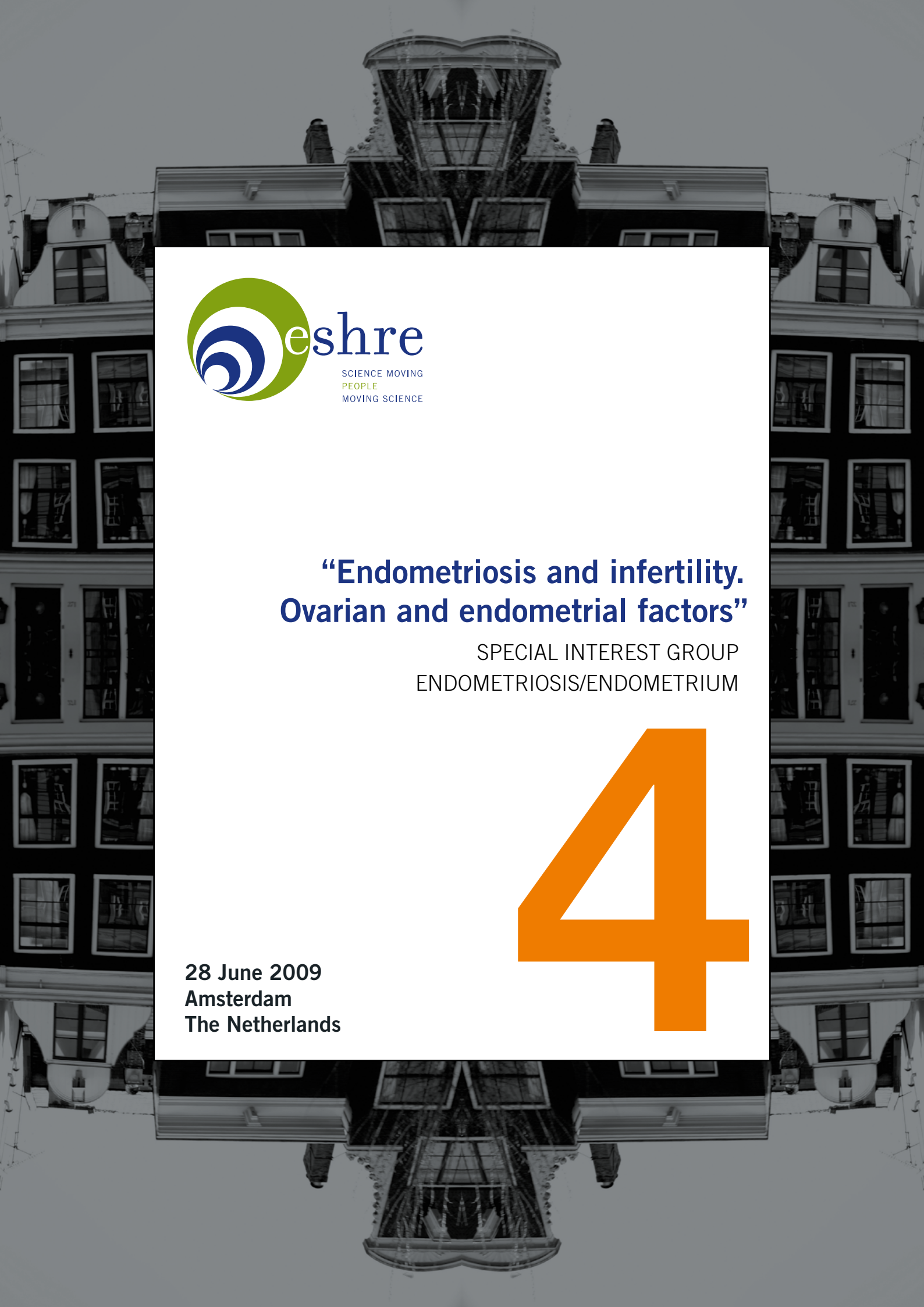

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SCIENCE MOVING
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MOVING SCIENCE

"Endometriosis and infertility. Ovarian and endometrial factors"

SPECIAL INTEREST GROUP
ENDOMETRIOSIS/ENDOMETRIUM

4

28 June 2009
Amsterdam
The Netherlands

PRE-CONGRESS COURSE 4

Organised by the Special Interest Group Endometriosis/Endometrium

Table of contents

PRE-CONGRESS COURSE 4 - PROGRAM

Endometriosis and infertility
Ovarian and endometrial factors

Organised by the Special Interest Group Endometriosis/Endometrium

Course co-ordinators: Charles Chapron (France), Thomas D 'Hooghe (Belgium), Dominique de Ziegler (Switzerland)

Course description: To provide an overview of the links between endometriosis and infertility with particular emphasis placed on the effects exerted by endometriosis on gamete function (sperm-oocyte interaction) and endometrial receptivity to embryo implantation. The former constitutes the primary impact of endometriosis on the in vivo process of fertility whereas the latter is clinically relevant with respect to the effect of endometriosis on IVF outcome. With these objectives set, the course will focus on offering a comparative and critical overview of modern surgical and medical therapeutic options in case of endometriosis and ways to optimize innovative combinations of both approaches. Ultimately, the course intends to draw state of the art algorithms that will be very useful for physicians who care for women affected by endometriosis and steer them through existing options in order to optimize clinical management. This will be conducted in an evidence-based spirit, while deeply anchoring therapeutic choices into the patho-physiology of endometriosis.

Target audience: Gynecologist with vested interest in endometriosis, reproductive endocrinology and infertility and/or reproductive surgery, trainees in these specialties and subspecialties and physician as well as scientists actively involved in the clinical management and study of endometriosis with emphasis on its impact on fertility

09:00 – 09:30	Pathogenesis of endometriosis - ***Paolo Vercellini (Italy)***
09:30 – 09:45	Discussion
09:45 – 10:15	Nonhuman primate models for translational research in endometriosis - ***Thomas D'Hooghe (Belgium)***
10:15 – 10:30	Discussion

10:30 – 11:00 Coffee break

Endometrial factors:

11:00 – 11:30 Impaired steroid hormone action in endometriosis - ***Aydin Arici (USA)***

11:30 – 11:45 Discussion

11:45 – 12:15 Uterine factor infertility in endometriosis - ***Hugh Taylor (USA)***

12:15 – 12:30 Discussion

12:30 – 13:30 Lunch

Pelvic and ovarian factors:

13:30 – 14:00 Inflammatory and immunological aspects - ***Mauricio Abrao (Brazil)***

14:00 – 14:15 Discussion

14:15 – 14:45 Oocyte quality in endometriosis - ***Alain Audebert (France)***

14:45 – 15:00 Discussion

15:00 – 15:30 Coffee break

Treatment:

15:30 – 16:00 Medical treatment and ART - ***Dominique de Ziegler (Switzerland)***

16:00 – 16:15 Discussion

16:15 – 16:45 Principles and results of surgical treatment - ***Charles Chapron (France)***

16:45 – 17:00 Discussion

17:00 –17:30 Synthesis and final conclusions

Pre-Congress Course
"Endometriosis and infertility.
Ovarian and endometrial factors".
ESHRE 25th Annual Meeting
Amsterdam, 28 June-1 July 2009

Pathogenesis of endometriosis

Paolo Vercellini & Giussy Barbara
University of Milan and
Center for Research in
Obstetrics and Gynecology
Milan, Italy

LEARNING OBJECTIVES

1. Interpret the available data on anatomic distribution of endometriotic lesions in terms of compatibility with different pathogenic theories
2. Describe the mechanism leading to anatomic distortion of several pelvic organs affected by endometriosis
3. Define the anatomic and pathologic characteristics of ovarian endometriomas and of rectovaginal and vesical endometriosis

Variation in menstrual and reproductive factors over the past century

Variable	Foremothers	Modern women
Age at menarche (y)	16	12
Age at first birth (y)	19	24-30
Pregnancies (*n*)	6	1-2
Breast feeding	Years	Months
Ovulations and menstruations	30-160	450

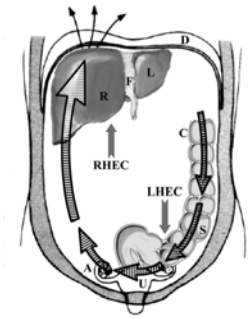
Data from: Thomas, BMJ 1993
Eaton et al., Quart Rev Biol 1994
Thomas & Ellertson, Lancet 2000

PATHOGENESIS OF ENDOMETRIOSIS

• The presence of the sigmoid colon creates a hidden microenvironment around the left adnexa, so that implantation of endometrial cells regurgitated through the left tube is facilitated.

• The large bowel does not provide the right hemipelvis with this sort of anatomical shelter as the cecum is more cranial

THE CLOCKWISE PERITONEAL FLUID CURRENT



A= adnexa
C= descending colon
D= diaphragm
F= falciform ligament
L= left hepatic lobe
R= right hepatic lobe
S= sigmoid
RHEC= right hypocondrium endometriotic complex
LHEC= left hemipelvis endometriotic complex

From Vercellini et al., Hum Reprod, 2007

PATHOGENIC PATHWAY LEADING TO ANATOMIC DISTORTION

1. Superficial implantation of endometrial cells
2. Strong inflammatory stimules
3. "Protective" response with adhesion of pelvic structures to exclude the irritating lesion from the peritoneal environment
4. Fibroblast partecipation in the "burial" of endometriotic foci
5. Scar retraction
6. Duplication and invagination of adjacent surfaces

PATHOGENESIS OF ENDOMETRIOMAS

• The endometrioma is formed by invagination of the cortex and active implants are located at the site of invagination

• Endometriomas have the ovarian cortex as their wall. This explains the frequent combination of endometrial cysts with cystic corpora lutea and lutein cysts

Brosens et al., Fertil Steril 1994

PATHOGENESIS OF ENDOMETRIOMAS

• The majority of endometriomas are not intraovarian but extraovarian

• The wall of endometriomas is lined by ovarian cortex

• Inversion of ovarian cortex produces an extraovarian pseudocyst

• The stigma of inversion is usually found on the anterior or lateral side of the ovary

Brosens et al., Fertil Steril 1996

Lateral distribution of ovarian endometriomas
Literature data.

	Left ovary		Right ovary		Bilateral lesions	
Author, year	*n*	(%)	*n*	(%)	*n*	(%)
Vercellini *et al.*, 1998	255	(45.5)	148	(26.4)	158	(28.1)
Ghezzi *et al*., 2001	58	(47.9)	41	(33.9)	22	(18.5)
Prefumo *et al*., 2002	178	(52.5)	98	(28.9)	63	(18.6)
Vercellini *et al.*, 2002	64	(45.6)	404	(28.7)	362	(25.7)
Al-Fozan and Tulandi, 2003	90	(48.6)	59	(31.9)	36	(19.5)

ESTIMATED LIFETIME NUMBER OF OVULATIONS AND INCIDENCE OF ENDOMETRIOSIS
The Nurses' Health Study II

No. of ovulations (quartiles)	Cases	Never used OCs RR	95% CI	Ever used OCs RR	95% CI
<174	465	1.0	Referent	1.0	Referent
175-234	472	2.0	0.9-4.9	1.2	1.0-1.4
235-291	387	2.6	1.0-7.1	1.2	1.0-1.5
>291	312	6.0	2.0-17.5	1.4	1.1-1.8
		P<.001		*P*<.001	

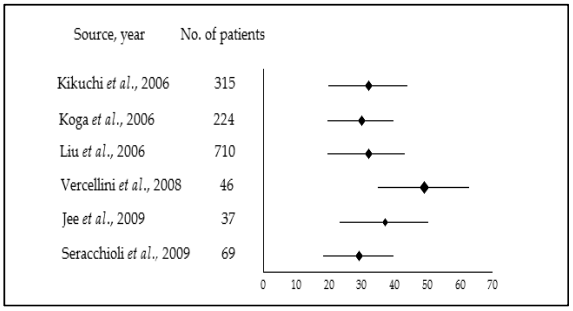
From Missmer et al., Obstet Gynecol 2004

REPRODUCTIVE HISTORY AND ENDOMETRIOSIS
The Nurses'Health Study II

"Our observation that the ovulatory cycle-associated risk of endometriosis was greatest among never users of OCs may suggest that prescription of OCs before disease onset is a valid public health intervention"

From Missmer et al., Obstet Gynecol 2004

Reported incidence of postoperative endometrioma recurrence. Literature data, 2006-2009



“BLOOD ON THE TRACKS” STUDY

US DIAGNOSIS OF HEMORRHAGIC CORPUS LUTEUM CYST

Ovarian cyst with a diameter > 3 cm, thin, well defined, regular walls, posterior enhanced through-transmission, with:

a) fishnet wave or reticular appearance due to fine interdigitating septations without flow

b) anechoic content with triangular or curvilinear echogenic areas and occasional fluid-debris levels

Jain, J Ultrasound Med 2002

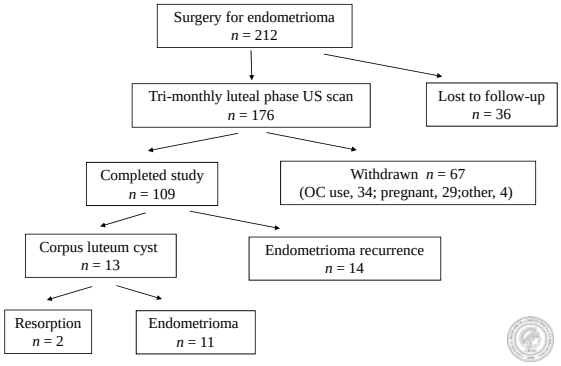
“BLOOD ON THE TRACKS” STUDY

US DIAGNOSIS OF OVARIAN ENDOMETRIOMA

Round-shaped cystic mass with a minimum diameter of 2 cm, with thick walls, regular margins, homogeneous low echogenic fluid content with scattered internal echoes, and without papillary proliferations

Vercellini et al., Am J Obstet Gynecol 2008

“BLOOD ON THE TRACKS” STUDY



"BLOOD ON THE TRACKS" STUDY

CLINICAL IMPLICATIONS

1. Endometrioma peculiar to ovary
2. Sudden apperance instead of slow, progressive growth
3. No endometrial layer within the "cystic wall"
4. Blood content does not originate from ectopic micromenstruation
5. Possibility of tertiary prevention

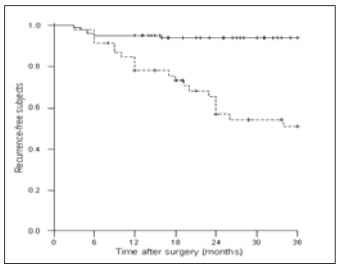
MEDICAL TREATMENT OF ENDOMETRIOSIS

If ovulation is causally related to endometriotic cyst development, ovarian suppression after conservative surgery for endometrioma would greatly reduce the risk of lesion recurrence

POSTOPERATIVE OC EXPOSURE AND RISK OF ENDOMETRIOMA RECURRENCE

After conservative surgery for endometriomas, patients not seeking pregnancy were offered long-term oral contraception with a cyclic, low-dose, monophasic OC containing EE 0.02 mg and desogestrel 0.15 mg

Vercellini et al., Am J Obstet Gynecol 2008



36-month endometrioma recurrence-free survival analysis after conservative laparoscopic surgery according to the treatment modality adopted:
(——) oral contraception for the entire follow-up period (n = 102);
(- - -) expectant management (n = 46) (log-rank test, $\chi^2_1 = 36.2$; $P < .001$)

Vercellini et al., Am J Obstet Gynecol 2008

POSTOPERATIVE OC EXPOSURE AND RISK OF ENDOMETRIOMA RECURRENCE

- Adjusted OR: 0.04 (95% CI, 0.02-0.13)
- Only OC use was associated with a significant reduction in recurrence risk

Vercellini et al., Am J Obstet Gynecol 2008

POSTOPERATIVE OC EXPOSURE AND RISK OF ENDOMETRIOMA RECURRENCE

Relative Risk Reduction:	80%
Absolute Risk Reduction:	47% (95% CI , 37-57)
NNT:	2 (95% CI , 0.2-7)

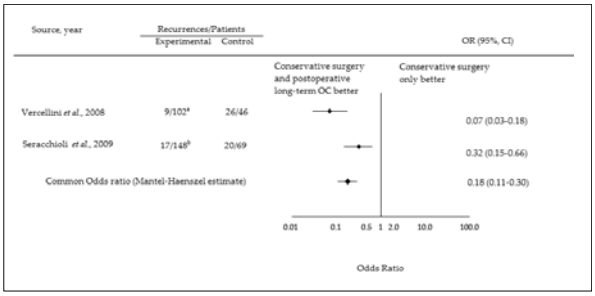
Vercellini et al., Am J Obstet Gynecol 2008

POSTOPERATIVE OC EXPOSURE AND RISK OF ENDOMETRIOMA RECURRENCE

Current OC use is associated with a dramatic reduction in the risk of ovarian endometriotic cyst recurrence

If RCTs confirm the observation of this cohort study, postoperative long-term ovarian suppression with OCs could be routinely offered, especially to women seeking conception in the future

Vercellini et al., Am J Obstet Gynecol 2008



Overview of trials that compared conservative surgery for ovarian endometriomas with or without postoperative long-term OC use. *Diamonds* represent odds ratio of cyst recurrence, and *horizontal lines* are 95% C.I.

[a] Only "always users" are considered.
[b] "Cyclic users" and "continuous users" are combined.

Anatomic location of unilateral peritoneal endometriosis in the left and in the right hemipelvis

	Left		Right	
Site	*n*	(%)	*n*	(%)
Peritoneum	52	(70.3)	22	(29.7)
Posterior cul-de-sac	34	(63.0)	20	(37.0)
Adhesions	NR	(16.6)	NR	(6.9)

NR = not reported

Data from Al-Fozan and Tulandi, Obstet Gynecol 2003

Lateral distribution of deep endometriosis infiltrating the uterosacral ligaments

	n	(%)
Left US ligament	69	(53.1)*
Right US ligament	38	(29.2)
Bilateral lesions	23	(17.7)
Total	130	(100.0)

*Unilateral lesions = 64.5%

Data from Chapron et al., BJOG 2001

Deeply infiltrating endometriosis: anatomical distribution of pelvic lesions

Unilateral and bilateral pelvic DIE lesions (*n* = 425 patients; *n* = 730 DIE lesions)

	n	Left	Median	Right
USL	400	227	-	173
Vagina	123	-	123	-
Bladder	48	-	48	-
Intestine	143	30	113	0
Ureter	16	11	-	5
Total	730	268*(36.7%)	284*(39.0%)	178*(24.3%)
		268*(60.0%)		178*(40.0%)

* *P* < .001

From Chapron et al., Hum Reprod 2006

Lateral distribution of obstructive ureteral endometriosis

*Literature data, 1980-2000**

	n	(%)
Left ureter	72	(55.0†)
Right ureter	40	(30.5)
Bilateral lesions	19	(14.5)
Total	131	(100.0)

*After exclusion of 54 women who had had previous pelvic surgery
†Unilateral lesions only = 64% (95% CI, 55% to 73%)

From Vercellini et al., BJOG 2000

Distribution of obstructive lower intestinal tract endometriosis

Literature data, 1980-2003

	n	(%)
Left lesion*	245	(72.7)
Right lesion†	84	(24.9)
Bilateral lesions	8	(2.4)
Total	337	(100.0)

*Descending and sigmoid colon
†Terminal ileum cecum and ascending colon

From Vercellini et al., Obstet Gynecol 2004

INGUINAL ENDOMETRIOSIS

Literature data, 1925-1987

No. of articles	26	
No. of patients	31	
No. with unilateral lesions	30	(100%)
Right side	27	(90%)*

*95% CI, 73%- 98%

From Clausen & Nielsen, Int J Gynecol Obstet 1987

DIAPHRAGMATIC ENDOMETRIOSIS

Literature data, 1954-2007

No. of articles	17	
No. of patients	52	
No. with unilateral lesions	40	(77%)
Right side	36	(90%)*

*95% CI, 76% to 97%

PLEURAL AND PULMONARY ENDOMETRIOSIS

Literature data, 1951-1981

No. of articles:	41	
No. of patients:		
pleural lesions	54	
pulmonary lesions	11	
Right side:		
pleural lesions	50	(93%)*
pulmonary lesions	7	(64%)†

*95% CI, 82% to 98%
†95% CI, 31% to 89%

From Foster et al., Obstet Gynecol 1981

PATHOGENESIS OF RECTOVAGINAL ENDOMETRIOSIS

1. Inflammation in the most dependent portion of the pouch of Douglas
2. Adhesion between anterior rectal wall and posterior fornix
3. Fibrosis and infiltration of the muscolar layers of the rectum and vagina
4. Formation of a sort of desmoid tumor which is a fibrotic “cast” of what was the bottom of the posterouterine pouch

Clinical characteristics and anatomic measurements of the 209 women studied.

