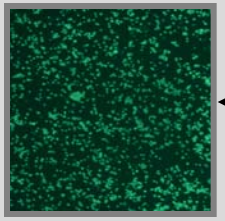
Beaudry P et al., Clin Cancer Res 2005

GFP +/+ bone marrow

Wild type

+



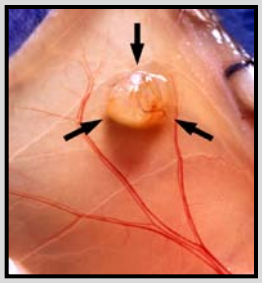


GFP +/+

WT GFP+/+ bm

+

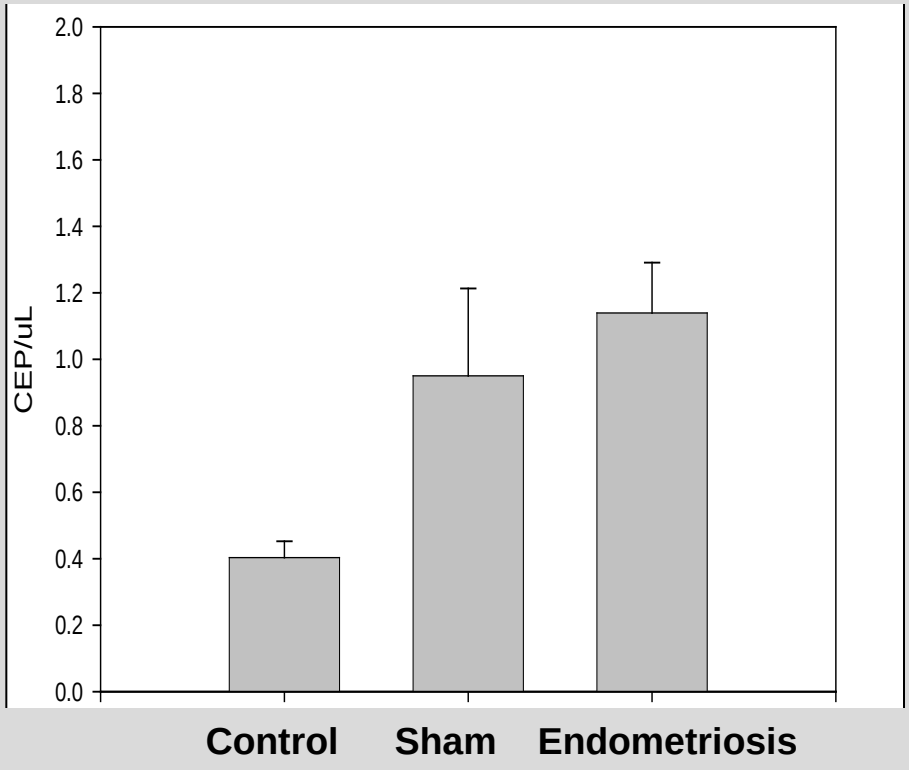
Wild type



WTGFP+/+ bm
WT endo

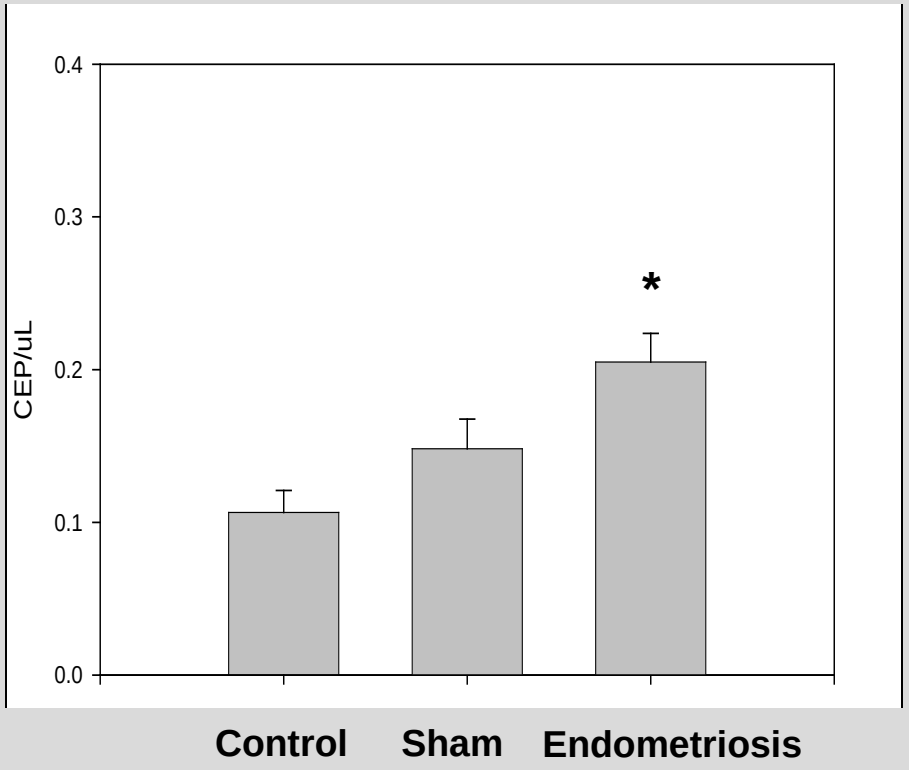
CEP in endometriosis model
(C57BL/6 mice)

4 Lesions



1 week post surgery

6 Lesions



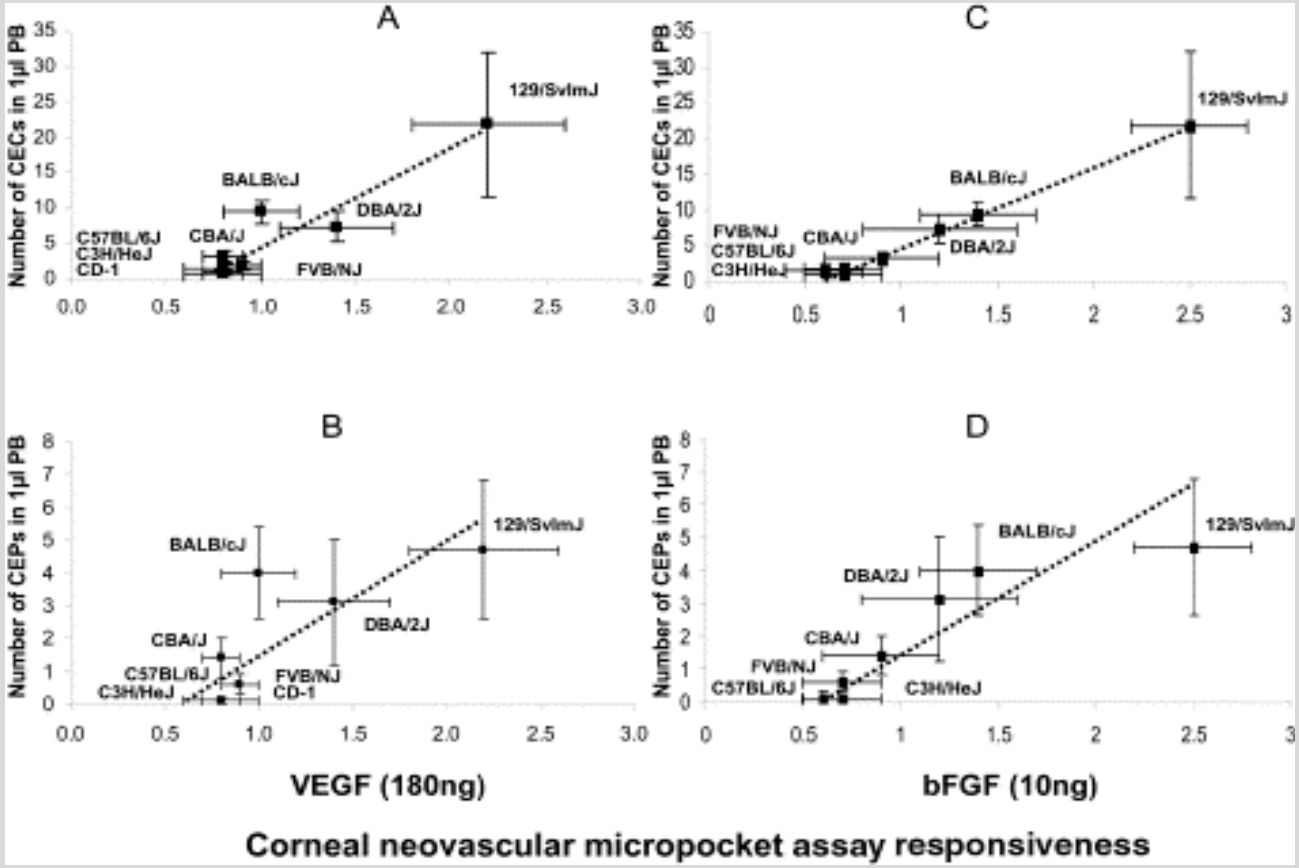
1 week post surgery

Becker CM et al., Am J Pathol 2011

ARTICLE

Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis: Implications for cellular surrogate marker analysis of antiangiogenesis

