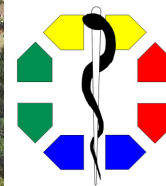
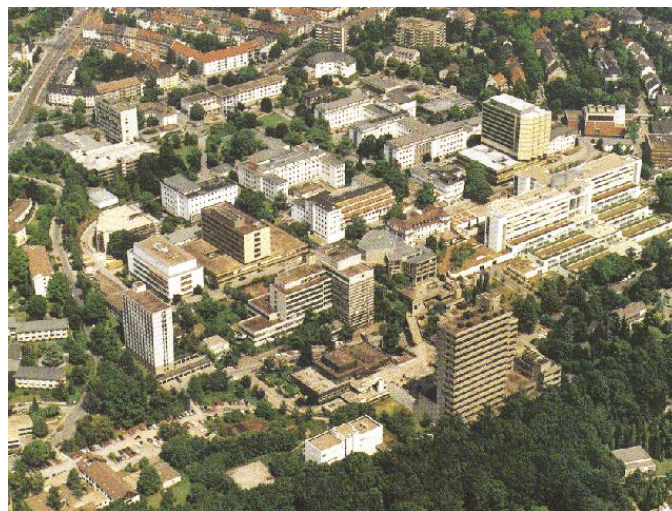


Rodent models for endometriosis research

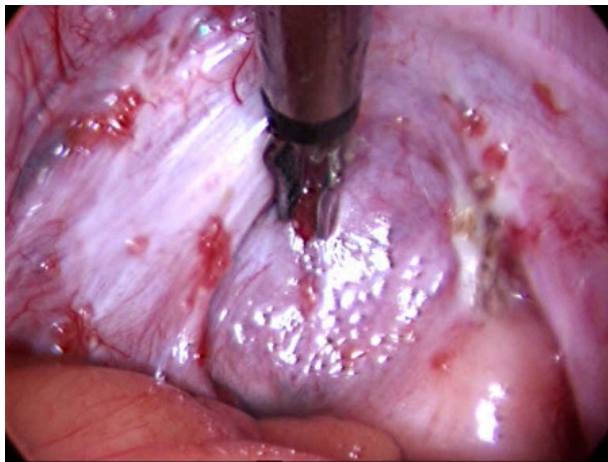
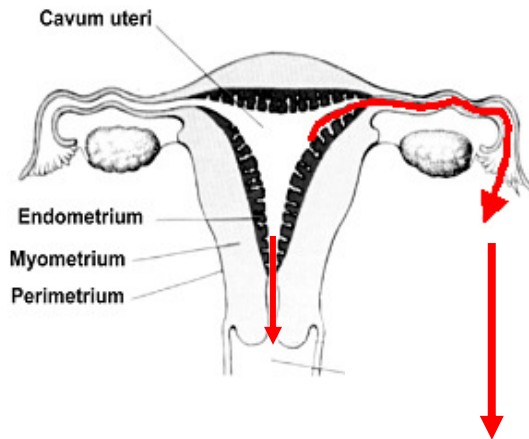
Prof. Ruth Grümmer, PhD

**University Hospital Essen, Germany
Institute of Molecular Biology**



University Hospital Essen

Treatment of Endometriosis



Current treatment:

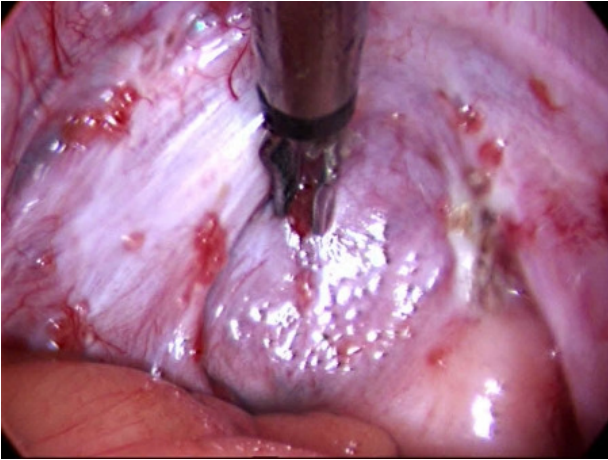
- Surgical removal of endometriotic lesions
- hormonal treatment
(mostly induction of hypoestrogenic environment)
 - Oral contraceptives
 - GnRH-agonists / antagonists
 - Danazol
 - Progestins

Side effects:

- high recurrence rate
- symptoms of menopause
- impairment of fertility

→ Novel therapeutical approaches are needed

Targets for novel therapeutical approaches



- **adhesion / invasion**
- **angiogenesis**
- **estrogen metabolism**
- **immune system**

→ **need for experimental models**

Rodent models for endometriosis research

Autologous

Transplantation of
autologous endometrium



•rats / mice



Heterologous

Transplantation of
human endometrium

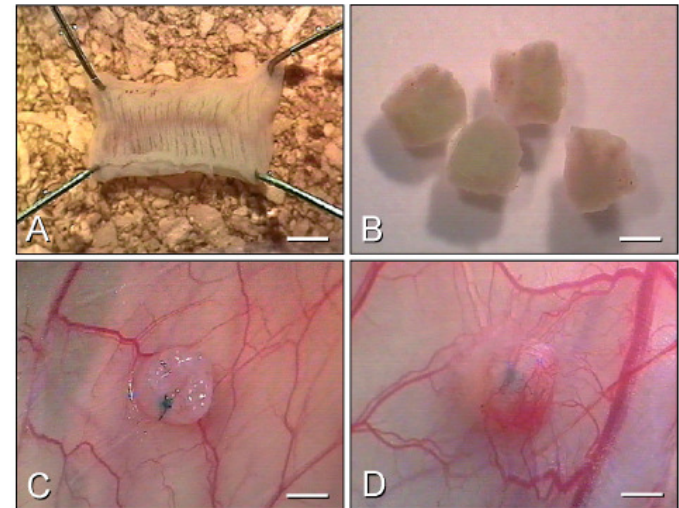
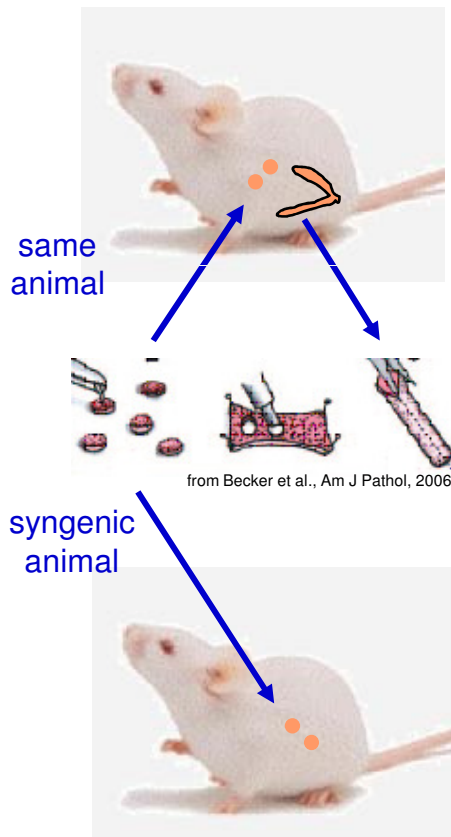


immunodeficient
mice

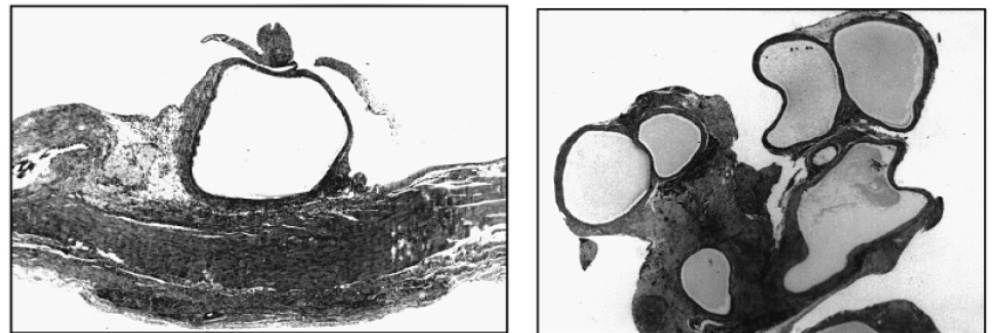


Autologous animal model

Transplantation of autologous uterine tissue



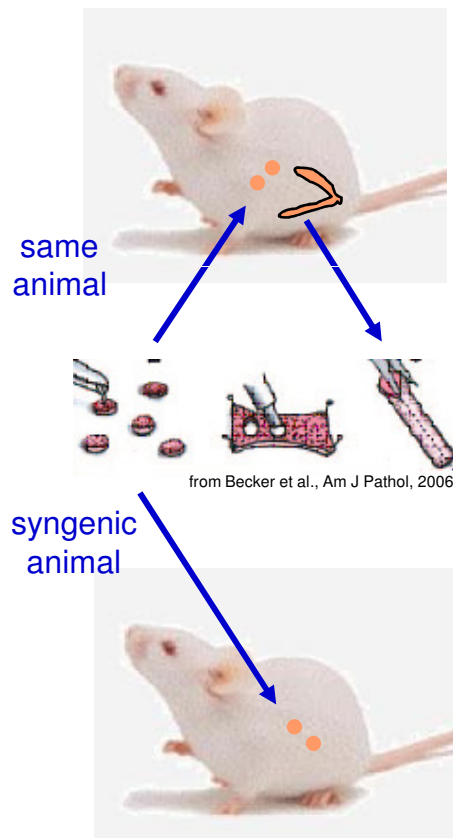
Laschke + Menger, Hum Reprod Update, 13, 331-342, 2007



Somigliana et al., Hum Reprod. 14: 2944-2950, 1999

Autologous animal model

Transplantation of autologous uterine tissue



Good availability / low costs

Study in large groups of genetically similar animals

No rejection of autotransplanted tissue → long term studies

Use of transgenic mouse models

Immunocompetent mice

→ Testing of immune modulating drugs / anti-inflammatory agents



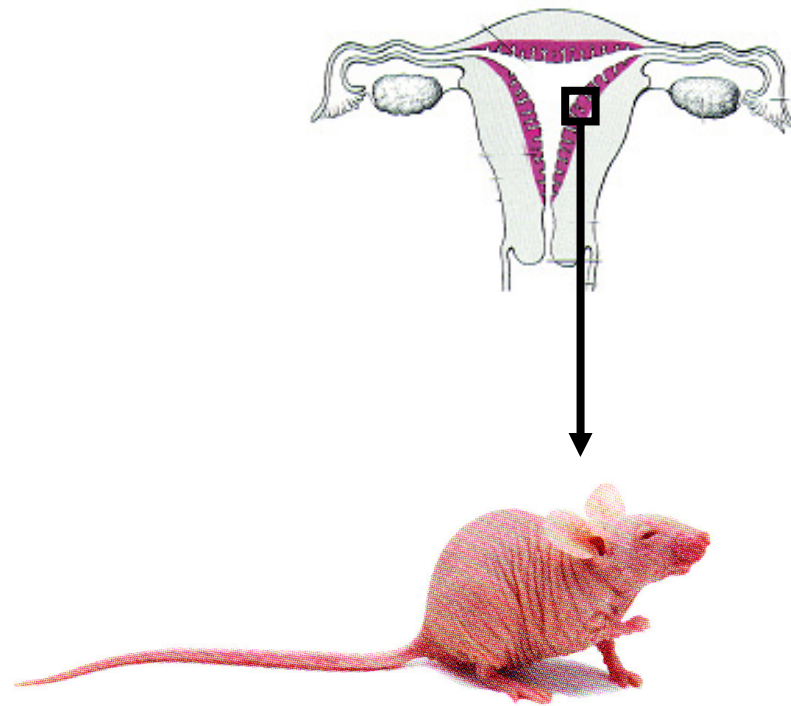
Mostly transplantation of endometrium + myometrium

Separation of endometrium only after E₂-treatment

No development of spontaneous endometriosis → causal factors for the development of endometriosis may not be present in the rodent endometrium

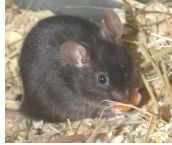
Heterologous models

Transplantation of human endometrium



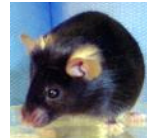
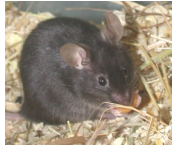
immunodeficient
mice

Immunodeficient mouse models






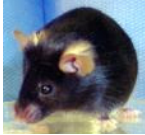

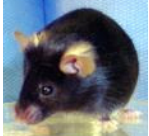
	wildtype	nude mouse
T-Lymph.	+	-
B-Lymph.	+	+
NK-cells	+	+

