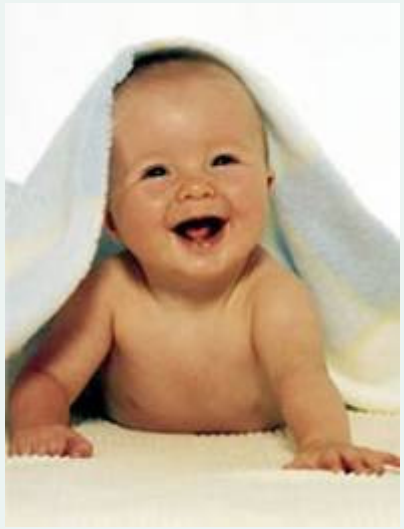

In Vitro Models in Endometriosis Research

Patrick Groothuis
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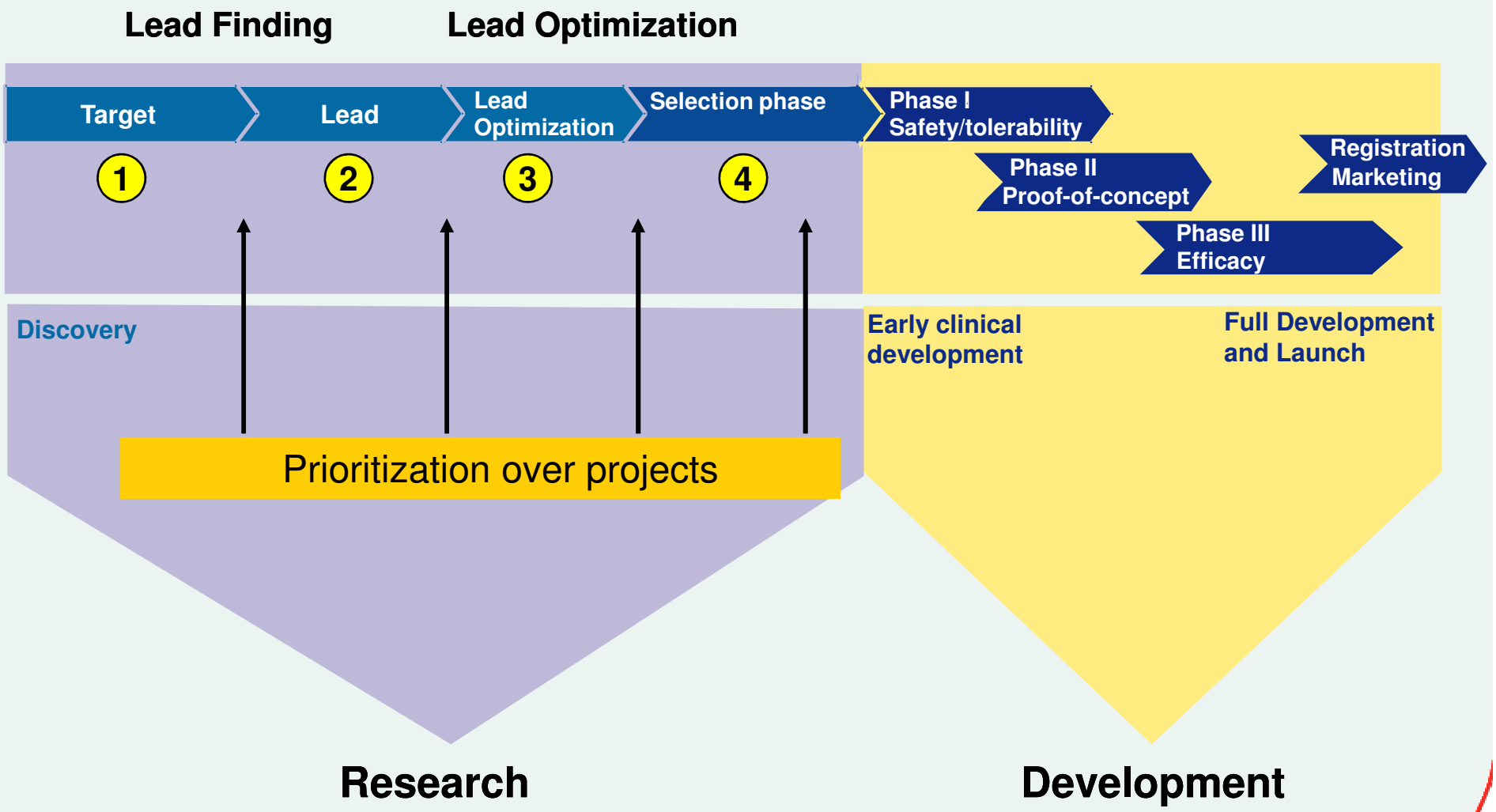
Women's Health Department
Section Endometriosis and Hormone Dependent Disorders

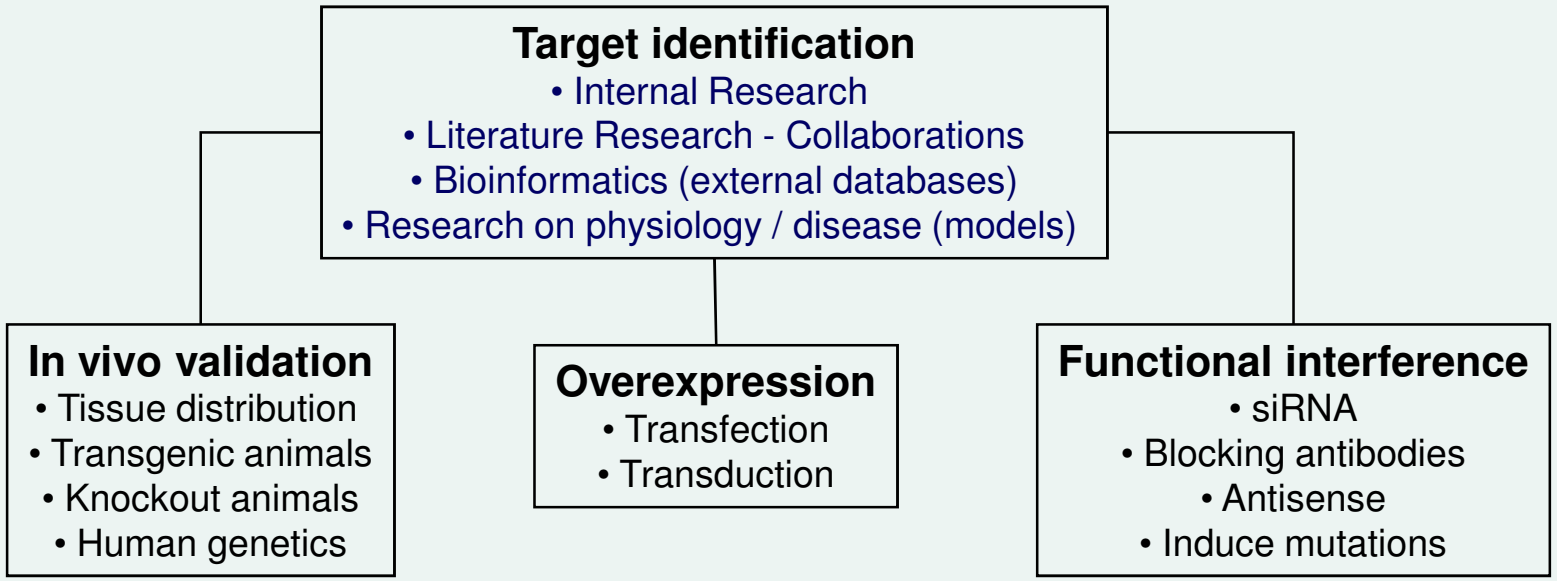
Schering-Plough

ESHRE Campus Course, Leuven 2009

General R&D process



Target discovery



Flow chart LO

• Phase 1a	In vitro efficacy in screening assay
• Phase 1b	In vitro efficacy in biologically relevant assay
• Phase 2a	PK, In vivo efficacy
• Phase 2b	In vivo efficacy in therapeutic/predictive model, PK/PD
• Selection phase	PK dog/monkey, bioavailability, safety, tolerability

“ Priorities for endometriosis research:

recommendations from an international consensus workshop “

- Xth World Congress on Endometriosis, Melbourne, Australia
- Moderator Peter Rogers, Monash University
- Problem:
  - Disease with high social and healthcare impact
  - No non-invasive diagnostic tools available
  - Research is under-funded
  - Current therapies are not satisfactory: poor efficacy, high relapse rate
  - Practical difficulties to investigate this complex disease
  - Lack of novel targets and drugs

“ Priorities for endometriosis research:

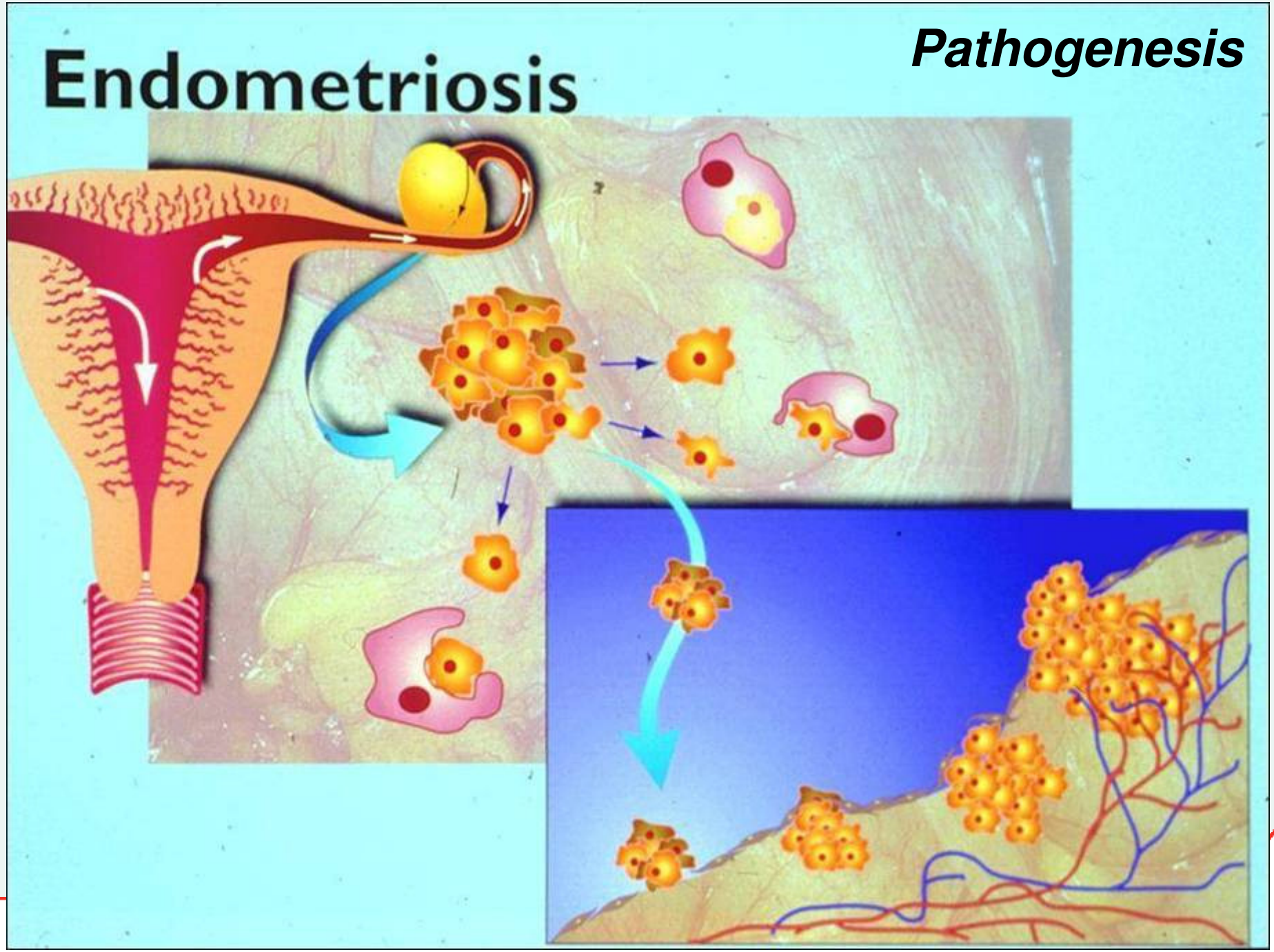
recommendations from an international consensus workshop “

- Recommendations:
  - A better understanding of the role of the eutopic endometrium in the establishment and continuation of endometriosis is required
  - Develop non-invasive diagnostic tools
  - Biomarkers are required
  - Appropriate in vitro and therapeutic models are needed for studying different aspects of endometriosis pathophysiology

What are appropriate in vitro models ?

Endometriosis

Pathogenesis



In vitro models to study endometriosis

- Endometrial cells/tissue from women without endometriosis
- Endometrial cells/tissue from lesions/cysts
- Immortalized cells from lesions/cysts

How should we employ them ?