	Endometriosis with deep lesion (*n*=16)	Endometriosis without deep lesion (*n*=127)	Miscellaneous anomalies (*n*=35)	Normal pelvis (*n*=26)
Age (y)	27.5 ± 2.9	31.2 ± 3.6	31.7 ± 4.0	32.4 ± 2.5
Nulliparous	15 (83)	99 (78)	27 (77)	28 (80)
Douglas pouch depth (cm)	3.6 ± 1.6*	5.3 ± 0.8	5.2 ± 0.9	5.5 ± 0.8
Douglas pouch volume (mL)	41.6 ± 19.3*	67.2 ± 18.1	67.6 ± 12.6	65.8 ±10.9

Data are presented as mean ± SD or *n* (%) **p* <0.001, one way-ANOVA

From Vercellini et al., Fertil Steril 2000

MRI and deeply infiltrating endometriosis (DIE)

- 8 women with histologically confirmed DIE
- DIE nodules located below the torus uterinum, level with the posterior vaginal fornix and the upper third of the posterior vaginal wall
- The DIE nodules were always located above the upper edge of the rectovaginal septum, with the latter appearing fine and regular
- DIE lesions do not originate from the rectovaginal septum

From Chapron et al., Gynecol Obstet Invest 2002

PATHOGENESIS OF RECTOVAGINAL ENDOMETRIOSIS

•What is called “rectovaginal septum” endometriosis may instead be massive disease of the deepest portion of the pouch of Douglas that has been buried and excluded from the remaining pelvis by adhesions

•The semilunar hard crest protruding through the posterior fornix could be the fibrotic “cast” of what was the bottom of the posterior cul-de-sac

Clinica Ostetrica e Ginecologica “Luigi Mangiagalli”, University of Milano, Italy

PATHOGENESIS OF RECTOVAGINAL ENDOMETRIOSIS

Endometriotic plaques and nodules are found in the posterior vaginal fornix, cranially with respect to the rectovaginal septum

Various forms of peritoneal and ovarian disease are usually present in patients with vaginal endometriosis, suggesting that the pathogenesis may not be different

Frequency of other forms of endometriosis in 93 patients with deep peritoneal endometriotic nodules

Forms of disease	*n*	%	95% CI
Superficial peritoneal implants	57	61.3	51.4-71.2
Endometriotic ovarian cysts	47	50.5	40.3-60.7
Pelvic adhesions	69	74.2	65.3-83.1
Overall	87	93.5	87.7-97.2

From Somigliana et al., Hum Reprod 2004

BLADDER DETRUSOR ENDOMETRIOSIS: ETIOLOGIC HYPOTHESES

1. Transtubal menstrual reflux of endometrial cells with implantation on the peritoneum covering the bladder dome
2. Metaplasia of subperitoneal mullerian remnants located in the vesicovaginal septum
3. Extension of adenomyosis from the anterior uterine wall to the bladder

The pathogenesis of bladder detrusor endometriosis

- 40 women evaluated between 1995 and 2000
- Histologically confirmed, full-thickness detrusor endometriosis
- With one exception, anterouterine pouch partially or totally obliterated
- Nodule in the posterior wall or dome of the bladder, well above the uterine isthmus, adherent to the anterior wall or fundus
- With one exception, pelvic US, cystoscopy, IV pyelography, MRI, and CT identified the lesion cranially with respect to the vescicovaginal septum and excluded uterine adenomyosis

From Vercellini et al., Am J Obstet Gynecol 2002

Frequency of extravesical endometriosis in 58 patients with bladder endometriotic nodules

Forms of disease	*n*	%	95% CI
Superficial peritoneal implants	34	58.6	45.2-71.2
Endometriotic ovarian cysts	26	44.8	32.2-58.2
Pelvic adhesions	47	81	68.4-89.6
Deep peritoneal implants	16	27.6	16.7-40.8
Overall	51	87.9	76.7-94.3

From Somigliana et al., Fertil Steril 2007

PATHOGENESIS OF VESICAL ENDOMETRIOSIS

1. Intraperitoneal seeding of regurgitated endometrium
2. Endometrial cells collect in the anterior cul-de-sac due to gravity
3. Implantation is favored by juxtaposition of prevesical peritoneum and anterior uterine wall
4. Inflammation and adhesion between adjacent surfaces
5. Reactive proliferation of fibroblasts and nodule formation with infiltration of detrusor muscle

SUMMARY

• Anatomic, surgical and pathologic findings suggest that “deep” endometriosis originates intraperitoneally

• Peritoneal, ovarian and “deep” lesions may be diverse manifestations of one disease with one origin (i.e., regurgitated endometrium)

PATHOGENESIS OF ENDOMETRIOSIS

CONCLUSIONS I

1. Findings regarding anatomic, menstrual, and reproductive factors consistently support the role of pelvic endometrial contamination as the major determinant of disease development.
2. Available data on OCs use suggest that ovulation, is the major determinant of disease progression.

PATHOGENESIS OF ENDOMETRIOSIS

CONCLUSIONS II

3. Future studies should verify if the actual manifestations of endometriosis are partly due to dramatic modifications in modern women's reproductive habits.

CENTER for RESEARCH in OBSTETRICS & GYNECOLOGY · 2006 · C.R.O.G.

Nonhuman primate models for translational research in endometriosis

Thomas M.D'Hooghe, MD, PhD
-Coordinator Leuven Univ Fertil Ctr (B),
-Chair, Int'l Advisory Board, Institute of Primate Research (WHO Collab Ctr), Nairobi, Kenya

INSTITUTE OF PRIMATE RESEARCH
NATIONAL MUSEUMS OF KENYA
WHO COLLABORATING CENTRE

Learning Objectives: NHPmodels for translational research in endometriosis

1. **Introduction**
2. **Endometriosis cost**
3. **NHPrimate >< rodent models**
4. **Development baboon model endo**
5. **Unicity/validation baboon model endo: 20 relevant points**
6. **Endo research baboon model: 5 relevant observations**

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European Board and College of Obstetrics and Gynaecology (EBCOG)

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CERTIFICATE OF ACCREDITATION

Leuven University Fertility Center / Department of Obstetrics and Gynaecology
"UZ Gasthuisberg", Leuven, Belgium

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President EBCOG & UEMS Section

Prof. J.W. Wladimiroff
Chair of the Subspeciality Recognition Committee

Prof. J. Geraedts
Chairman of ESHRE

B.C. Tarlatzis
Chairman of the ESHRE Subspecialist Recognition Committee

CERTIFICATE OF APPROVAL

This is to certify that the Quality Management System of

Leuvens Universitair Fertiliteits Centrum (LUFC)
Leuven, Belgium

has been approved by Lloyd's Register Quality Assurance to the following Quality Management System Standards:

ISO 9001 : 2000

The Quality Management System is applicable to:

Multidisciplinary and evidence based management of couples with fertility problems.

Approval Certificate No: 459041

Original Approval: 30 November 2004
Current Certificate: 30 November 2004
Certificate Expiry: 30 November 2007

Issued by LRQA (Rotterdam)

This document is subject to the provision on the reverse

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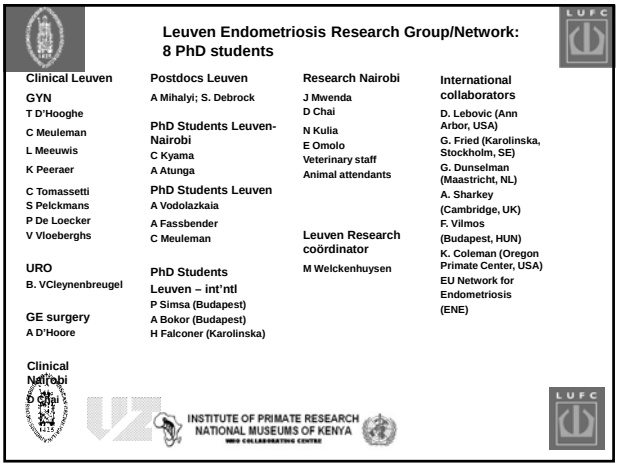

Leuven Endometriosis Research Group/Network: 8 PhD students

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F. Vilmos (Budapest, HUN)
K. Coleman (Oregon Primate Center, USA)
EU Network for Endometriosis (ENE)

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Disclosure

- Full Professor and Merck Serono Chair (2005-09) Reproductive Medicine (Leuven University)
- Clinical Head Leuven University Fertility Center
- Chair ESHRE Special Interest Group for Endometriosis
- PI ENDOCOST study

Disclosure

- Board member, WERF
- Editor-in Chief Gynecologic and Obstetric Investigation
- Research Associate and Chair International Advisory Board, Institute of Primate Research, Kenya
- Fundamental Clinical Investigator for endometriosis, Belgian Research Foundation FWO

http://www.esnre.com

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Endometriosis

- **EM (glands/stroma) outside the uterus + chronic inflammation**
- **Retrograde menstruation (Sampson 1927)**
- **Variable phenotype, localization and extent**
- **Subfertility, pelvic pain, reduced QOL**
- **Prevalence**
  7-15% of reproductive age women
  up to 50% patients with pelvic pain/infertility
- **Estrogen dependent**
  rare before menarche or after menopause
- **Progressive:**
  >50% women/baboons after 1-2 years

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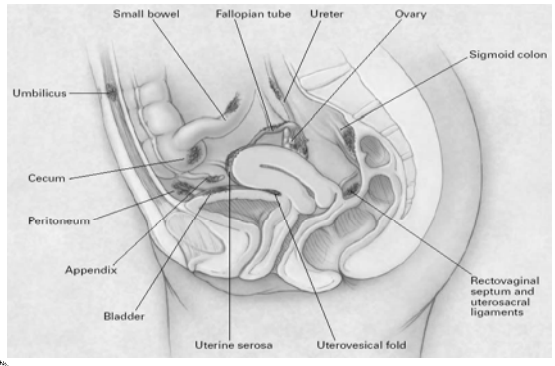

Endometriosis treatments

- Pain killers
- Oral contraceptives
- Progestins
- GnRH-agonists
- Surgery
- Assisted reproductive therapies
- Hysterectomy
- Yet little investment in causal research

- **Often more than one**
- **Hit and miss**
- **All have side effects**
- **No cure**

Learning Objectives: NHPmodels for translational research in endometriosis

1. **Introduction**
2. **<u>Endometriosis cost</u>**
3. **NHPrimate >< rodent models**
4. **Development baboon model endo**
5. **Unicity/validation of baboon model: 20 relevant points**
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The cost of endometriosis

DRUGS	DIAGNOSTICS	SURGERY	HEALTH CARE	OTHER
NSAIDs	Ultrasound scan	Laparoscopy	GP	ART
Progestagens	Internal scan	Laparotomy	Gynaecologist	A&E visits
c-OCP	MRI	Hysteroscopy	Nurse	Hospitalisation
Danazol	Blood tests	Hysterectomy	Urologist	Alternatives
Gestrinone	Swabs	Endometrial ablation	Gastro-enterologist	Transportation
GnRH-a	Barium enema		Anaesthetist	Child care
Add-back HRT	Sigmoidoscopy	Theatre costs	Radiologist	Work absence
Mirena coil	Endoscopy		Theatre staff	↓ productivity
Antibiotics	Bone scans		Haematologist	↓ education
Anti-depressants	X-rays		Counsellor	↓ activities
			Physiotherapist	
			Psychiatrist	

COMPARATIVE COST: ENDOMETRIOSIS versus OTHER CHRONIC DISEASES

- Review of endo-related cost estimates in USA (Simoens et al, 2007)

  1. annual (2002) healthcare costs + costs of productivity loss:
  = about $ 4000 per patient per year

  2. USA cost per year for endo (2002)
  $22 billion per year
  (at 10% prevalence of endo among women of reproductive age)

  3. **Endo cost considerably higher than cost related to Crohn's disease or to migraine in the USA for 2002**

COMPARATIVE COST:
ENDOMETRIOSIS versus OTHER CHRONIC DISEASES

- **Retrospective review of administrative data for commercial payers of a US insurance company (Mirkin et al, 2007):**

  Extrapolated cost per patient per month (PPPM):

  $ 791: endo
  $ 500: hypertension
  $ 916: diabetes
  $ 1.121: rheumatoid arthritis

  explained by high hospital admission rate/ surgical procedures.


COMPARATIVE COST:
ENDOMETRIOSIS versus OTHER CHRONIC DISEASES

- **Retrospective review of administrative data for commercial payers of a US insurance company (Mirkin et al, 2007):**

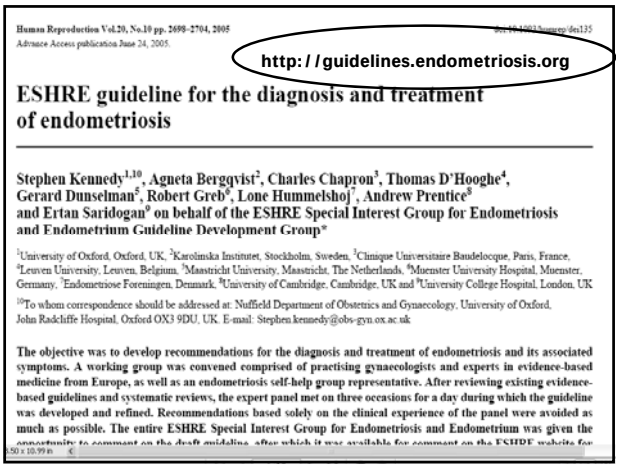
  Women with endometriosis: total direct medical costs: 63% higher than average women

  Explained by added cost due to comorbid conditions:
  interstitial cystitis,
  depression,
  migraine,
  irritable bowel syndrome,
  chronic fatigue syndrome,
  abdominal pain and infertility,...


CALCULATION OF
ENDOMETRIOSIS COST IN EU
IS NEEDED FOR

INCREASED AWARENESS OF
ENDOMETRIOSIS IN

POLITICS DETERMINING
HEALTH POLICY
+ RESEARCH FUNDING

Human Reproduction Vol.20, No.10 pp. 2698–2704, 2005
Advance Access publication June 24, 2005

http://guidelines.endometriosis.org

ESHRE guideline for the diagnosis and treatment of endometriosis

Stephen Kennedy[1,10], Agneta Bergqvist[2], Charles Chapron[3], Thomas D'Hooghe[4], Gerard Dunselman[5], Robert Greb[6], Lone Hummelshoj[7], Andrew Prentice[8] and Ertan Saridogan[9] on behalf of the ESHRE Special Interest Group for Endometriosis and Endometrium Guideline Development Group*

[1]University of Oxford, Oxford, UK, [2]Karolinska Institutet, Stockholm, Sweden, [3]Clinique Universitaire Baudelocque, Paris, France, [4]Leuven University, Leuven, Belgium, [5]Maastricht University, Maastricht, The Netherlands, [6]Muenster University Hospital, Muenster, Germany, [7]Endometriose Foreningen, Denmark, [8]University of Cambridge, Cambridge, UK and [9]University College Hospital, London, UK

[10]To whom correspondence should be addressed at: Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK. E-mail: Stephen.kennedy@obs-gyn.ox.ac.uk

The objective was to develop recommendations for the diagnosis and treatment of endometriosis and its associated symptoms. A working group was convened comprised of practising gynaecologists and experts in evidence-based medicine from Europe, as well as an endometriosis self-help group representative. After reviewing existing evidence-based guidelines and systematic reviews, the expert panel met on three occasions for a day during which the guideline was developed and refined. Recommendations based solely on the clinical experience of the panel were avoided as much as possible. The entire ESHRE Special Interest Group for Endometriosis and Endometrium was given the

Role of ESHRE Special Interest Group for Endometriosis (SIGEE)

- Education and training
- ESHRE Guidelines for endometriois: Annual update via Working Group
- ESHRE endometriosis cost working group: 2007-10

ESHRE Endometriosis Cost Working Group

- Initiative for ENDOCOST study
- 8 countries, 10 centers:
  Germany, Hungary, UK, Italy, Denmark, France, Netherlands, Belgium, Switzerland, USA (2)
- Retrospective/Prospective study (2009)
- Team per center: 1 gynecologist + 1 health economist
- Travel/lodging supported by ESHRE
- Collaboration with ASRM SIG Endometriosis
- Sponsored by World Endometriosis Research Foundation

European Network on Endometriosis

First ever EU research grant for endometriosis

1. Pan European epidemiological study
2. Internet based endometriosis gateway
3. Consolidate and formalise the European Alliance

- 8 Associate partners and 4 Collaborating partners
  - Endometriosis UK lead partner
  - Belgium, Denmark, Italy, UK
- Application scored very highly – 87/100 and received funding 300.000 Euro (2007-9)

Learning Objectives: NHPmodels for translational research in endometriosis

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LACK OF PROGRESS IN ENDOMETRIOSIS RESEARCH

1.Unknown duration of endo at diagnosis

2.Inadequate study design: nl controls needed

- **pelvic condition (endo, nl pelvis, other)**
- **symptoms (none, infertility, pain, other)**

3.Endometriosis>surgical gynecological disease. Need for multidisciplinary clinical and research teams.

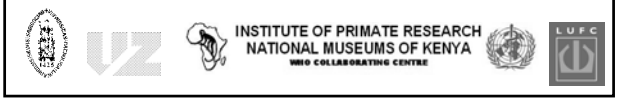
4. Need for good animal models.