Immunodeficient mouse models



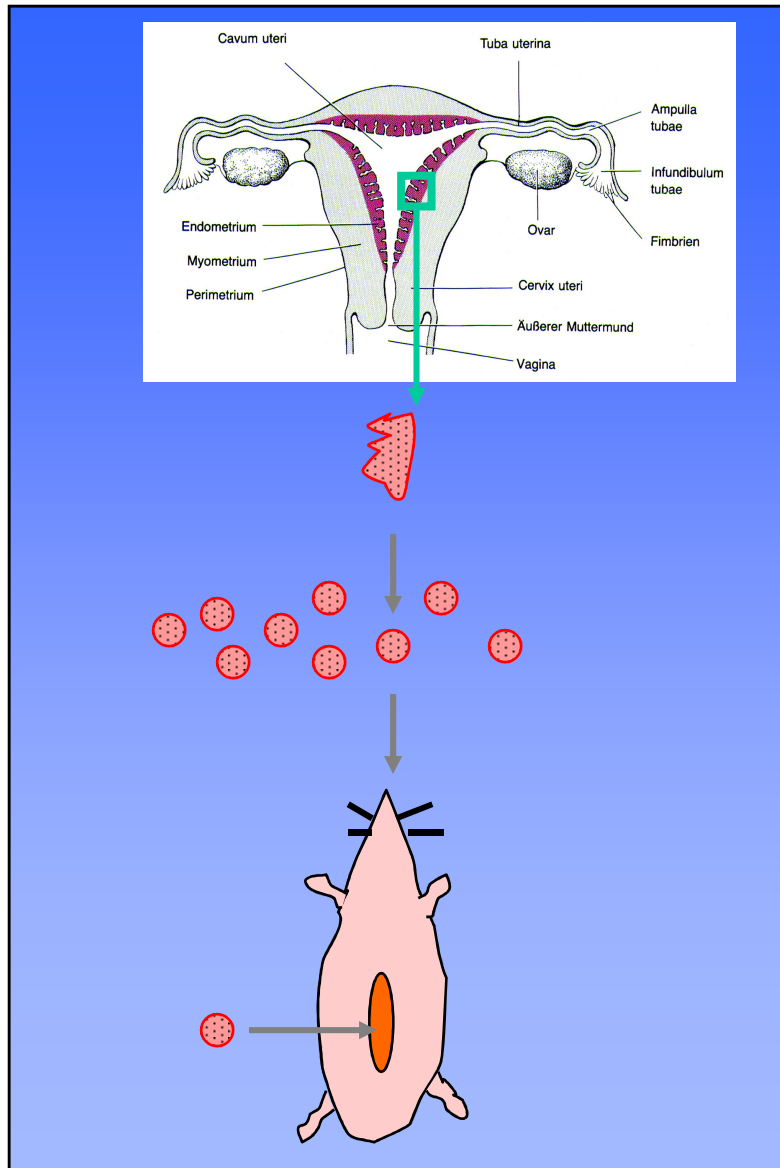
	wildtype	nude mouse	SCID	Rag-1 ^{null} Rag-2 ^{null}
T-Lymph.	+	-	-	-
B-Lymph.	+	+	-	-
NK-cells	+	+	+	+

Immunodeficient mouse models

						
	wildtype	nude mouse	SCID	Rag-1 ^{null} Rag-2 ^{null}	NOD/SCID/ γ C ^{null} (NOG)	Rag-2/ γ C ^{null}
T-Lymph.	+	-	-	-	-	-
B-Lymph.	+	+	-	-	-	-
NK-cells	+	+	+	+	-	-

no cytokine receptor gamma-chain
Interfering with signaling of cytokines
IL-2,-4,-7,-9 und -15

Heterologous animal model



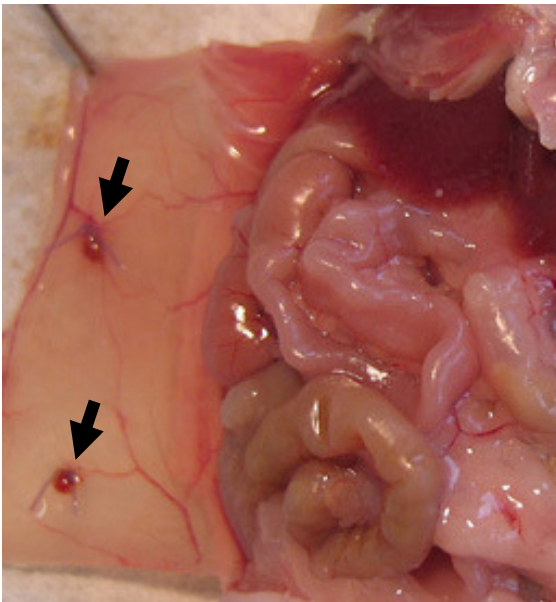
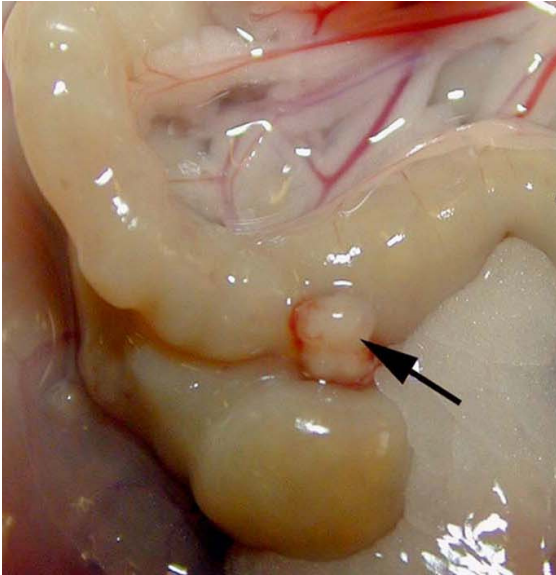
Human endometrial tissue

- fragments
- cell suspension

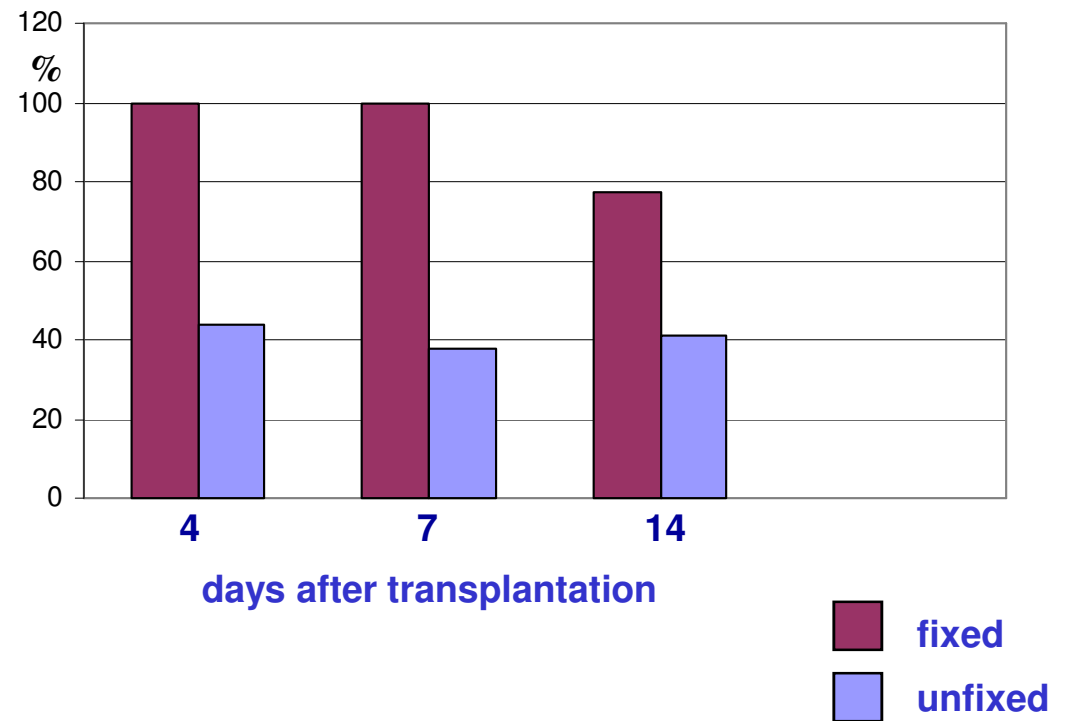
Transplantation into immunodeficient mice

(subcutaneous, intraperitoneal, kidney capsule)

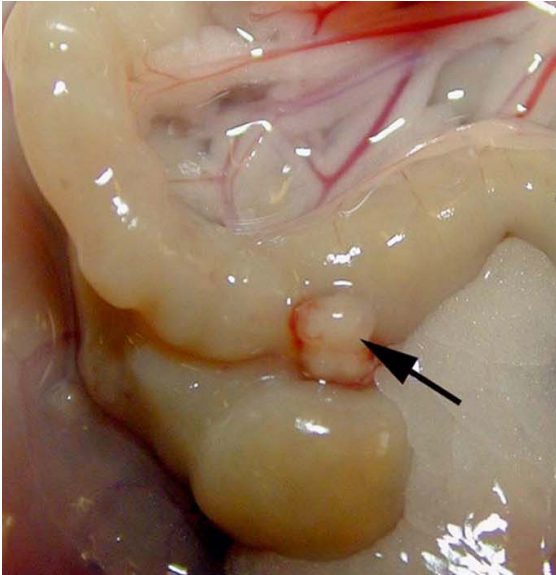
Human endometrial lesions in nude mice



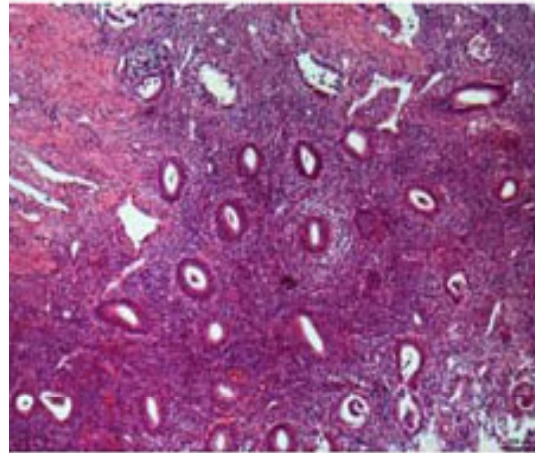
Recovery rate of fragments



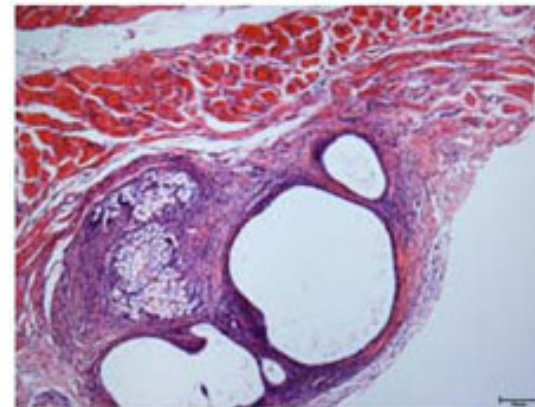
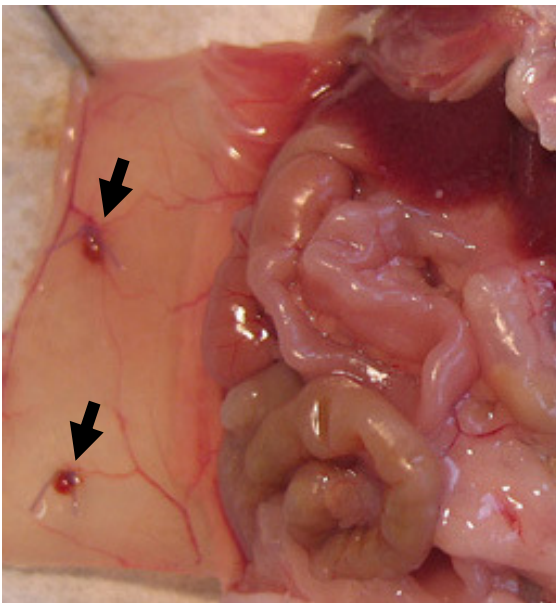
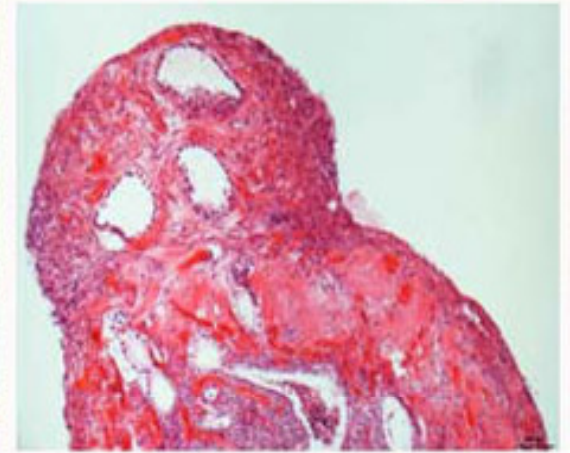
Human endometrial lesions in nude mice