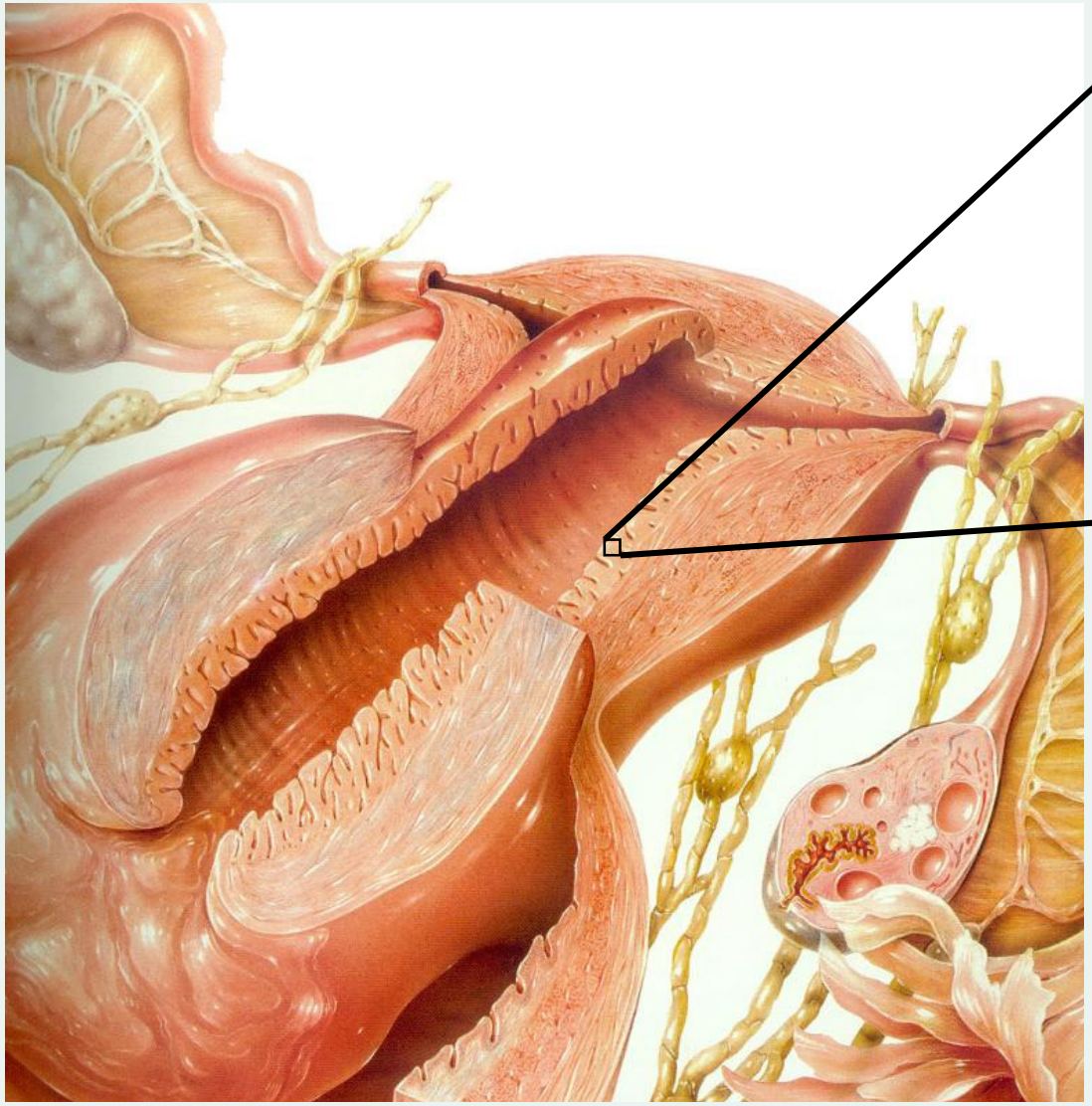
Focus on particular biological processes that contribute to the total complexity of the disease, i.e.:

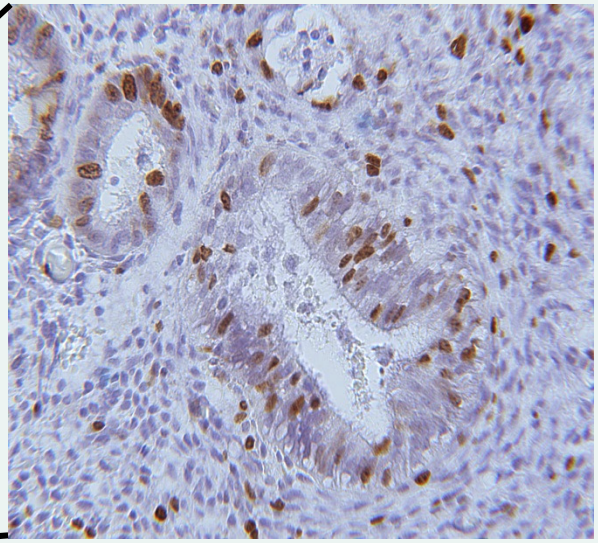
- adhesion
- angiogenesis
- proliferation/apoptosis
- progesterone resistance
- estrogen metabolism

Create conditions mimicking in vivo situation by combining primary cells/tissues with other cellular/tissue components, i.e.:

- menstrual endometrium
- (Activated) macrophages, lymphocytes
- ECM components
- peritoneum/mesothelium
- chick chorioallantois membrane model (model for peritoneum)

Primary cell/tissue cultures from endometrium (from women without endometriosis)

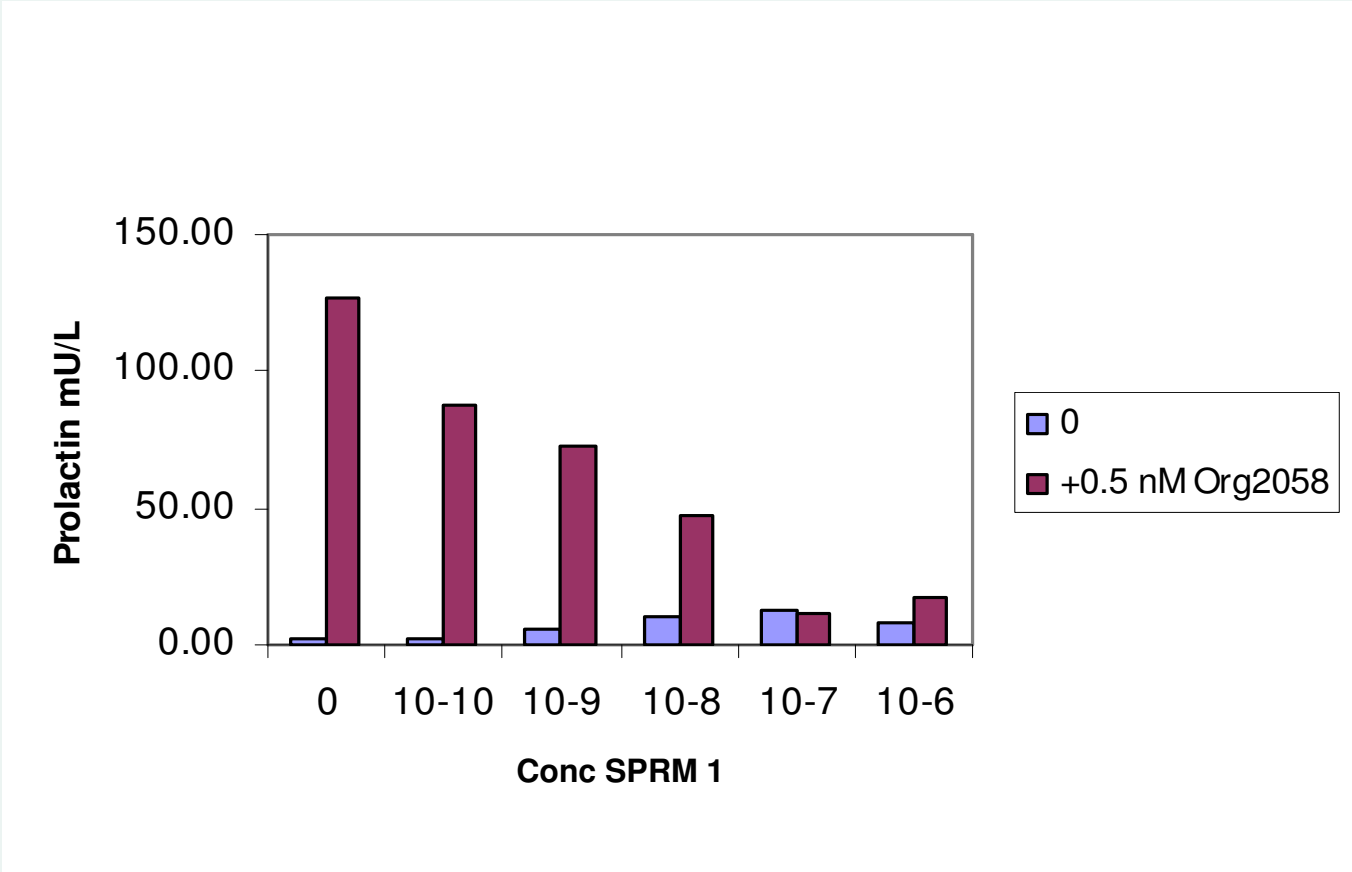




Endometrium function in part dictated by paracrine, Juxtacrine and matricrine interactions

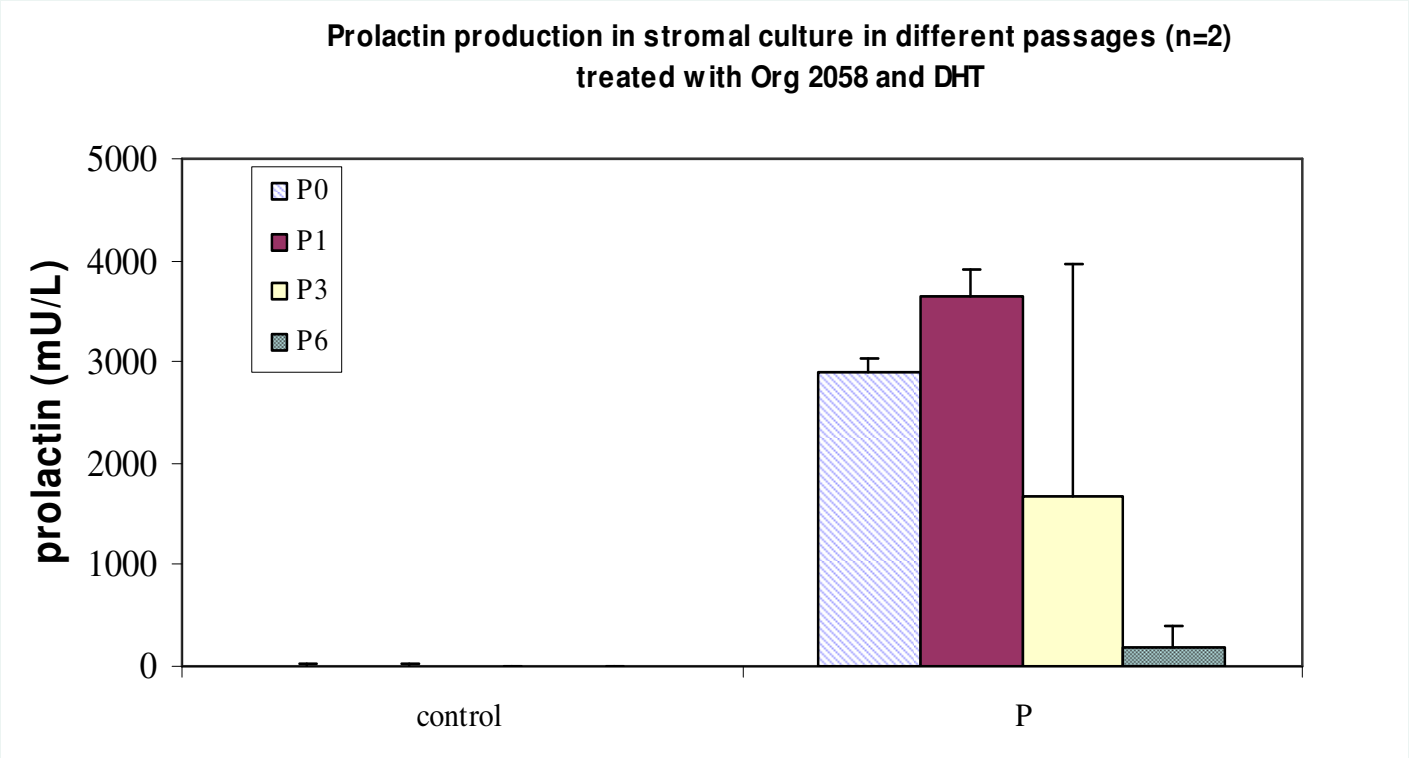
(E. Marbaix; Punyadeera et al., 2004 JSBMB)

Primary cultures of endometrial stromal cells are steroid-hormone responsive



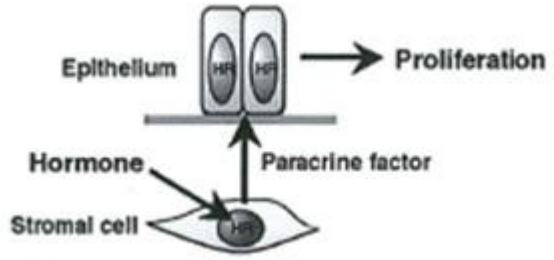
Regulation of prolactin production by SPRMS in primary endometrial stromal cells