NEED FOR NHP MODELS FOR THE STUDY OF ENDOMETRIOSIS

Rodents:

- **Advantages:**

1.Low cost
2.Easy handling
3.Genetic manipulation possible (cost!):
KO mice, K-ras transgenic mice (Dinulescu et al, 2006)



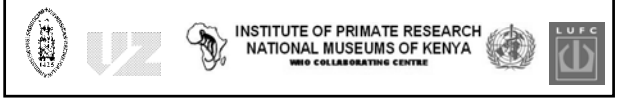
	Rodents	NHP's	Humans
Genetic ally close to humans	-	+	+
Repro anatomy close to humans	-	+	+
Estrus behavior	+	-	-
Repro cycle	5 days	28-33 days	28-30 days
Embryonic aneuploidy	-	?	+
Optional diapause	+	-	-
Multiple implantations	+	-	-
Embryonic control of endometrium	+	-	-
Invasive implantation	-	+	+
Menstruation	-	+	+
Spont Endo	-	+	+
Spt+Ind Endo similar to humans	-	+	+
Spont PF	-	+	+

NEED FOR NHP MODELS FOR THE STUDY OF ENDOMETRIOSIS

Rodents:

- **Disadvantages:**

1. wide phylogenetic gap with humans
2. different reproductive endocrinology and anatomy,
3. no menstruation
4. no peritoneal fluid
5. no spontaneous endometriosis,



NEED FOR NHP MODELS FOR THE STUDY OF ENDOMETRIOSIS

Rodents:

6. Induced endo: unphysiological induction by uterine square autotransplantation (→ adhesion formation)

7. Induced endo: unphysiological "endometriotic lesions" with limited phenotypes

8. ?human EM-murine peritoneal interaction in nude/SCID: extrapolation possible to human endometriosis?

9. ? Preclinical model for studies testing new drugs: extrapolation not always possible to human endometriosis (Interferon alpha 2b: + in mice, - in women)

NEED FOR NHP MODELS FOR THE STUDY OF ENDOMETRIOSIS

NHPs:

- **Disadvantage:**

1. High cost (affordable outside EU and US)
2. Handling requires special expertise/infrastructure
3. Ethically sensitive research

NEED FOR NHP MODELS FOR THE STUDY OF ENDOMETRIOSIS

NHPs:

- **Advantages when compared to humans:**

1. Very narrow phylogenetic gap
2. Comparable reproductive endocrinology/anatomy,
3. Menstruation (baboon, rhesus, not all other NHPs)
4. Spontaneous endometriosis,
5. Induced endometriosis by autologous seeding or injection of eutopic EM in pelvis (baboons, rhesus, cynomolgus)
6. Both spontaneous and induced endometriosis: similar phenotype as human endometriosis

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Baboon model endo

Institute of Primate Research
Nairobi, Kenya

WHO Collaborating Center

Research areas:
Reproduction
Infectious Diseases
Ecology and Conservation

20 yrs research collaboration Leuven-Nairobi

- **1990-1993 Baboon model for Endometriosis, Institute Primate Research, Nairobi, Kenya**
- **1993-1995 Fellowship Reproductive Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, (JA Hill/ DJ Anderson) Endometriosis in baboons and women**
- **PhD Leuven 1994 (Promotors: PR Koninckx, CS Bambra) Baboon as model for endometriosis**
- **1996-present: coordinator Center Reproductive Medicine, Leuven University Hospital, Belgium (ISO 9001-2000 certified 11/04)**

20 yrs research collaboration Leuven-Nairobi

1998-2008: 50% fundamental clinical investigator (Flemish fund scientific research)

Clinical Leuven: biobank frozen tissue and DNA + clinical database since 1998

Preclinical IPR Nairobi:

Baboon model: pathogenesis and testing of new drugs (prevention/treatment of endometriosis)

IPR International Advisory Board

- **Established 2007**
- **Initiative by NMK/IPR + supported by WHO (P. Van Look)**
- **Aim:**
- **advise Kenyan leaders about long term development of IPR into African Center of Excellence**
- **increase international research collaboration**

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IPR International Advisory Board

- 12 experts in areas of reproduction, infectious diseases ecology and conservation
- Chair T. D'Hooghe
- Annual meetings, (August + December 07, February 09)

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Slide 36

x1 x292720; 9/11/2007

Learning Objectives: NHPmodels for translational research in endometriosis

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UNICITY OF BABOON MODEL

1. Cost affordable outside EU or USA
2. Ethical issues

***Institute of Primate Research*, Nairobi, Kenya**

Cost per baboon
Purchase: $ 450
Per diem: $ 3
Surgery: $ 60/hour

Proof of concept RCT
15-20 baboons, 6/12:
$ 100.000 USD

2. Ethics of endometriosis research in baboons at IPR

2.1. Baboons are not an endangered species but represent a threat to agriculture in Africa

2.2 Baboons live in their natural habitat at IPR

2.3. Lack of other clinically relevant preclinical animal models to study cause-effect relationships:

Only NHPs do have spontaneous/induced endo similar to the disease in women

2.4. Ethical need to show safety + efficiency of new drugs before application in women

Ethics of endometriosis research in baboons at IPR

2.5. For each project: double approval by ethical committees from both IPR and from Leuven University

2.6. Global level: capacity building of Primate Research Center in poor resource country could/should be seen as relevant effort in the context of North-South collaboration

UNICITY OF BABOON MODEL

3. Noninvasive monitoring of menstrual cycle:

- Perineal inflation= Foll. Phase
- Perineal deflation=Luteal phase
- Ovulation = perineal deflation minus 2 days

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UNICITY OF BABOON MODEL

4. Continuous breeding in captivity
(>< rhesus)

5. Size (12-15kg) and Strength
(>rhesus>cynomolgus)
-repetitive blood sampling
(hourly during 24 hr in chair; daily)
-repetitive surgery
(every 2-3 days; D'Hooghe et al, 1996)

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6. Spontaneous peritoneal fluid (PF)
about 2 mL after ovulation (>< rhesus)
(D'Hooghe et al, 1991)

7. Vaginal transcervical uterine access.
-endometrial biopsy (D'Hooghe et al, 1991)
-embryo transfer
-preimplantation embryo flushing
-hysteroscopy
(D'Hooghe et al, 1991; 1995; 1996; 2004; Nyachieo et al. 2007, Chai et al., 2007).

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BABOON MODEL for non-endometriosis
REPRODUCTIVE RESEARCH

- **HCG exposure –EM implantation model (oviductal minipump HCG)- Fazleabas**
- **Embryo- EM implantation model (hysteroscopic interventions) –Leuven/IPR**
- **Reproducible IVF system in baboons (Embryonic stem cell development)- Leuven/IPR**
- **Prevention heterosexual transmission SHIV (vaginal immunology) –Leuven/IPR/BU**

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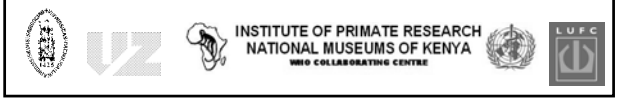
8. **Spontaneous endo similar to human endo:**

   laparoscopic appearance,
   pelvic localization,
   microscopic aspects

   [D'Hooghe, 1997]



UNICITY OF BABOON MODEL

9. **AFS/ASRM endo classification system adapted for baboon** (D'Hooghe et al, 1995)

10. **Full spectrum of spontaneous endo:**
    minimal endo (prev 25%, D'Hooghe et al, 1991**)**
    to severe endo →
    bowel obstruction/death

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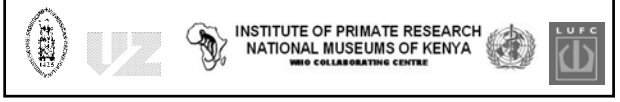
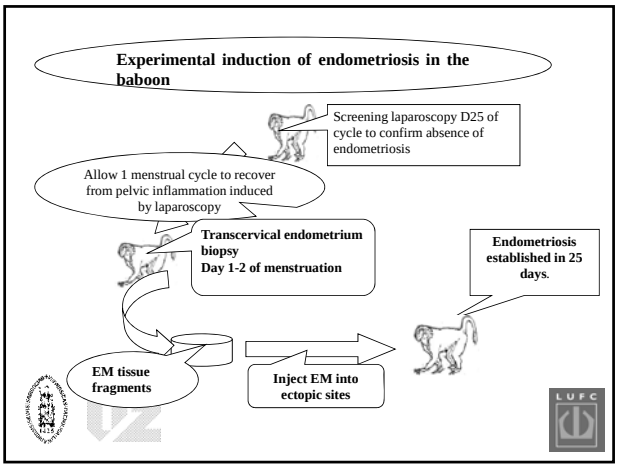
11. **Induced endo similar to human endo:**

    laparoscopic appearances,
    pelvic localization,
    microscopic aspects

    [D'Hooghe et al, 1995]





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12. In vivo culture model for study of early endometrial-peritoneal interaction (after induction)

- EM pellet versus EM supernatant
- Early establishment of endo: D1-3-6-10-15-25

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13. Preclinical model for study of cause-effect relationships in endometriosis (after induction)

Design:
- longitudinal observation in same baboon
- before, during and after induction

- interventions at well defined times of the cycle

- assess local effects: EM, PF, nl peritoneum, endo lesions
- assess systemic effects: PB

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IDEAL ANTI-ENDOMETRIOSIS DRUG

1. Prevent the development of endometriosis
2. Cures existing endometriosis, also after cessation of treatment
3. No interference with menstrual cycle
4. No side effects
5. Safe for women who wish to become pregnant

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14. Evaluate new drugs for prevention of endometriosis

Aim: prevent endometrial-peritoneal attachment after IP injection of menstrual EM

3 groups, n=5 each,
test drug, - control, + control

a. Pretreatment of baboons N days→induction
b. Pretreatment of EM at time of induction
c. Combination of a+b

(TNF-alpha inhibitors, D'Hooghe et al, 2006)

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15. Evaluate new drugs for treatment of endometriosis

Aim: reduction of existing endometriotic lesions (after induction using IP injection of menstrual EM)

3 groups, n=5 each,
test drug, - control, + control

1. **Induction laparoscopy (D1-2)**
2. **Staging laparoscopy pre-treatment (D25)**
3. **RCT 3 groups and treat during 1-3 months**
4. **Staging laparoscopy post-treatment**

(TNF-alpha inhibitors, Falconer et al, 2006; ROSI, Lebovic et al, 2007)

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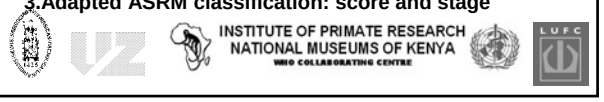
16. Endometriosis outcome variables in prevention or treatment trials

(D'Hooghe et al, 2006; Falconer et al, 2006; Lebovic et al, 2007)

1. Endometriosis Lesions: N, surface area, depth, volume
2. Phenotype of endo lesions: black, red, white,….
2. Adhesions: N and surface area
   - endo-related versus non endo-related
   - Integrated in >< independent from ASRM staging
3. Adapted ASRM classification: score and stage



UNICITY OF BABOON MODEL

17. General and reproductive safety in prevention or treatment trials

(D'Hooghe et al, 2006; Falconer et al, 2006; Lebovic et al, 2007)

1. General: side effects
2. Cyclicity
   -cycle length, length follicular phase/luteal phase
   -E2/P assays
3. Fertility and miscarriage
4. Offspring: congenital abnormalities

UNICITY OF BABOON MODEL

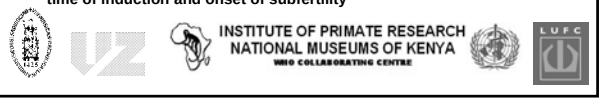
18. Model Endometriosis-associated infertility

(D'Hooghe et al, 1994 and 1996)

1. Normal MFR in baboons with minimal endo
2. Reduced MFR in baboons with
mild, moderate or severe endo (spontaneous and induced),
- related to an increased incidence and recurrence of the Luteinized Ruptured Follicle Syndrome
- also in the absence of ovarian endometriotic cysts

(D'Hooghe, 1997; D'Hooghe et al, 1996 several studies).

- ? Causal role of EM changes (Fazleabas)
- ? Temporal relationship between time of induction and onset of subfertility



UNICITY OF BABOON MODEL

19. Model for Treatment of endometriosis-associated subfertility

(Falconer et al, 2007)

with standardization for:

1. **Degree of endometriosis (amount EM for Ipseeding)**
2. **Ovulation (perineal cycle),**
3. **Male factors (proven fertility, nl sperm)**
4. **Sexual intercourse**
   - **timing**
   - **behavioral observation**
   - **postcoital test**

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20.? Endometriosis -associated pain

- **Under investigation at IPR**
- **Pilot study in 5 baboons with 24 hour camera surveillance before-after induction**
- **Collaboration Dr Coleman (Oregon Primate Center, USA)**

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VALIDATION OF BABOON ENDOMETRIOSIS MODEL

- **Pub Med (updated 28th Jan 2009):**
- **Baboon AND Endometriosis N=62**
  - **34 Leuven-IPR Nairobi group (T. D'Hooghe)**
  - **14 Chicago group (A. Fazleabas)**
  - **6 San Antonio Group (B. Barrier)**
  - **8 others**

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Learning Objectives: NHPmodels for translational research in endometriosis

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5 observations BABOON ENDOMETRIOSIS MODEL

- **Uninterrupted retrograde menstruation causes endometriosis**
- **Endometriosis causes pelvic inflammation + systemic immunomodulation**
- **Endometriosis causes secondary endometrial changes**
- **General immunosuppression does not cause or cure endometriosis**
- **Specific immunomodulation may prevent and/or cure endometriosis**

UNINTERRUPTED RETROGRADE MENSTRUATION CAUSES ENDOMETRIOSIS

1. **Prevalence of spontaneous endometriosis increases with duration of captivity (D'Hooghe et al, 1996a).**
2. **Spontaneous endometriosis is progressive when followed during 2 years (D'Hooghe et al, 1996b)**
3. **Baboons with an initially normal pelvis develop in 64% histologically proven minimal endometriosis after 32 months (D'Hooghe et al, 1996c)**
4. **Positive correlation between weight of EM tissue used for intrapelvic seeding and extent of endometriosis in baboons (D'Hooghe et al, 1995)**

UNINTERRUPTED RETROGRADE MENSTRUATION CAUSES ENDOMETRIOSIS

5. **Iatrogenic obstruction of the cervix (supracervical ligation) leads to diminished antegrade menstruation + pelvic endometriosis within 3 months**
   (D'Hooghe et al, 1994)
6. **Menstrual EM: higher capacity than secretory EM in endo induction**
   (D'Hooghe et al, 1995)

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ENDOMETRIOSIS CAUSES
PELVIC INFLAMMATION + SYSTEMIC IMMUNOMODULATION
(D'Hooghe et al., 2001, Kyama et al., 2003)

1. PF: Increased volume, WBC conc, inflamm cytokines:
- during spontaneous retrograde menstruation
- following intrapelvic injection of endometrium (within 1/12)
[D'Hooghe et al.,1999, D'Hooghe et al., 2001].

2. PF: Increased WBC concentration, increased % of macrophages and cytotoxic T cells:
- in PF of baboons with spontaneous endometriosis [D'Hooghe et al 1996a, D'Hooghe et al 1997a].

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ENDOMETRIOSIS CAUSES
PELVIC INFLAMMATION + SYSTEMIC IMMUNOMODULATION
(D'Hooghe et al., 2001, Kyama et al., 2003)

3. PB:
increased % of CD4+ and IL2R+ cells in baboons with stage II-IV endo (both spontaneous long term endo and induced endo)
>< recent spontaneous endometriosis (Stage I) or nl pelvis.

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Endometriosis causes secondary EM changes

- Research Group A. Fazleabas (Chicago)
- ? Clinical relevance to endometriosis-associated subfertility

General immunosuppression does not cause or cure endometriosis

3/12 high dose immunosuppression with azathioprin and methylprednisolone

1. No effect on:
   - the incidence of spontaneous endometriosis
   - the extent of induced endometriosis,
2. Only marginal stimulatory effect on: progression of spontaneous endo

[D'Hooghe et al., 1995c]

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Specific immunomodulation may prevent and/or cure endometriosis

- PPAR-gamma activators reduce and prevent induced endometriosis (Lebovic et al, 2007; 2009)
- TNF alpha antagonists prevent and reduce spontaneous or induced endometriosis, mainly via an effect on active red peritoneal lesions (3 independent studies Barrier et al, 2004; D'Hooghe et al, 2006; Falconer et al, 2006)
- MAJOR CONCERN: GENERAL AND REPRODUCTIVE SAFETY

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WHO COLLABORATING CENTRE

Overall conclusions

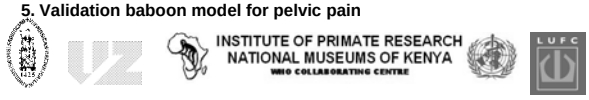
- NHPs = most relevant preclinical models for endo research

- Among NHPs, baboons represent
- the most relevant and
- the best validated model for endo research

Overall conclusions

Most important areas of endometriosis research in baboons:

1. Early pathogenesis
2. Cause-effect relationship studies
   may lead to discovery of new biomarkers
   and therapeutic targets
3. Test new drugs in prevention or treatment of endometriosis and endometriosis-associated subfertility
4. Test new endometriosis drugs with respect to general and reproductive safety
5. Validation baboon model for pelvic pain



Overall conclusions

Long term support for IPR, Nairobi, Kenya

1. Increasing international collaboration
2. Role of IPR International Advisory Board, Kenya Government and WHO

GLOBAL RESEARCH EFFORT TO STUDY CAUSE-EFFECT RELATIONSHIPS OF ENDOMETRIOSIS IN BABOON MODEL AT IPR

1. Sufficient N baboons with long term follow-up (+ pain)
2. Paired comparisons before+after induction (+ pain)
3. Building biobank for international collaborative research

Acknowledgments of mentors

- Institute Primate Research, Nairobi, Kenya: CS Bambra, PhD
- Harvard Medical School, Boston, USA (93-95) JA Hill, MD; DJ Anderson, PhD

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F. Vilmos (Budapest, HUN)
K. Coleman (Oregon Primate Center, USA)
EU Network for Endometriosis (ENE)

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European Network on Endometriosis

First ever EU research grant for endometriosis

1. Pan European epidemiological study
2. Internet based endometriosis gateway
3. Consolidate and formalise the European Alliance

- 8 Associate partners and 4 Collaborating partners
  - Endometriosis UK lead partner
  - Belgium, Denmark, Italy, UK
- Application scored very highly – 87/100 and received funding 300.000 Euro (2007-9)

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D Willemen
H Devroe
H Afschrift
O De Maeght
L Hollanders
A Velaers
F Vynckier
P Bols
E Vergison
K Bullens
B Quintens

International Collaboration

- Institute of Primate Research, Nairobi, Kenya, WHO Collaborating Center
- WHO
- University of Milwaukee, WI, USA (D. Lebovic)
- Oxford and Cambridge Universities, UK
- European Network Endometriosis
- Karolinska University, Stockholm, Sweden (H. Falconer)
- Semmelweis University, Budapest, Hungary (A.Bokor)
- Endometriosis Association, Milwaukee, USA
- World Endometriosis Research Foundation, London, UK

Funding since 1998

- Leuven University Research Council
- Leuven IRO (International Council for Development Collaboration)
- Leuven University Hospital Clinical Research Foundation

- Belgian Fund for Scientific Research (FWO)
- Belgian Institute for Science/Technology (IWT)
- Flemish Government (endocrine disrupters)

- Endometriosis Association USA
- University Michigan Ann Arbor; University Milwaukee, WI, USA
- World Endometriosis Research Foundation
- EU Public Health Grant

- Merck Serono Pharmaceuticals
  Serono Chair Reproductive Medicine 2005-2010

Impaired Steroid Hormone Action in Endometriosis

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Department of Obstetrics, Gynecology & Reproductive Sciences
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Disclosure

I have no relevant financial relationship with any commercial interest related to this lecture.