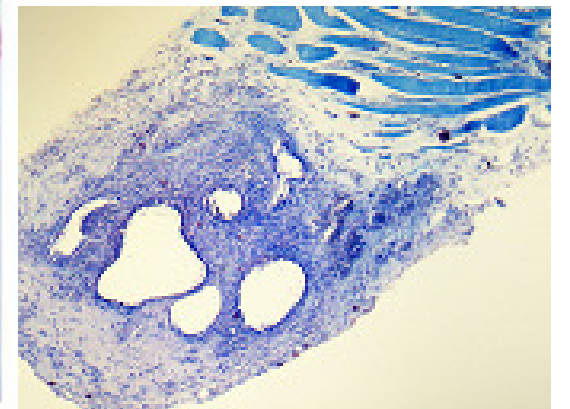
native tissue



1 week

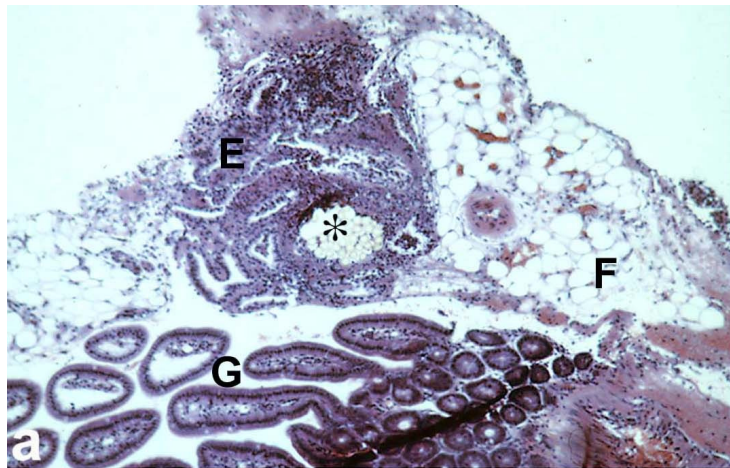


4 weeks

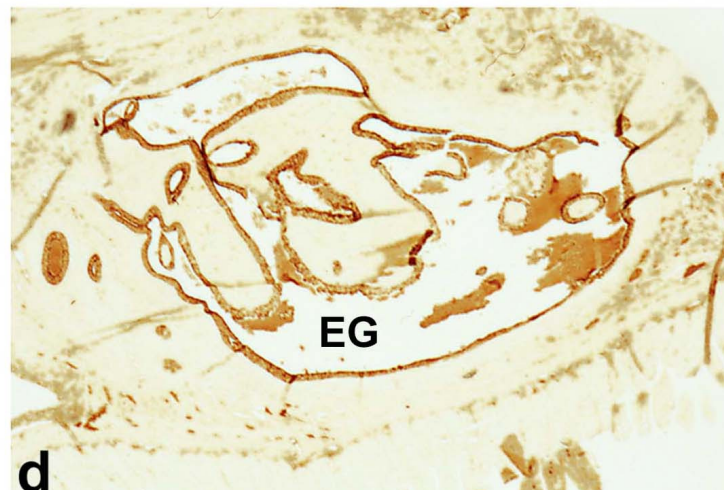
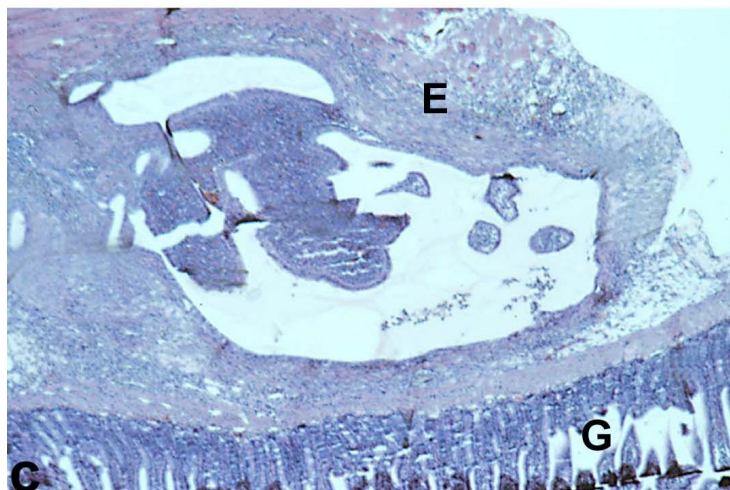


12 weeks

Expression of cytokeratin in endometriotic lesions

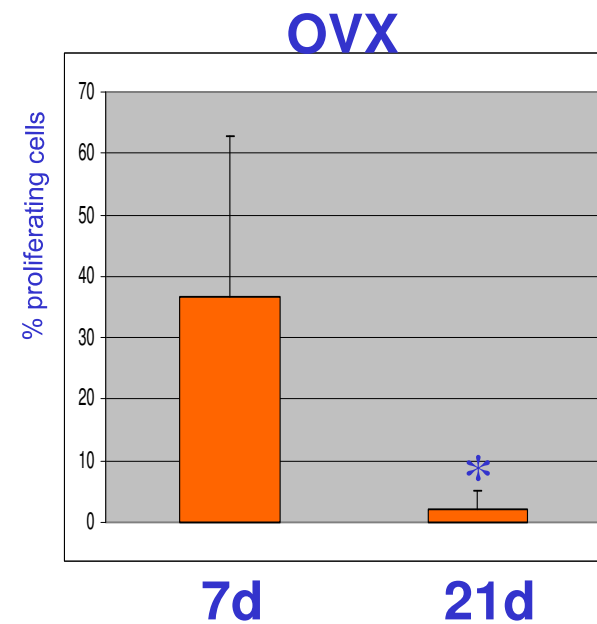
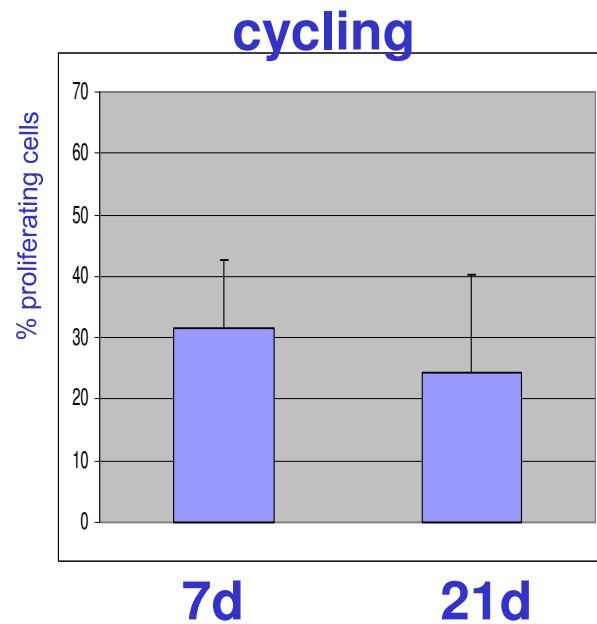
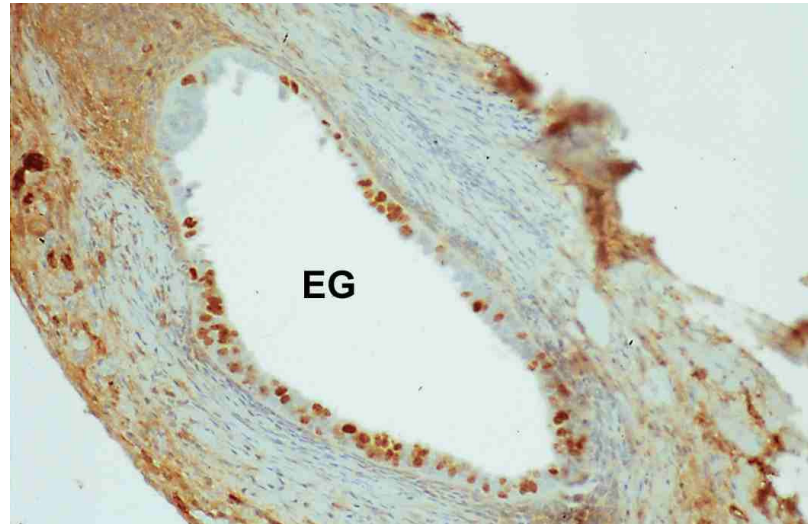


2 days



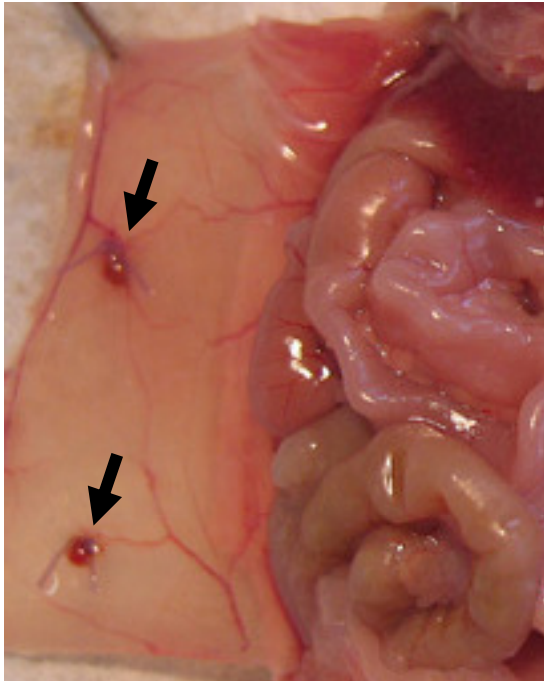
14 days

Proliferation of human endometriotic lesions

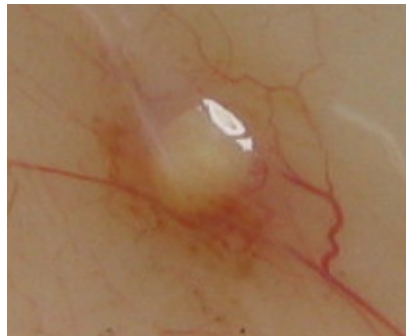


* $p \leq 0.05$

Angiogenesis in ectopic lesions



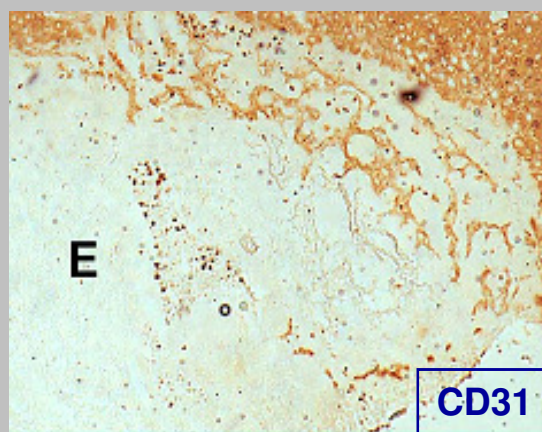
peritoneum without lesion



peritoneum + endometriotic lesion



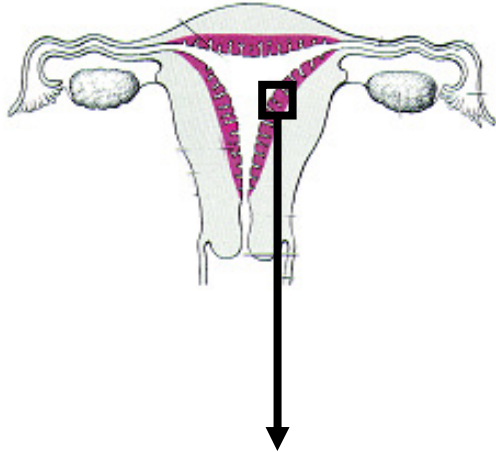
HE



CD31

Heterologous animal model

transplantation of
human endometrial tissue



immunodeficient
mice



Low costs

Use of human endometrium

Evaluation of drug effects in a large cohort of patients

Comparison of endometrium from women with and without endometriosis

Differentiation between effects of host versus graft

Human hormonal cycle can be mimicked in OVX animals



Availability of tissue

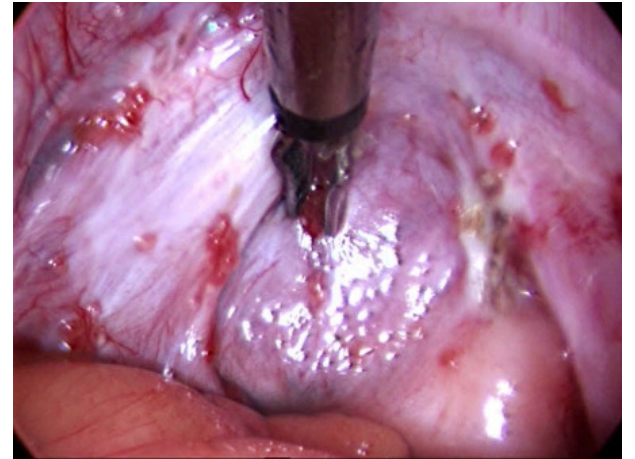
Limited duration of maintenance of transplants

No intact immune system

Possible species interactions

Targets for novel therapeutical approaches

- **adhesion / invasion**
- angiogenesis
- estrogen metabolism
- immune system

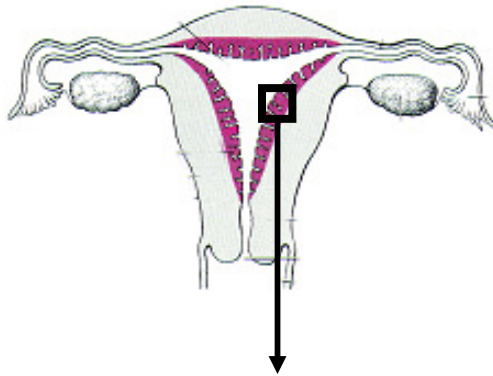


Matrix metalloproteinases upregulated in endometriosis

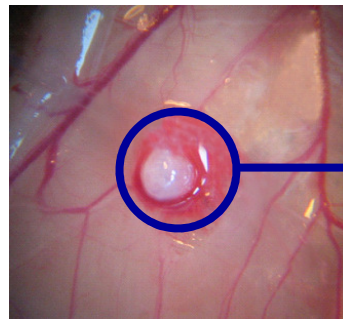
- **MMP-1** collagenase (Kokorine et al. 1997)
- **MMP-2** gelatinase A (Wenzl and Heinzl 1998)
- **MMP-3** stromelysin (Cox et al. 2001)
- **MMP-7** matrilysin-1 (Osteen et al. 1996)
- **MMP-9** gelatinase B (Chung et al. 2001)