Responsiveness of primary stromal cells to steroid hormones reduces in time



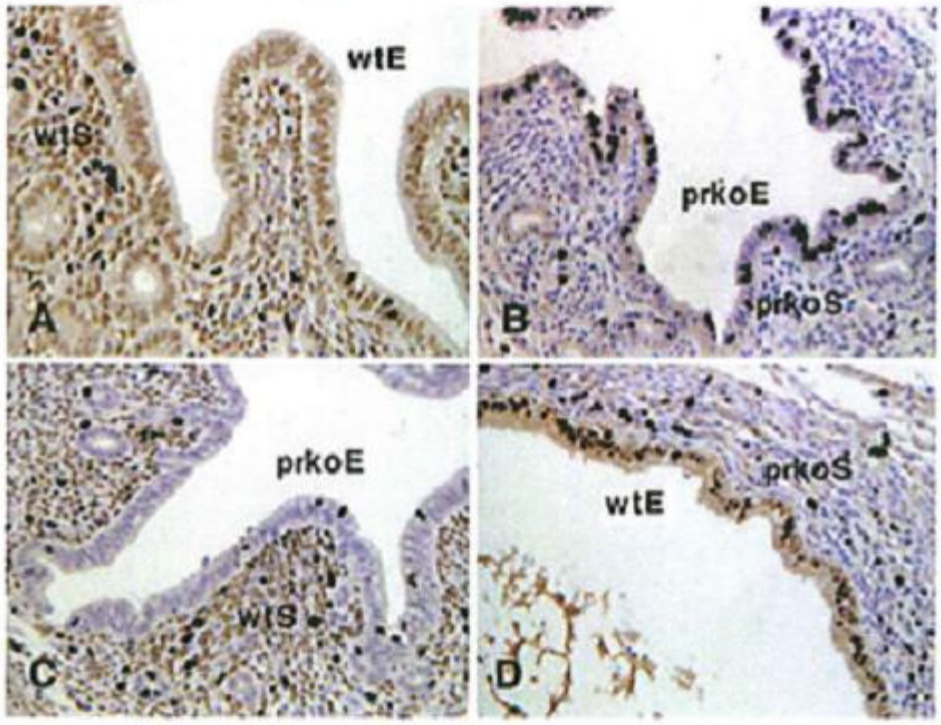
NRs in stroma determine proliferative response

Stroma	Epithelium	Proliferative response
Wild-type	Wild-type	++++
Knockout	Knockout	-
Knockout	Wild-type	-
Wild-type	Knockout	++++



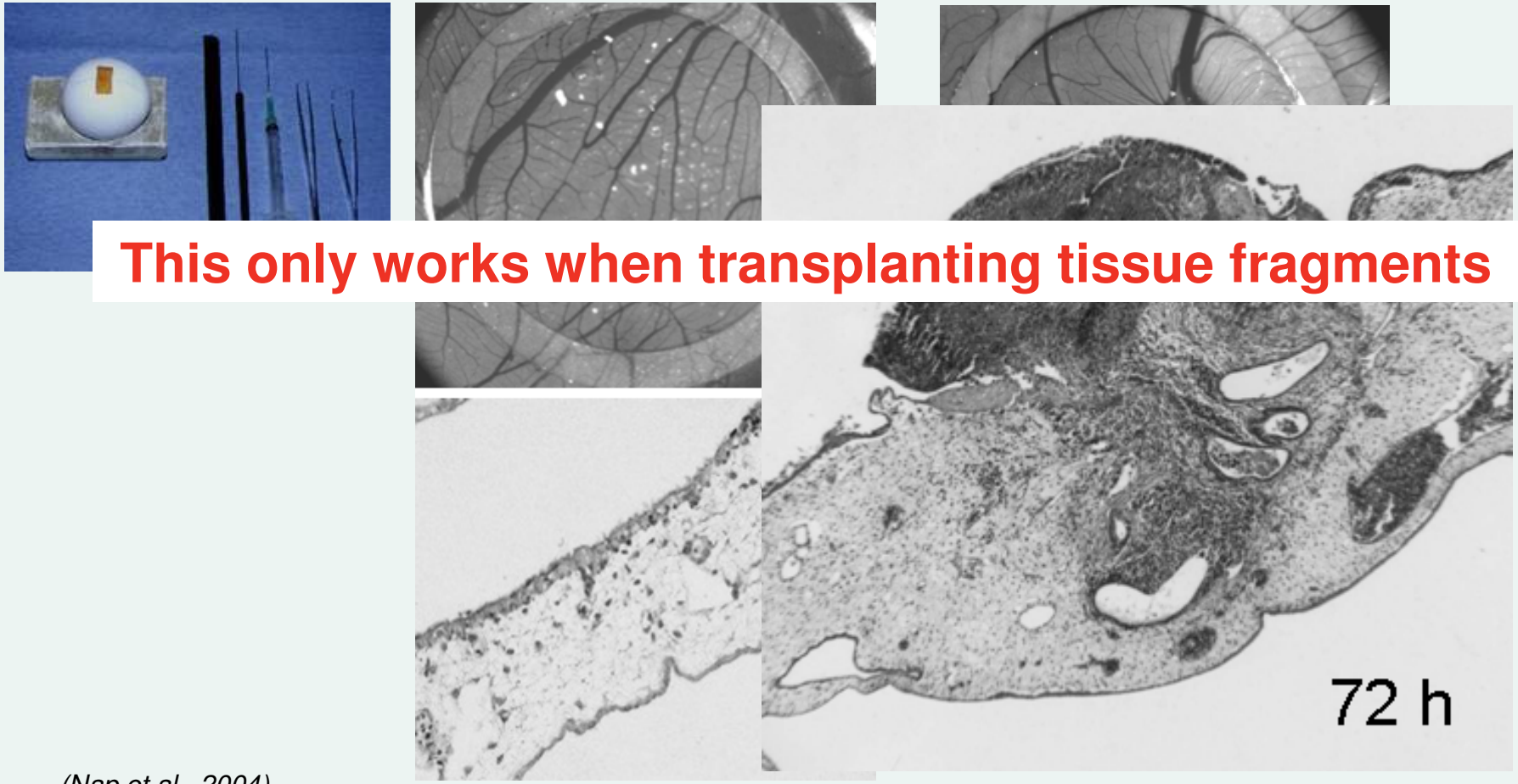
Recombination and
renal capsule transplantation

Stroma	Epithelium	Proliferative response to E2	Proliferative response to E2+P
Wild-type	Wild-type	++++	-
PRKO	PRKO	++++	++++
PRKO	Wild-type	++++	++++
Wild-type	PRKO	++++	-



(Cunha et al., 2004 Arch Histol Cytol)

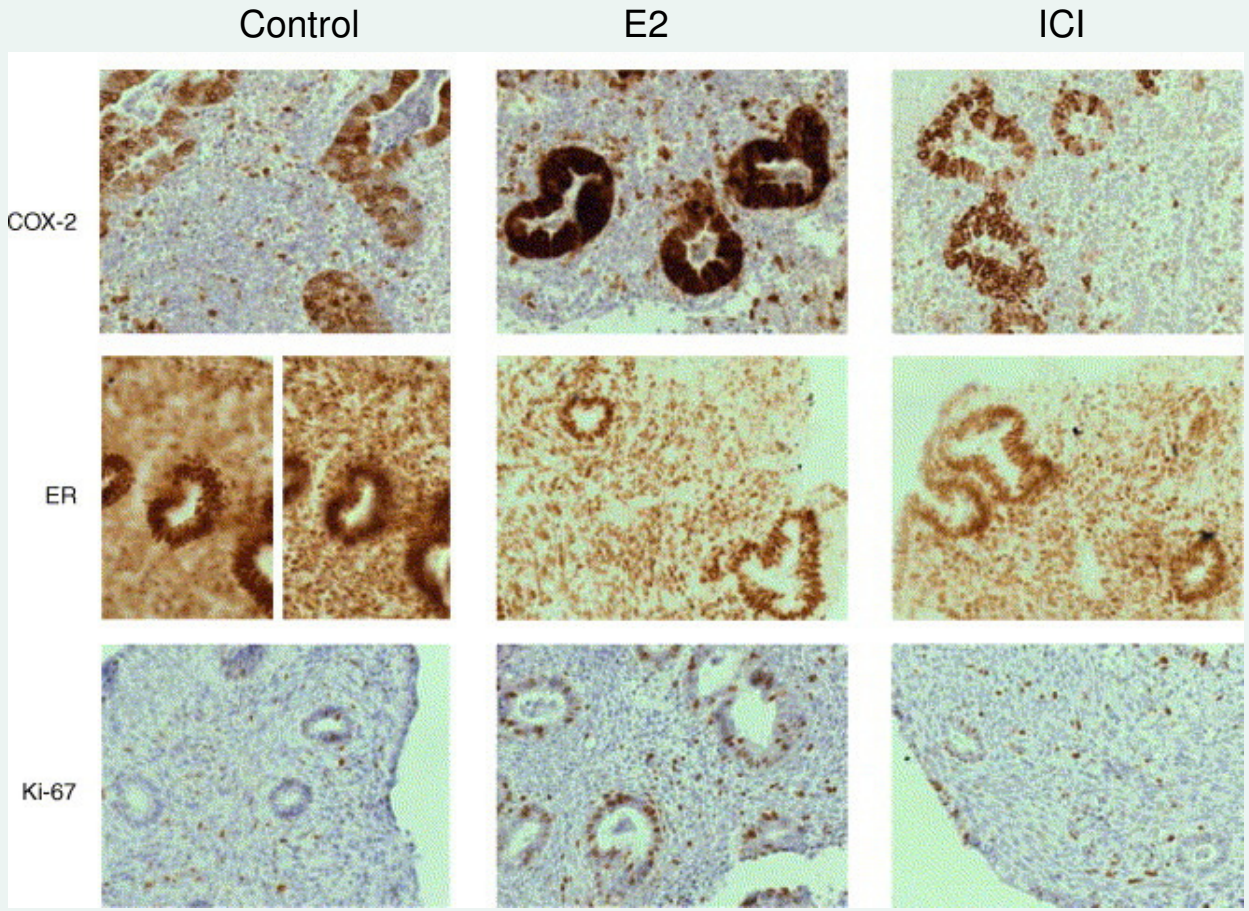
Chick chorioallantoic membrane model



This only works when transplanting tissue fragments

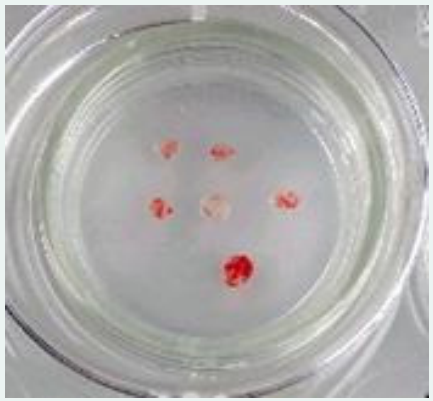
(Nap et al., 2004)

Short term explant cultures human endometrium are responsive to estrogen

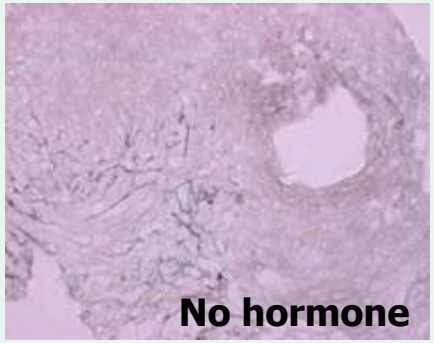


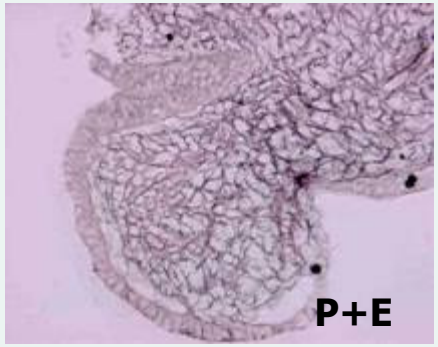
(Punyadeera et al., 2004 JSBMB)

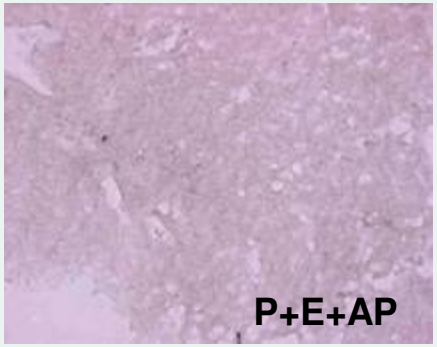
Effect of progestins/SPRMs on MMP activation in cultured explants of pre-menopausal endometrium