Yuval Shaked,[1] Francesco Bertolini,[2] Shan Man,[1] Michael S. Rogers,[3] Dave Cervi,[1,4] Thomas Foutz,[2] Kimberley Rawn,[1] Daniel Voskas,[1,4] Daniel J. Dumont,[1,4] Yaacov Ben-David,[1,4] Jack Lawler,[5] Jack Henkin,[6] Jim Huber,[7] Daniel J. Hicklin,[7] Robert J. D'Amato,[3] and Robert S. Kerbel[1,4,*]

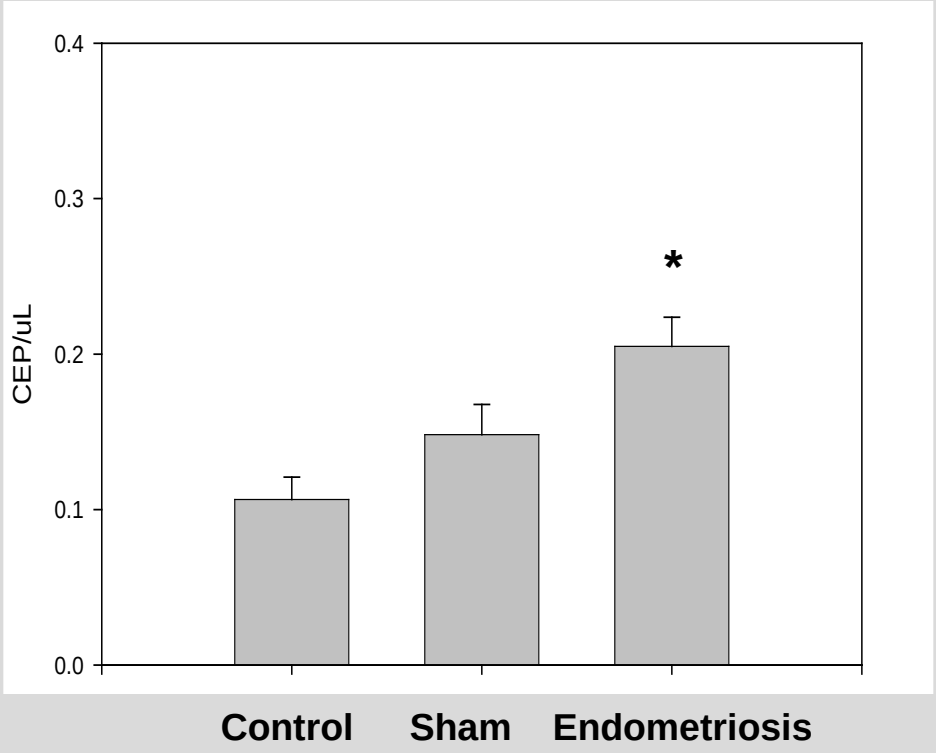


Corneal neovascular micropocket assay responsiveness

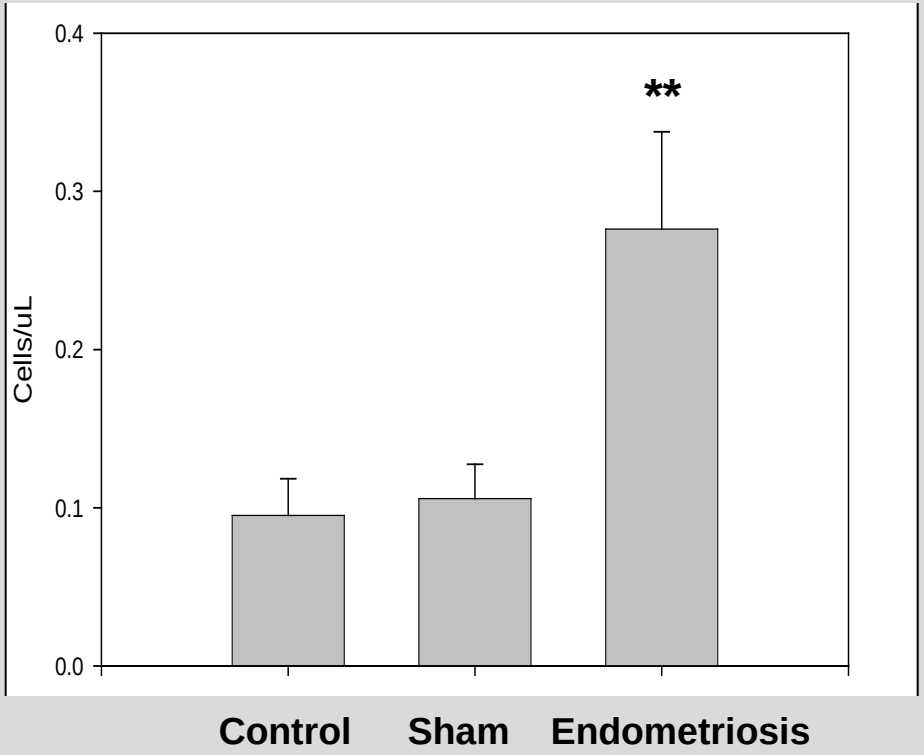
Shaked Y et al., Cancer Cell 2005

CEP in endometriosis model (6 lesions)