MMPs in ectopic lesions

transplantation of
human endometrium



7 days



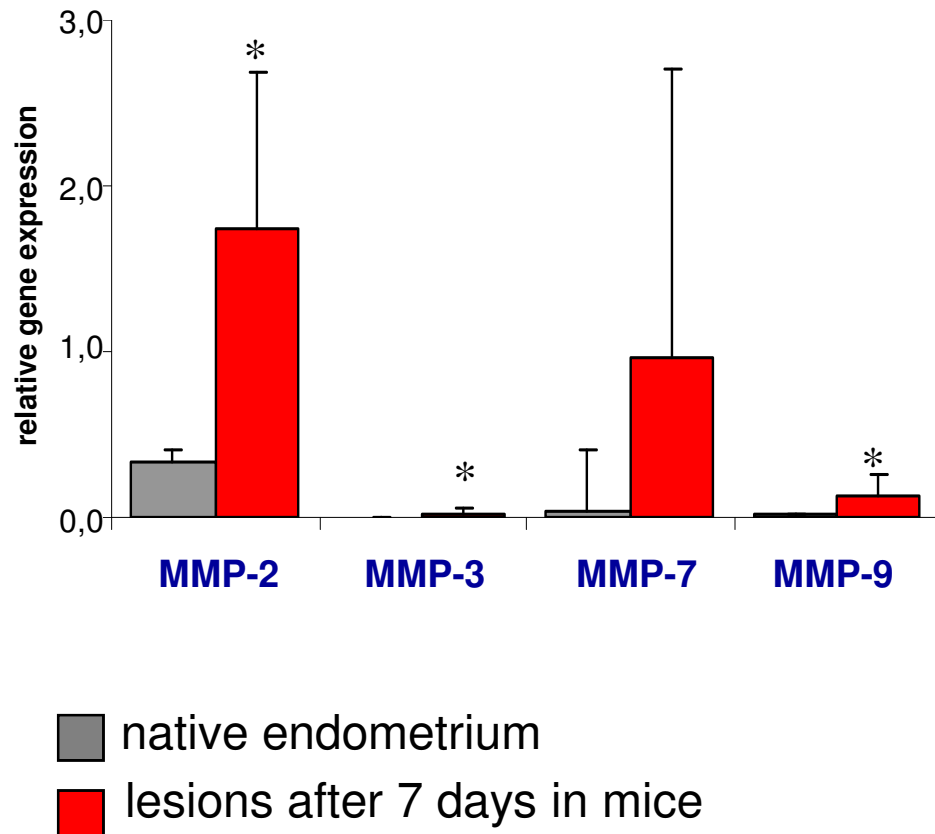
qPCR

MMP-2
MMP-3
MMP-7
MMP-9

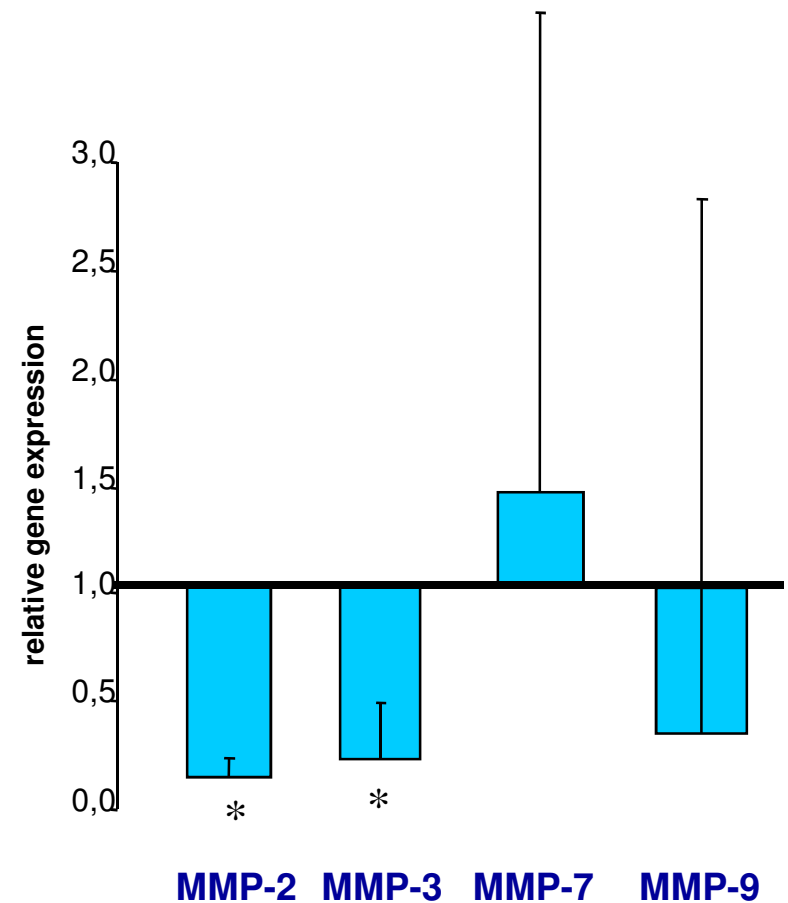
analysis of lesions

MMP expression in ectopic lesions in nude mice

MMPs are upregulated
7 days after transplantation

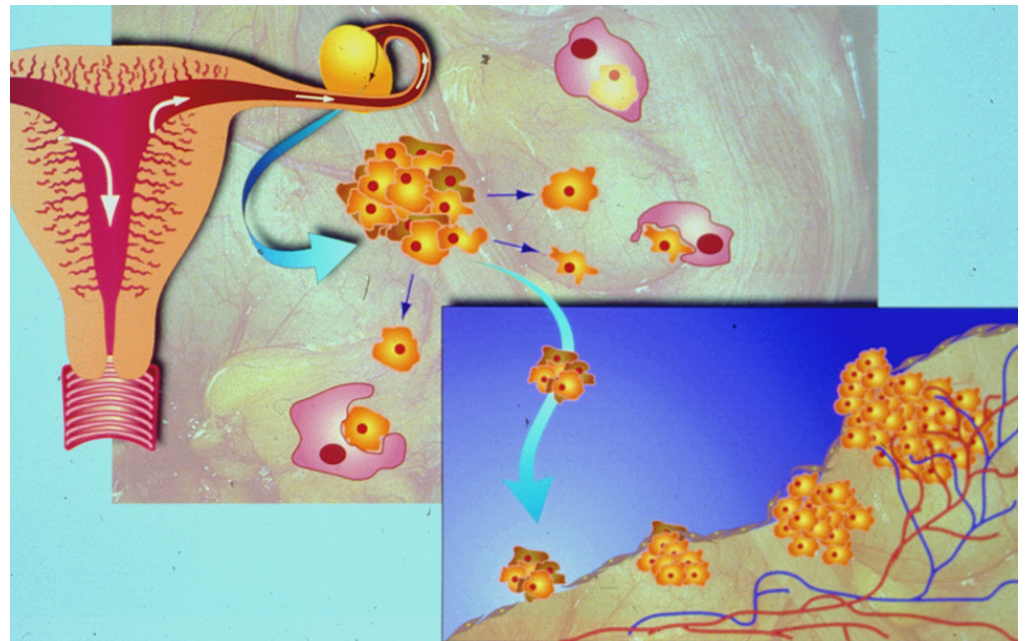
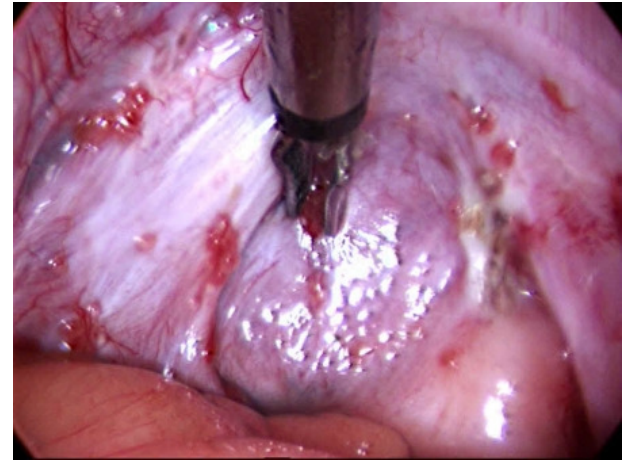


Expression of MMP-2 and -3
is suppressed by progesterone



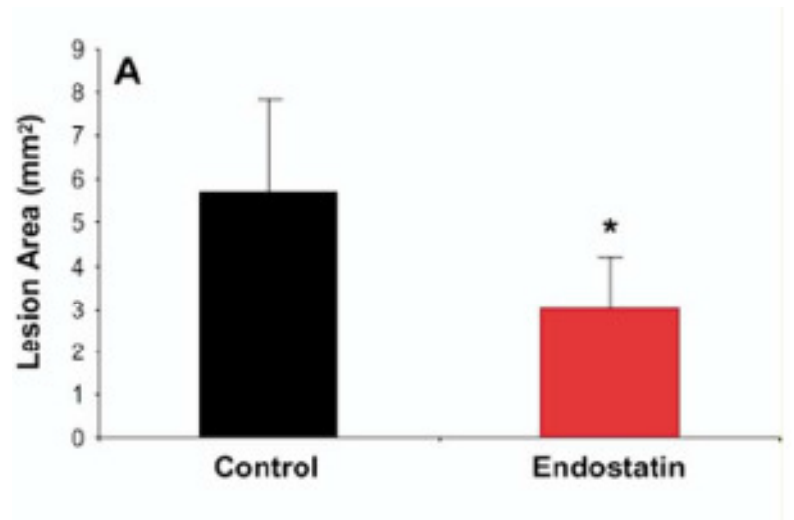
Targets for novel therapeutical approaches

- adhesion / invasion
- **angiogenesis**
- immune system
- estrogen metabolism



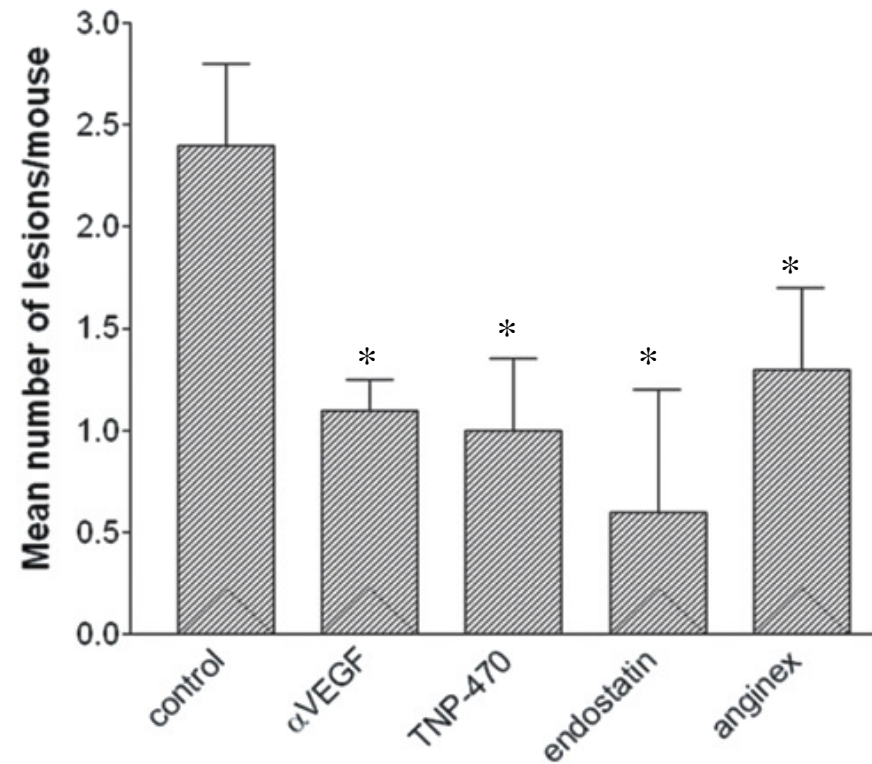
Inhibition of angiogenesis

Autologous model



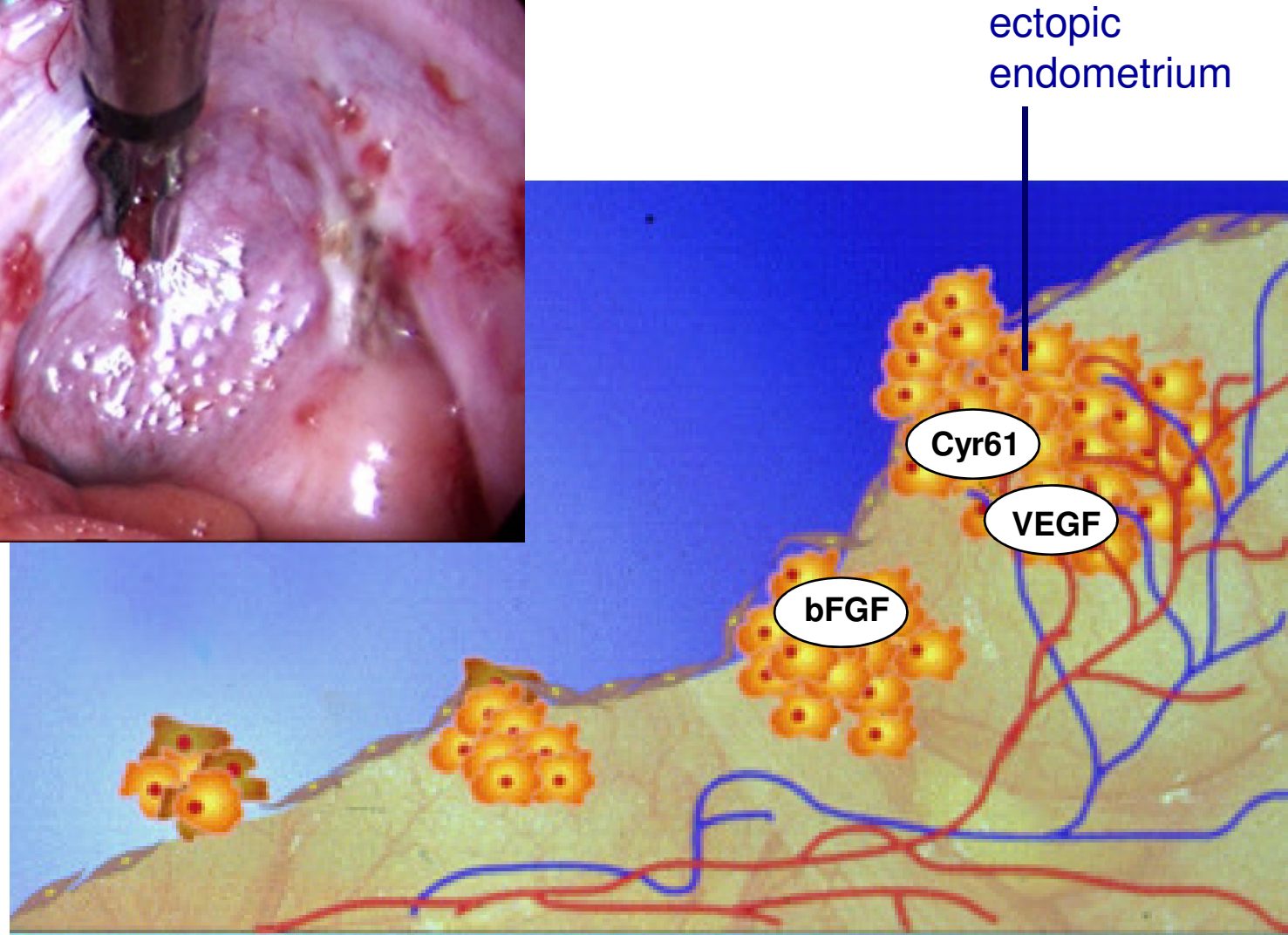
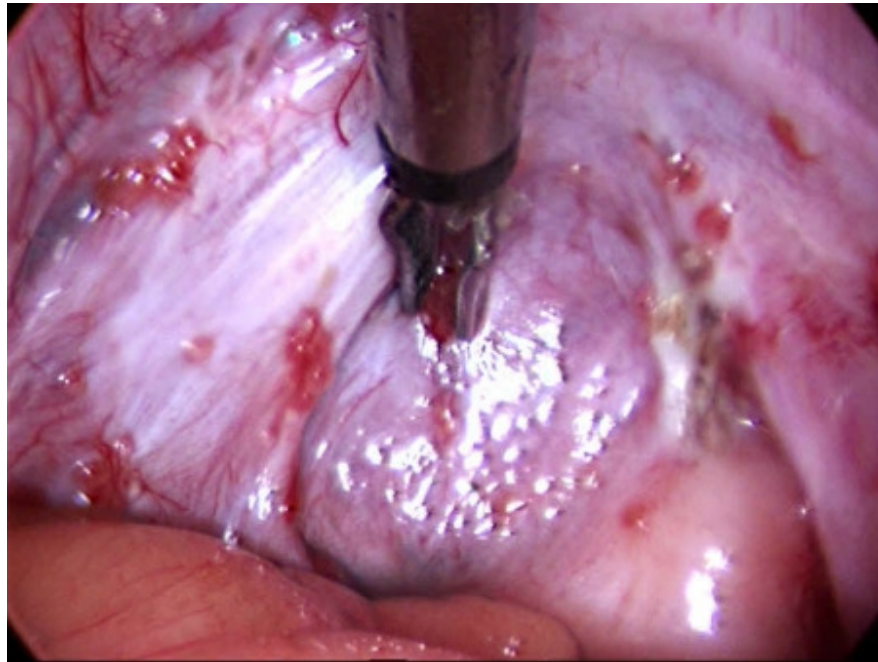
Becker et al., Fertil Steril, 2005