3 day incubation

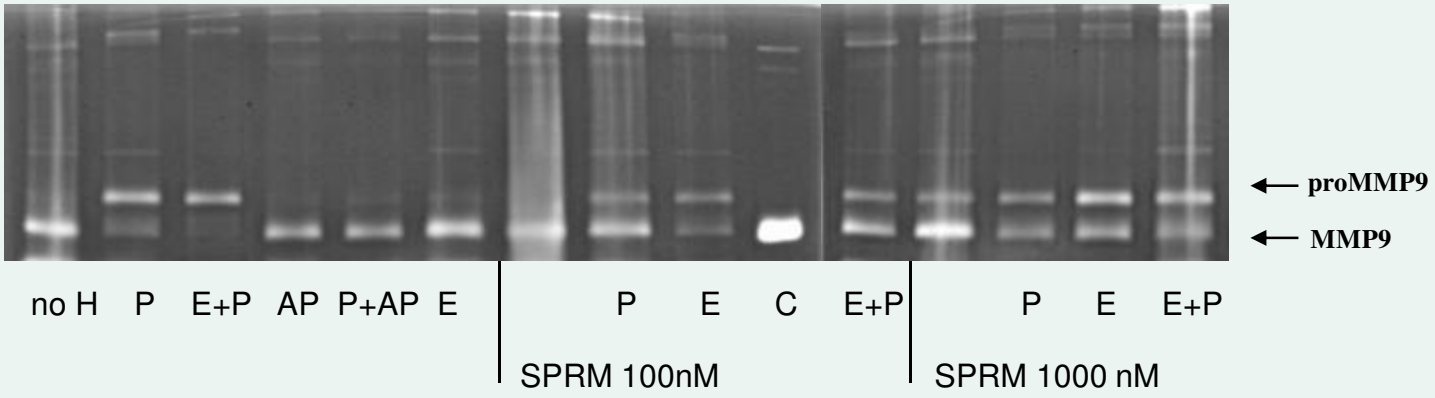






Silver-stained reticulin (collagen) fibers

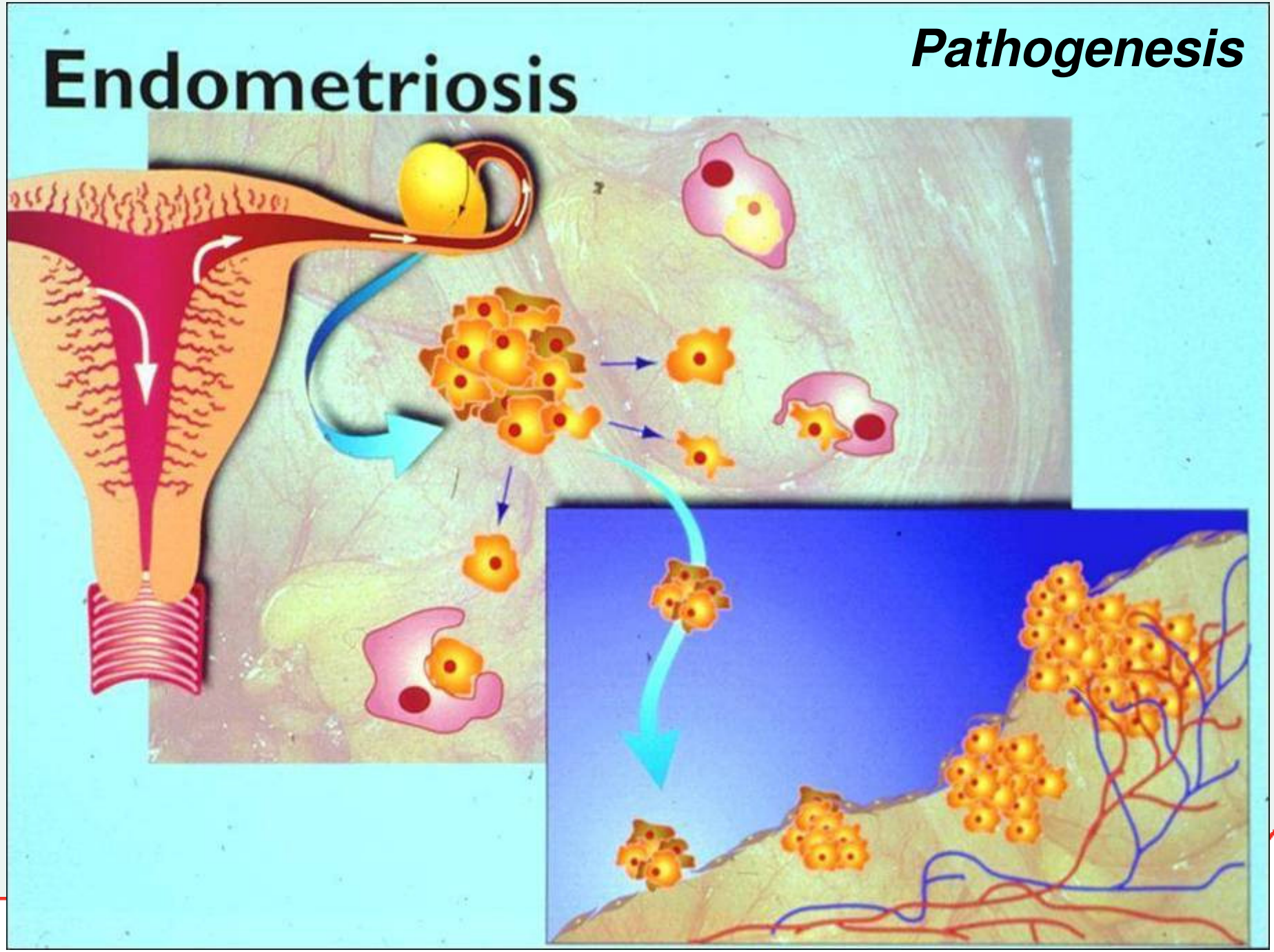
Effect of progestins/SPRMs on MMP activation in cultured explants of post-menopausal endometrium



Gelatin zymography 8%, day 3

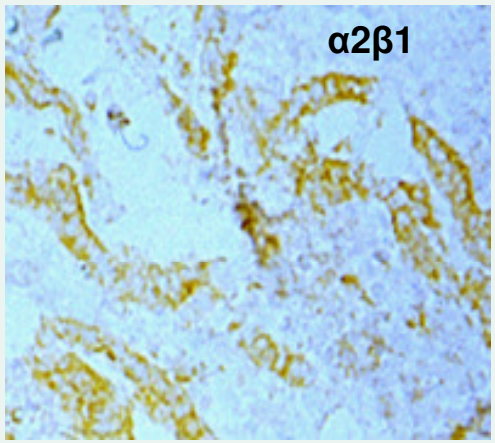
Endometriosis

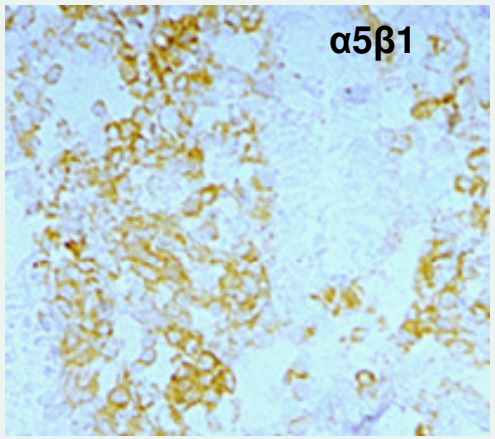
Pathogenesis

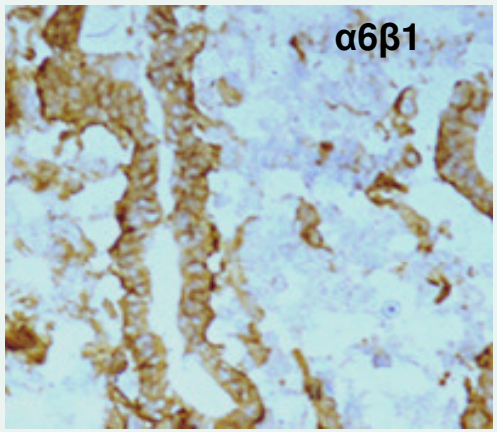


Preferably shed menstrual endometrium should be used

Integrin expression on shed menstrual endometrium

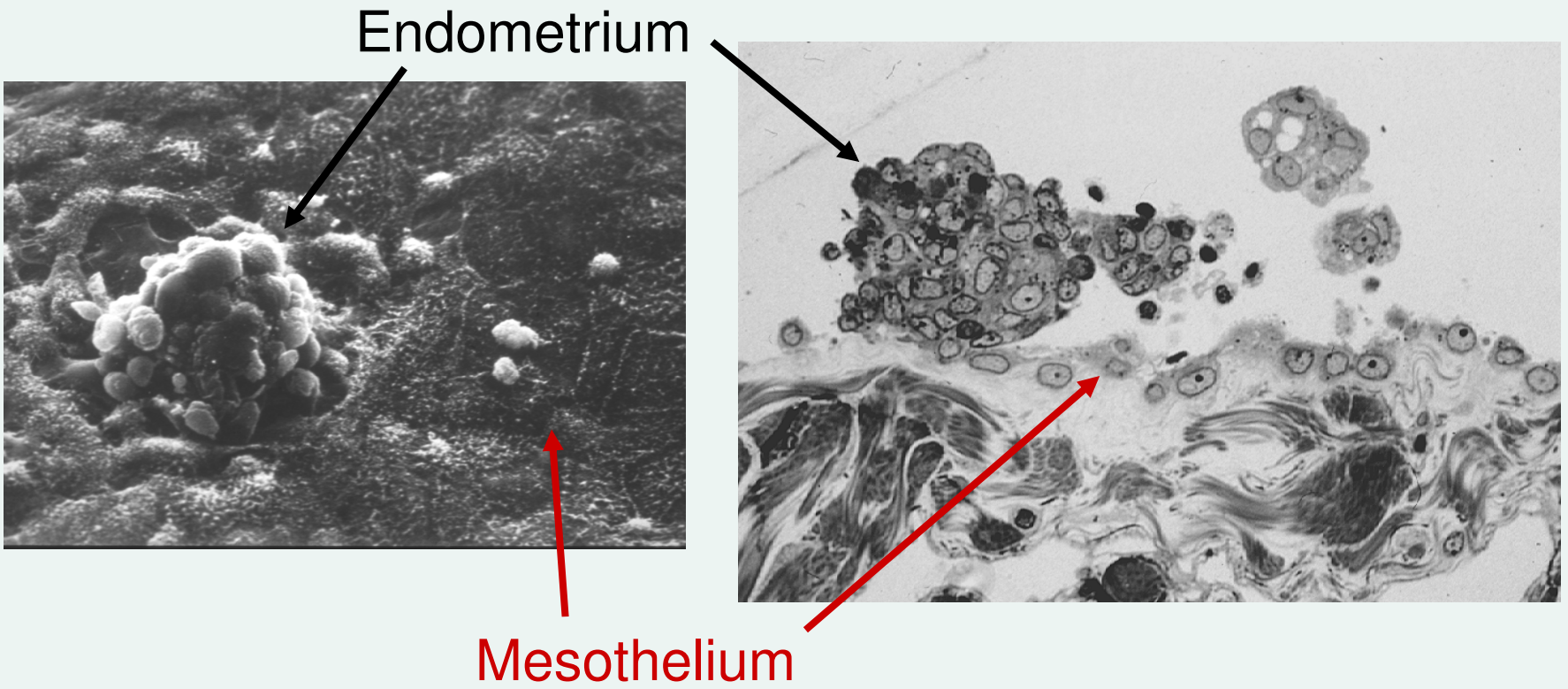






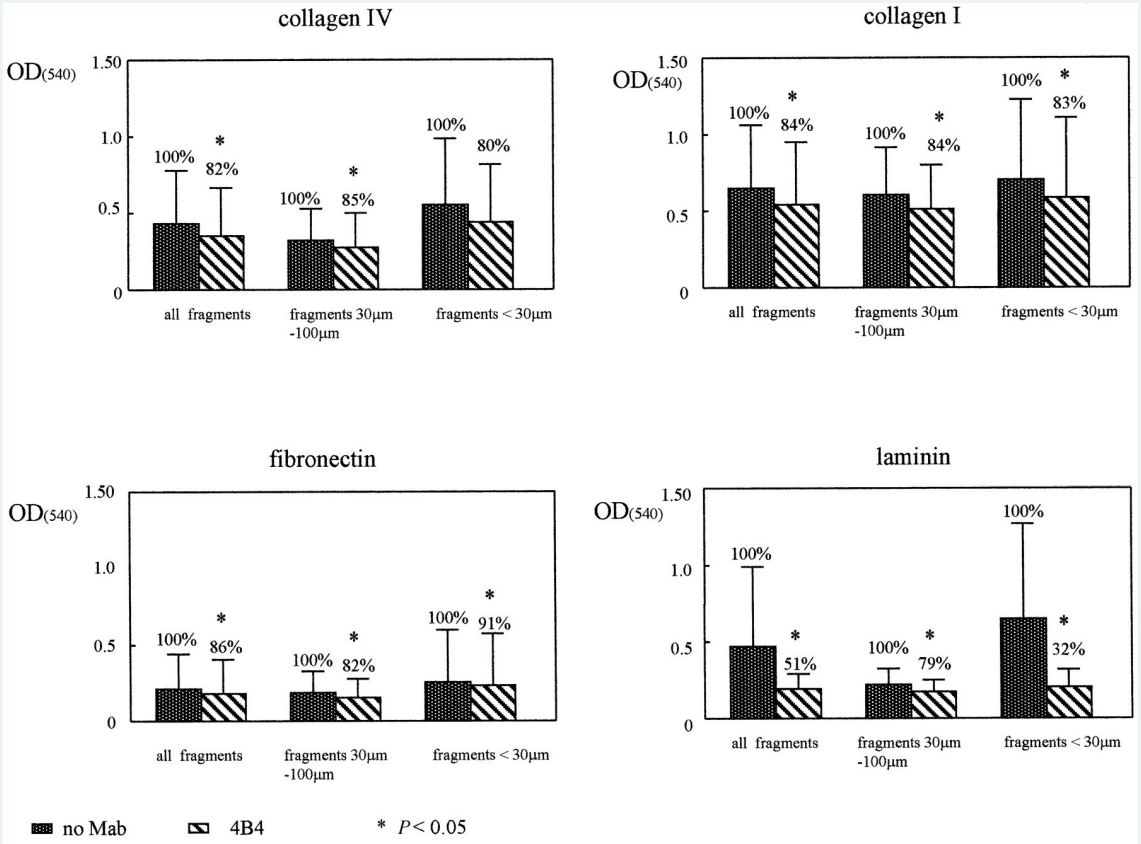
(Koks et al., 2000 Mol Hun reprod)