C57BL/6 mice

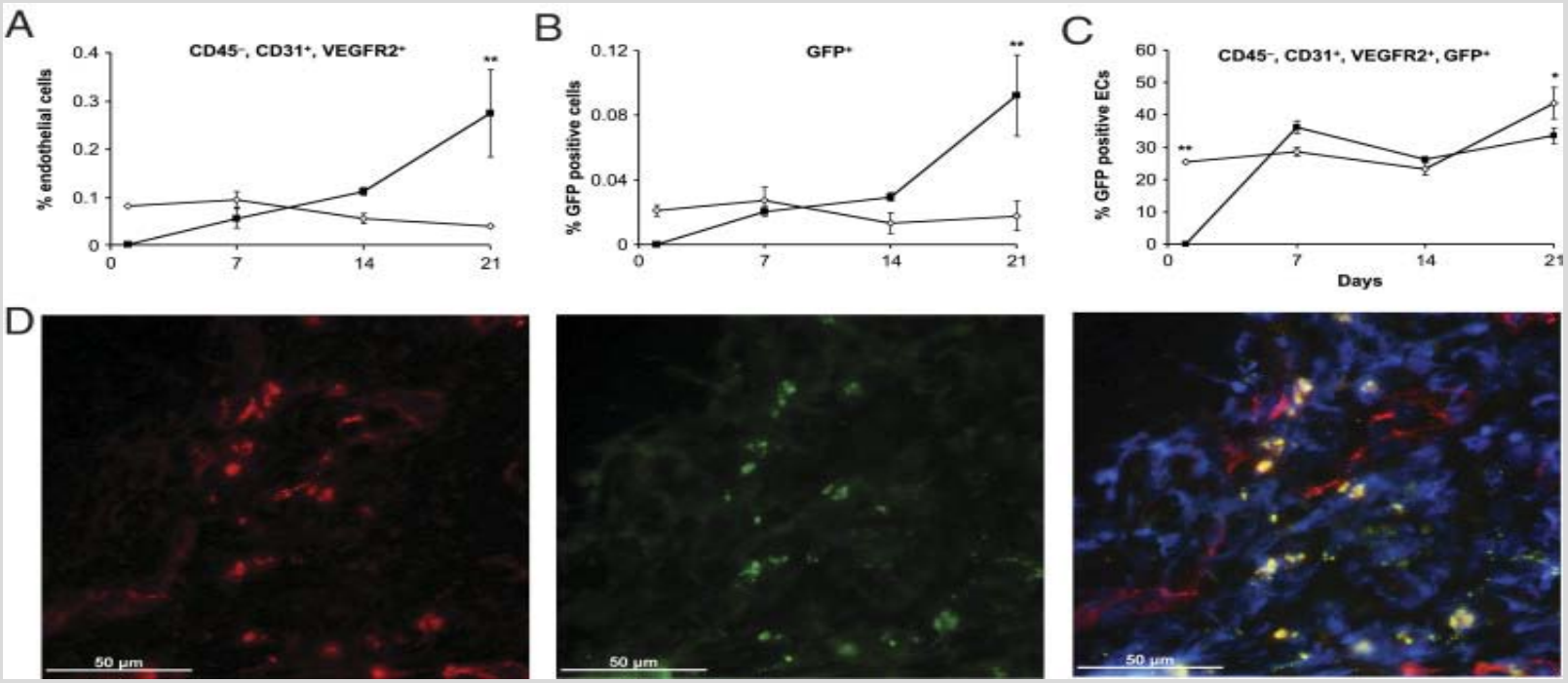


129SvJ mice



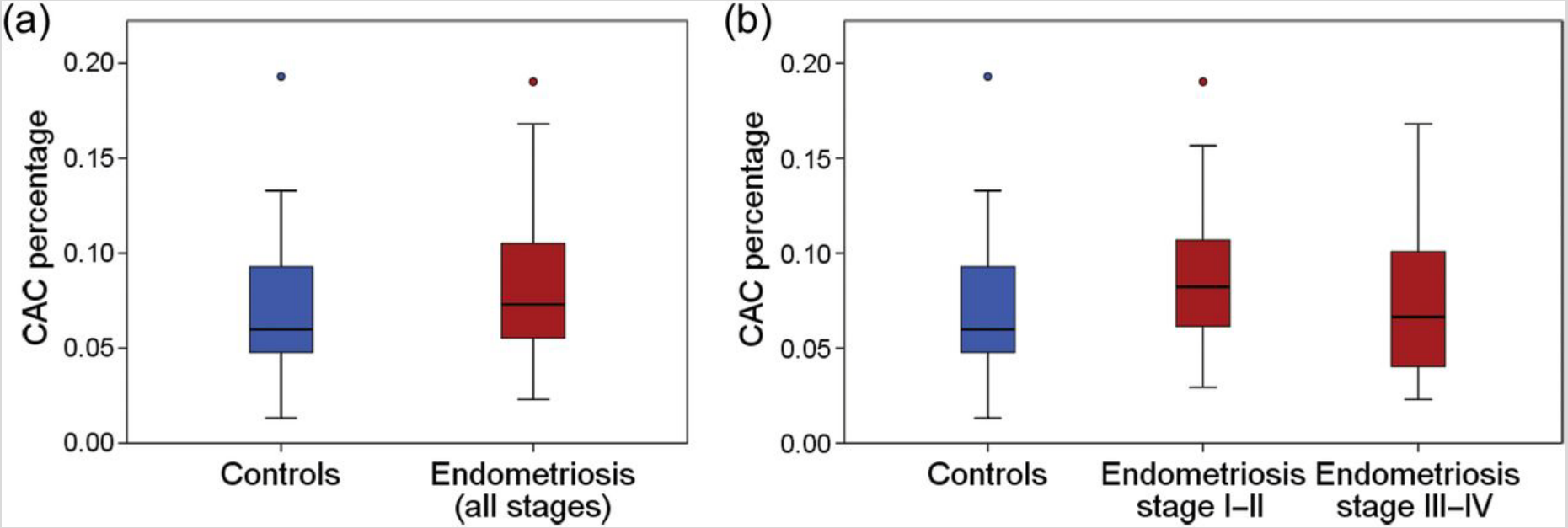
Becker CM et al., Am J Pathol 2011

Bone marrow origin



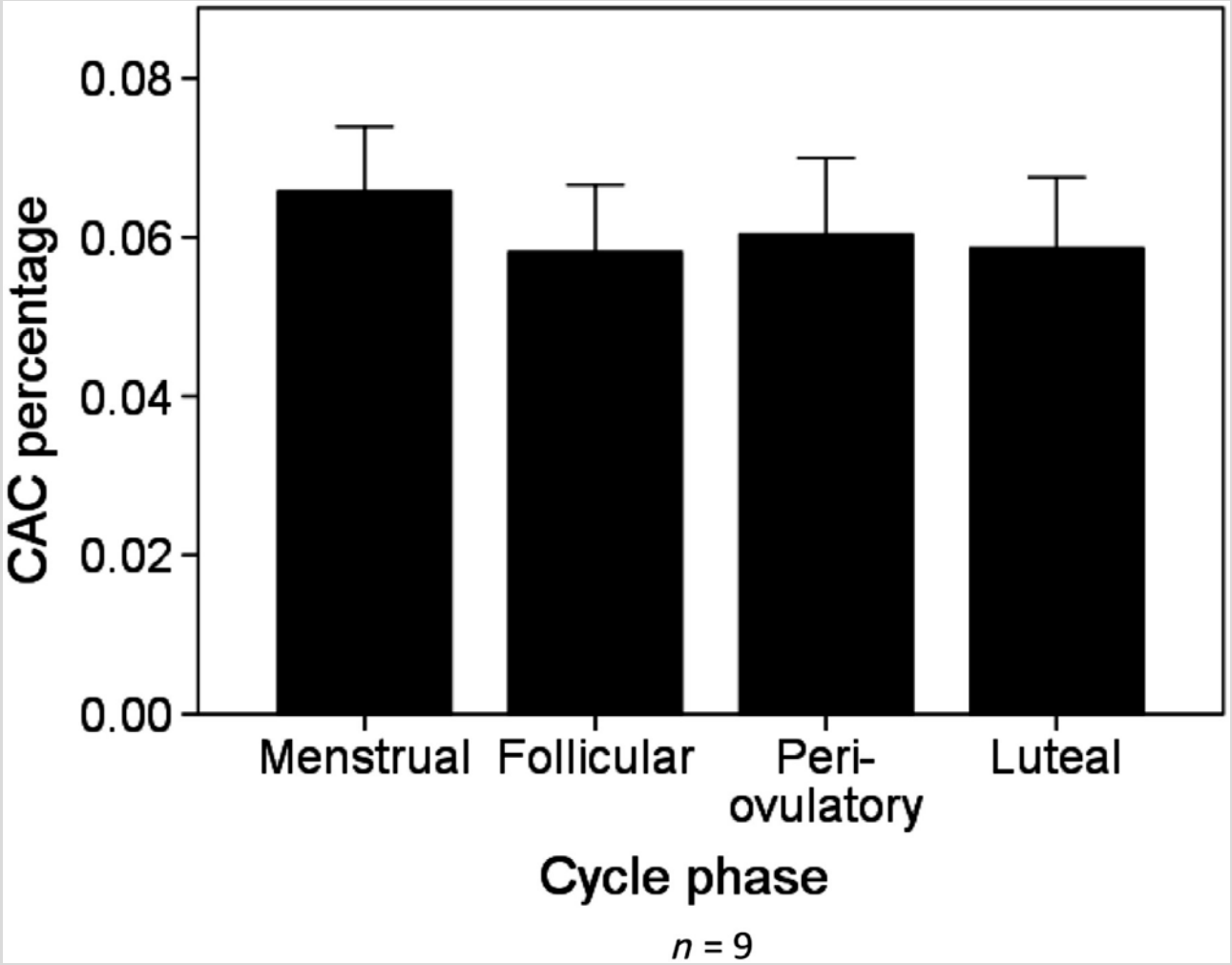
Becker CM et al., Am J Pathol 2011

CAC levels in women with and without endometriosis



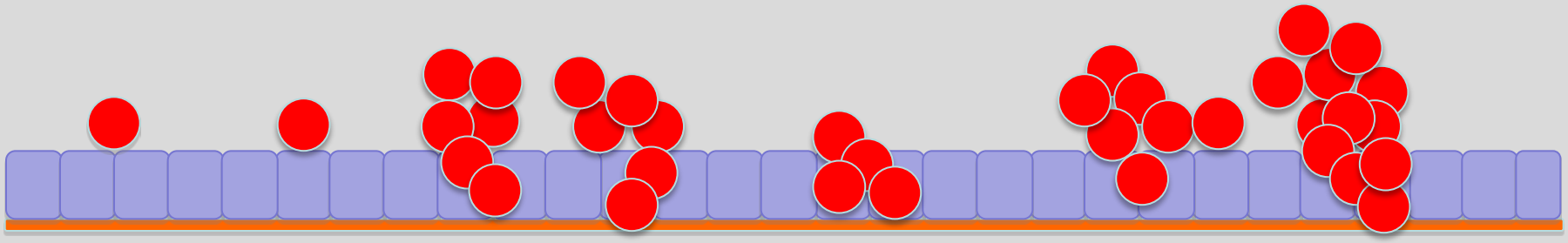
Webster K at al., Hum Reprod 2013

CAC levels in healthy women throughout the menstrual cycle

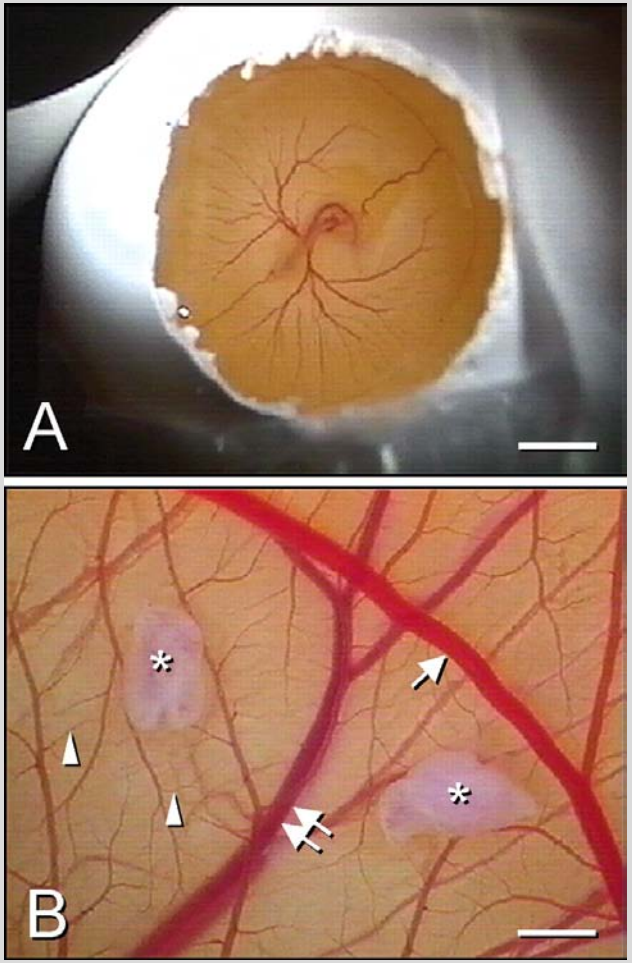


Webster K at al., Hum Reprod 2013

Mechanism



Mesothelial-epithelial interaction



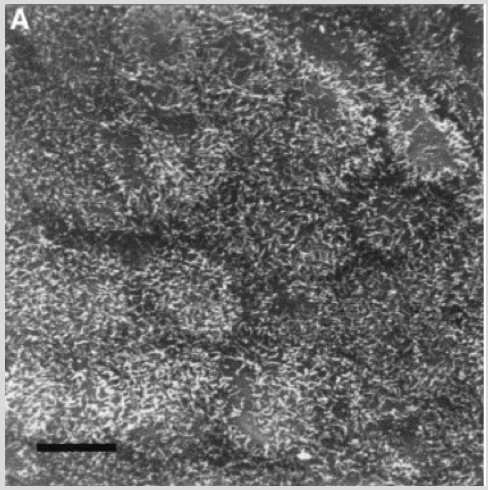
Laschke MW , Menger MD, Hum Reprod Update 2007