Learning Objectives

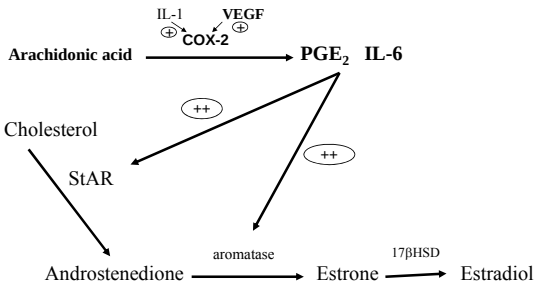
At the end of this presentation, the participant will be able:

- To discuss the interactions between steroid hormones and intracellular signal pathways, and
- To appreciate the relevance of these interactions in the pathogenesis of endometriosis.

Endometriosis

- Endometriosis is an estrogen-dependent disease that affects 5-10% of women of reproductive age.
- Endometriosis is characterized by cell survival, inflammation, excessive estrogen formation, and progesterone resistance.

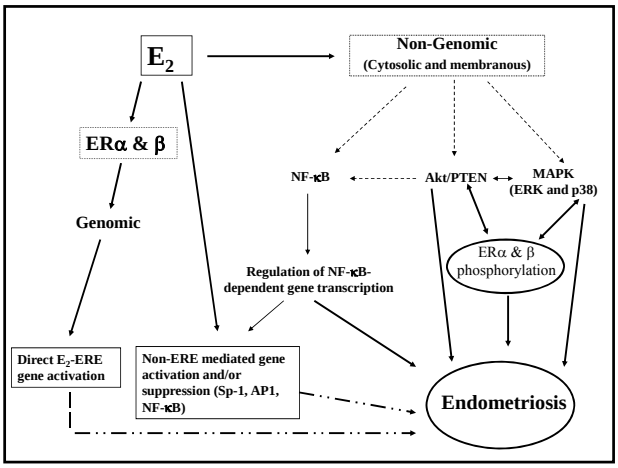
Immune-Steroid Hormone Interactions



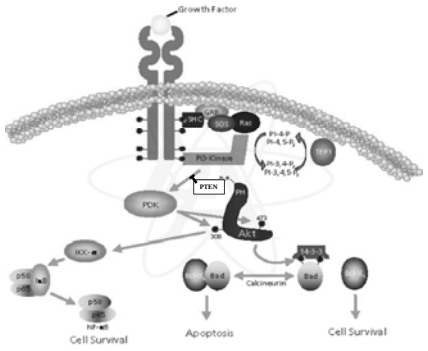
Noble et al. JCEM 1997

Possible Intracellular Regulatory Steps in Immune-Steroid Hormone Interactions

- **PTEN pathway**
- **Akt pathway**
- **MAPK pathway**
  - p38
  - ERK1/ERK2
  - JNK
- **NF-κB pathway**



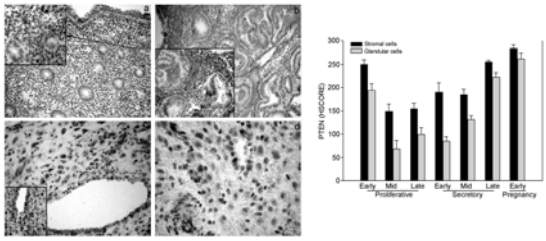
PTEN/Akt/NF-κB Signaling



PTEN

- PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a tumor-suppressor protein that inhibits PI3K/Akt signaling
- PTEN increases the activity of pro-apoptotic molecules such as Bad and caspase-9, and inhibits cell cycle progression by down-regulating cyclin D1.
- Estrogen typically stimulates cell proliferation by activating genes that promote cell cycle progression, such as cyclin D1 and c-myc.
- Estrogen may down-regulate PTEN activity by increasing its phosphorylation in endometrial cells.

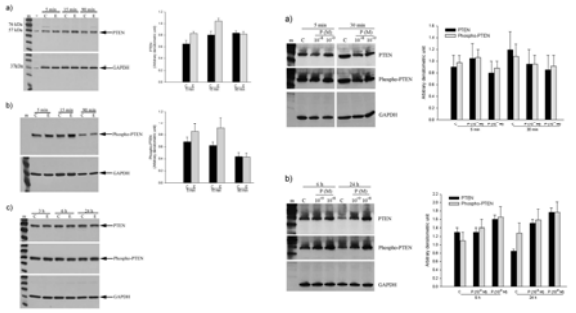
PTEN Expression in Human Endometrium



A: Mid-prolifeartive; B: Late-secretory
C: Early decidua; D: Negative control

Guzeloglu-Kayisli et al. JCEM 2003

Regulation of PTEN by Estradiol and Progesterone



Guzeloglu-Kayisli et al. JCEM 2003

K-Ras and *PTEN* Mutations

PTEN mutations in human endometrial cancer

↓

50-80 %

PTEN mutations in → 20% of Ovarian endometrioid carcinomas

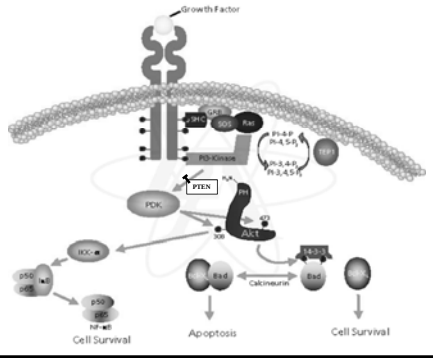
→ 8.3% of Ovarian clear cell carcinomas

K-Ras and *PTEN* Mutations

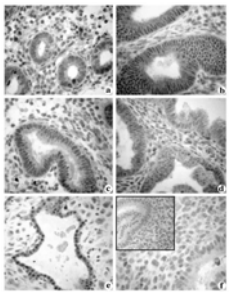
- Activation of *K-ras* or inactivation of *PTEN* was sufficient for formation of 'benign' endometriotic ovarian lesions.
- Endometriosis developed in the *K-ras* transgenic mice - the first genetic model of this disease.
- Whereas cancer was not seen in these mice with a single 'hit', mice with a double hit (*K-ras* activation and *PTEN* inactivation) developed invasive and metastatic ovarian endometrioid adenocarcinomas - the first mouse model of this ovarian cancer subtype.

Dinulescu et al. 2005

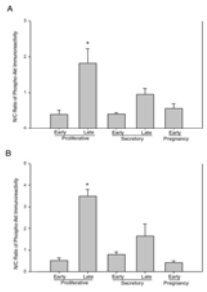
PTEN/Akt Signaling



Akt Expression in Human Endometrium

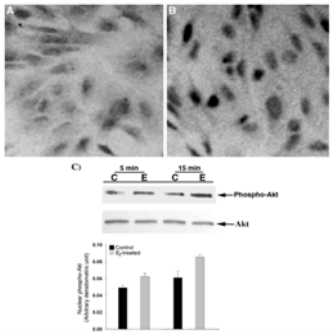


a: early; **b**: late proliferative phase
c: early; **d**: late secretory phase
e: early pregnancy, **f**: neg control



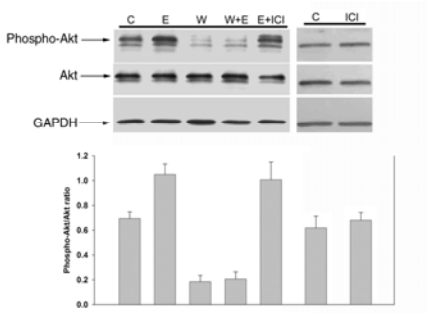
Guzeloglu-Kayisli et al. JCEM 2004

Regulation of Akt Phosphorylation by Estradiol



Guzeloglu-Kayisli et al. JCEM 2004

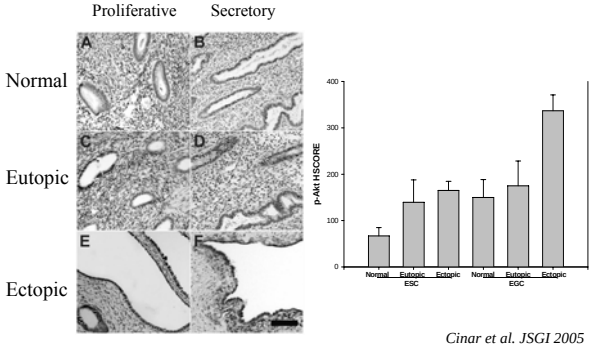
Regulation of Akt Phosphorylation by Estradiol



W: Wortmannin 10^{-5} M (PI3K inhibitor)

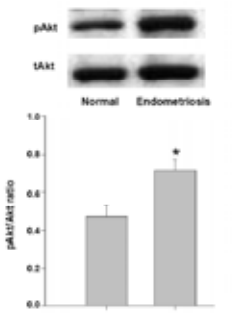
Guzeloglu-Kayisli et al. JCEM 2004

Phospho-Akt in normal, eutopic, and ectopic endometrium

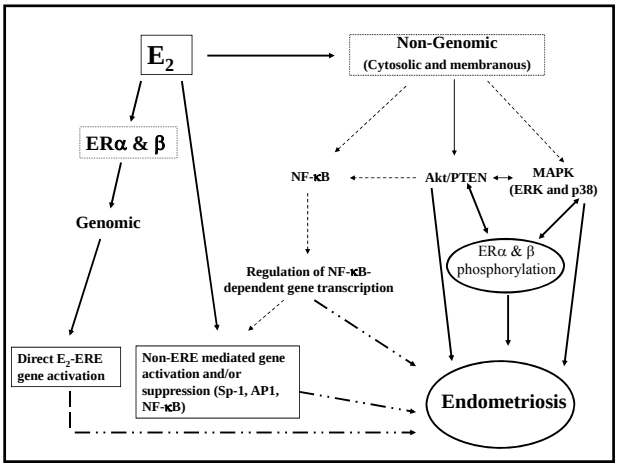


Cinar et al. JSGI 2005

Akt Activity in Endometriotic cells



Cinar et al. JSGI 2005



Mitogen-activated protein kinase (MAPK)

- All eukaryotic cells possess MAPK pathways
- ERK, p38, and JNK are most relevant MAPK families
- MAPKs are evolutionarily conserved and regulate diverse biological activities:
  - gene expression
  - mitosis
  - metabolism (motility, survival, apoptosis)
  - differentiation

Hypothesis

Endometriosis requires growth and sustained viability of ectopic endometrial tissue.

+

MAPK signaling regulates cell proliferation, differentiation, and apoptosis

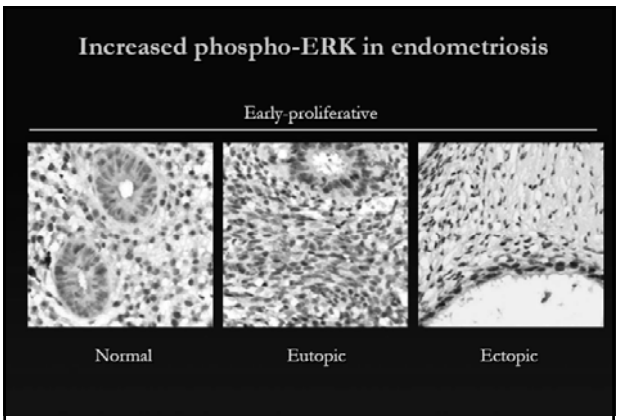
⇩

MAPK is involved in the pathogenesis of endometriosis

Extracellular signal-regulated kinase (ERK)

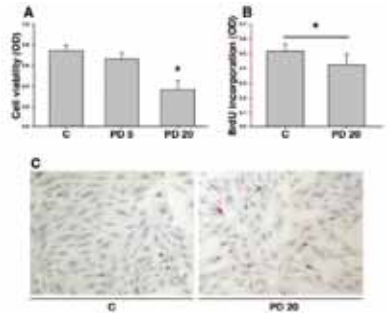
- 2 main isoforms, ERK1 and ERK2
- Activated by phosphorylation
- Stimuli include steroids, growth factors, cytokines, growth factors, and carcinogens
- Involved in cell proliferation, differentiation, survival, and motility

Increased phospho-ERK in endometriosis



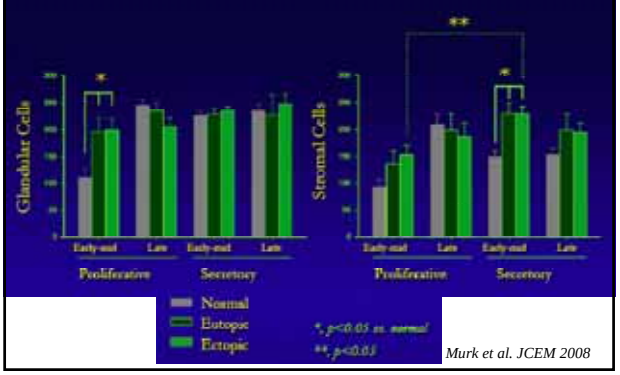
Murk et al. JCEM 2008

p-ERK is Involved in HESC Viability and Apoptosis



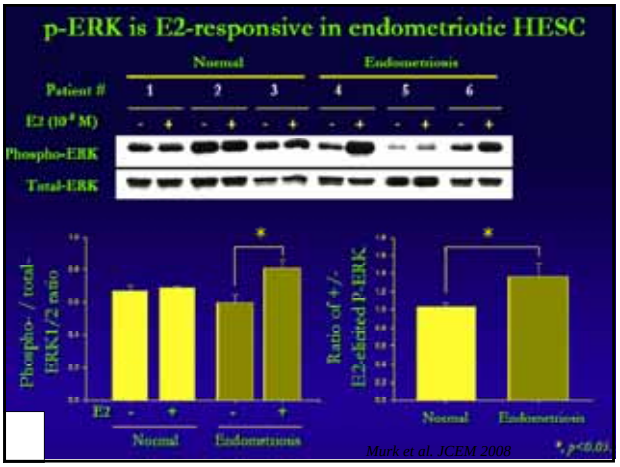
Murk et al. JCEM 2008

Increased phospho-ERK in endometriosis (HSCORE results)



Murk et al. JCEM 2008

p-ERK is E2-responsive in endometriotic HESC

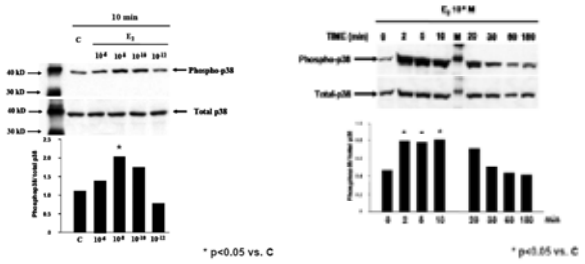


Murk et al. JCEM 2008

ERK and Endometriosis

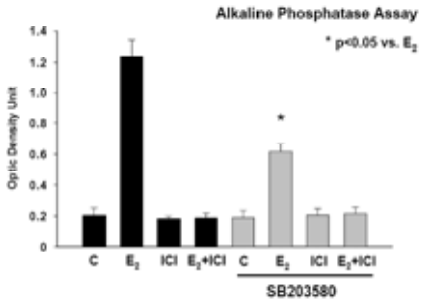
- *In vivo:*
  - ERK activity is increased in eutopic and ectopic endometrial tissues compared to normal endometrium
- *In vitro:*
  - ERK activity is involved in stromal cell proliferation and apoptosis.
  - ERK phosphorylation is increased in response to E2 in cells isolated from women with endometriosis, but not in those from normal women.

E_2 Induces p38 MAPK Activation



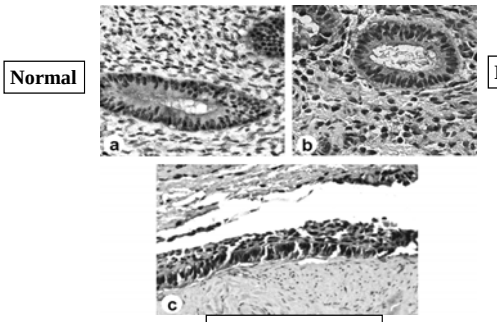
Seval et al. JCEM 2006

Effect of p38 MAPK on Estrogenic Activity



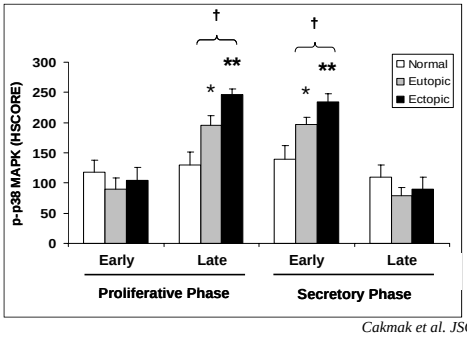
Seval et al. JCEM 2006

Phospho-p38 MAPK Expression in Eutopic and Ectopic Endometrium



Cakmak et al. JSGI 2006

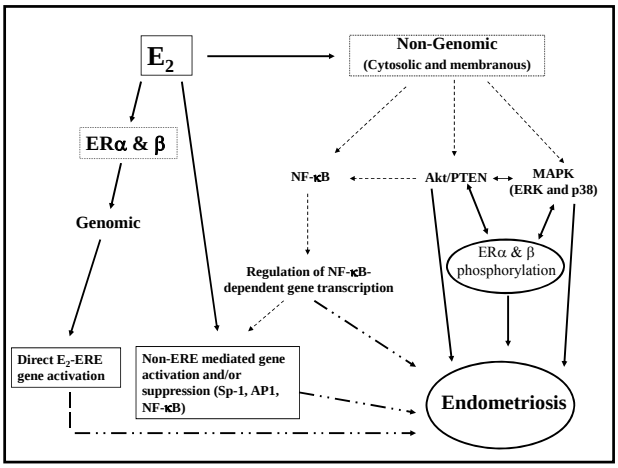
Phospho-p38 MAPK Expression in Eutopic and Ectopic Endometrium



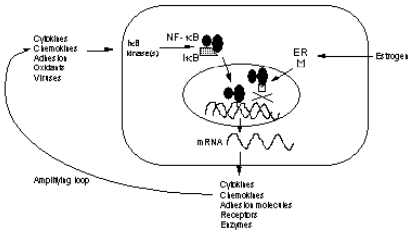
Cakmak et al. JSGI 2006

p38 MAPK and Endometriosis

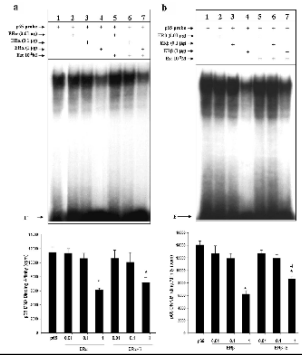
- *In vivo:*
  - p38 MAPK activity is higher in superficial endometriosis compared to deeper implants of the same patient.
  - Active p38 MAPK levels correlate positively with IL-8 expression (an inflammatory marker) but do not correlate with the level of apoptosis.
- *In vitro:*
  - Both p38 MAPK and E_2 affect each other's response suggesting a bidirectional interaction (positive enhancement):
    - » Estrogen activates p38 MAPK
    - » p38 MAPK mediates some of the estrogen's effects



ER and NF-κB interaction

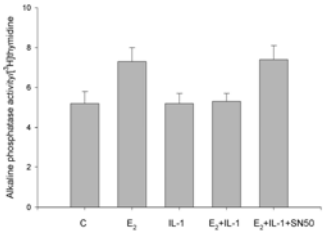


Effect of ERα and ERβ on p65-DNA binding



Guzeloglu-Kayisli, et al. Reprod Sciences 2008

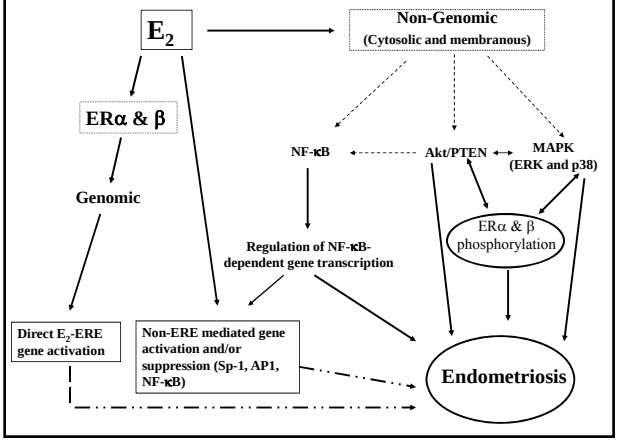
Effect of NF-κB activation on estrogen response



Alkaline phosphatase activity in Ishikawa cell line.