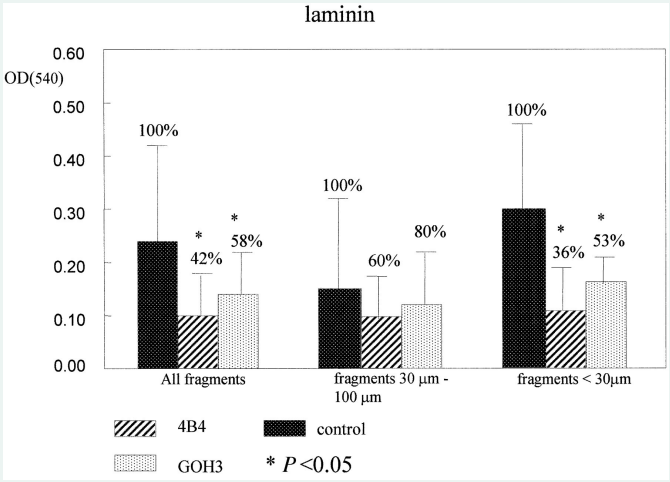
Coculture menstrual endometrium and peritoneum



(Groothuis et al., 1999; Koks et al., 2000)

Coculture menstrual endometrium and ECM

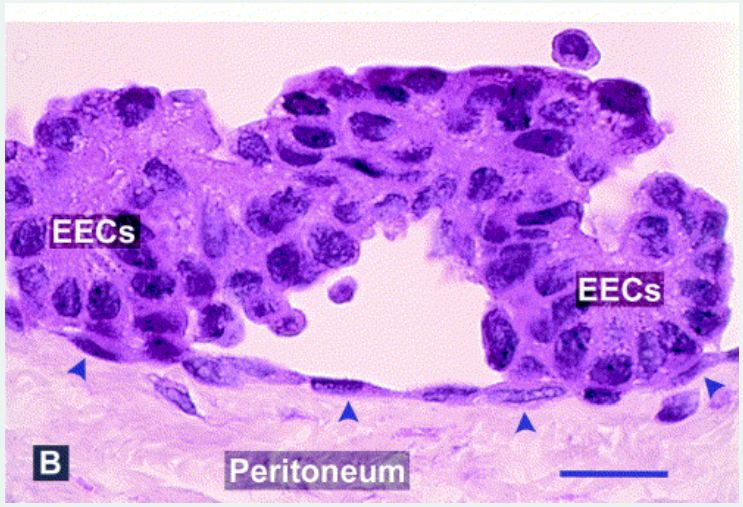


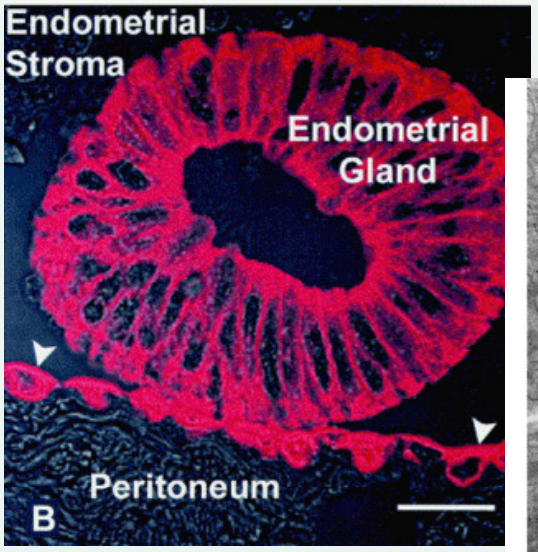


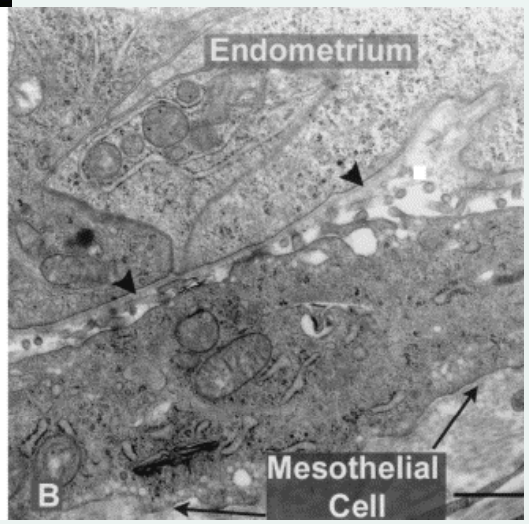
Adhesion of menstrual endometrium to extracellular matrix is largely mediated by integrin alpha(6)beta(1) and laminin interaction.

(Koks et al., 200 Mol Hum Reprod)

Endometrium also adheres to mesothelial surface

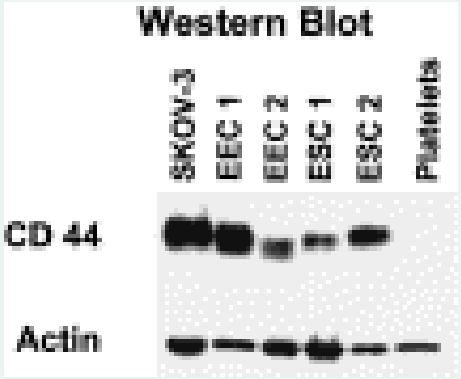


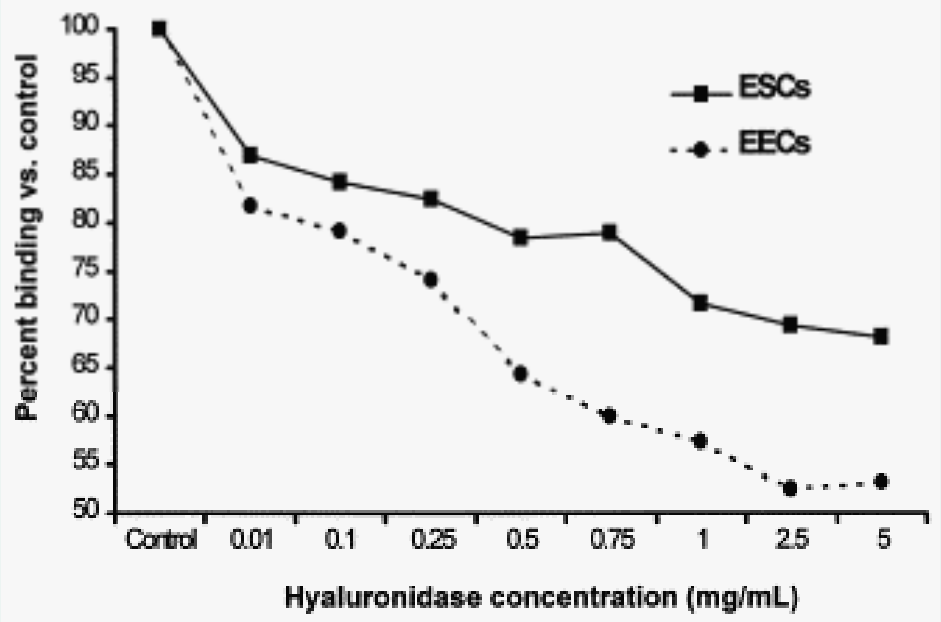




(Witz et al., 2002 Fertil Steril)

Coculture menstrual endometrium and mesothelial cells

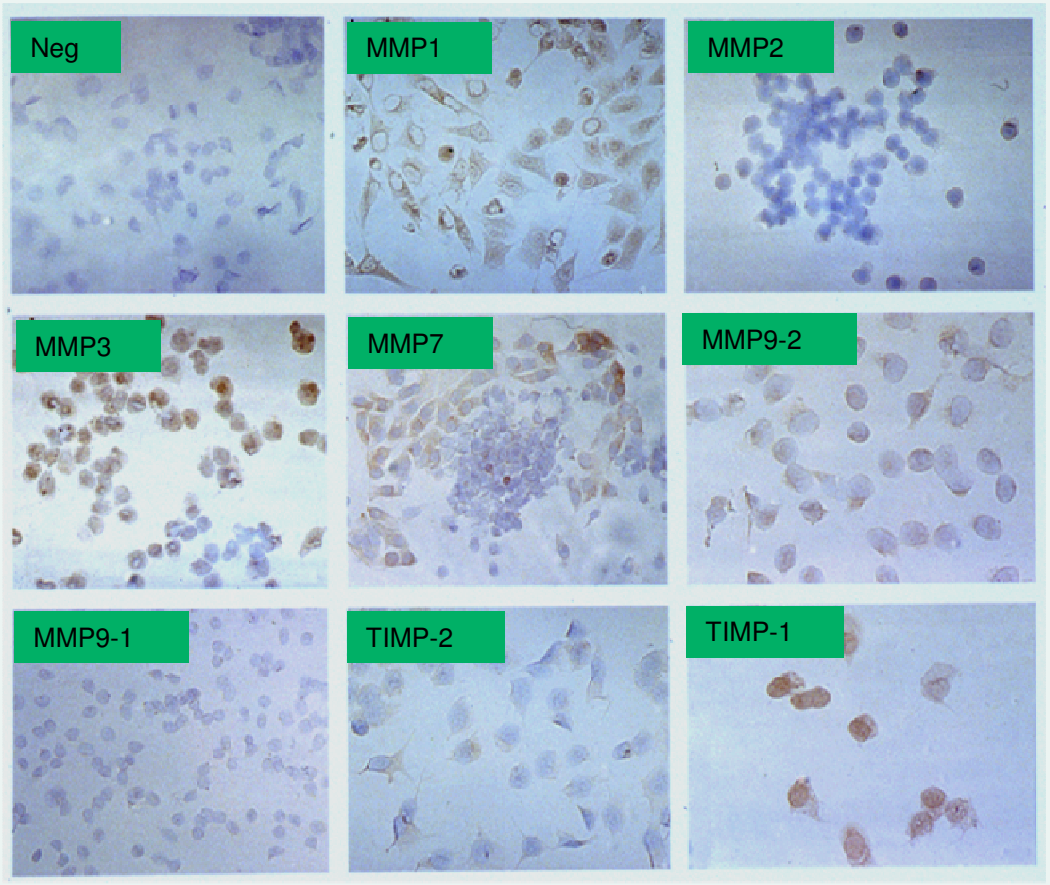




Binding is mediated by CD44 – hyaluronic acid interaction

(Dechaud et al., 2001 Fertil Steril)

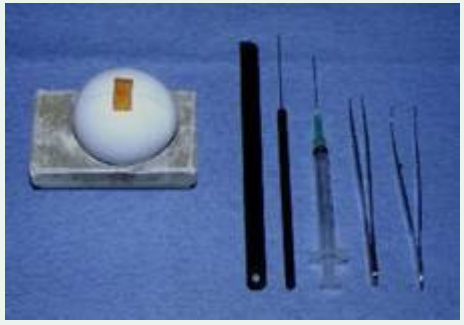
Shed endometrium contains viable cells that express MMPs and TIMPs

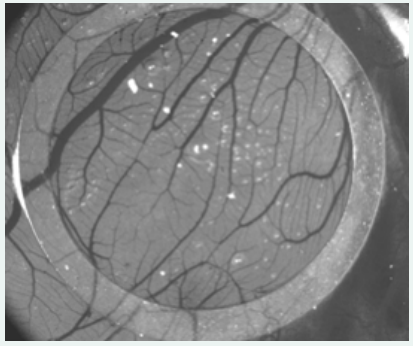


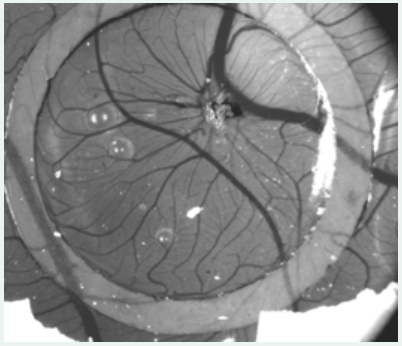
(Koks et al., 2000 Fertil Steril)