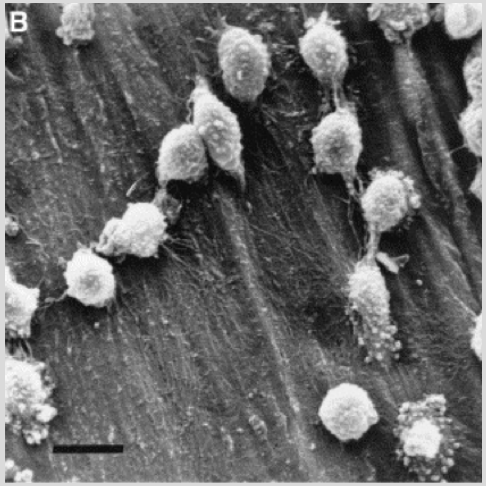
Impact of tissue integrity on infiltration and endometriosis-like lesion formation in the chorioallantoic membrane (CAM)

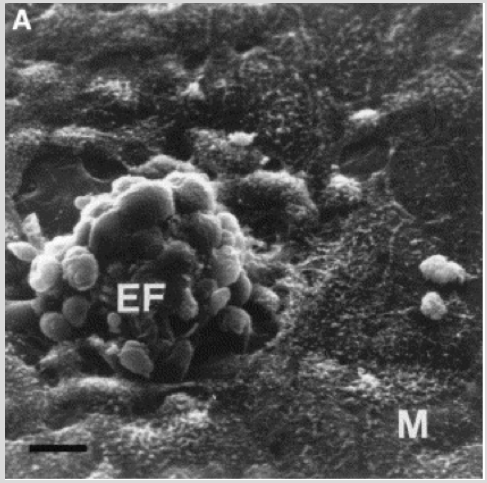
Endometrium	No. of CAM	Infiltration	Lesion
Biopsied cyclic endometrium	19	14 (74)	13 (68)
Biopsied menstrual endometrium	18	14 (78)	12 (67)
Endometrial cells isolated from menstrual effluent collected in Keeper	50	0 (0)*	0 (0)*
Tissue fragments in menstrual effluent collected in Keeper	16	10 (63)	7 (44)**
Collagenase digested biopsied cyclic endometrium	45	24 (53)**	1 (2)*
Biopsied menstrual endometrium, stored in Keeper	13	10 (77)	9 (69)
Biopsied menstrual endometrium, stored in Keeper, processed as menstrual effluent	11	6 (55)	4 (36)**