Cells were incubated for 24h with either estradiol (10^{-8} M), IL-1α (2 ng/ml)or in combination of estradiol and IL-1α. Combination of estradiol with IL-1α revealed a significant decrease in the level of estrogen-induced alkaline phosphatase activity ($p<0.01$). Addition of SN50 (5 μg/ml), an inhibitor of nuclear translocation of NF-κB reversed the inhibition.

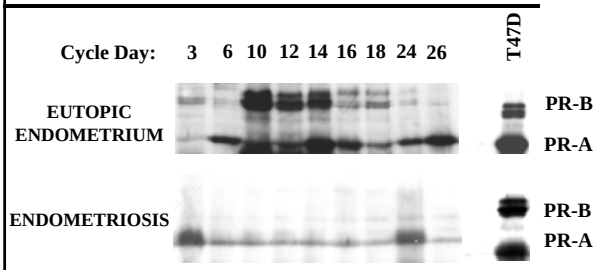
Guzeloglu-Kayisli, et al. Reprod Sciences 2008



Progesterone

- Progesterone receptors (PR) are expressed as A and B isoforms and they differ functionally.
- Progesterone action on target genes is conferred primarily by PR-B homodimer.
- Progesterone induces the expression of 17β-hydroxysteroid dehydrogenase 2, which catalyzes the conversion of biologically potent estradiol to the less estrogenic estrone.
- PR-A represses the function of the B isoform.

Progesterone Receptors PR-A and PR-B



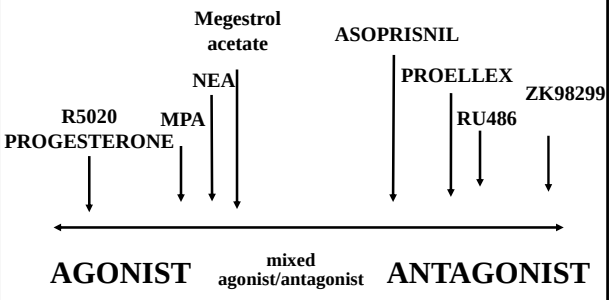
Attia, et al, JCEM 2000

Progesterone Resistance

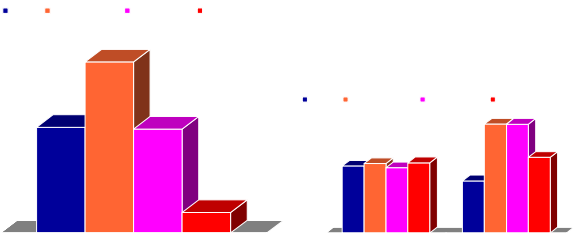
	17β HSD-2 activity	Progesterone receptors
Eutopic tissue	High levels in secretory phase	Both PR-A and PR-B present
Ectopic tissue	Absent	No PR-B Low levels of PR-A No cyclic variation

Bulun, NEJM 2009

Selective Progesterone Receptor Modulators (SPRMs)



SPRM and Endometriosis



Pain Score x Pain Days
Baseline to Month 3

Endometrial Thickness
Baseline vs Month 3

Repros Inc. Phase II Study

Summary

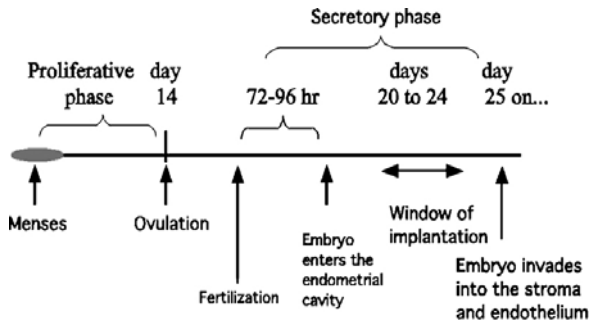
- Akt, ERK, and p38 MAPK activities are increased in endometriosis implants.
- Akt and ERK stimulate endometrial cell proliferation and inhibit apoptosis; p38 MAPK increase inflammation – both characteristics of endometriosis.
- Akt, ERK, and p38 MAPK activities are more responsive to E_2 in women with endometriosis.
- The regulation of inflammation involves also protein-protein interaction between ER and NF-κB.

Summary

- E_2 activates Akt, ERK, and p38 MAPK – possibly through a non-genomic pathway.
- These inflammatory mediators not only stimulate aromatase activity, but also simulate estrogenic activity; therefore, creating a vicious circle.
- Elevated ratio of ERβ/ERα suppresses PR-B levels, resulting in progesterone resistance in endometriosis.
- SPRMs, Akt and p38 MAPK inhibitors may provide novel treatment alternatives in endometriosis.

Uterine Factor Infertility in Endometriosis

Hugh S. Taylor
Yale University School of Medicine



From Donaghay and Lessey,Semin Reprod Med 2007; 25: 461-475

Implantation Defects

Endometriosis-Associated Infertility

- ↓ IVF Implantation Rates
  Simon, 1994; Arici, 1996; Pellicer, 1998
- ↓ HOXA10, HOXA11
  Taylor, 1999
- ↓ Integrin $\alpha_v\beta_3$
  Lessey, 1994

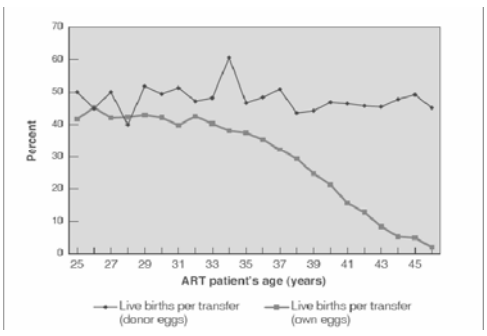
Endometriosis and Implantation

Endometriosis	Control	
12.7 %	18.0%	Barnhart et al, Fertil Steril 2002

Endometriosis is not detrimental to embryo implantation in oocyte recipients

Sung et al Journal of Assisted Reproduction and Genetics 1997

Live Births per transfer using a woman's own or donor eggs



2002 Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinic Report. CDC

What causes endometrial defects and how can we detect them ?

Endometrial Biopsy

Reproductive Medicine Network
trial shows poor predictive value.

Out of phase biopsy:

midluteal

- Fertile: 49.4%
- Infertile: 43.2%

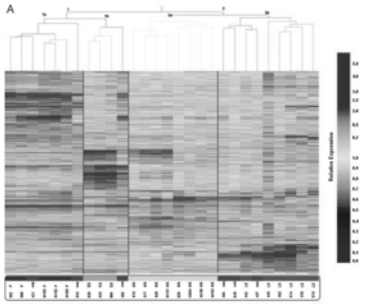
late luteal

- Fertile: 35.5%
- Infertile: 23.0%

Coutifaris, et al. Fertil Steril. 2004; 82:1264-72

Endometrial Molecular Defects

Molecular Profiling



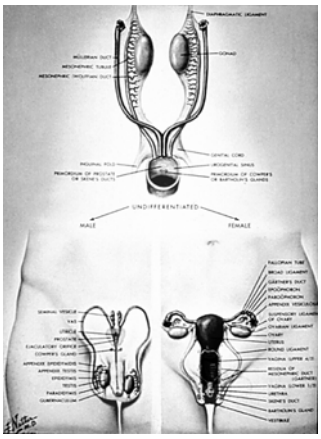
Giudice L et al: Endocrinology 2006:147(3):1097-121

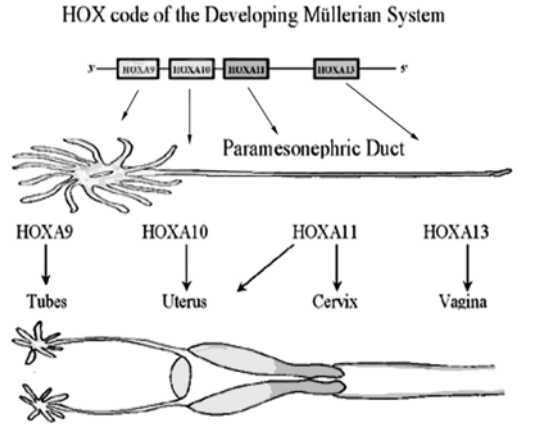
Genes with Well Characterized Endometrial Receptivity Phenotype

- Hoxa10
- Hoxa11
- LIF

HOX Genes

- Present in all Multicellular Animals
- Highly Conserved Between Species
- Encode transcription factors
- Essential Role in Embryonic Axial Developmental Patterning





Taylor et al, Biol Reprod. 1997; 57:1338

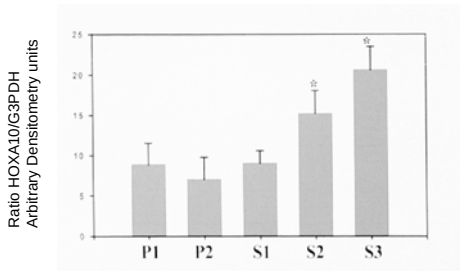
Hoxa10 Deficient Mice

- Females sterile
- Endometrial histology normal
- Subtle abnormalities in uterus
- Normal ovulation
- Failure of implantation, loss of blastocyst after E3.5
- E0.5 zygotes transferred to wt recipient are viable

Satokata et al, Nature 1995; 374:460

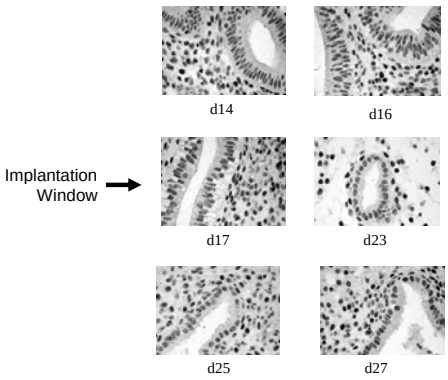
HOXA10 Expression

human endometrium



Taylor et al, J Clin Invest 1998; 101:1379-1384

HOXA10 Expression



Taylor et al J Clin Invest 1998, 101:1379-1384, Sarno and Taylor JCEM 2005, 90: 533-528.

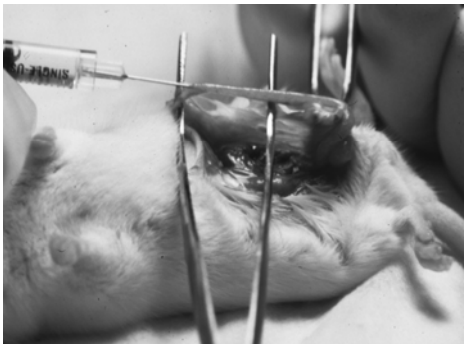
HOXA10 in the Human Endometrium

- HOXA10 is expressed in the adult
- HOXA10 expression varies with menstrual cycle; epithelial expression dramatically rises at the time of implantation
- Estrogen and Progesterone regulate HOXA10

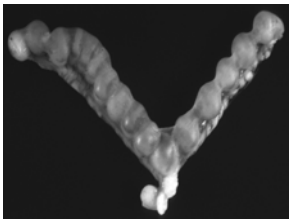
Taylor et al, J Clin Invest 1998; 101:1379

What is the Role of HOX Expression in the Adult Uterus ?

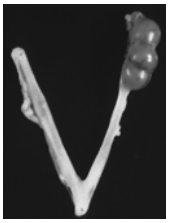
HOXA10 Antisense or pCDNA/HOXA10 Transfection



Pregnant Mouse Uteri



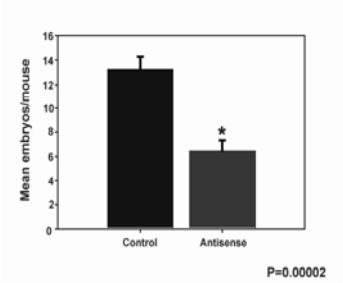
Control



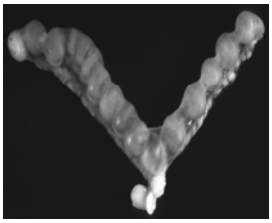
Antisense

Bagot and Taylor, Gene Therapy 2000; 7:1378

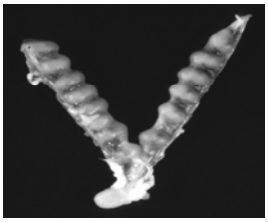
Implanted Embryos - Day 9
Control vs. Antisense



Pregnant Mouse Uteri

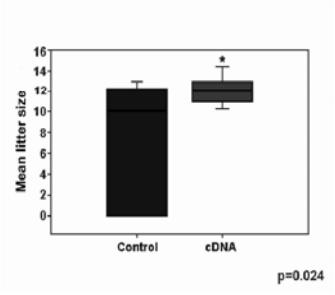


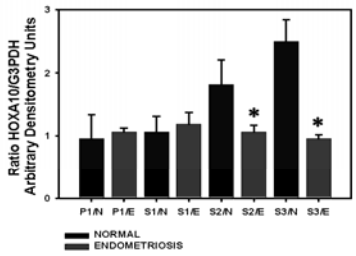
Control



HOXA10 cDNA

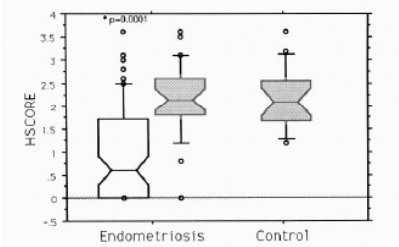
Term Litter Size
Control vs. HOXA10 cDNA





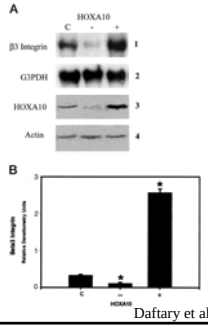
Taylor et al, Hum Reprod 1999; 14:1328

Endometriosis Reduces Endometrial Beta 3 Integrin



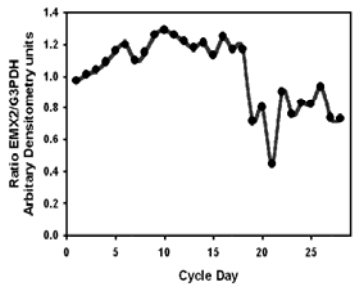
Lessey et al, JCEM 1994 79(2): 643-649.

Altered HOXA10 expression leads to corresponding changes in Beta 3 integrin



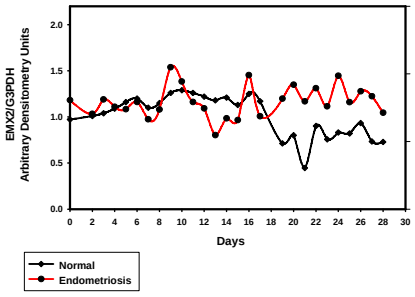
Daftary et al, Mol Endocrinol 2002; 16: 571

EMX2 Expression in the Menstrual Cycle



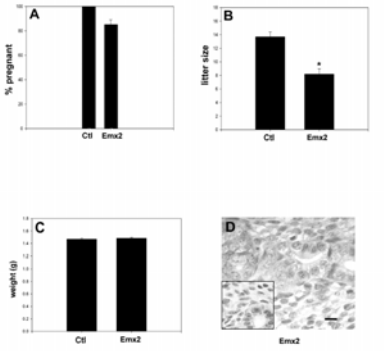
Troy et al, Mol Cell Biol, 2003; 23:1

EMX2 Expression: Endometriosis vs. Normal Cycling



Daftary and Taylor J Clin Endocrinol 2004; 89:2390

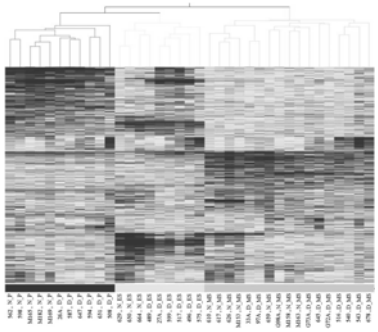
EMX2 Gene Transfer



* P<0.0006

Fei X et al Mol Endocrinol 2005; 146:3445

Hierarchical clustering analysis of endometrium from subjects with moderate/severe endometriosis (D) and subjects without disease (N) in the P (red), ES (gold), and MS (light blue) phases

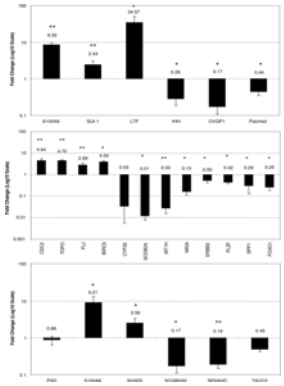


Burney, R. O. et al. Endocrinology 2007;148:3814-3826

Number of significantly differentially expressed genes in endometrium of endometriosis *vs.* normal subjects

	1.5X		2.0X		4.0X	
Menstrual phase	Up	Down	Up	Down	Up	Down
Proliferative	252	447	24	14	2	0
Early secretory	747	1741	213	521	26	59
Midsecretory	428	293	4	22	0	0

Expression of selected genes per cycle phase in the endometrium of women with endometriosis relative to women without endometriosis using real-time PCR



Burney, R. O. et al. Endocrinology 2007;148:3814-3826

LIMITATIONS IN UNDERSTANDING THE PATHOPHYSIOLOGY OF ENDOMETRIOSIS IN HUMANS

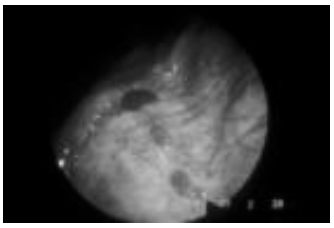
- Randomized studies with appropriate controls are not feasible
- Clinical experiments in vivo to determine etiology and pathology are difficult
- Events surrounding the establishment of the disease are difficult to study since in women at the time of diagnosis the disease has been prevalent for extended periods time

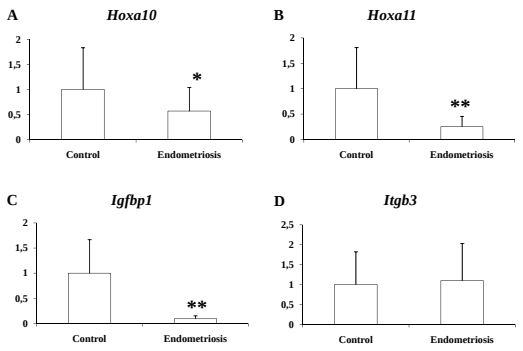
Animal Models of Endometriosis

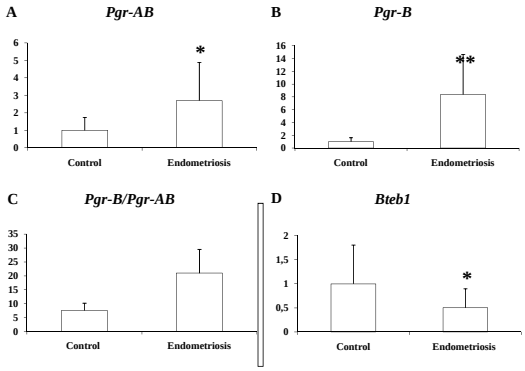
Allows determination of cause and effect