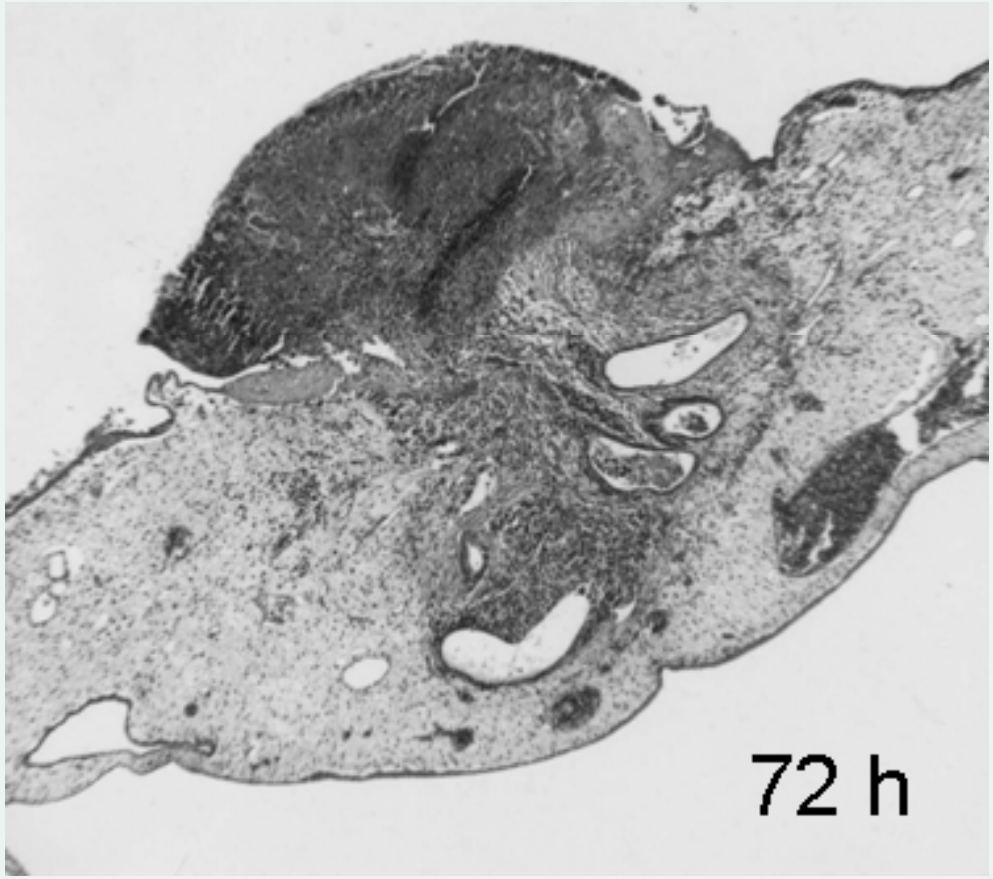
Chick chorioallantoic membrane model

Antegradely shed menstrual endometrium forms lesions in CAM





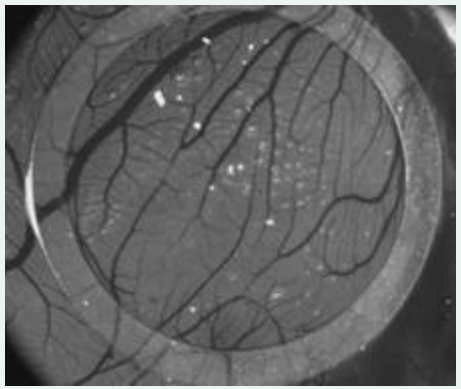


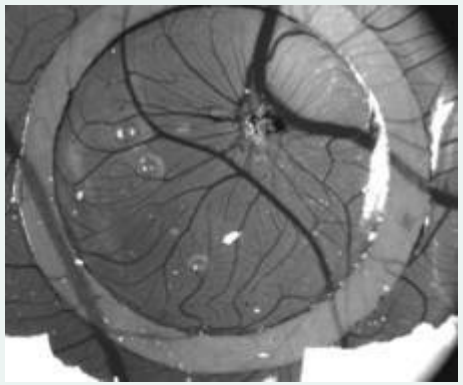


(Nap et al., 2004)

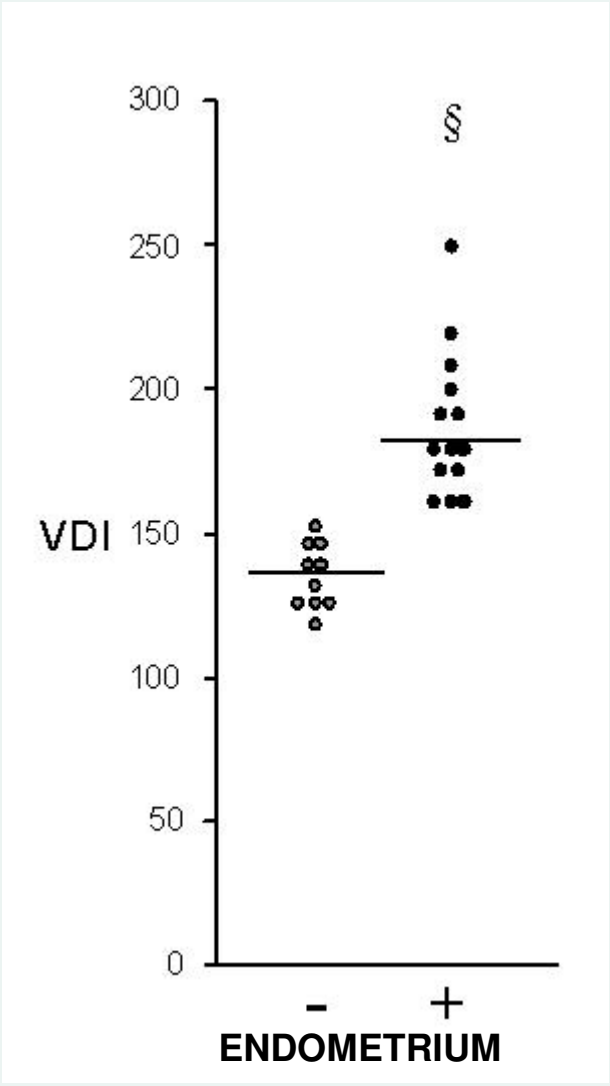
Chick chorioallantois membrane model

Endometrium is angiogenic



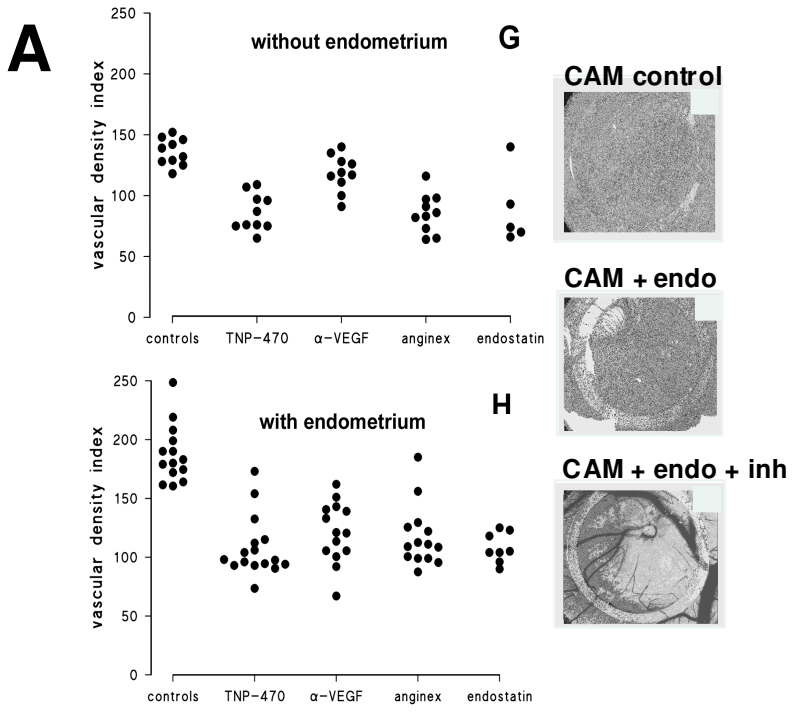


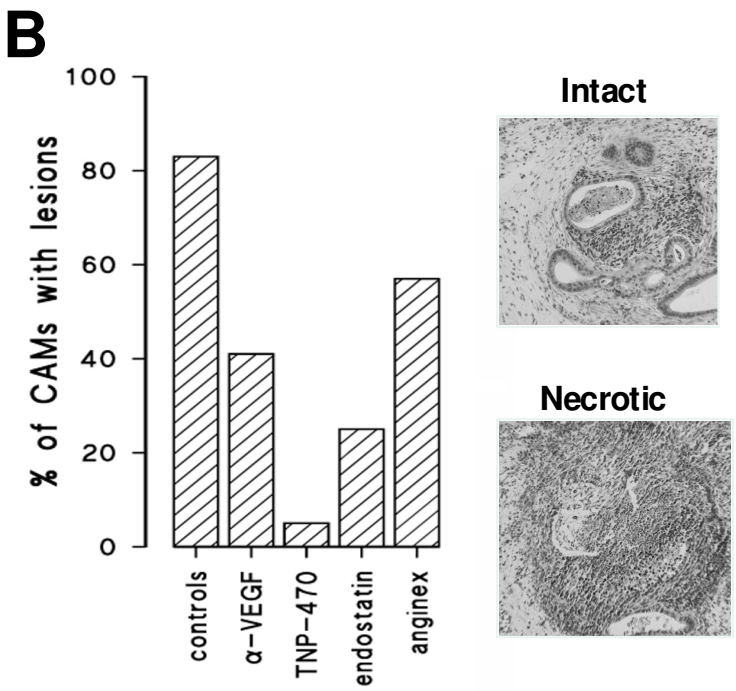
(Maas et al., 2001; Nap et al., 2005)



Chick chorioallantois membrane model

Angiostatic therapy inhibits lesion formation in the CAM





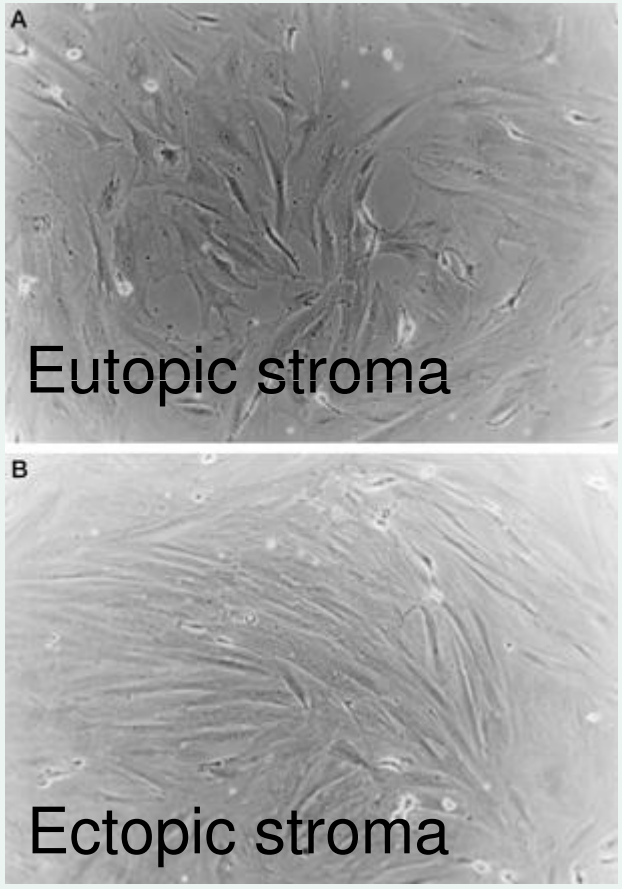
(Nap et al., 2005)

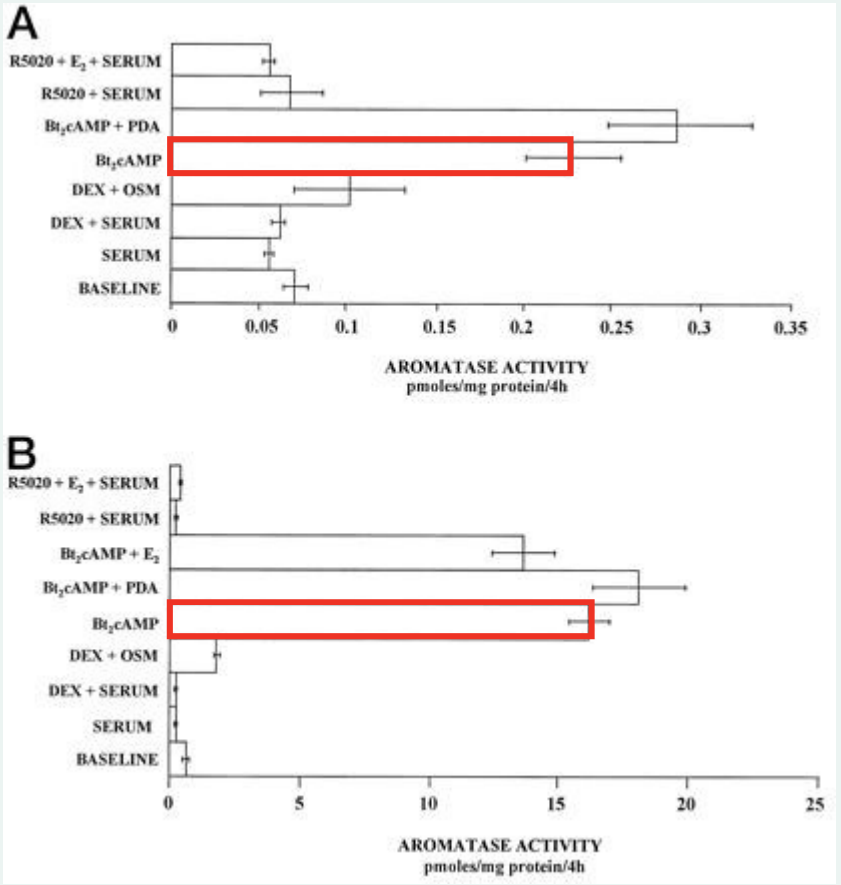
Summary

- Endometrium tissue is suitable to study basic processes involved in the early pathogenesis of endometriosis
- The tissue context is critical, particularly with regard to steroid hormone responsiveness and implantation
- Endometrium tissue is not likely suitable for the generation of therapeutic models, many endometriosis-related characteristics are lacking
  - i.e. changes in cell endometrial physiology, altered immune system