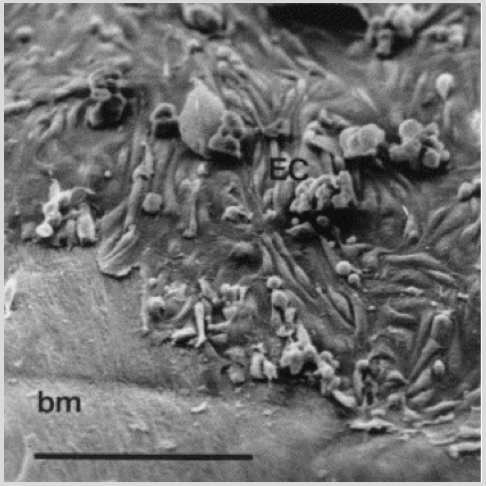
Nap AW et al., Hum Reprod 2003

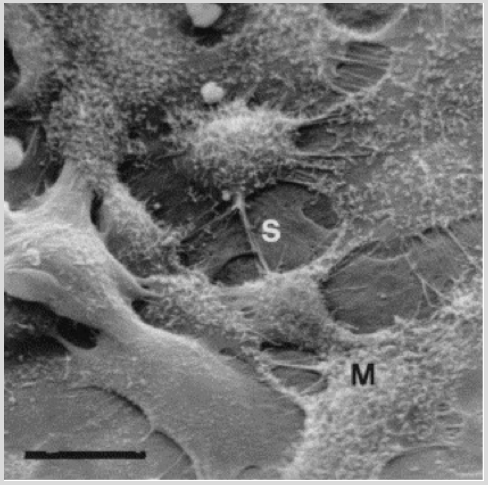
Mesothelial-epithelial interaction





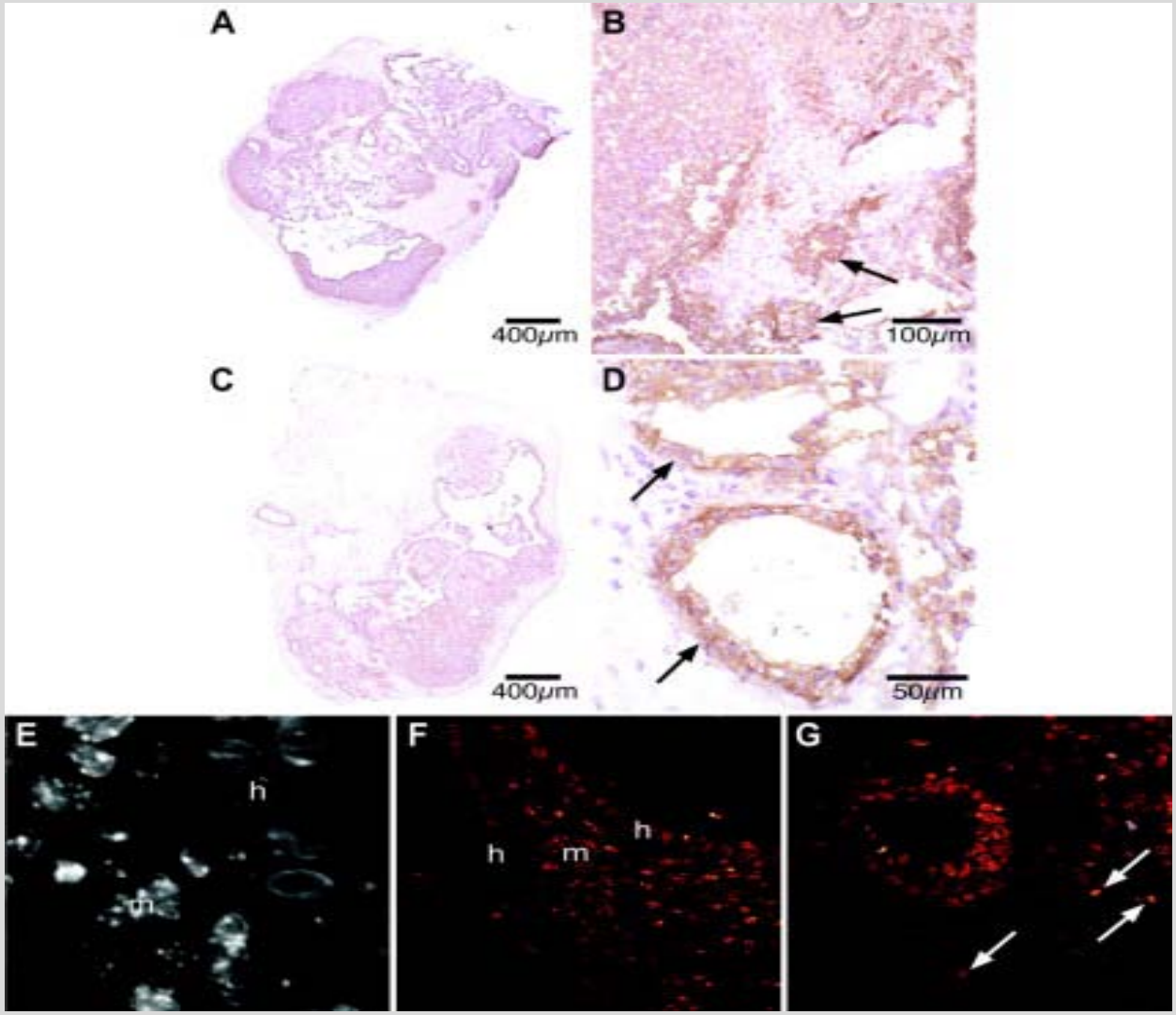






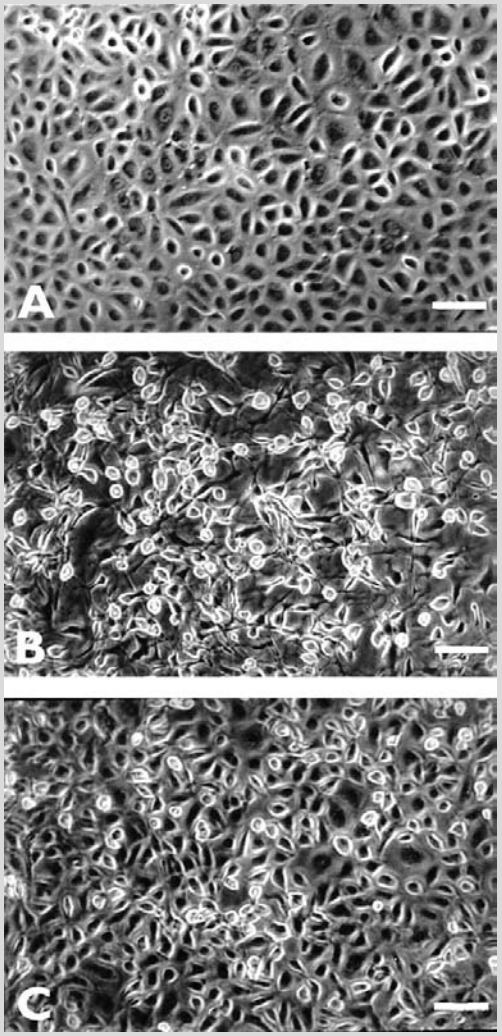
Groothuis PG et al., Fertil Steril 1999

Mesothelial-epithelial interaction

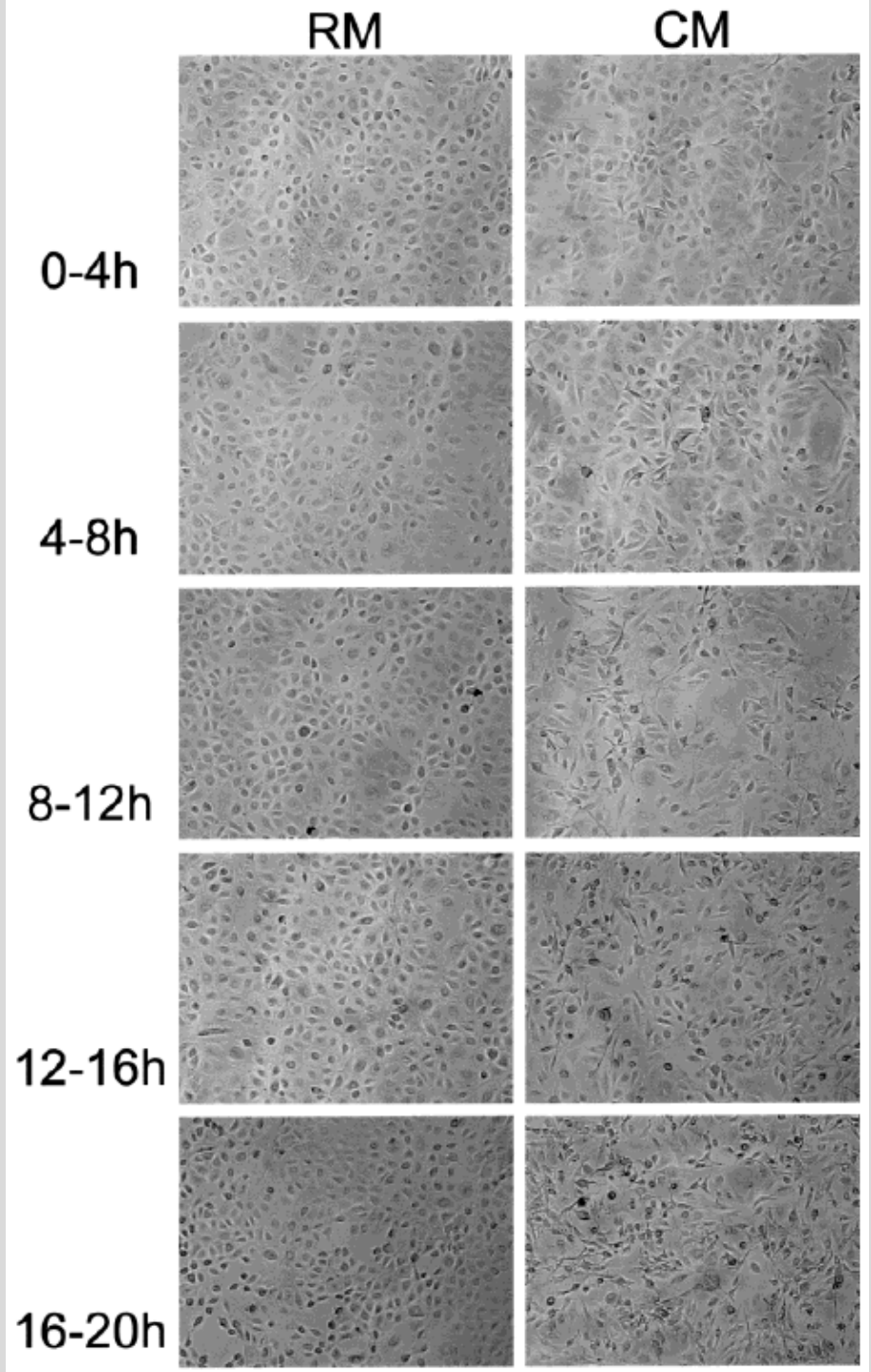


Hull ML et al., Am J Pathol 2010

Light micrographs of HOMEC after overnight co-incubation with conditioned medium.



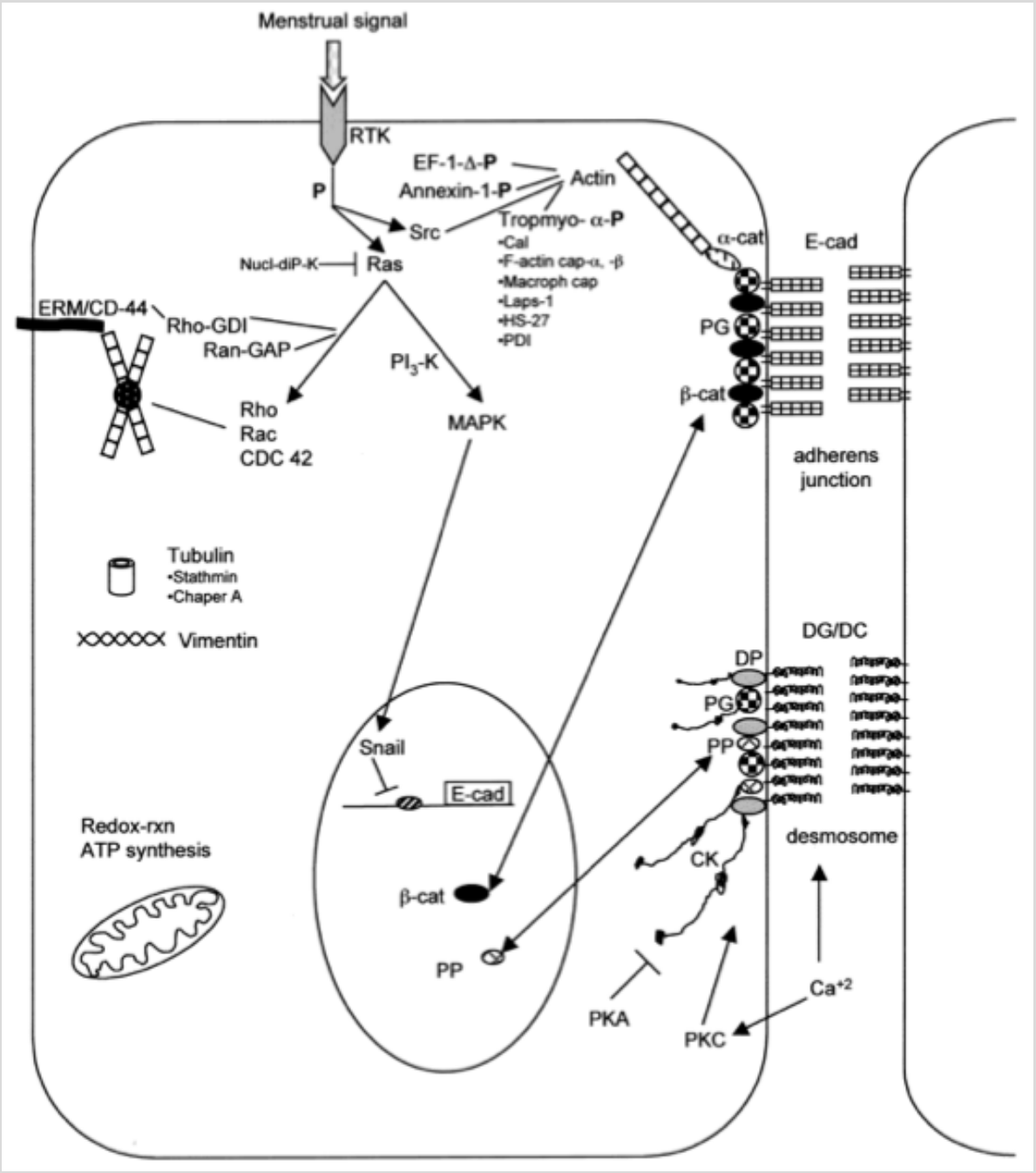
Demir Weusten AY et al., Hum. Reprod. 2000



Epithelial mesenchymal transformation (EMT)

Omental mesenchymal cells incubated with normal or conditioned media (from antegrade menstruation blood)