- Mouse
- Non-Human Primate

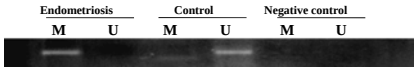
Murine Experimental Endometriosis





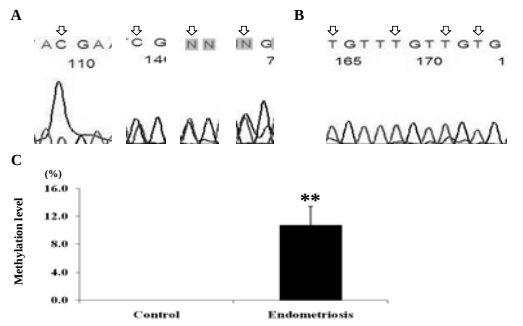


Hoxa10 DNA Methylation



Epigenetic alteration of gene expression in endometriosis

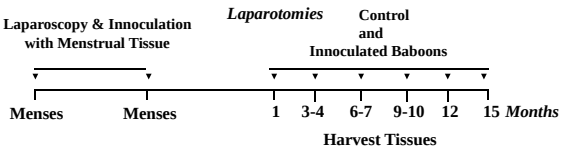
Lee and Taylor, In Press

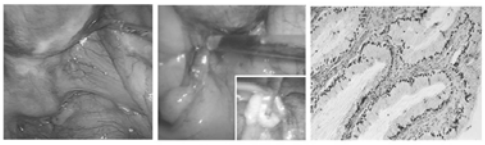


Lee et al. In Press

Baboons with experimentally induced endometriosis

EXPERIMENTAL DESIGN
INDUCTION OF ENDOMETRIOSIS

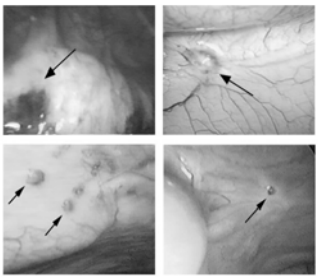




Pelvic cavity prior to inoculation | *Pelvic cavity during to inoculation* | *Histology of endometrial tissue in menstrual effluant*

LAPROSCOPIC AND HISTOLOGICAL EVALUATION OF LESIONS

Uterus Peritoneum

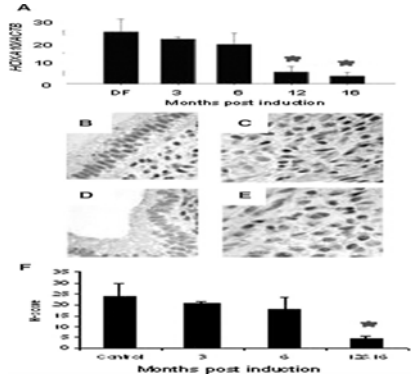


Upper Panel – *One Month*
Lower Panel – *Four Months*

MICROARRAY ANALYSES
One month post inoculation

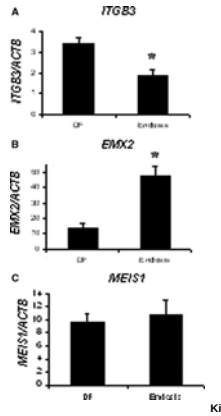
- **Eutopic Endometrium from baboons with induced endometriosis (n=4) compared with controls on day 8 PO on an Affimetrix Gene Chip HUM199A**
- **134 genes were upregulated and 115 genes were downregulated >2fold**
- **17 of the upregulated genes and 19 of the downregulated genes are associated with implantation and/or decidualization**
- **HOXA 10 was downregulated 2.4 fold**

Expression of HOXA10 in the eutopic endometrium of baboons with endometriosis



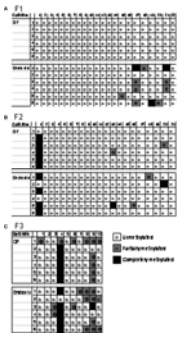
Kim, J.J. et al. Mol. Hum. Reprod. 2007 13:323-332

Expression of ITGB3, EMX2 and MEIS1 in eutopic endometrium of baboons with endometriosis



Kim, J.J. et al. Mol. Hum. Reprod. 2007 13:323-332

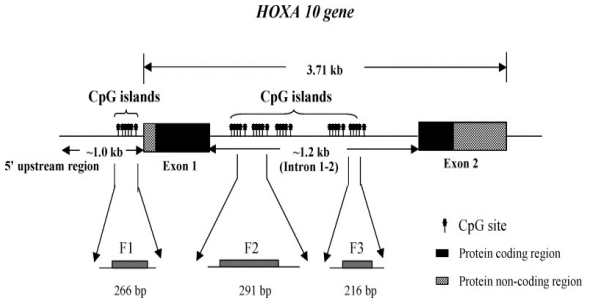
Methylation of the HOXA10 gene



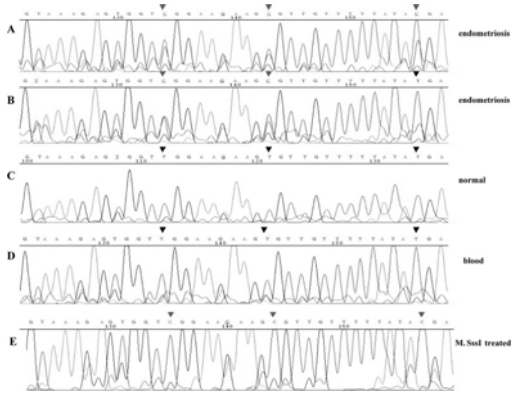
Kim, J.J. et al. Mol. Hum. Reprod. 2007 13:323-332

Normal Endometrium Gives Rise to Endometriosis and Aberrant Endometrial Gene Expression.

The HOXA10 Gene

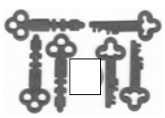


Epigenetic changes in HOXA10 in women with endometriosis

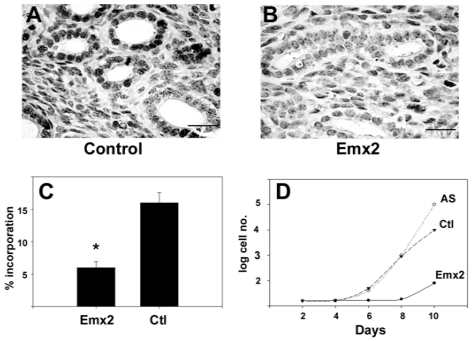


Wu et al, Am J Obstet Gynecol. 2005;193(2):371-80.

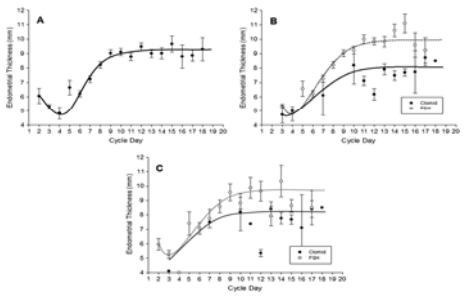
Looking in the wrong place?



Is a Luteal Phase Implantation Defect Determined in the Proliferative Phase?

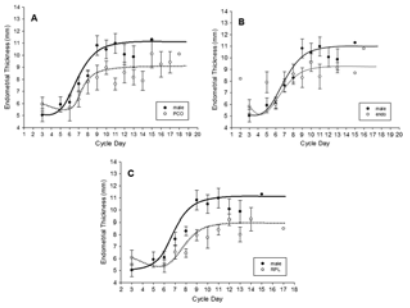


Defining the 'PROLIFERATIVE PHASE DEFECT'



Bromer J et al, Fertil Steril 2008

The Proliferative Phase Defect



Bromer J et al, Fertil Steril 2008

Can we treat endometriosis associated implantation defects?

ENDOCAN

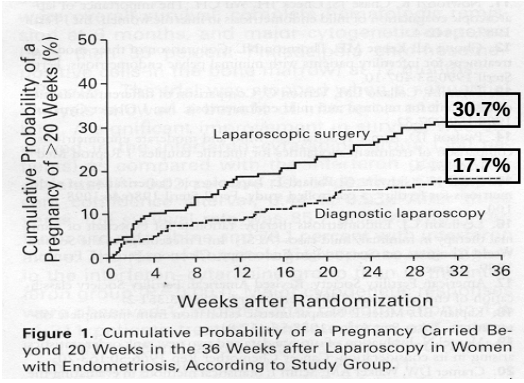
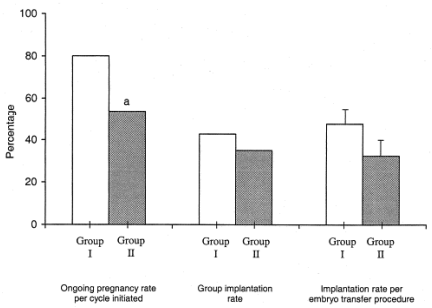


Figure 1. Cumulative Probability of a Pregnancy Carried Beyond 20 Weeks in the 36 Weeks after Laparoscopy in Women with Endometriosis, According to Study Group.

Marcoux, Maheux, Berube NEJM 1997

Prolonged GnRHa treatment of endometriosis prior to IVF



Surrey, Silverberg, Surrey and Schoolcraft, *Fertil Steril*, 2002 78; 699-704

Conclusions:

- Endometriosis is associated with diminished implantation.
- Altered gene expression decreases endometrial receptivity
- The presence of ectopic endometrium leads to the altered gene expression
- Implantation defects may have their roots in the proliferative phase.

Treatment Options

- Surgery
- GnRHa
- ? Methylation inhibitors
- ? Proliferative phase support

Acknowledgements

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- Ivan Penna
- Erin Wolff
- Banghuyn Lee

Collaborators:
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-Julie Kim
-Sun-Wei Guo

- NIH R01 HD036887
- NIH R01 ES010610
- NIH U54 HD052668

ENDOMETRIAL RECEPTIVITY

Objectives:

- 1. To demonstrate the clinical relevance of reduced endometrial receptivity.
- 2. To identify the molecular determinants of endometrial receptivity
- 3. To define how endometriosis affects endometrial receptivity.

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STEM CELLS

Objectives:

- Stem cells:
- 1. To understand the characteristics and types of stem cells
- 2. To define the role of stem cells in normal reproductive physiology
- 3. To explore the role of stem cells in endometriosis

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Course 4 - Endometriosis and Infertility

Inflammatory and immunological aspects

Mauricio S Abrao

2009

Endometriosis Division, Sao Paulo University, Brazil

President, SBE – Brazilian Endometriosis and Minimally Invasive Gynecology Society

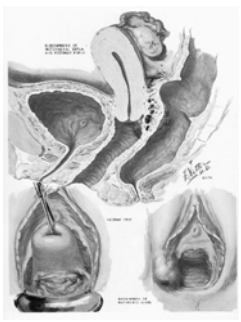
www.endometriose.net

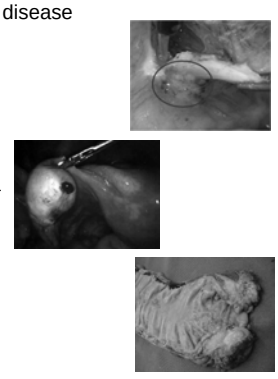
Learning Objectives

To present the immunological and inflamatory aspects of endometriosis and infertility, from the pathogenesis to the terapeutic aspects of the disease.

Endometriosis

More than one disease





Pathogenesis

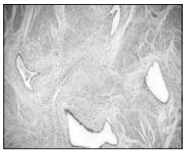
Von Rokitansky, 1860
First description

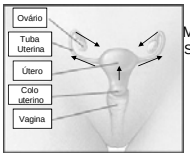
Cullen, 1896
Ectopic endometrium

Russel, 1899
Embrionary rests

Pathogenesis

Celomic Metaplasya
Meyer, 1919





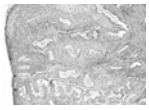
Retrograde Menstrual flow
Sampson, 1927

Environment
Genes
Immune Response
Hormones

Endometriosis

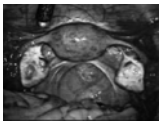
Weed & Arquembourg, 1980; Harada, 2001; Missmer, 2003; Ulkus, 2005

Immune Factor
Immune Response



CELL IMMUNITY

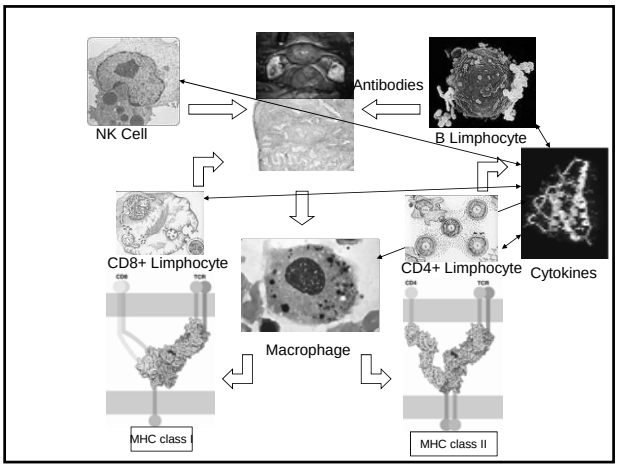
CD8+ Limphocytes
CD4+ Limphocytes
NK Cells
Macrophages
Cytokines



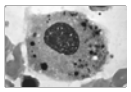
HUMORAL IMMUNITY

B Limphocytes
Antibodies
Cytokines

Harada, Fertil Steril 76:1, 2001



Cell Immunity: Macrophages



- Increase in number and activity
  - Hill JA, Faris HMP, Schiff I, Anderson DJ. Characterization of leukocyte subpopulations in the peritoneal fluid from women with endometriosis. Fertil Steril 1998, 50:216-22.
- Cytokines and growth factors
  - Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. Fertil Steril 2001, 75:1-10.
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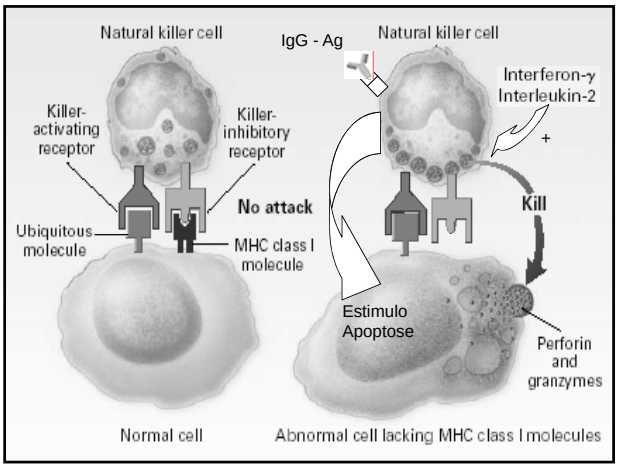
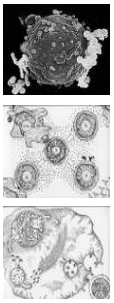
Cell Immunity: NK Cells

- Decreased activity
  - Wilson TJ, Hertzog PJ, Angus D, Wood EC, Kola I. Decreased natural killer cell activity in endometriosis patients. Fertil Steril 1994, 62:1086-8.
- Peritoneal fluid and sera of patients with endometriosis: decreased activity of NK cells
  - Ho HN, Wu MY, Yang YS. Peritoneal celular immunity and endometriosis. Am J Reprod Immunol 1997, 38:400-12.
- Increased number in peritoneal fluid
  - Gomez-Torres MJ, Acien P, Campos A, Velasco I. Embryotoxicity of peritoneal fluid in women with endometriosis. Its relation with cytokines and lymphocyte populations. Hum Reprod 2002 Mar;17(3):777-81
- Killer cell inhibitory receptors
  - Wu MY, Yang JH, Chao KH, Hwang JL, Yang YS, Ho HN. Increase in the expression of killer cell inhibitory receptors on peritoneal natural killer cells in women with endometriosis. Fertil Steril 2007, 74:1187-91



Cell Immunity: T Lymphocytes

- Decreased antigenic response
- Decreased citotoxicity
- T helper / T supressor ratio?
- Scientific methods



Nothnick WB. Fertil Steril, 76(2):223-31, 2001

Humoral Immunity

- B Lymphocytes activity
  Startseva NV. Clinical immunological aspects of endometriosis. Akush Gynecol, 3:23-6, 1980
- Complement activity
  Weed JC, Arguembourg PC. Endometriosis: can it produce an autoimmune response resulting in infertility? Clin Obstet Gynecol, 23:885-93, 1980
- Auto-antibodies against endometrial tissue
  - Yes
    Switchenko AC, Kaufman RS, Becker M. Are there anti-endometrial antibodies in sera of women with endometriosis? Fertil Steril, 56:235-41, 1991
  - No
    Taylor PV, Maloney MD. Autoreactivity in women with endometriosis. Br J Obstet Gynecol, 98:680-94, 1991

Endometriosis and Cytokines

Macrophage migration

Stromal cell adhesion to fibronectine

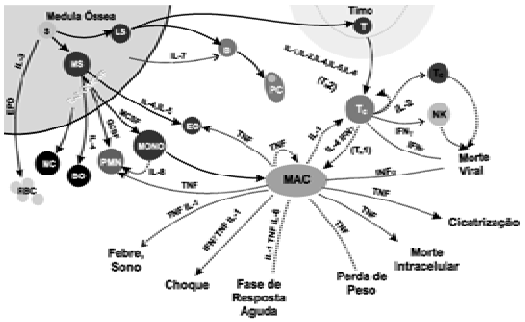
Extracellular matrix decay (MMP): adhesion

Endometrial cell proliferation

Angiogenesis

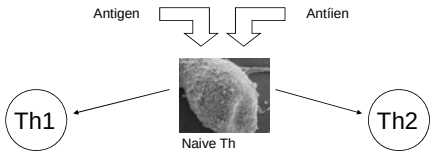
Seli et al., Obstet Gynecol Clin N Am.2003

Cytokines

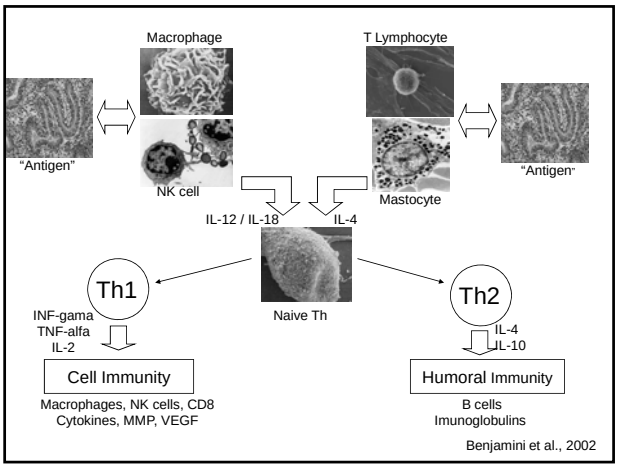


Th1 Th2 Response

- Th1/Th2: two types of T *helper* cells with differences in the cytokine secretion patterns