Primary cell/tissue cultures from lesions/cysts

Stromal cell cultures from ovarian cysts

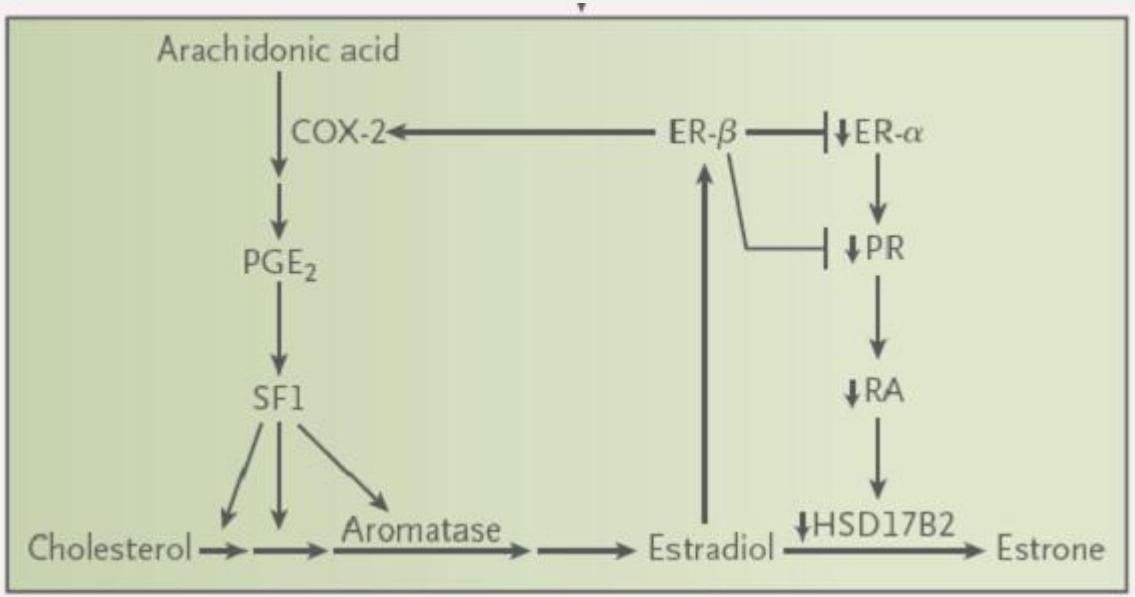




(Noble et al., 1997 JCEM)

Bulun hypothesis

- ERβ and SF1 are upregulated in ovarian endometriosis (demethylation)
- COX-2 is induced and consequently PGE2 and aromatase production



- Not validated by others; only shown in stromal cells from ovarian cysts

(Bulun, 2009, NEJM)

Explant cultures endometriosis tissue

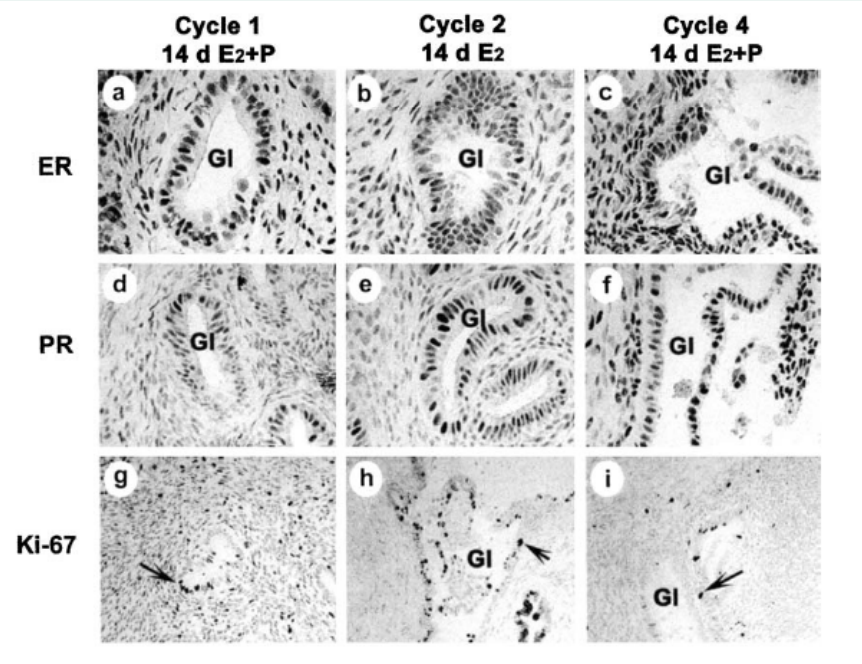
- One publication: Sharpe-K et al., Fertil Steril 1993
- Proteomics study which led to the discovery of the endometriosis-specific haptoglobin isoform

(Bulun, 2009, NEJM)

Xenografts with explants of endometriotic tissue

Human primary xenografts:

- Tissue from ovarian cysts and peritoneal endometriosis is transplanted in pockets made in abdominal wall
- OVX RAG-2γ(c) Knockout mice, E2 and P4 pellets, to support four menstrual cycles



(Greenberg and Slayden, AJOG 2004)

Table I Summary of human endometriotic samples

Sample type	Biopsy characteristic		Samples grafted (n)	Take (%)
	Color	Description		
Ovarian endometrioma	Red	Stroma	8	0
Peritoneal stage II	Red	Glands + stroma	36	88
	Red	Stroma only	4	75
	Brown	Stroma only	8	0
	White	Adhesions	8	25
	Red	Glands + stroma	24	91
Peritoneal stage III	Red	Stroma	12	50
	Brown	Stroma	4	25
	White	Adhesions	8	0
Peritoneal stage IV	Red	Glands + stroma	12	100
	Brown	Stroma	4	0
	White	Adhesions	8	25

Immortalised cells from lesions/cysts

Examples

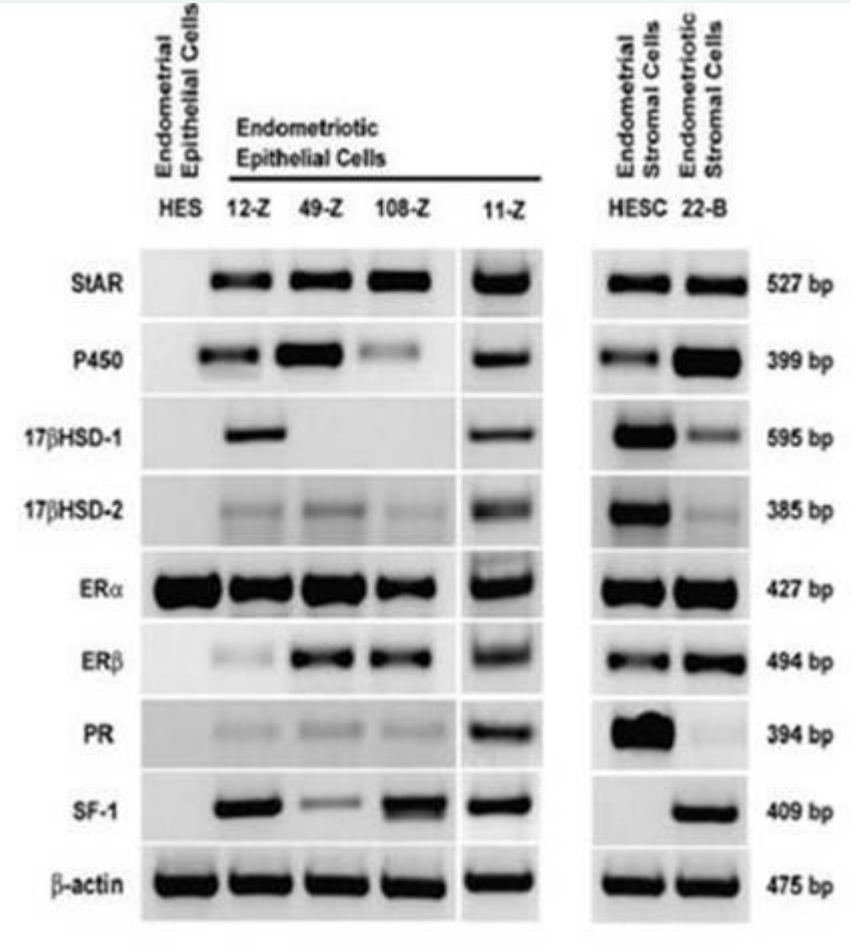
SV40 T-antigen transformed epithelial cells from peritoneal endometriosis *(Akoum et al., 1999 Am J Pathol)*

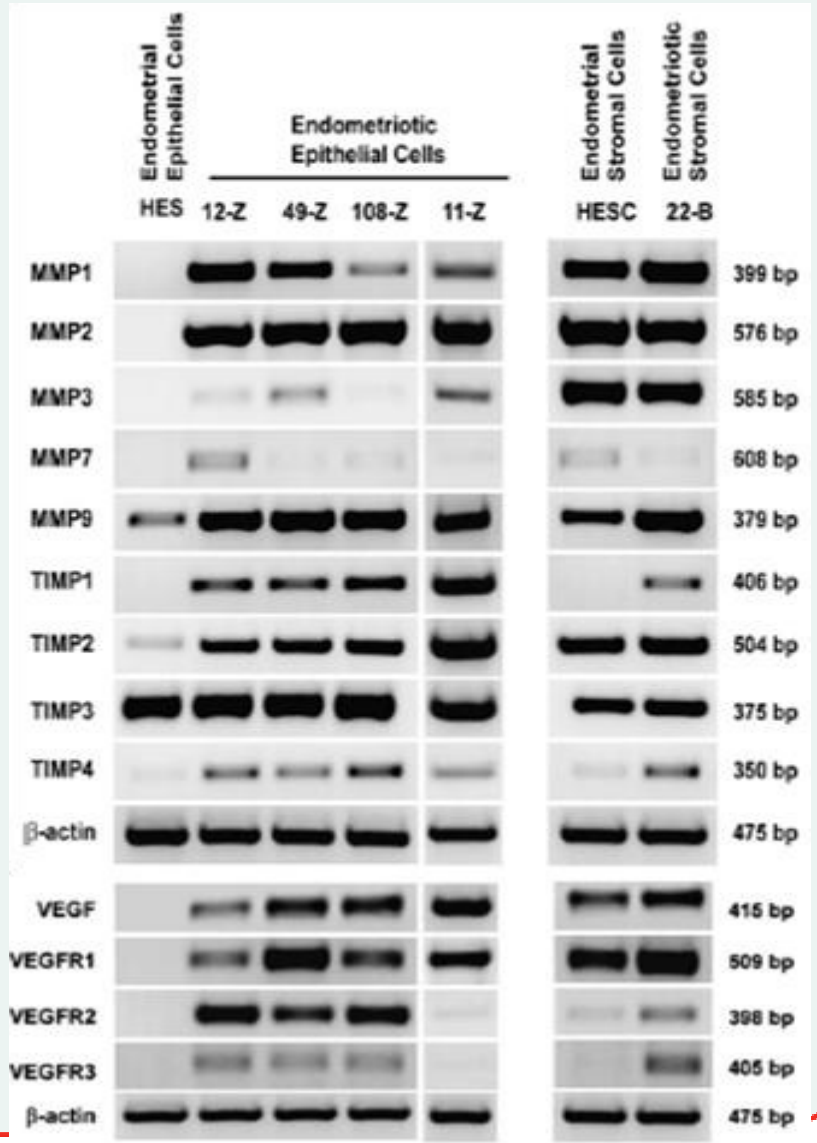
- Nuclear receptor status unknown, many chromosomal aberrations

hTERT-immortalized endometriotic stromal cells *(Annunziata et al., 2009 Fertil Steril)*