Demir AY et al., Proteomics 2004

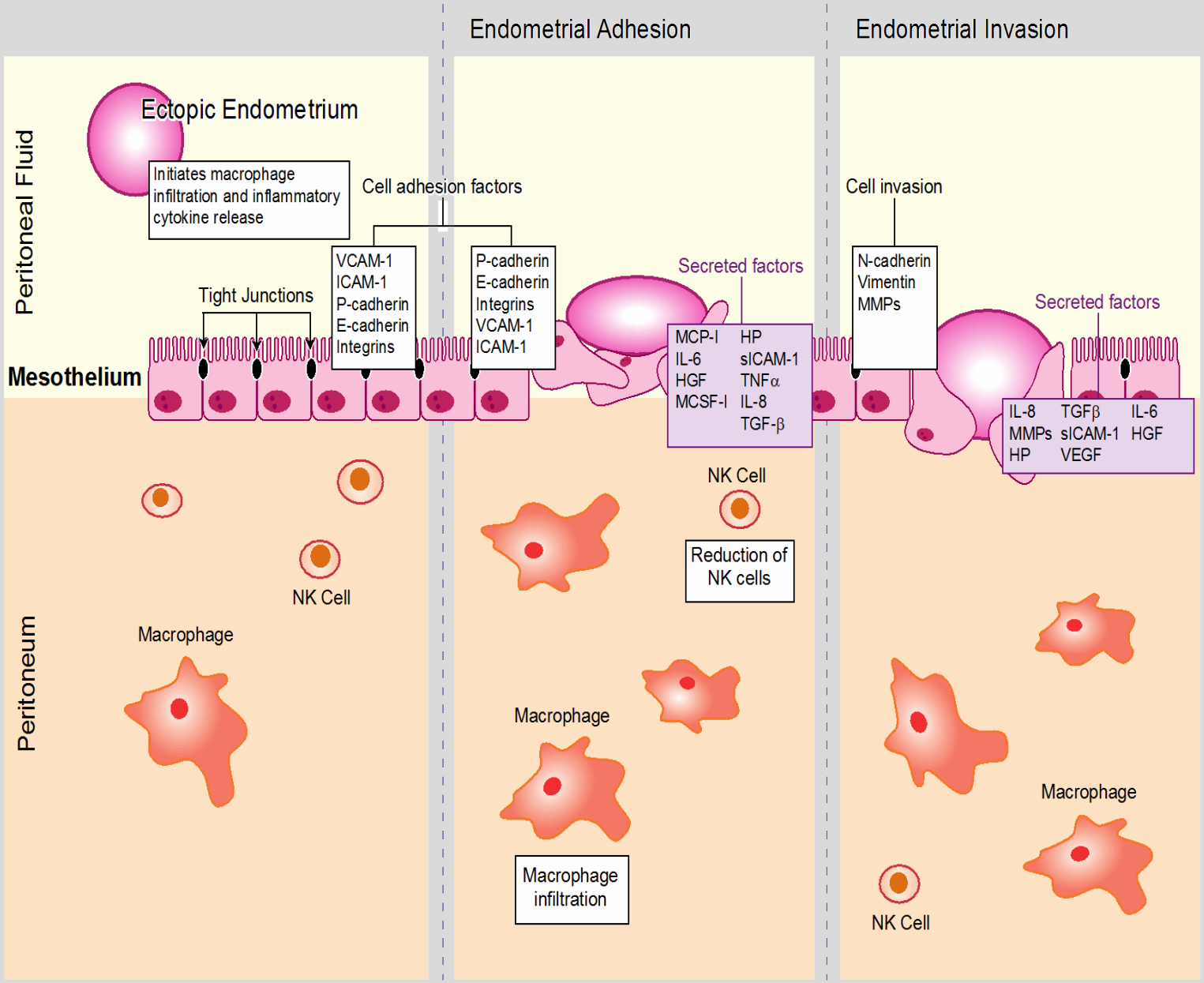


Differential expression and phosphorylation of mesothelial proteins.

Expression of 35 proteins was altered:

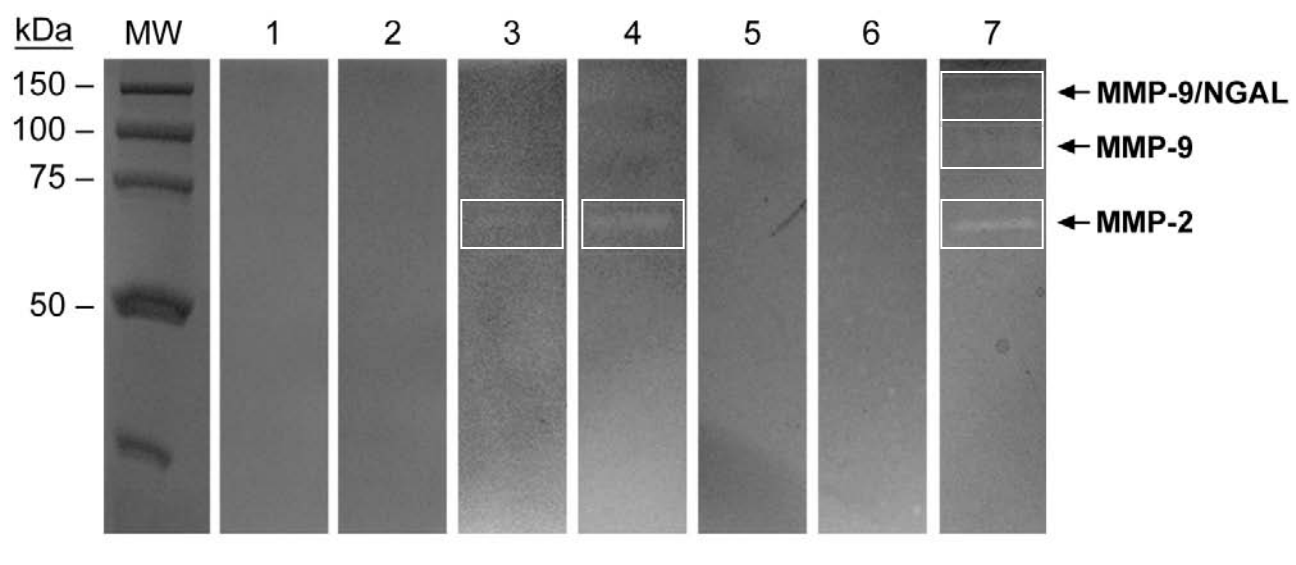
- organization of the cytoskeleton
- signal transduction
- regulation of the redox state
- production of ATP

Demir AY et al., Proteomics 2004



Young VJ et al., unpublished

Urinary MMPs in endometriosis patients



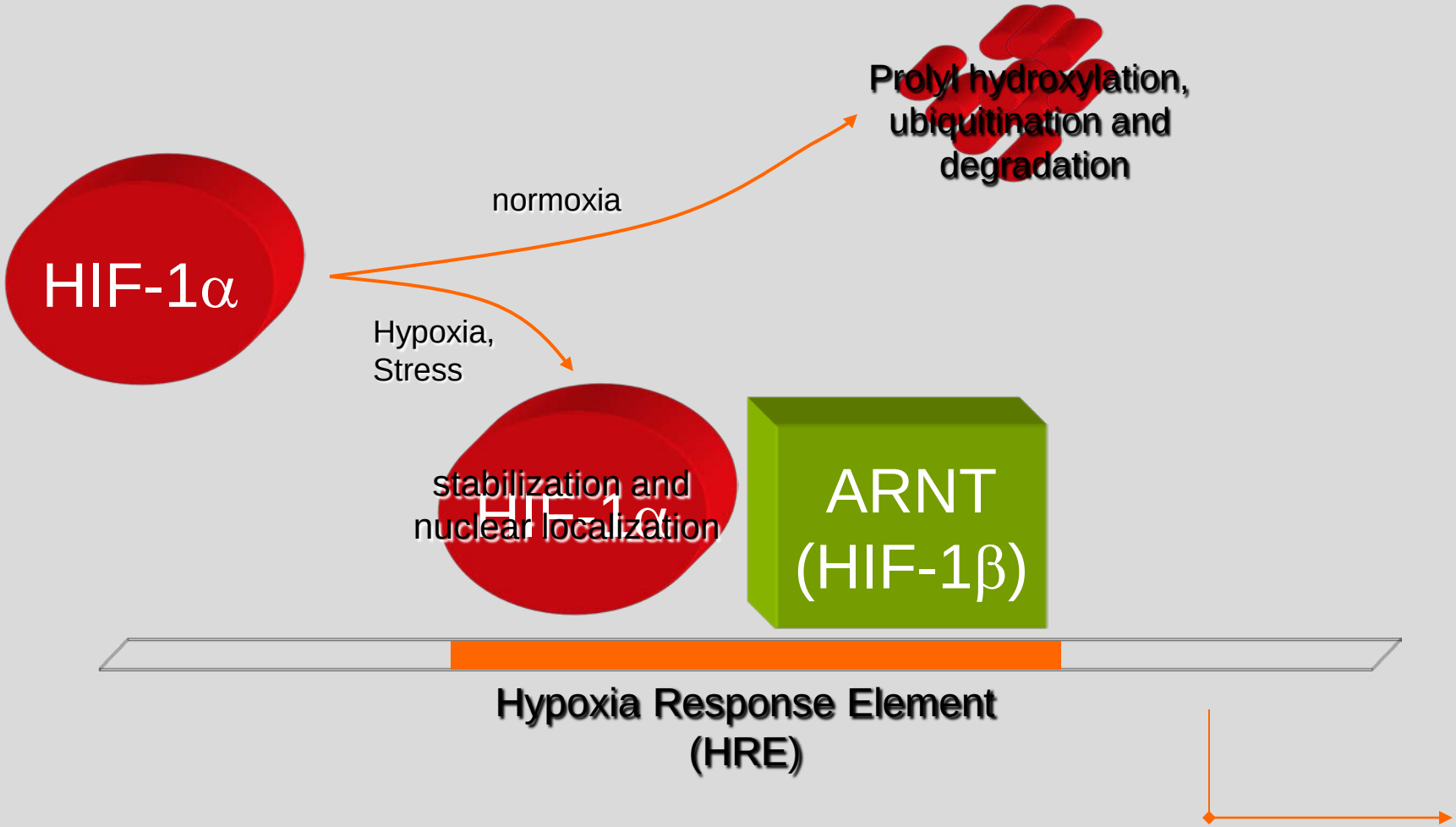
MMP Biomarker	Odds Ratio	95% CI	*P* value
MMP-9/NGAL	6.3	1.7 – 22.8	<0.001†
MMP-9	7.8	2.5 – 25.1	<0.001†
MMP-2	4.8	1.8 – 13.2	<0.001†
Any MMP above	8.3	3.0 – 22.7	<0.001†