Heterologous model



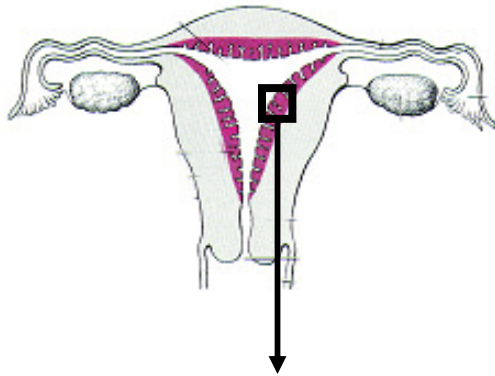
Nap et al., J Clin Endocrin Metab, 2004

Angiogenic factors

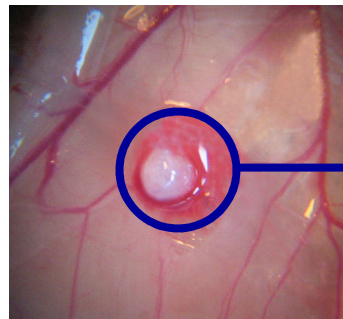


Angiogenic factors in ectopic lesions

transplantation of
human endometrium



7 days

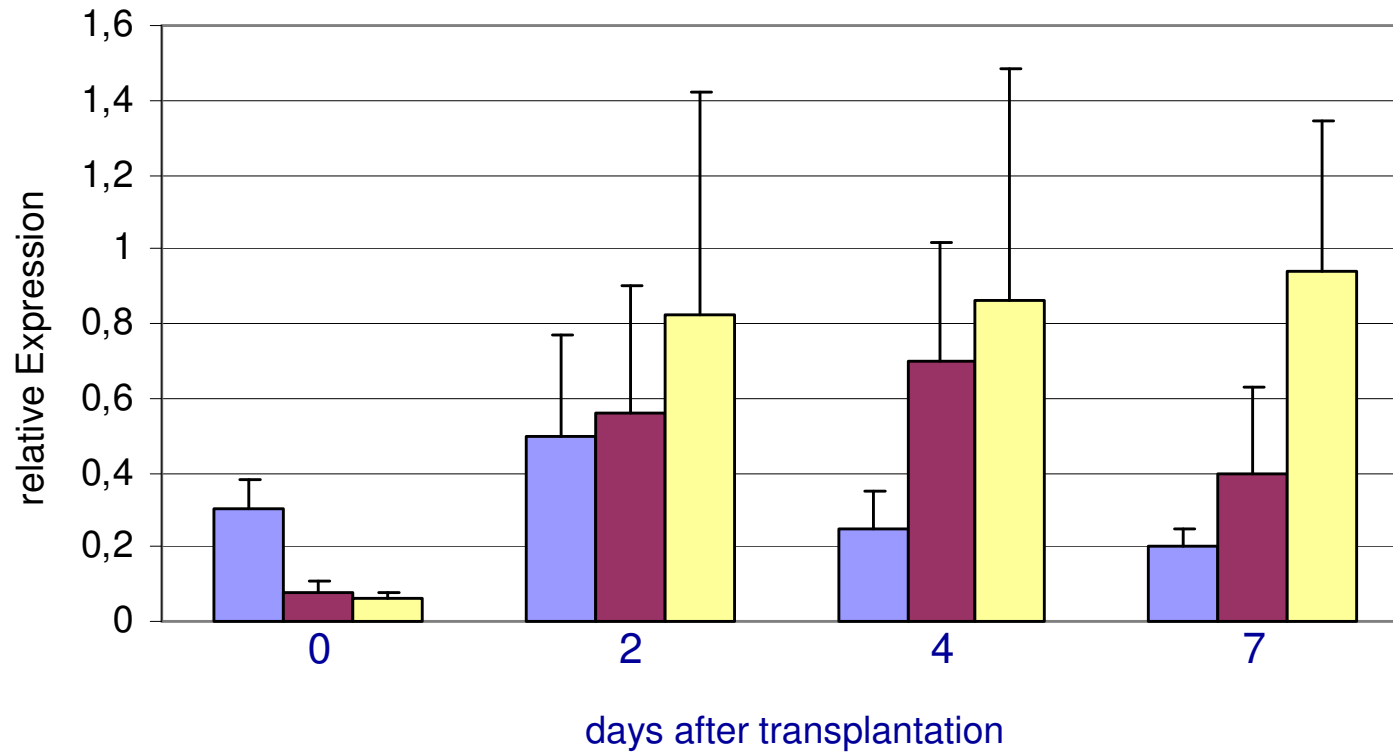


qPCR

VEGF
bFGF
CYR61

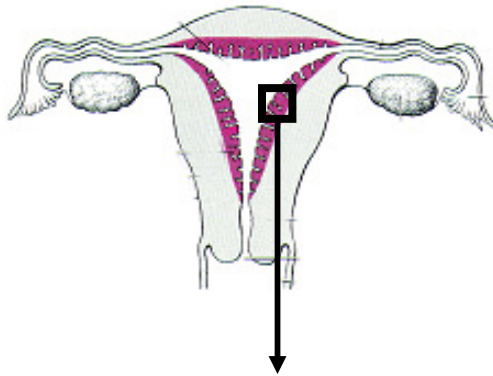
analysis of lesions

Angiogenic factors in ectopic lesions

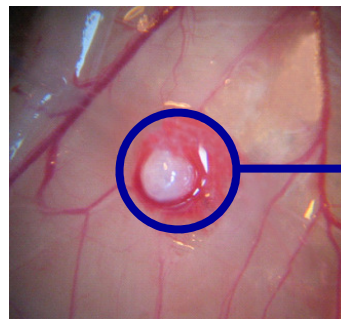


Angiogenic factors in ectopic lesions

transplantation of human endometrium



7 days



qPCR

VEGF
bFGF
CYR61

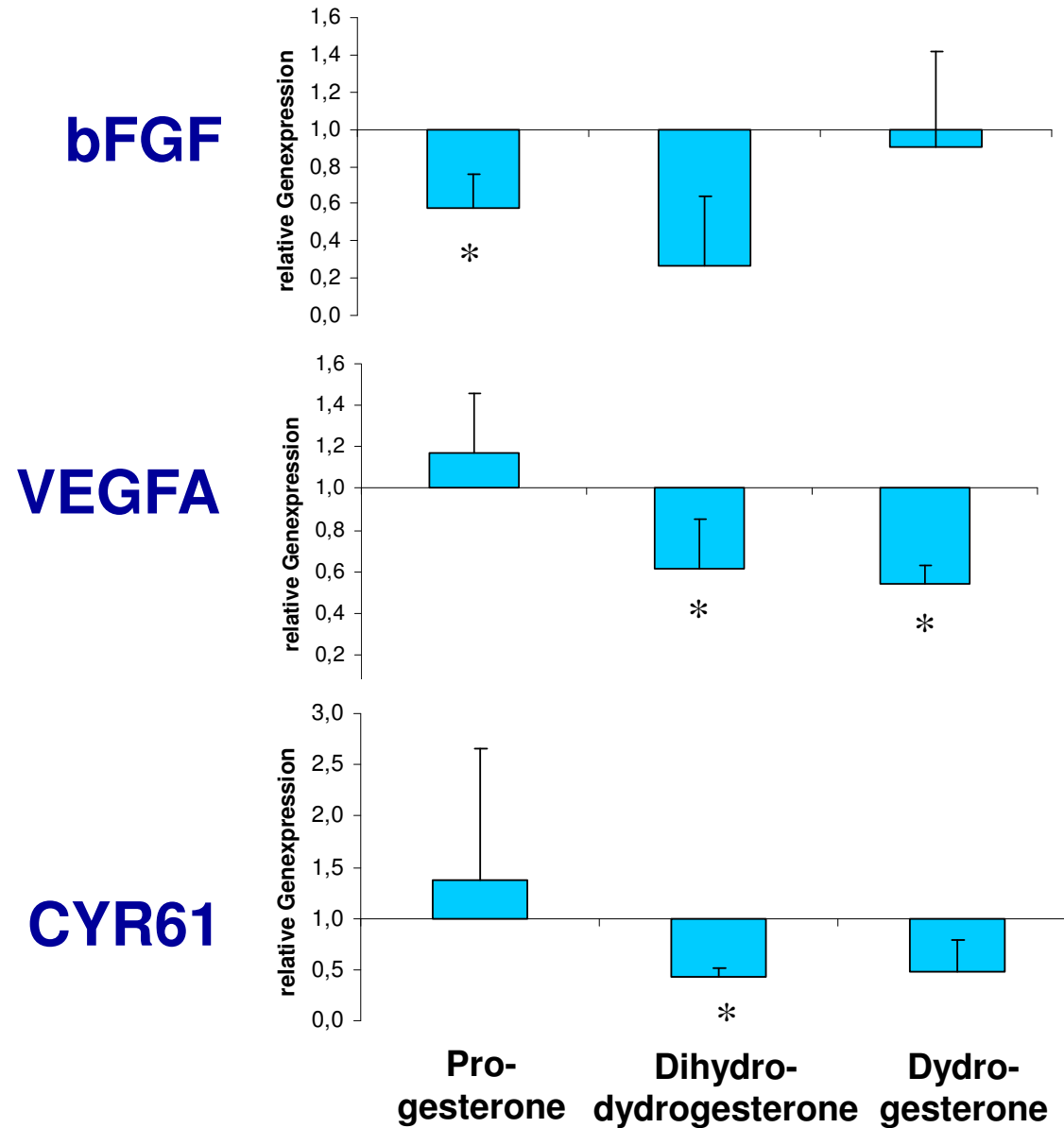
analysis of lesions

Progestins: Progesterone
Dydrogesterone
Dihydrodydrogesterone (DHD)

Control: Vehicle

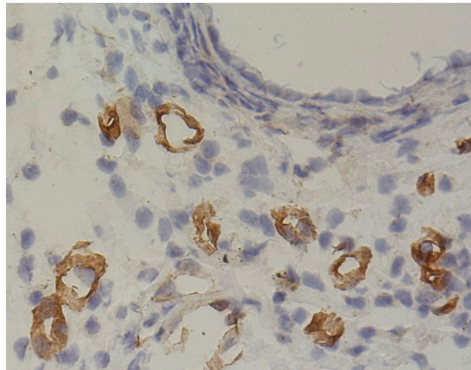
subcutaneous
50 µg/mouse/day
starting day 1 after transplantation

Effect of progestins on angiogenic factors

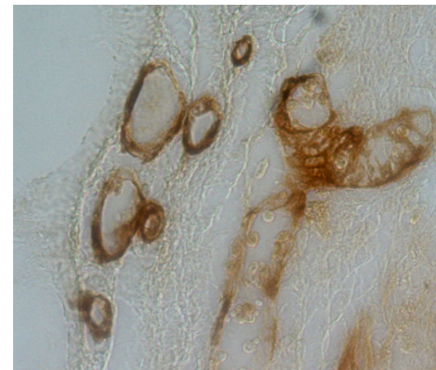


Effect of progestins on angiogenesis

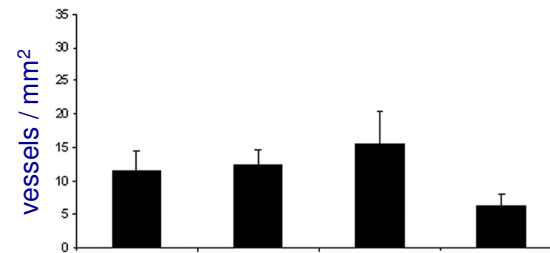
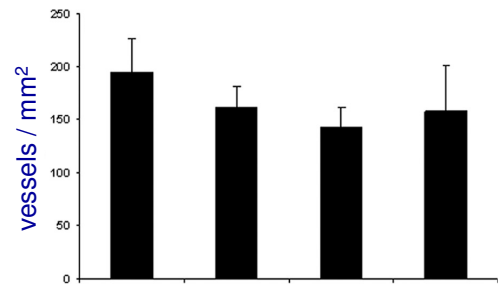
CD31