- No details on lesion type/location, no characterization of cell line

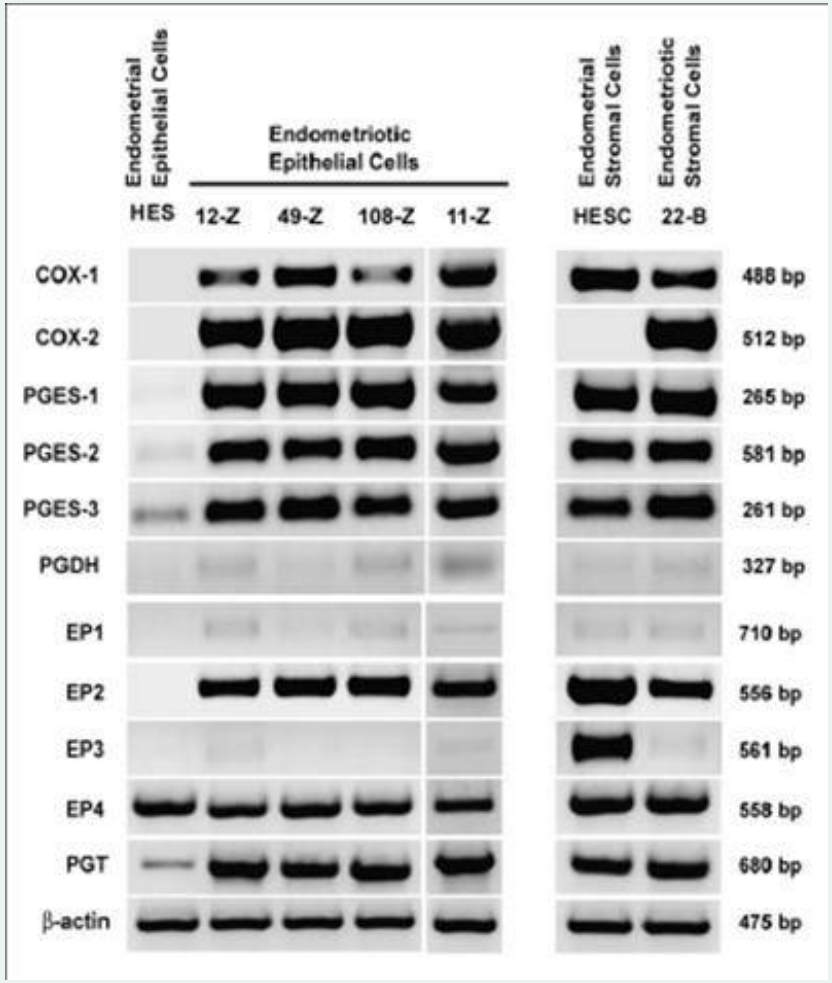
SV40 T-antigen transformed epithelial and stromal cell lines from peritoneal endometriosis





(Banu et al., Fertil Steril, 2008)

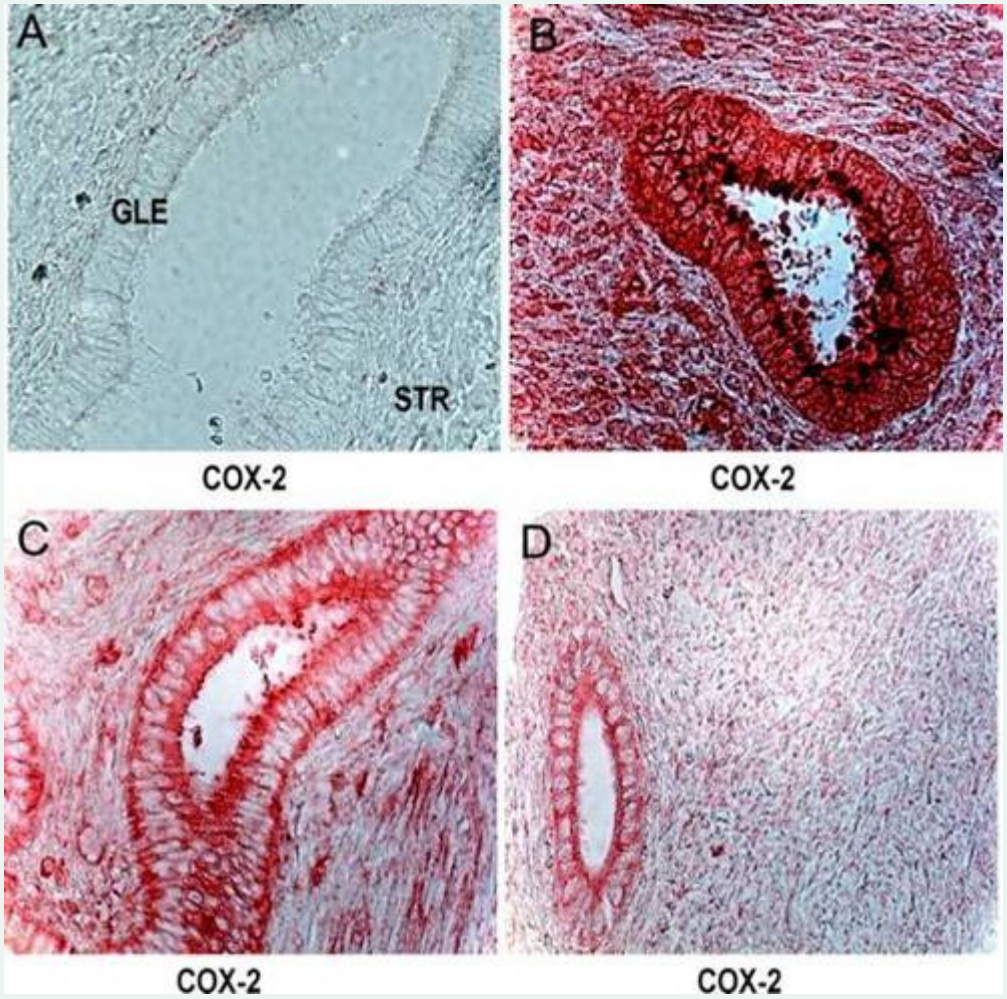
SV40 T-antigen transformed epithelial and stromal cell lines from peritoneal endometriosis



(Banu et al., Fertil Steril, 2008)

COX2 overexpression in endometriosis

Neg control



Ectopic

Eutopic patient

Eutopic control

Banu et al., 2008

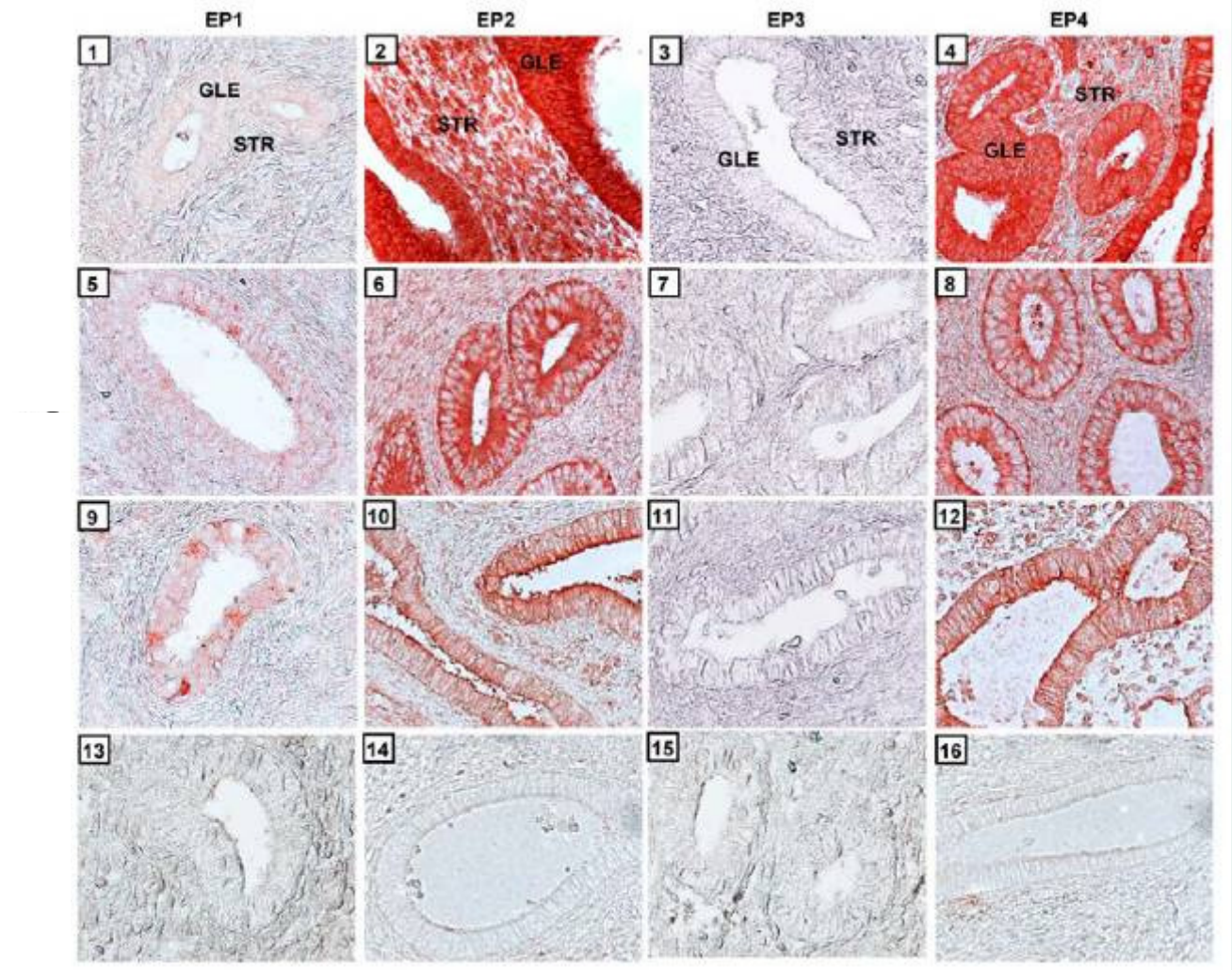
Prostaglandin receptor overexpression in endometriosis

Ectopic

Eutopic patient

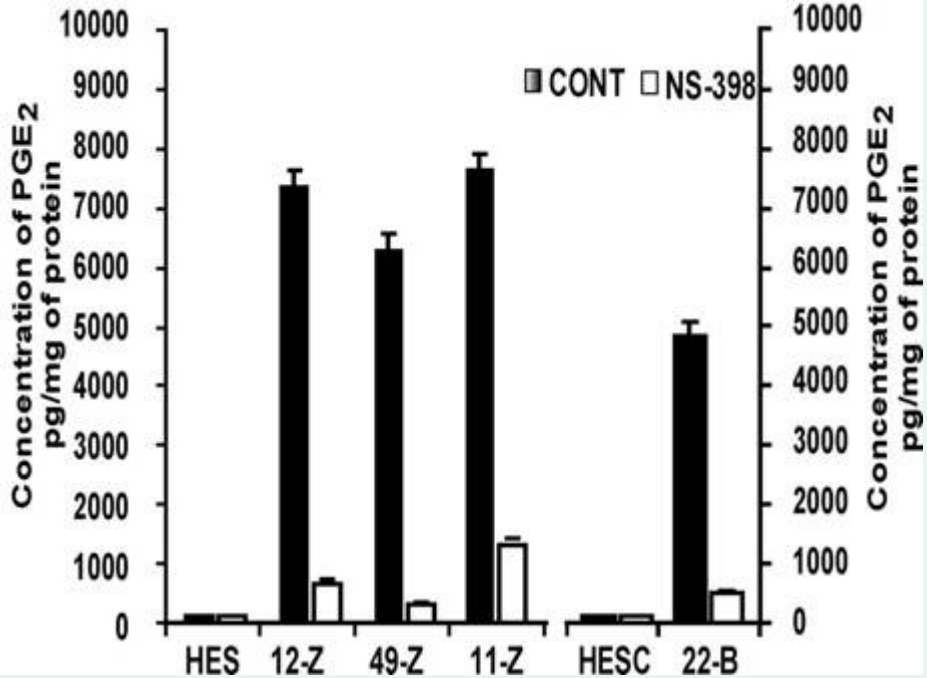
Eutopic control

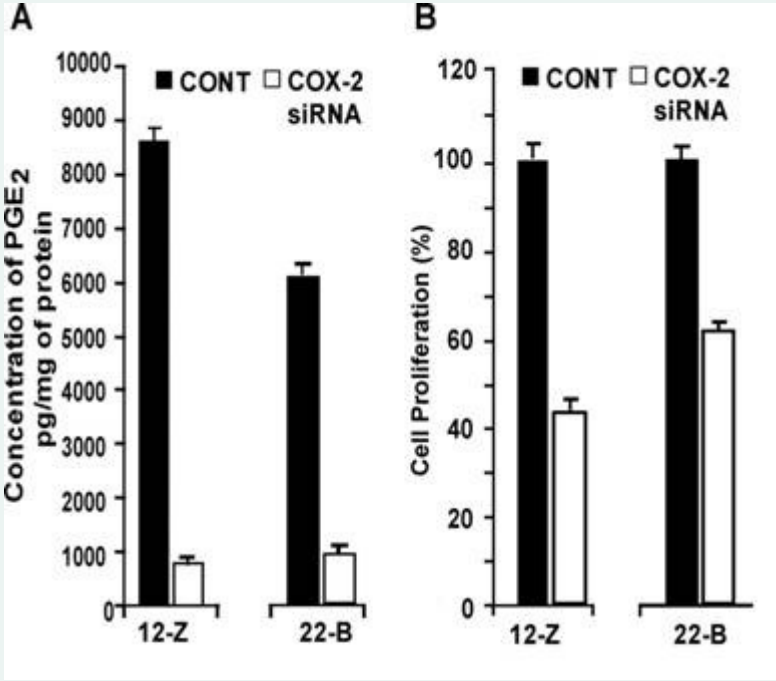
Neg control



Banu et al., 2008

Prostaglandins regulate cell survival in endometriosis





Conclusions

- The choice of in vitro model for target validation and to demonstrate mechanism of action, depends on the target and the biological mechanism of interest
- There is a need for more studies using explant cultures of endometriotic tissue as well as for more cell lines derived from endometriotic tissues
- Models should be validated with reference drugs

In vitro assays in LO

- Factors dictating selection assays HO and LO are different
- Reproducibility may be compromised
  - biological variation
  - variation expression target
- Biological relevance
- Biological connection in vitro assays and therapeutic models
  - tissue context
- Predict clinical efficacy
  - demand for returning therapeutically relevant data
  - check/search for biomarkers