Becker CM et al., Fertil Steril 2010

Factors of mesothelial damage

- Mechanical trauma
- Toxic insult
- Hypoxia
- Hyperoxia
- Desiccation

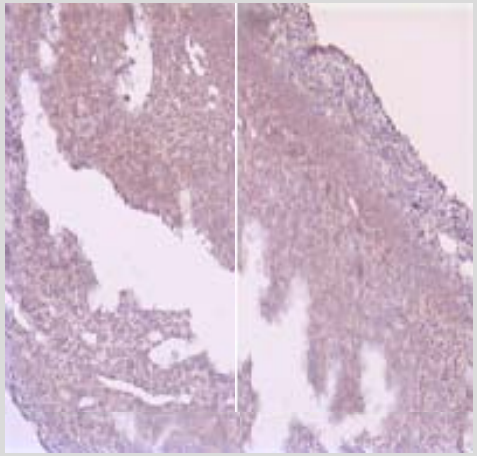
Inflammation



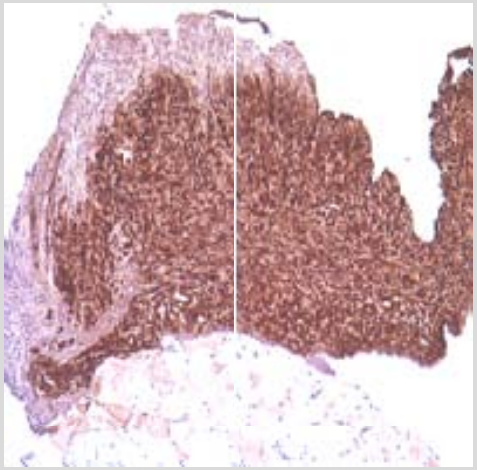
HIF-1α expression is regulated at the protein level

Glycolytic enzymes
Angiogenic factors
Apoptosis control proteins
EPO

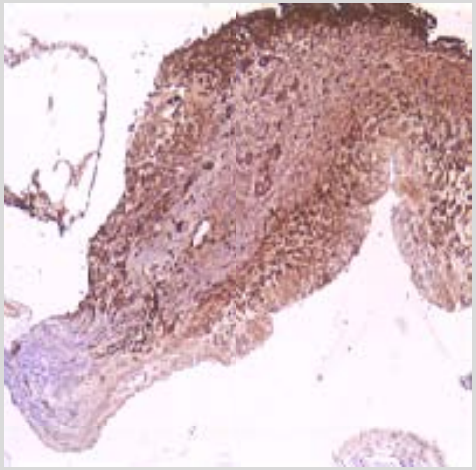
Hypoxia in uterine and endometriosis tissue (Hypoxyprobe HRP immunohistochemistry)



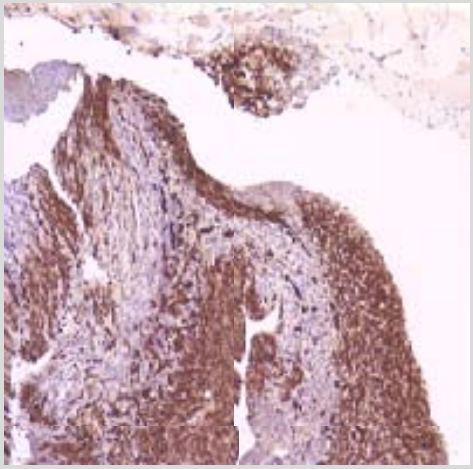
Uterus 1h



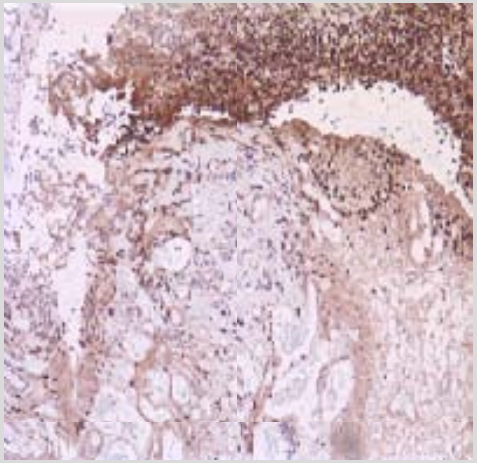
Lesion 1h



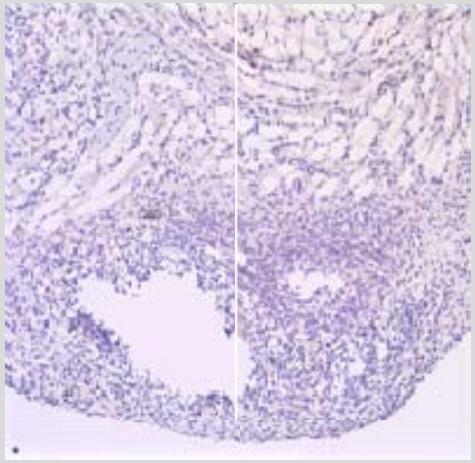
Lesion 4h



Lesion 24h

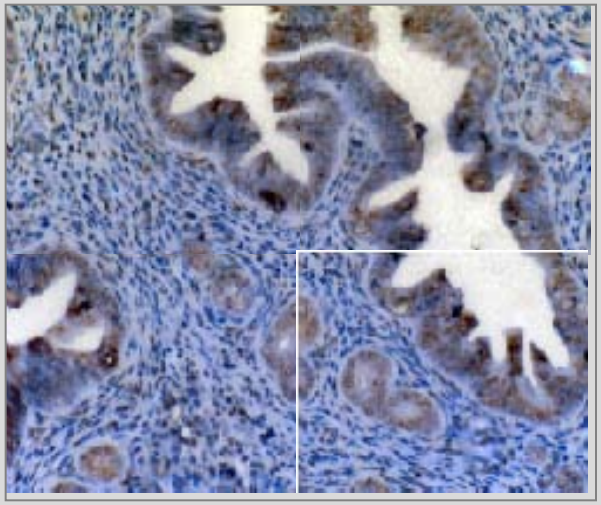


Lesion 48h

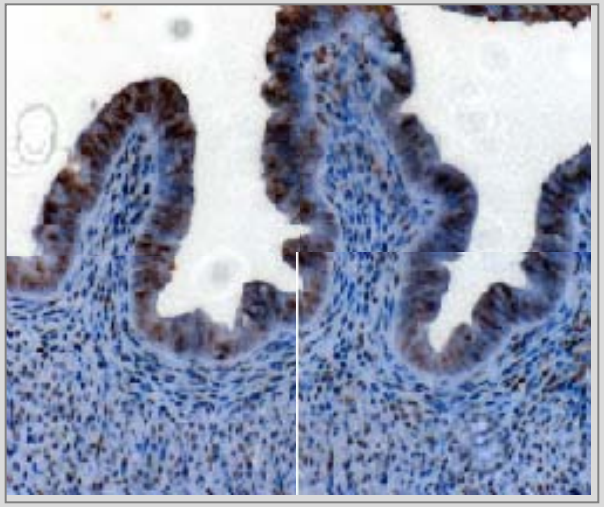


Lesion 1wk

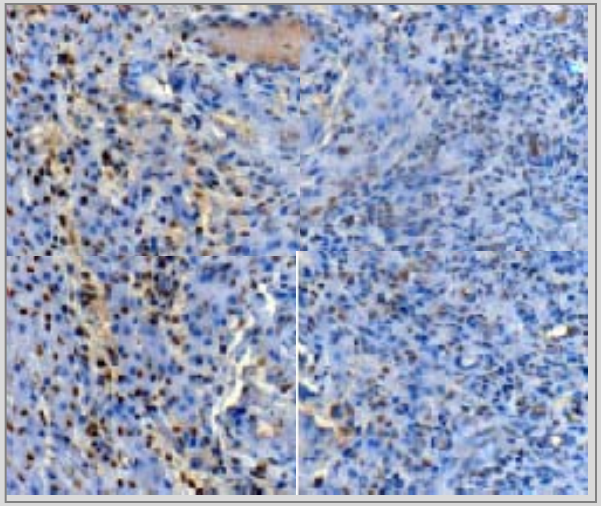
HIF-1α expression in endometriotic tissue (HRP immunohistochemistry)