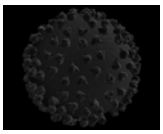
Mosmann et al.,J Immunol. 1986



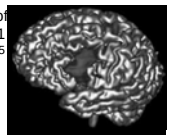
Benjamini et al., 2002

Th1 Th2 Response

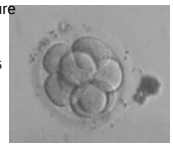
HIV: Th1 and viral activity
Norris & Rosenberg, 2001



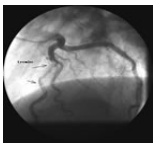
Vascularization of affected site: Th1
Arumugan et al., 2005



Implantation failure or repeated abortion: Th1
Kwak-Kim et al., 2005



Vascular thrombosis: Th1
Adler et al., 2005



Cytokine concentration (pg/ml): serum and peritoneal fluid

Cytokines	Group A	Group B	p
TNF-alfa (serum)	2,3	3,7	0,188
IFN-gama (serum)	1,6	2,1	0,571
IL-2 (serum)	7,4	8,3	0,447
IL-4 (serum)	1,9	2,0	0,731
IL-10 (serum)	3,2	3,1	0,904
TNF-alfa (peritoneal fluid)	3,1	1,4	0,364
IFN-gama (peritoneal fluid)	0,5*	0,1	**0,039***
IL-2 (peritoneal fluid)	0,2	0,2	0,072
IL-4 (peritoneal fluid)	1,7	0,9	0,557
IL-10 (peritoneal fluid)	28,6*	15,7	**0,035***

* p<0,05

Podgaec et al., Hum Reprod. 2007

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***** p<0,05

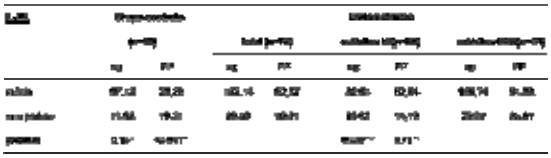
Hsu, 1997 – mRNA IL-4
Antsiferova, 2005 – mRNA IL-4 e IL-10

Pasoto, 2005 - FAN

Podgaec et al., Hum Reprod. 2007

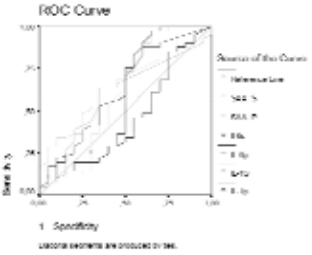
IL-12 e IL-18

- Peritoneal fluid IL-12: increase in endometriosis patients in relation to control group
- Serum IL-12: increase in advanced stages in relation to initial stages



Fairbanks et al, Fertil Steril. 2008

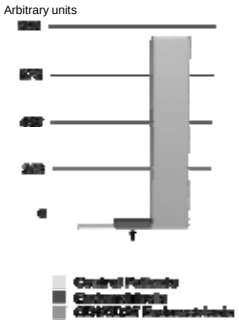
IL-1, IL-6, SAA



- Serum IL-6 and peritoneal fluid SAA: diagnosis of endometriosis
- Best acuracy: serum IL-6
- Cut-off of3.45pg/ml
- sensibility: 52.6%
- specificity: 61.5%

Ejzenberg et al., WCE 2008

Treg Cells – Foxp3

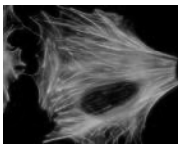


- Foxp3 presence in peritoneal cells: RT-PCR
- CD4+CD25+ peritoneal fluid cells of endometriosis patients: high levels of Foxp3
- Treg cells

Podgaec et al., WCE 2008

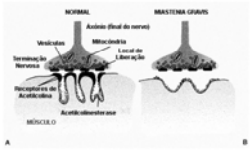
Auto-immune disease definition

- Direct proof
  - Auto-antibodies transfer to a host reproduces the disease
  - Mother with miastenia transfers auto-antobodies to the fetus: fetus is born with neonatal myastenia gravis
  - Ethics in reproduction of this model



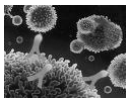
Auto-immune disease definition

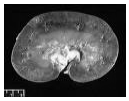
- Indirect proof
  - To identify target-antigen and to reproduce in experimental model: tireoglobulin (tireoiditis), acetilcolin receptor (myastenia)
  - To identify auto-antibodies: anti-DNA (lupic nephritis)

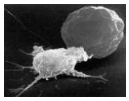


Auto-immune disease definition

- Circumstance evidence
  - Clinical data
  - Family predisposition, MHC association, lymphocite infiltration
  - Immunosupressive drugs: clinical improvement
  - Common criteria in human auto-immune diseases







Is endometriosis an auto-immune disease?

- Several organs
- Family / genes
- Women
- Environment factors
- Tissue damage
- Other diseases association
- Apoptosis
- Lymphocyte abnormalities

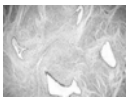
Nothnick, Fertil Steril. 2001

Endometriosis Treatment X Immunity

- Simon et al. Glucocorticoid treatment decreases sera embryotoxicity in endometriosis patients. Fertil Steril. 1992
- D'Hooge et al. The effects of immunosuppression on development and progression of endometriosis in baboons (Papio anubis). Fertil Steril. 1995
- Dmowski et al. The effect of endometriosis, its stage and activity, and of autoantibodies on in vitro fertilization and embryo transfer success rates. Fertil Steril. 1995
- Kim et al. The immunotherapy during in vitro fertilization and embryo transfer cycles in infertile patients with endometriosis. J Obstet Gynaecol Res. 1997
- Shakiba & Falcone. Tumour necrosis factor-alpha blockers: potential limitations in the management of advanced endometriosis? A case report. Hum Reprod. 2006
- Kyama et al. Non-steroidal targets in the diagnosis and treatment of endometriosis. Curr Med Chem. 2008

Endometriosis: disease of endometrium?

- Endometrium from endometriosis patients is abnormal in comparison to the endometrium of normal patients?
  - Higher estrogenic production
  - Survive in the peritoneum
  - Proliferation and Invasion
  - Auto-protection against phisiologic apoptosis
  - Increased Angiogenesis



Vinatier et al., Eur J Obstet Gynecol Reprod Biol. 2000
Ulukus et al., J Societ Gynecol Invest. 2006

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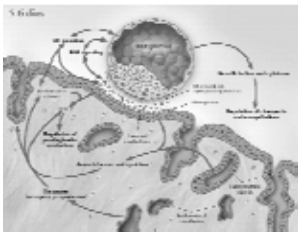
Endometriosis and Infertility

Adherences

tubal obstruction



Hormonal
luteal phase
ovarian reserve

Imunological
citocynes Th1 / Th2
NK cells
Macrophages

Endometrial receptivity

Oocyte quality

Schenken RS, et al. Fertil Steril 1984

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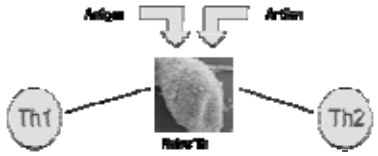
Endometriosis and Infertility



Hormonal
luteal phase
ovarian reserve

Imunological
citocynes Th1 / Th2
NK cells
Macrophages

Endometrial receptivity

Oocyte quality

Gleicher N. Am J Reprod Immunol 2002

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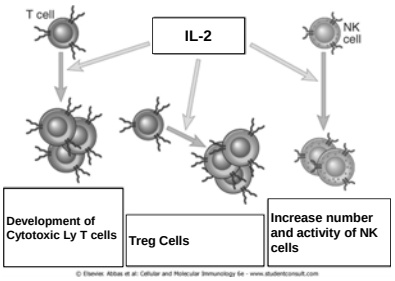
Endometriosis and Infertility



Hormonal
luteal phase
ovarian reserve

Imunological
citocynes Th1 / Th2
NK cells
Macrophages

Endometrial receptivity

Oocyte quality

32

TABLE 1

Role of altered immunologic factors in endometriosis-associated infertility.

Affected immunologic factors	Levels of the immunologic factor in women with endometriosis	Mechanistic actions of affected immune factors	Effect of altered immune factor levels on fertility	Reference
Follicular fluid studies				
VEGF	Decreased	Decreases follicle health and vascularization	Decreased embryo quality and implantation rates	(31)
IL-6	Increased	Decreases aromatase activity within follicles	Decreased intrafollicular E_2 levels, leading to decreased fertility and fertilizing capacity	(50)
Peritoneal fluid studies				
RANTES	Increased	Attracts monocytes and memory T-cells to inflamed areas	Increased inflammation, cytotoxic effects on healthy cells, and OS produced, leading to decreased fertility	(101)
IL-10	Increased	Prevents p27 down regulation in developing granulosa cells	G_0 arrest of granulosa cell cycle, resulting in low-quality oocytes	(55)
VEGF	Increased	Induces the formation of angiogenesis promoting fibrin matrix in the peritoneal cavity	Increased adhesion of free endometrial tissue within the peritoneal cavity	(57)
TNF-α	Increased	Causes increased prostaglandin production by endometrial epithelial cells	Increased adhesion of free endometrial tissue within the peritoneal cavity, and increased inflammation, leading to subfertility	(28)
TNF-α	Increased	Decreases the effect of TIMP	Increase effects of MMP, leading to increased endometriotic tissue invasiveness	(59)
PAPP-A	Increased	Increases follicular androstenedione syntheses	Increased conversion of androstenedione to E_2 by endometriotic tissue, leading to increased tissue proliferation	(54)
Cathepsin D	Increased	Initiates harmful proteolytic events	Degradation of basement membrane and extracellular matrix components	(51)

Grupta et al., Fertility & Sterility - 2008

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Endometriosis and Infertility

IL X Endometriosis AND Infertility

Dimitriadis *et al*. Interleukin-11, IL-11 receptoralpha and leukemia inhibitory factor are dysregulated in endometrium of infertile women with endometriosis during the implantation window. **J Reprod Immunol. 2006**
Yoshida *et al*. A combination of interleukin-6 and its soluble receptor impairs sperm motility: implications in infertility associated with endometriosis. **Hum Reprod. 2004 .**
Gomez-Torres *et al.* Embryotoxicity of peritoneal fluid in women with endometriosis. Its relation with cytokines and lymphocyte populations. **Hum Reprod. 2002**

Hormonal
luteal phase
ovarian reserve

Imunological
citocynes Th1 / Th2
NK cells
Macrophages

Endometrial receptivity

Oocyte quality

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Endometriosis and Infertility

PF citocynes in Patientes with DIE and Infertility

	TNF-a	INF-gama	IL-2	IL-4	IL-10
Endometriosis (n=42)	2.5	1.9	5.9	2.6	1.5
Control (n=9)	2.2	1.1	0.7	1.5	3.0

Podgaec et al., IX World Congress Endometriosis 2008

Hormonal
luteal phase
ovarian reserve

Imunological
citocynes Th1 / Th2
NK cells
Macrophages

Endometrial receptivity

Oocyte quality

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Endometriosis and Infertility

peripheral NK Cells and Endometriosis

	Controls	EDT I/II	EDT III/IV	p
n=56	19	14	23	
NK (%)	**11,9 (+-6,2)**	**8,9 +-4,9**	**18,6 +-10,2**	**0.01**

Dias JA Jr et al., Eur J Obst Gynecol Reprod biol 2005

Hormonal
luteal phase
ovarian reserve

Imunological
citocynes Th1 / Th2
NK cells
Macrophages

Endometrial receptivity

Oocyte quality

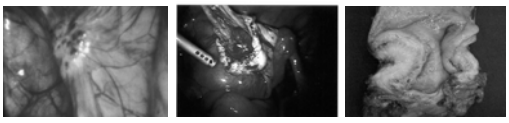
Endometriosis

Environment factors

Genetics

Immune system: Vigilance failure

Progesterone resistance



Oocyte quality in endometriosis

ESHRE Pre Congress Course
Amsterdam June 2009

Alain Audebert, MD
IGF1-Institut Greenblatt France-Bordeaux

Nothing to disclose

Learning objectives

The aim of this lecture is to review :

- Impact of endometriosis on fecundity
- Reported mechanisms
- Why to suspect oocyte quality alteration ?
- Oocyte quality assessement
- Oocyte quality in endometriosis
- Potential physiopathogenic mechanisms
- Conclusive remarks

Introduction

- Endometriosis is a chronic inflammatory disease…..
- « *……studies suggested that 25% to 50% of infertile women have endometriosis and that 30% to 50% of women with endometriosis are infertile……* » (1)
- **Poor quality of oocyte** reported as a possible cause of subfertility associated with endometriosis as early as 1983 and 1985 (2, 3)
- « ***Age = egg quality*** » (4)

(1) Counseler VS. Am J Obstet Gynecol 1938;36:877
(2) Mahadevan MM. Fertil Steril1983;40:755
(3) Wardle PG. Lancet 1985;ii:236
(4) Toner JP. Fertil Steril 2003;79:491

Impact of endometriosis on fecundity

Impact on fecundity

- Epidemiological data
- Experimental data
- Clinical data
  - Spontaneous pregnancy rates
  - Results of treatments
- Conclusive remarks

Epidemiology (1)

Incidence of endometriosis

- General population : 2-10 %
- Tubal sterilisation : 6-43 %
- Infertile couples : 0,4-70 %

Final identified cause of infertility

- Thonneau (1) (1686 couples) : 4 %
- Collins (2) (2198 couples) : 6,6 %

(1) Thonneau P. Int J. Fertil 1993;38:37
(2) Collins JA. Fertil Steril 1995;64:22

Epidemiology (2)

Incidence and stage of endometriosis in infertile women

All cases : n=5000 (1)

- Incidence : 14,7 %
- Stage I-II : 62 %
- Tubal indications : 0.4 %

Normal ovulation & sperm: n=221 (2)

- Incidence : 47 %
- Stage I-II : 63 %

(1) Audebert A. 1986
(2) Meuleman C. Fertil Steril 2008 Aug 4

Experimental models

- **Rat model (1) :**
  - Reduced fecundity
- **Non human primate model (2):**
  - Minimal : normal fecundity
  - Other stages : reduced fecundity

(1) Stilley JA Biol Reprod 2008 Nov 19
(2) D'Hooghe TM. Fertil Steril 1996;66:809

Monthly Fecundity Rate (MFR)

Depends on age and cycle range

- Normal couples : 4-30 % (1)
- Endometriosis : 2-10 % (2)

(1) Schwartz D. New England J Med 1982;306:404
(2) Hughes EG. Fertil Steril 1993;59:963

3 years Estimated CPR
according stage and treatment

Stage	Expectant	Laparoscopy	Laparotomy
• Stage I-II	67 %	68 %	74 %
• Stage III-IV	-	62 %	44 %
• Endometrioma	-	52 %	46 %
• CDS Obliteration	-	30 %	24 %

(1) Adamson GD. Am J Obstet Gynecol 1994;172:1488

Estimated MPR in endometriosis-associated infertility

Treatment	I-II	III	IV
Expectant	3	3	0
Medical	3	4	1
Surgical	5	5	3
IVF	35	34	33

(1) Adamson D. ESRHE – Barcelona 2008

Stage I & II :
Arguments in favor of a reduced fecundity

- Prevalence of endometriosis in subfertile women
- Animal studies (Stage II and >) (1)
- Fecundity of women treated expectantly
- Lower fecundity vs unexplained infertility
- Lower conception rate in a donor insemination program
- Lower conceptions rates in an IVF program (2)
- Pregnancy rate increased by surgical treatment

(1) D'Hooghe TM. Sem Reprod Med 2003;21:243
(2) Barnhart K. Fertil Steril 2002;77:1148

Spontaneous pregnancy rates (stage I & II)
for patients in RCTs managed expectantly

Author (year)	number	Follow-up (month)	% Pregnancy
Seibel (1982)	28	6	50
Thomas (1987)	17	12	24
Bayer (1988)	36	12	47
Fedele (1992)	25	6	24
Fedele (1922)	36	18	37
Marcoux (1997)	169	9	18
Parazzini (1999)	45	12	22

Spontaneous pregnancy rates

Endometriosis (I & II) vs Unexplained infertility

Author (year)	N. couples		Follow-Up	% Pregnancy	
	endo	UI	(months)	Endometriosis	Unexplained
I.					
Collins (1995)	224	562	36	20	33,3
Berube (1998)	168	263	9	18,2	23,7
Akande (2004)	75	117	36	36	55

Fecundity rate per cycle in women undergoing donor insemination treatment

Probability	Controls	Endometriosis	
Jansen (1986)	0.11	0.02	0.02
Bordson (1986)	0.15	0.07	0.06
Rodriguez (1988)	0.20	0.06	-
Barrat (1990)	0.18	0.09	0.01

(1) Cahill DJ. Hum Reprod Update 2000;6:56

Stage I & II :
Meta-analysis of IVF results

Number of women = 2602

Outcome	Endometriosis	Control	Crude OR (95 % CI)
Pregnancy rate	21.11	27.71	0,70 (0.56-0.87)
Fertilisation rate	58.38	66.09	0.93 (0,92-0.94)
Implantation rate	11.31	18.08	0.80 (0.78-0.82)
Mean n. of oocytes	8.19	7.30	1.11 (1.06-1.14)

(1) Barnhart K. Fertil Steril 2002;77:1148

Natural IVF cycle
results according to indication

Indication	N.	Fertilization	Clin.Pregn/C	Clin.Pregn/ET
Endometriosis (I-II)	30	8O %	10.4 %	23.5 %
Unexplained Infert.	33	62.2 %	2.6 %	16 %
Tubal factor	24	68.6 %	5.8 %	16 %

(1) Omland AK. Hum Reprod 2001;16:2587

Stage I & II :
Results of Laparoscopic ablation

	Marcoux (1997)	Parazzini (1998)	Jacobsen (2000)
Number	341	101	442
% Pregnancy (12 m.)			
Ablation	30,4	24	OR=1,64
No treatment	17,7	29	

(1) Marcoux S. New England J Med 1997;337:217

Mechanisms of fecundity impairment

Etiopathogenesis of endometriosis related infertility

- It is generally accepted that **moderate/severe** endometriosis related sterility is due to mechanical factors, namely to the distortion/subversion of the regular pelvic anatomy (severity of adhesions)
- On the contrary, the factors behind infertility/subfertility related to minimal/mild endometriosis are less clear, as well as « pure » unilateral endometrioma eand deep lesion

Changes associated with endometriosis

- **Hormonal**
  LH surge, Hyperprolactinemia
- **Autoimmune/ Immune dysfunction**
- **Ovarian dysfunction**
  Follicular growth, Anovulation
  LUF syndrome, CL insuff.
- **Distorded pelvic anatomy**
- **Altered follicular function**
- **Altered Peritoneal fluid**
- **Altered tubal milieu**
- **Altered endometrial function**

Changes associated with endomeriosis may affect :

- Ovulation and corpus luteum function
- Oocyte quality
- Oocyte capture
- Sperm transport and function
- Fertilization
- Embryo development
- Implantation

Why to suspect reduced oocyte quality ?

Arguments to suspect reduced oocyte quality in endometriosis

- Experimental studies on amanimal models
- Reduced fertilization (IVF)
- Poor embryo quality
- Oocyte donation program

Studies on animal models

- **Rabbit Model :**

-Normal embryo development in animal with experimentally induced endometriosis (Dunselselman 1991)
-Reduced fertilization of oocytes exposed to high concentrations of PF from women with endometriosis (Dodds 1992)
-Accumulation of TIMP-1 negatively impact on oocyte quality (Stilley 2008)

- Endometriosis has no direct effect on oocyte
- …but ovarian follicular and/or oocyte dysfunction as a primary disorder can not excluded (Cahill 2000).