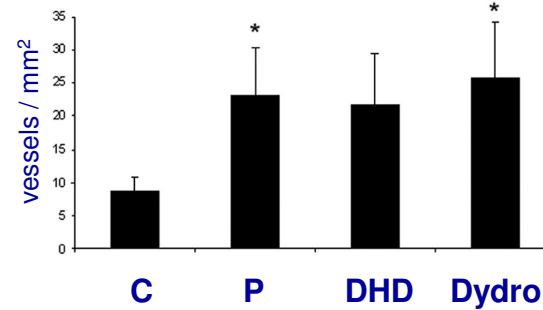
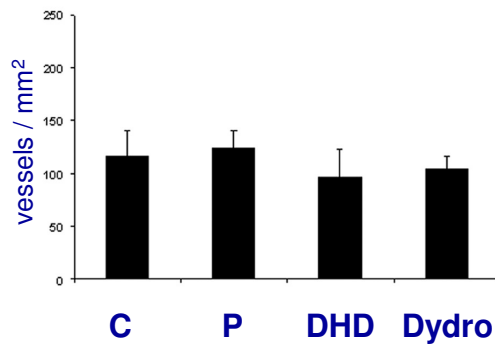
α SMA



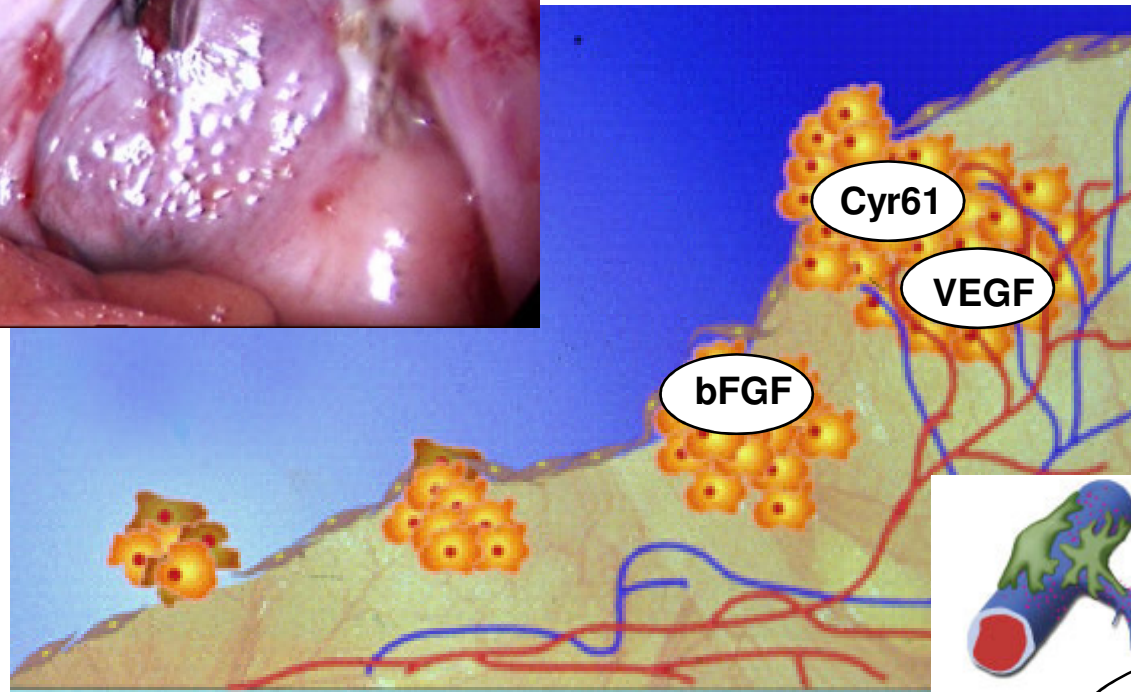
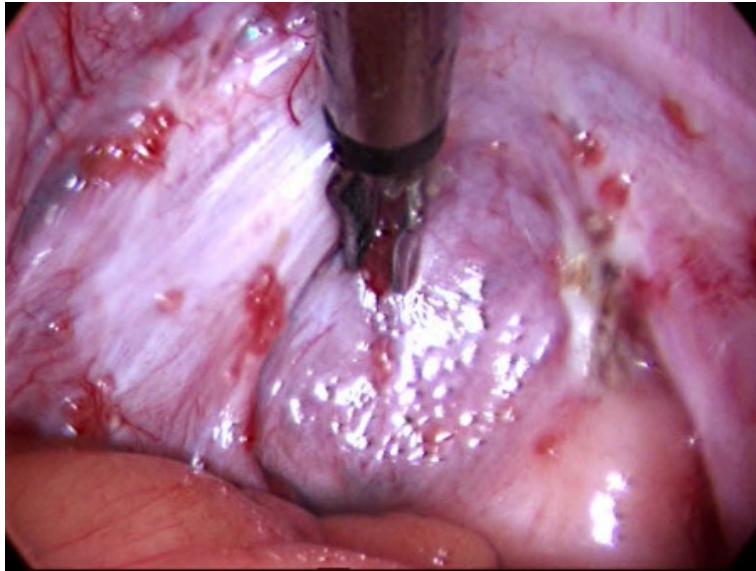
7 days



28 days



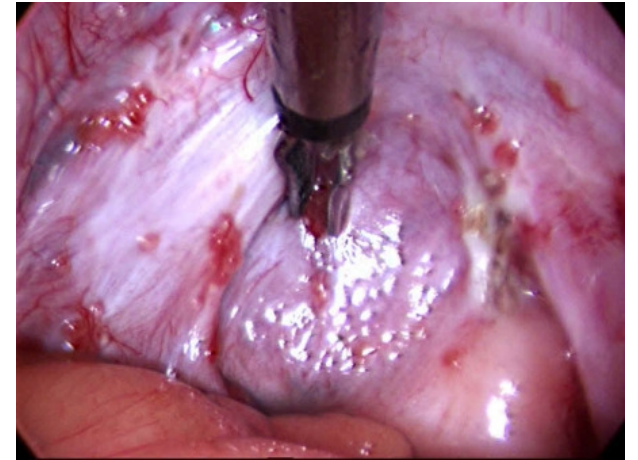
Possible targets of progestin action



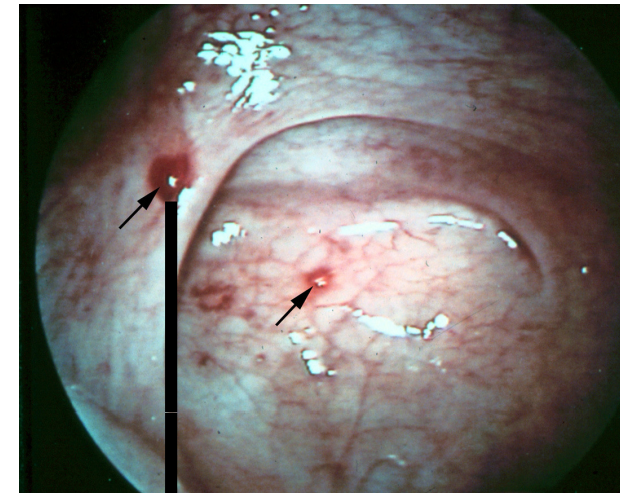
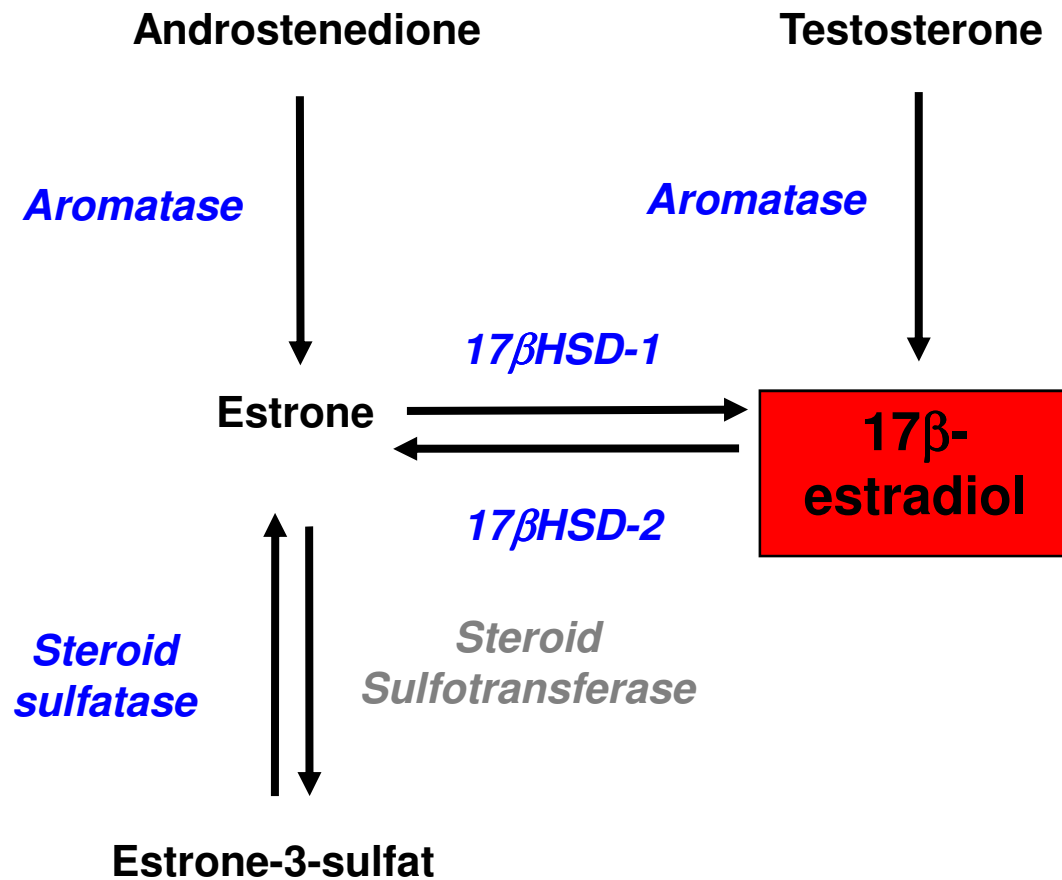
stabilizing of vessels
by pericytes

Development of novel therapeutical approaches

- adhesion / invasion
- angiogenesis
- **estrogen metabolism**
- immune system



17 β -Estradiol-Synthesis

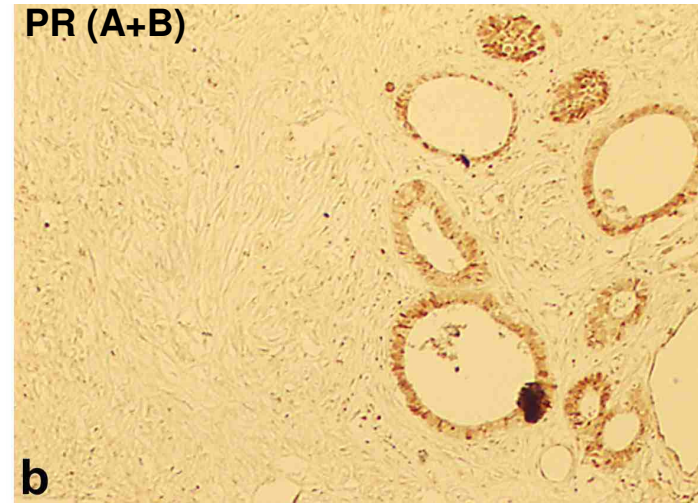
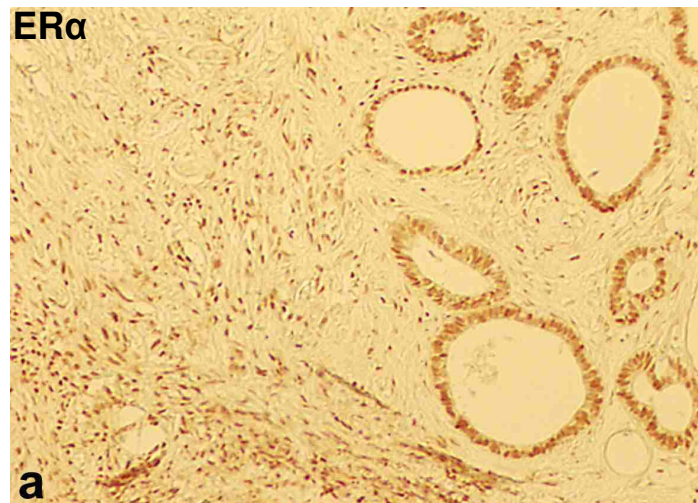
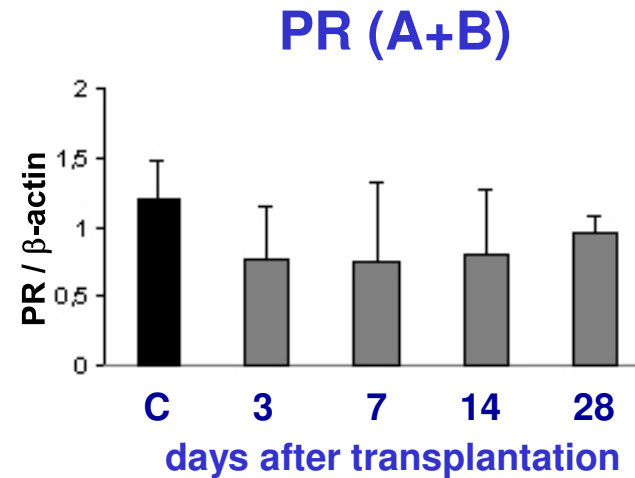
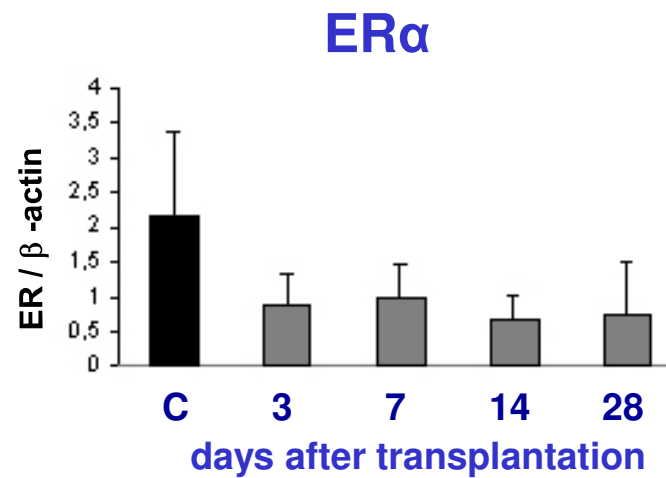