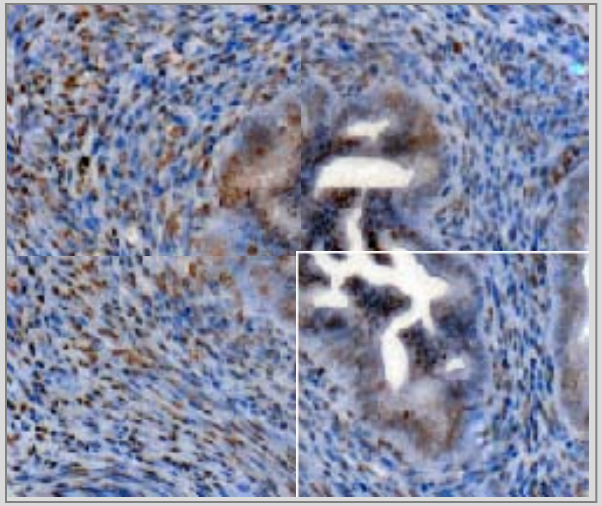
Lesion 0h



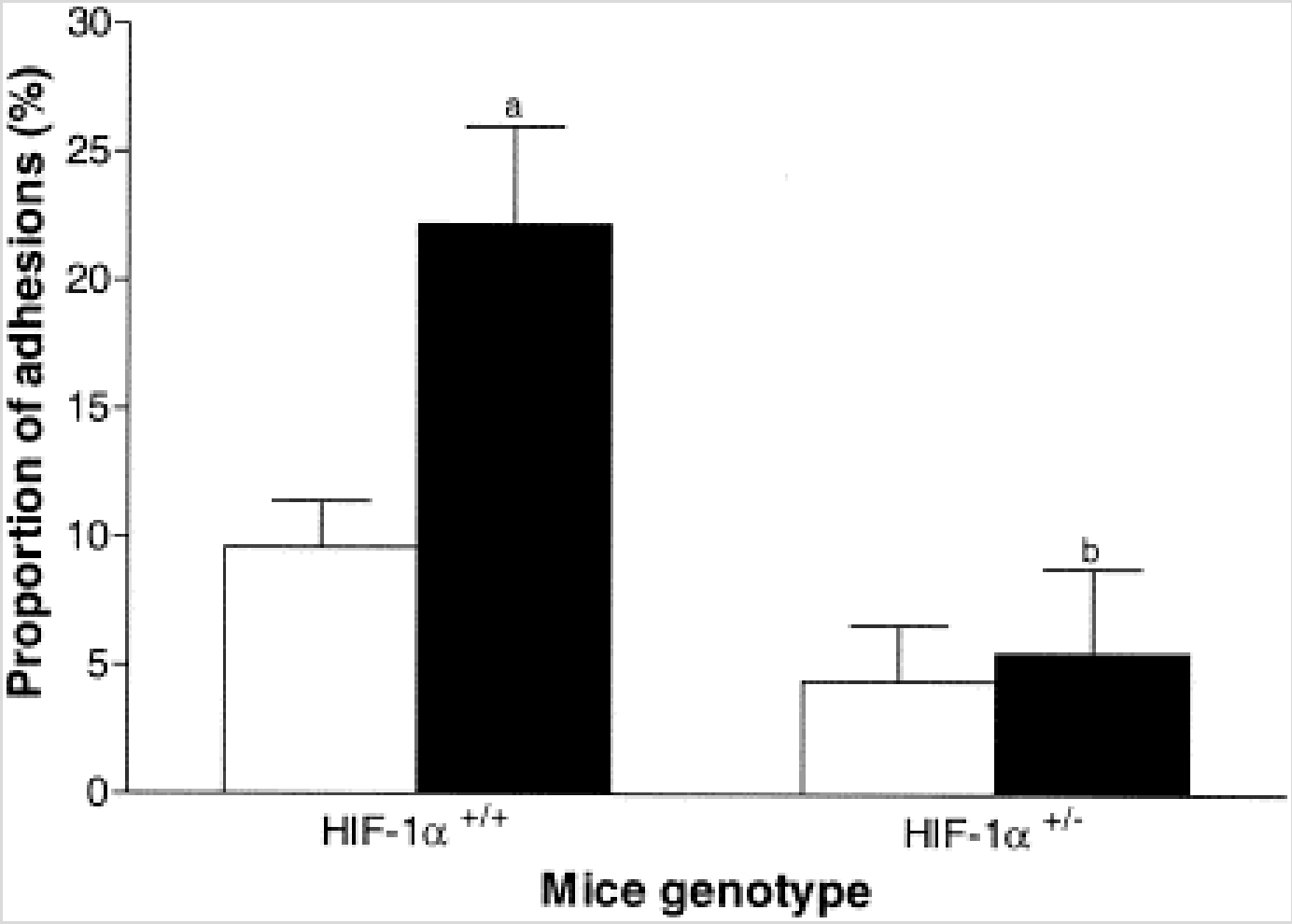
Lesion 1h



Lesion 3h



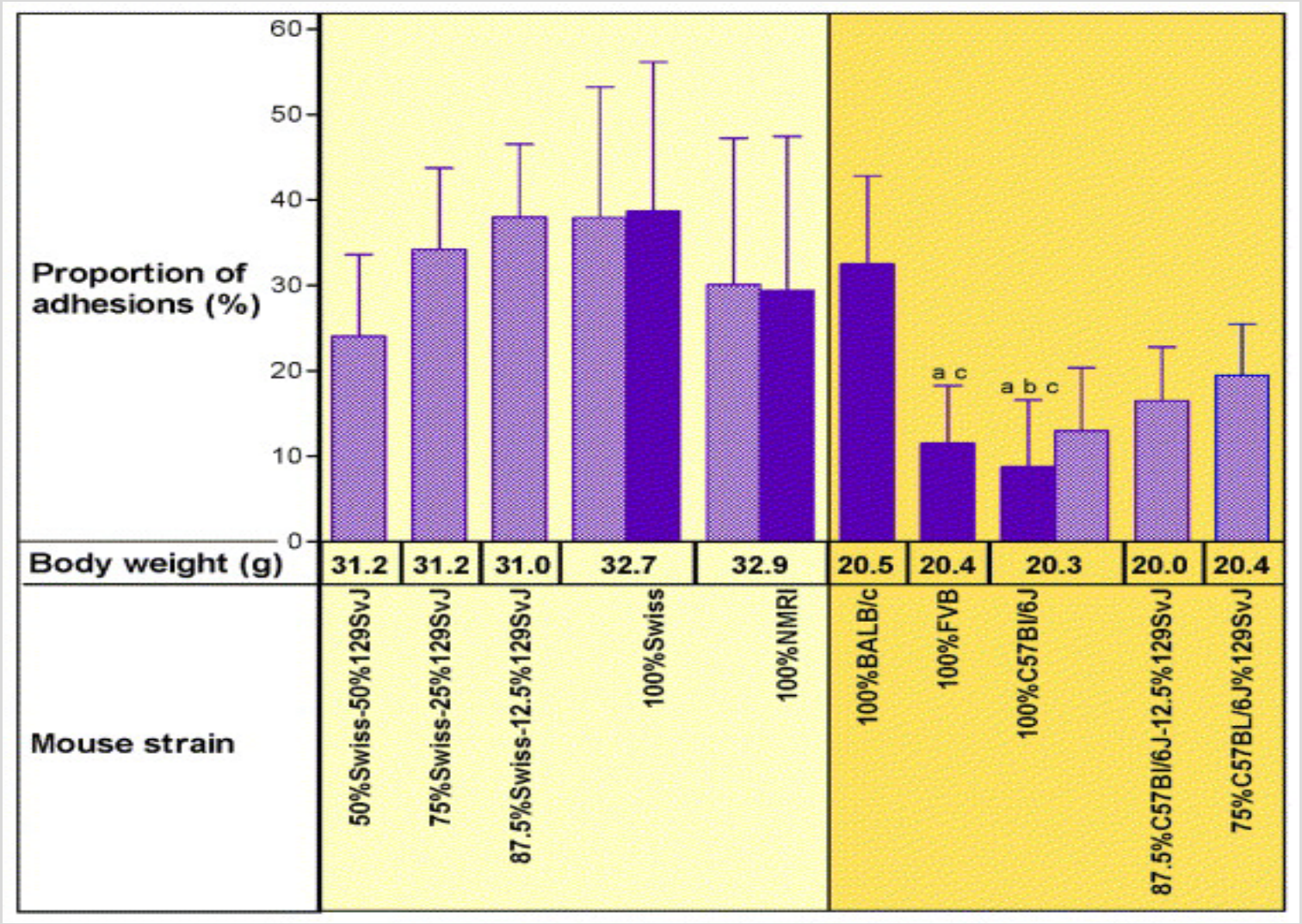
Lesion 6h



Adhesions after **10** min pneumoperitoneum

Adhesions after **60** min pneumoperitoneum

Molinas et al., Fertil Steril 2003



Molinas CR et al., Fertil Steril 2004

Conclusion

- Endometriosis remains an enigmatic disease.
- Stem cells or retrogradely menstruated endometrium or transformed mesothelial cells or a combination of all are likely to be involved in lesion formation.
- Mesothelial-endometrial interaction appears to be crucial for the development of endometriosis.