•Aromatase \uparrow
•17 β HSD-2 \downarrow



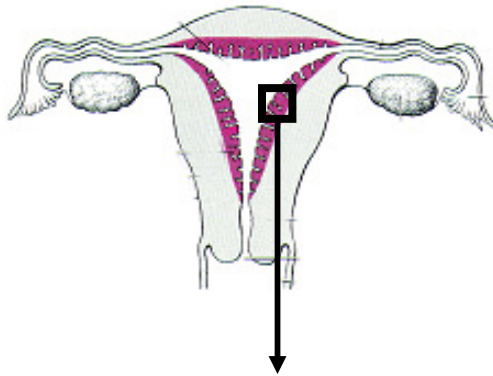
elevated local estradiol concentration

ER and PR in human ectopic lesions in nude mice

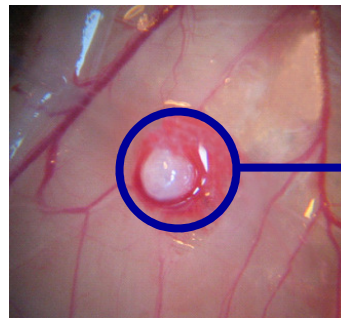


Estrogen metabolizing enzymes in ectopic lesions

transplantation of
human endometrium



7 days



qPCR

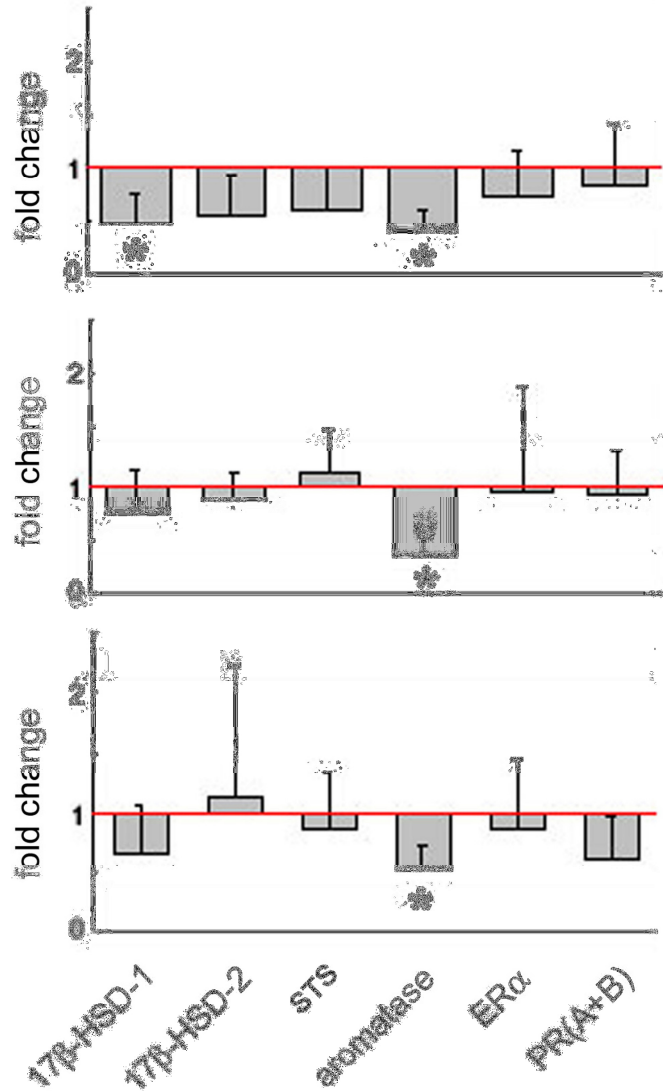
17 β -HSD-1
17 β -HSD-2
STS
aromatase
ER / PR

analysis of lesions

Progestins: **Dydrogesterone**
 MPA
Aromatase inhibitor: **Finrozole**
Control: **Vehicle**

Estrogen metabolizing enzymes in ectopic lesions

Q-PCR

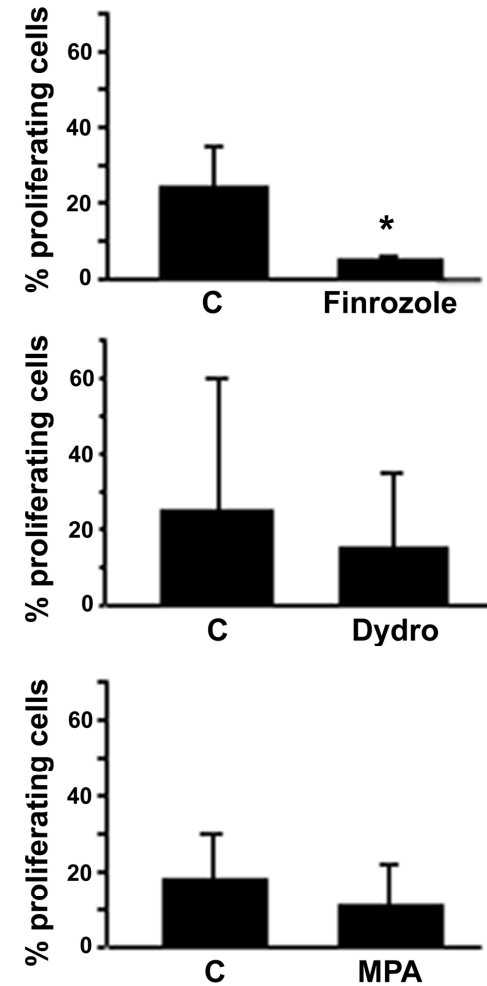


Finrozole

Dydrogesterone

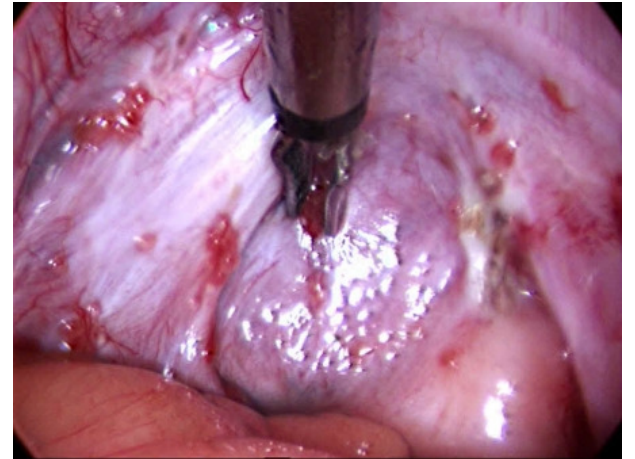
MPA

Proliferation

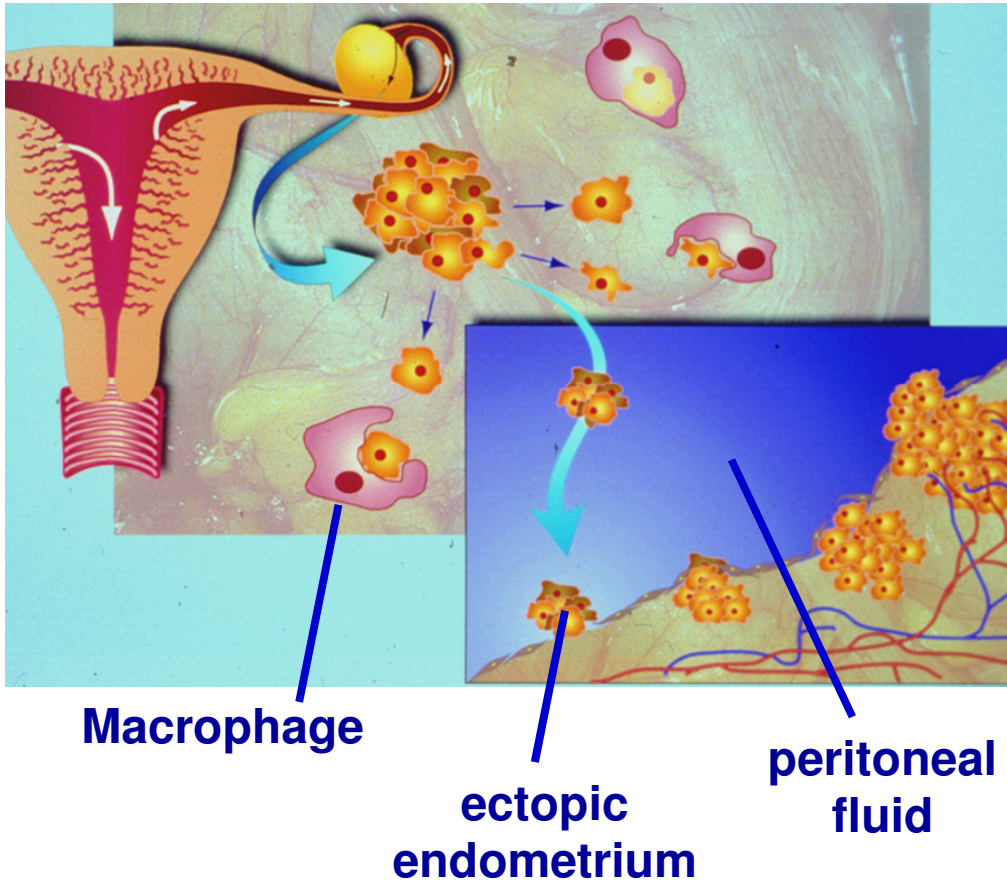


Targets for novel therapeutical approaches

- adhesion / invasion
- angiogenesis
- estrogen metabolism
- **immune system**



Inflammatory reaction

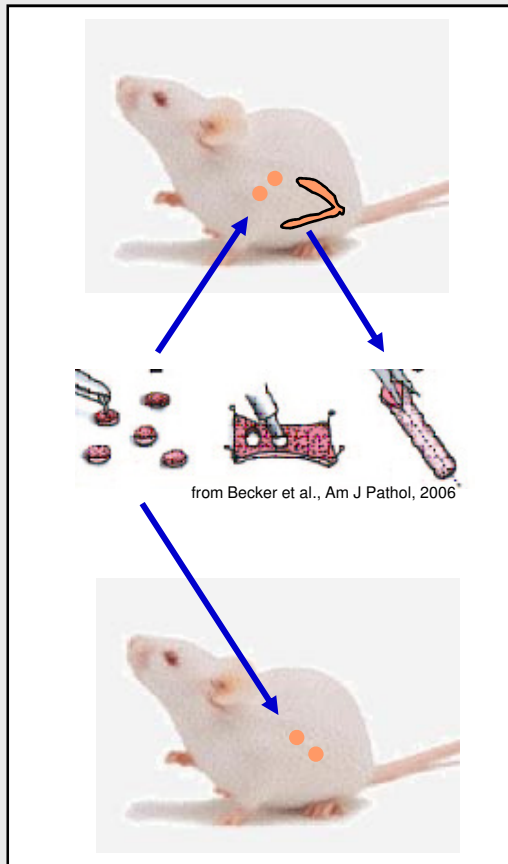


Elevated levels in the peritoneal fluid of endometriosis patients:

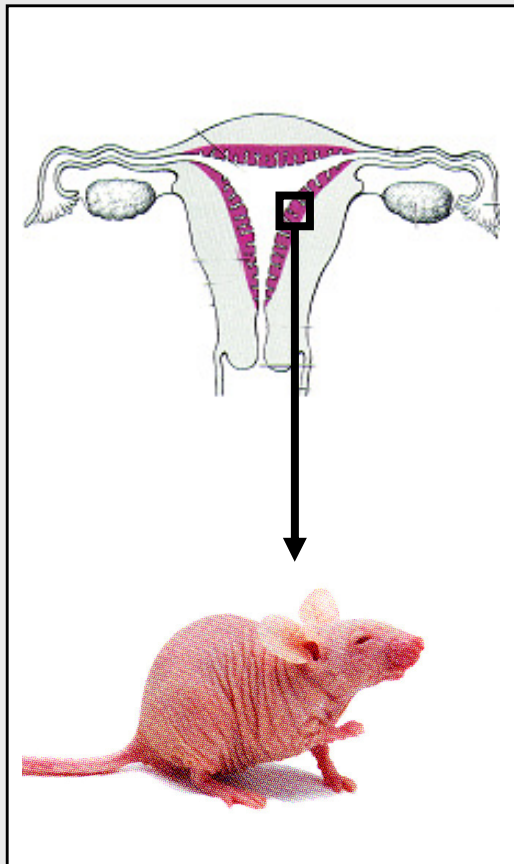
- IL-1
- IL-6
- IL-8
- TNF α

Evaluation of novel therapeutical approaches

autologous model



heterologous model



- adhesion / invasion
- angiogenesis
- immune system
- estrogen metabolism

In-vivo imaging of ectopic lesions

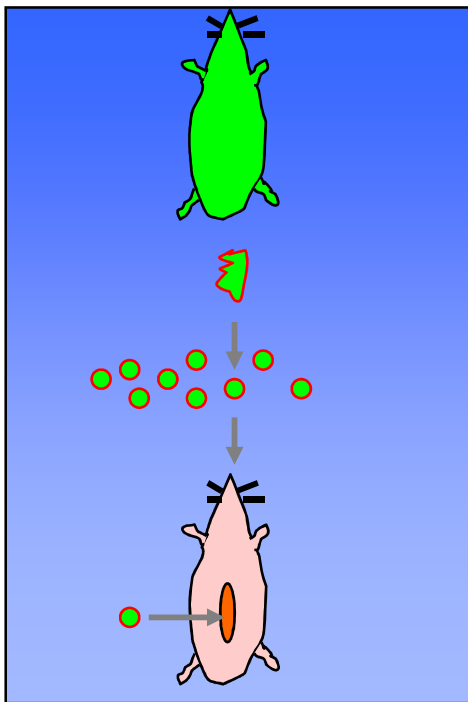
Human Reproduction Vol.20, No.8 pp. 2092-2096, 2005
Advance Access publication April 14, 2005

doi:10.1093/humrep/dei012

Development of an experimental model of endometriosis using mice that ubiquitously express green fluorescent protein

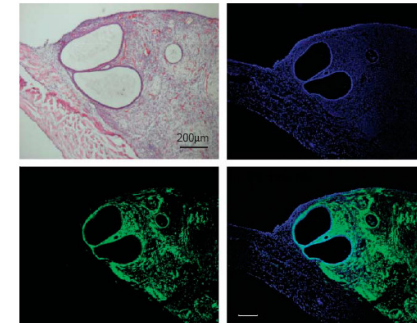
Tetsuya Hirata, Yutaka Osuga¹, Osamu Yoshino, Yasushi Hirota, Miyuki Harada, Yuri Takemura, Chieko Morimoto, Kaori Koga, Tetsu Yano, Osamu Tsutsumi and Yuji Taketani

autologous model (syngenic)

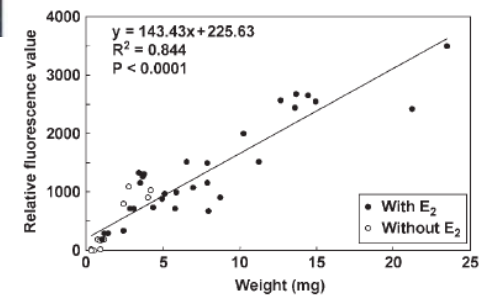


'green mice' : express green fluorescent protein (GFP)

E₂-supplemented
OVX mice
↓
minced endometrium
↓
i.p. injection



fluorescence
microscopy



In-vivo imaging of ectopic lesions

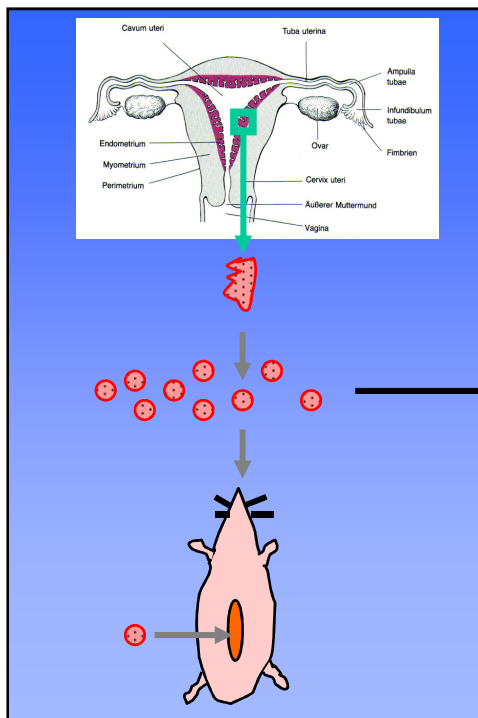
[Frontiers in Bioscience c4-9, July 1, 1999]

AN EXPERIMENTAL MODEL FOR THE ENDOMETRIOSIS IN ATHYMIC MICE

Tabibzadeh S¹, Miller S², Dodson WC² and Satyaswaroop PG²

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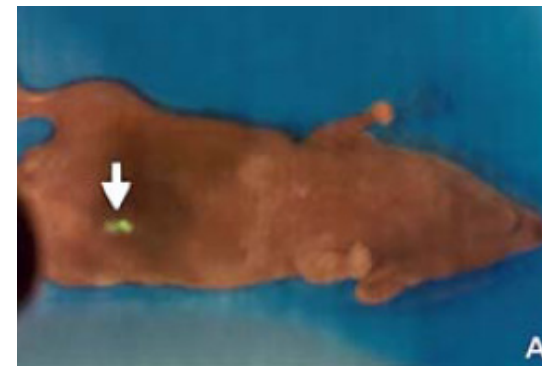
heterologous model



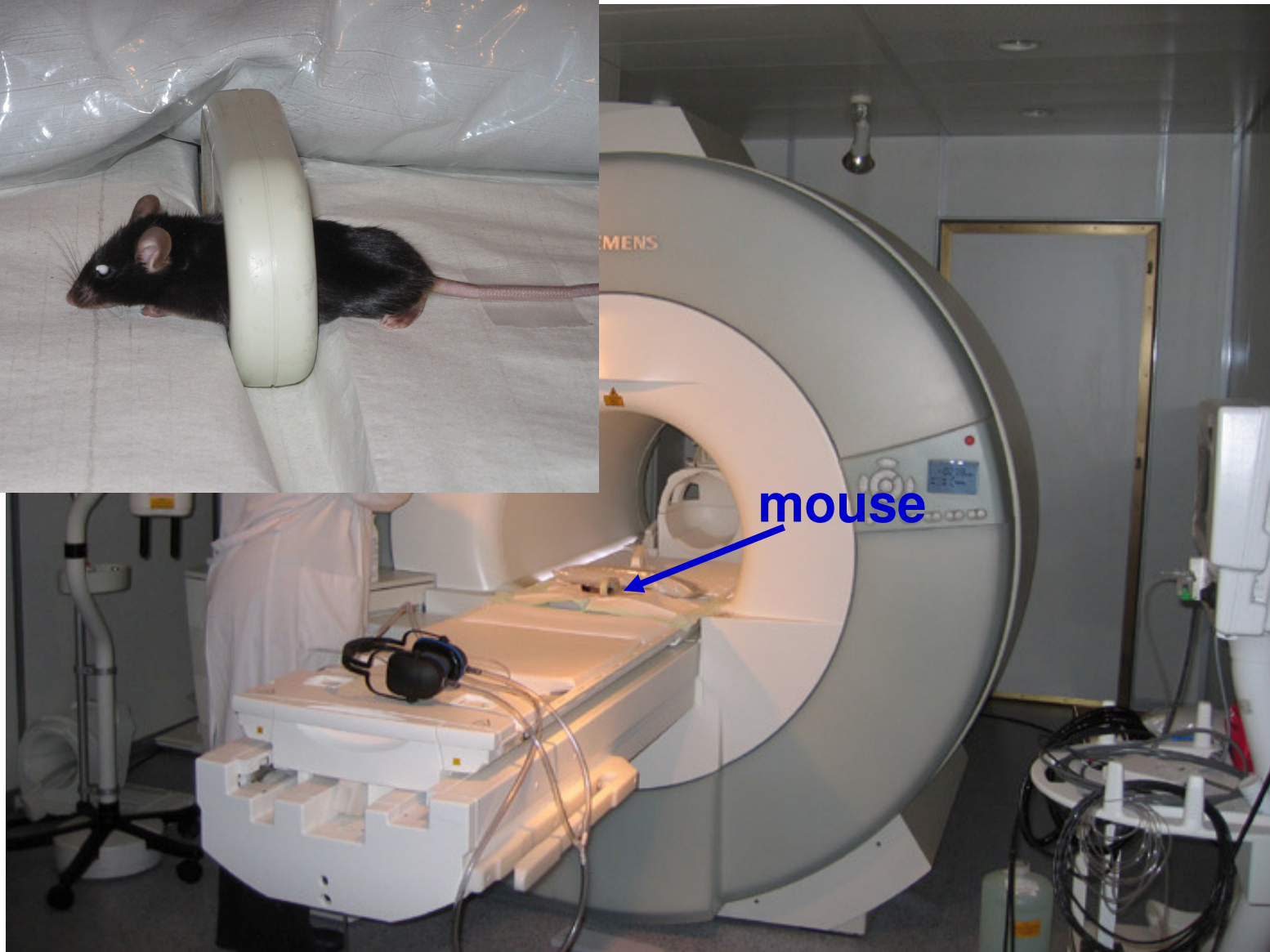
Human endometrium

• Incubation with DiO
(3,3'-dioctadecyloxycarbocyanine)

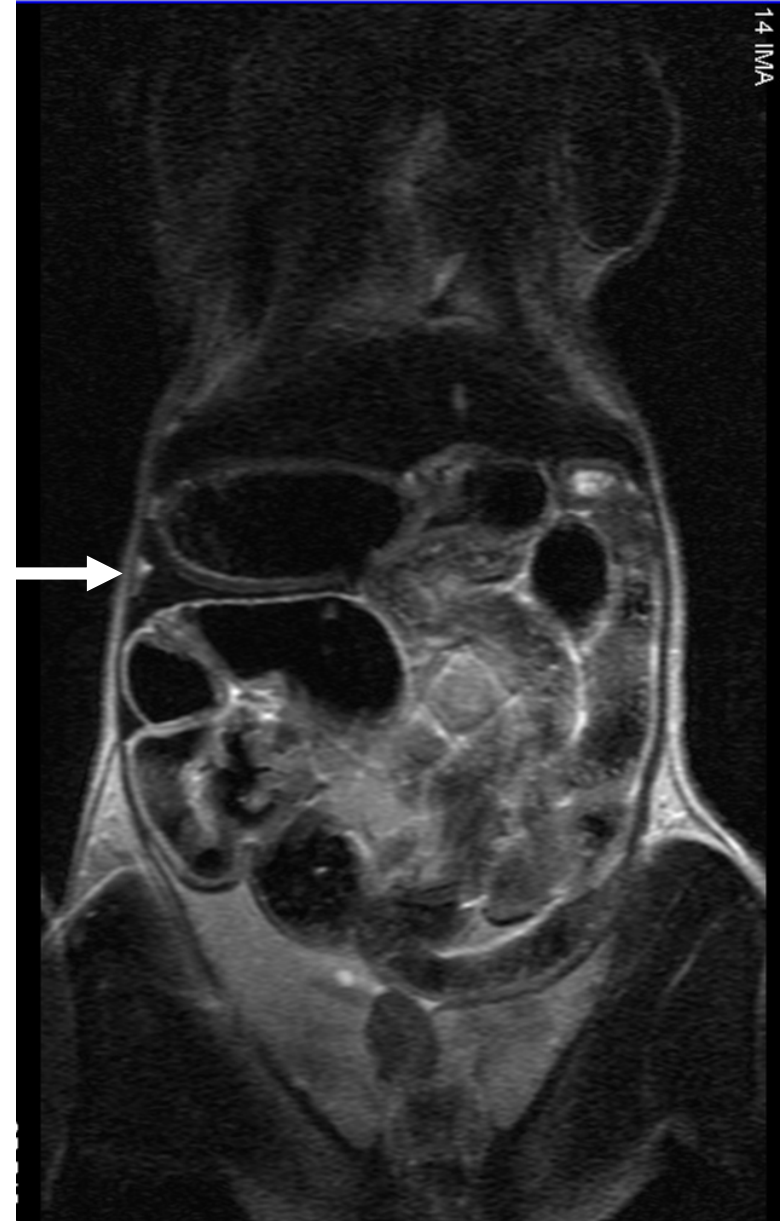
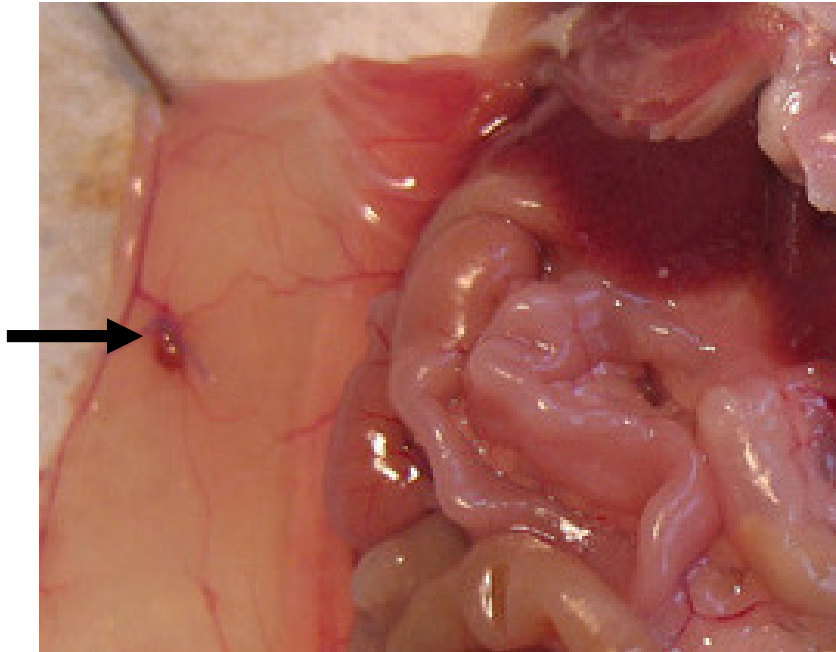
• i.p. injection into mice
• analysis after 60 days



In-vivo imaging of ectopic lesions: MRI



In-vivo imaging of ectopic lesions: MRI



Thanks!

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Michael Kumbartski**

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