Endometriosis : Fertilization rates

Reduced
Wardle (1985)
Matson (1986)
Dlugi (1989)
Mills (1992)
Lelaidier (1993)
Simon (1994)
Tanbo (1995) **(Stage I)**
Harlow (1996)
Huang (1997
Hull (1998)
Pal (1998)
Bergendal (1994)
Al Zemi (2000)
Gerber (2002) **(ICSI)**
Al-Fadhi (2006)

Similar
Mahadevan (1983)
Tanbo (1990)
Olivennes (1995)
Dmowski (1995)
Gerber (1995)
Arizi (1996)
Minguez (1997) **(ICSI)**
Matalliotakis (2008)

Natural cycle
Reduced
Cahill (1997)
Similar
Omland (2006)

Endometriosis :

Fertilizing ability of the oocyte

- **Severe disease** : reduced
- **Minor endometriosis :** controversial results but a review concludes a 25 % reduction in comparison with tubal infertility (54 % vs 69 % p<0.0001) (1)
- Control group characteristics

(1) Cahill DJ. Hum Reprod Update 2000;6:56

Embryo quality

Bad embryo quality as a result of poor oocyte quality (but role of sperm quality and gamete interaction)

- **Brizek (1995) :**
  - Increased rate of nuclear and/ or cytoplasmic aberration
- **Pouly (1998) :**
  - Less blastocysts at day 5
- **Pellicer (2000) :**
  - Less blastomeres at day 3
  - Decreased implantation rate

Oocyte donation program

Lower pregnancies rate than normal donor

- **Simon (1994)**
  - **Reduced implantation rate (p<0.05)**
- **Katsoff (2006)**
  - **Clin.Pregnancy rate/ET : 42.9 % 60.9 % NS**
  - **Implantation rate/ET : 20.4 % 33.2 % NS**

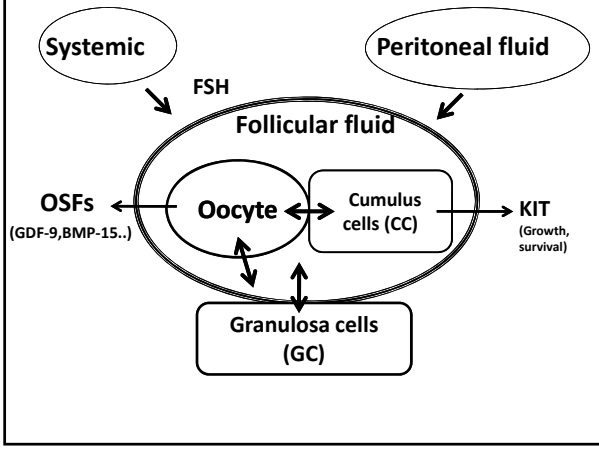
(1) Simon C. Hum Reprod 1994;9:725
(2) Katsoff B. Clin Exp Obst Gynecol 2006;33:201

Oocyte quality assessement

Oocyte quality

- Oocyte quality is a key limiting factor in female fertility, yet we have a poor understanding of what constitutes oocyte quality or the mechanisms governing it. The ovarian follicular microenvironment and maternal signals, mediated primarily through granulosa cells (GCs) and cumulus cells (CCs), are responsible for nurturing oocyte growth, development and the gradual acquisition of oocyte developmental competence (1)

(1) Gilchrist RB. Hum Reprod 2008;14:159



Oocyte quality assessement

- Major issue in IVF : permanent search, because too few oocytes have the ability to develop into viable embryo.
  > 50 % of retrieved oocytes may have underlying problems (1)
- Good quality oocyte results from multiple factors :
  - Intrinsic characteristics
  - Folliculogenesis
  - Oocyte-cumulus complex function
  - Follicular environment……
- Ideally, a method to measure of oocyte competence would enable better selection of the resulting embryo
- Focus on methods that can be used in the context of a clinical setting

(1) Scott L. Hum Reprod Update 2003;9:237

Oocyte morphology

- **Unfortunately morphologic criteria currently used are inadequate and ……operator dependant**
- **Selection system must be non invasive**
- **Negative parameters on pregnancy outcome :**
  - Presence of vacuoles, cytoplasmic pitting and particules in the perivitelline space
  - Zona pellucida appearance
  - Extruded first polar body : large, fragmentation (ICSI)
  - Cumulus cells quantity and expansion
  - Metaphase spindle formation (polarizing light microscope) : absence of birefringent spindle
  - Nulear precursor bodies (NPB) : distribution pattern and morphology…….

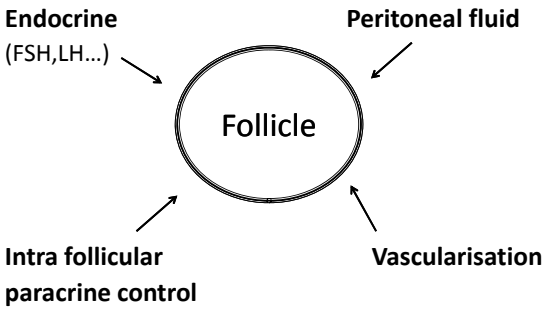
Pre ovulatory follicular fluid

- The major role of the pre ovulatory follicular fluid is to provide an optimal milieu for oocyte development, ensuring its nutrition, controling oocyte maturation, specifically cytoplasmic maturation, inhibiting nuclear maturation and preparing the process of of oocyte release, with the rupture of the follicular wall.

Regulation of folliculogenesis

- Ovarian folliculogenesis is regulated by both endocrine and intraovarian mechanisms that coordinate the processes of oocyte growth and somatic cell proliferation and differentiation
  - **Endocrine :** gonadotropins
  - **Locally :** Within the follicle, paracrine interactions between the oocyte and surrounding granulosa cells are critical for normal cell development and function : cytokines, growth factors, prostaglandins and steroid hormones.

Folliculogenesis regulation



Folliculogenesis assessement

- Follicular growth measurement using ultrasound
- Follicular blood flow evaluation by Doppler ultrasound
- Endocrine events regulating follicular growth
- Granulosa cells function
- Follicular fluid composition
- And……..Oocyte quality

Follicular fluid

- Compound from follicular secretions, plasmatic and peritoneal exudates
- Hormonal composition varies during menstrual cycle

Pre ovulatory follicular fluid composition : myriad of factors :

:

- Amino acids, carbohydrates, lipids…..
- Hypoxanthine, adenosine
- Estradiol, progesterone, androgens
- Proteins
- Cytokines
- Growth factors
- Enzymes
- OS

Potential follicular fluid indicators of oocyte quality

- E : marker of oocyte maturity and quality (Wunder 2005)
- Higher levels of of P, GH and IL-1 beta for oocytes that developped 2 PN (Mendoza 1999)
- P : No predictive value (Andersen 1992)
- P/E2 :higher ratio yields successfully fertilized oocytes (Enien 1995)
- A : Lower in conceptonal cycles (Andersen 1992)
- E/T : Higher ratio correlates with higher oocyte quality Andersen 1992, (Xia 2000)
- Androgens : higher levels correlated with abnormal oocyte (de Sutter 1991)
- AMH : higher level correlated with oocyte fertilizing ability (Cupisti 2007, Takahashi 2008)
- TNF-alpha : High concentration is correlated with poor oocyte quality (Lee 2000)
- IL-6 : Lower level in pregnant women (Baidawy 2007)
- TNF-alpha, IL-1 beta, IL-6, VEGF, Leptin, FGF, EGF, IGF-1 : concentration cannot predict fertilization outcome (Killic 2007, Asimakopoulos 2008)
- VEGF : Increased level associated with poor embryo development (Van Blerkom 1997)
- IL-12 : Higher levels in pregnant women (Badaiwy 2007)
- ENA-78 : deterioring effect on oocyte (Wunder 2006)……

Potential follicular fluid indicators of oocyte quality

But conflicting reports :

- Concentrations of E, P, T and PRL are not corelated with oocyte maturity and fertilizing ability (Rosenbush 1992)
- E2, P, IL-A beta, IL-6, TNF alpha, VEGF, leptin, FGF beta, EGF and IG1 were not associated with the fertilization outcome(Asimagopoulos 2008)……

Potential physiopathogenic mechanisms

Oocyte quality impairment
may in theory result from :

- **Inherent abnormalities :** inconsistant
- **Altered folliculogenesis**
- **Altered environment with :**
  - Altered peritoneal fluid
  - Impaired granulosa cells function
  - Follicular fluid abnormal composition

Oocyte quality impairment

In Vitro Maturation (IVM) model study (1)

- 14 women with endometriosis vs 8 without
- No difference was found in:
  - IVM
  - Meiotic anomalies
- But delay in the outcome of of oocyte meiosis I

(1) Santos Barcelos I. Rev Brasil Ginecol Obstet 2009

Granulosa cells function & endometriosis

Author	Year	Reported Abormalites
Kauppila	1982	Lower level of LH receptors in granulosa cells
Harlow	1996	Reduced aromatase activity and secretion of progesterone
Nakahara	1997	Increased granulosa cell apoptosis
Pellicer	1998	Impaired steroid production
Carlberg	2000	Elevated concentrations of TNF-alpha
Toya	2000	Alteration in the cell cycle
Saino	2002	Increase of oxydative stress markers
Yamashita	2002	Reduced VEGF gene expression
Cahill	2003	Decreased sentivity to LH stilumation
Yanlhara	2005	Higher expression of STS mRNA expression
Li	2008	Higher expression of TNF-alpha and IL-6 mRNA (rats)
Fujino	2008	Lower survivin gene expression

Granulosa cell function & endometriosis

Reported relevant abnormalities

- Impaired steroid production (lower aromatase activity) (1,2)
- In vitro granulosa cells secrete higher levels of IL-1 beta, IL-6,IL-8, and TNF alpha (3)
- Higher apoptosis of granulosa cells (4)
- Lower VEGF gene expression (5)

(1) Pellicer A. Fertil Steril 1998;69:1135
(2) Harlow CR. J Clin End Met 1996;81:426
(3) Carlberg M. Hum Reprod 2000;15:101
(4) Toya M. Fertil Steril 2000;73:344
(5) Yamashita Y. Fertil Steril 2002;78:865

Pre ovulatory Follicular fluid in endometriosis (1)
An inflammatory environment

	Modification		No change
	FF Level	Study	
Cellular content			
B lymphocytes	Elevated	Lachapelle (1996)	
NK cells,monocytes	Elevated	Lachapelle (1996)	
Cytokines			
Il-6	Elevated	Pellicer (1998)	Buyalos (1992)
		Carlberg (2000)	Hammadeh (2002)
			Pellicer (1998)
IL-1 beta			
TNF alpha	Elevated	Wunder (2006)	Kilick (2007)
		Carlberg (2000)	
VEGF	Decreased	Pellicer (1998)	
		Oliveira (2005)	
	Elevated	Attar (2003)	
	Elevated	Fuji (2008)	
Endotheline-I	Elevated	Abae (1994)	
PGF2 alpha			Bergqvist 1997
RANTES	Elevated	Xu (2006)	
ENA-78	Elevated	Wunder (2006)	
Neurotrophin	Decreased		
MCP-1	Decreased	Xu (2006)	
Oxydative stress			
ROS	Elevated	Bedaiwy (2003)	
Vitamine E	Decreased	Campos (2008)	

Pre ovulatory Follicular fluid in endometriosis (2)
Hormones and Growth factors

	Modification in FF		No change
Steroids			
Estradiol	Decreased	Wunder (2005)	
		Cahill (1997)	
Progesterone	Elevated	Pellicer (1998)	Wunder (2005)
Androgens			
Peptids			
LH	Decreased	Cahill (1997)	
Inhibine A	Elevated	Akade (2000)	
MIS			
			Fallat (1997)
Prolactin			Lima (2006)
Cortisol			Lima (2006)
Growth factors			
IGF-1			Cunha-Filhol (2003)
IGFBP-1	Decreased	Cunha-Filho (2003)	
FGF			Hammadeh (2002)

Follicular environment & endometriosis
Potential effects on oocyte quality

- Decreased ATP : Reduced oocyte viability
- Increased VEGF : Decreased embryo development
- TNF-alapha : Regulate apoptosis
- Higher level RANTES: Cytotoxic effects and OS produced
- Endothelin-I : Inhibition of granulosa cells (GC) steroidogenesis
- IL-10 : Cause arrest in the Go phase of GC
- Rantes : producd Increased inflamamtion, cytotoxic effects and OS

Oxidative stress & endometriosis

Oxydative stress (OS) and infertility

OS plays a significant role in infertility through multiple mechanisms and has a significant impact on fertility outcomes of ART

Effects of OS :
- -Local inflammation
- -Degradation of cell membrane
- -Inactivation of enzymes
- -Genomic & mitochondria DNA damage
- -Ultimatly cell death

⇨

Impairment of :
- -Granulosa cells function
- -Oocyte quality &function
- -Sperm integrity & function
- -Embryo integrity & dev.
- -Implantation

Oxydative stress (OS) in women with endometriosis :

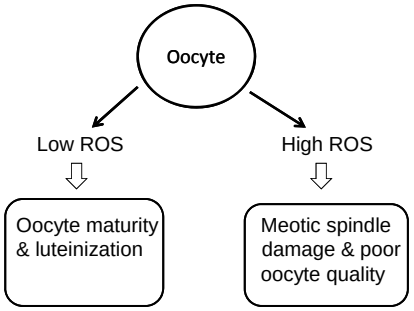
- Reduced antioxidant capacity (Szczepanska 2003)
- OS is increased in Granulosa cells (Saito 2002)
- ROS is increased in Peritoneal Fluid (Zeller 1987, Wang 1997)
- OS is increased in follicular fluid (Bedaiwy 2003)
- Antioxydant-oxidant imbalance (Gupta 2008)

Oxydative stress

- Low concentration of FF ROS is associated with higher blastocyst development and could be a good marker for predicting success of IVF (1)
- Intrafollicular high concentration of ROS is associated with a high rate of degenerate oocytes (2)
- Melatonin protects oocytes fromoxidative stress (2)

(1) Argawal A Fert Ster 2003 ;79:829
(2) Tamura H. J Pineal res 2008;44:280

Oxydative stress



Molecular methods for selection of the ideal oocyte….

« OMIC » for the assessement of oocyte

- Genomic
- Proteomic
- Metabolomic…..
- Analysis for GC, Follicular fluid, culture media….

Genomic assessement of oocyte

- Major advances on understanding the direct relationship between gene expression and developmental competence are being reported.
- Several studies have provided evidence that some gene expression levels could be used as objective markers of oocyte and embryo competence and capacity to sustain a successful pregnancy.
- A study, using microarray approach, identified new potential regulators and marker genes such as BARD1, RBL2, RBBP7, BUB3 or BUB1B, which are involved in oocyte maturation (1)

(1) Gasca S. Reprod Biomed Online 2007;14:175

Proteomic

- Proteomic analysis reflects cellular function or the complexity and diversity of the mammalian proteome with post-translational modifications or protein-protein interactions.
- The mature oocyte contains the full complement of maternal proteins required for fertilization, the transition to zygotic transcription, and the beginning stages of embryogenesis . A proteome reference database for the mouse oocyte was established (1). It will allow to expand our knowledge of the regulation of signaling in oogenesis, fertilization, and embryo development, while revealing potential mechanisms for epigenetic reprogramming. Some identified proteins may eventually serve as diagnostic biomarker candidates for ovarian function. (2).
- Proteomic profiling of mouse mature COC in order to identify proteins involved in ovarian follicular development and related to reproductive abnormalities (3).

(1) Ma M. J Proteome Res 2008;7:4821
(2) Satoh M. J Reprod Dev 2009 Mars 26
(3) MengY. Biochim Biophys Acta 2007;1774:1477

Metabolomic assessement of oocyte

- Metabolomic measurements correlate well with embryo development and morphology assessment. Furthermore, viability index on oocytes/embryos established by metabolomic tests may be a stronger predictor for implantation potential than traditional morphological assessment.
- A study on 412 oocytes culture samples with NIR spectroscopy-generated metabolomic (1)
  - Oocytes that developed to grade A embryos on day 3 demonstrated significantly higher viability indices
  - Metabolomic profiling from spent culture medium of the oocyte is related to nuclear maturity, is able to predict embryo development at day 3 and day 5 stages, and relates to embryo viability.

(1) Nagy ZP. Reprod Biom Online 2009;18:219

Conclusions

- Reduction of fertility associated with endometriosis appears to be at least partially due to reduced fertilizing ability of the oocyte.
- Reduced fertilizing ability of the ocyte may be due to :
  - Granulosa cells dysfunction
  - Non optimal (steroids) and inflammatory follicular environment, including increased OS…..
- There is a need for non invasive methods of oocyte quality assessement.

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Course 4: Endometriosis and Infertility Ovarian and Endometrial Factors

Organised by the Special Interest Group Endometriosis/Endometrium

Course co-ordinators:
Charles Chapron (France),
Thomas D'Hooghe (Belgium),
Dominique de Ziegler (France)

ART @ Cochin
Global management

Course 4: Endometriosis and Infertility Ovarian and Endometrial Factors

Patho-physiology:
09:00 – 09:30 Pathogenesis
Paolo Vercellini (Italy)
09:30 – 09:45 Discussion
09:45 – 10:15 Preclinical relevance of animal models
Thomas d'Hooghe (Belgium)
10:15 – 10:30 Discussion
10:30 – 11:00 Coffee break
Endometrial factors:
11:00 – 11:30 Impaired progesterone action
Aydin Arici (USA)
11:30 – 11:45 Discussion
11:45 – 12:15 The homebox genes and E2 effects
Hugh Taylor (USA)
12:15 – 12:30 Discussion
12:30 – 13:30 Lunch

Pelvic and ovarian factors:
13:30 – 14:00 Inflammatory and immunological aspects
Mauricio Abrao (Brazil)
14:00 – 14:15 Discussion
14:15 – 14:45 Oocyte quality in endometriosis
Alain Audebert (France)
14:45 – 15:00 Discussion
15:00 – 15:30 Coffee break
Treatment:
15:30 – 16:00 **Medical treatment and ART**
Dominique de Ziegler (France)
16:00 – 16:15 Discussion
16:15 – 16:45 Surgical treatment
Charles Chapron (France)
16:45 – 17:00 Discussion
17:00 –17:30 Synthesis and final conclusions

ART @ Cochin
Global management

Course 4: Endometriosis and Infertility Ovarian and Endometrial Factors

Medical treatment and ART

Dominique de Ziegler, MD
Professor and Head
Div. Reprod Endocr and Infertility
Université Descartes – Cochin Médical Center
Paris France

Disclaimer:

Sat on advisory boards of Ferring, IBSA and Vantia pharmaceuticals.
Holds stocks in Ultrast, LLC

ART @ Cochin
Global management

Course 4: **Endometriosis and Infertility**
Ovarian and Endometrial Factors

Medical treatment and ART

1. Endometriosis and reproduction
2. Medical vs. surgical treatment: What comes first?
3. Endometriosis and ART?
4. Medical treatment and/or surgery before ART?

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Course 4: **Endometriosis and Infertility**
Ovarian and Endometrial Factors

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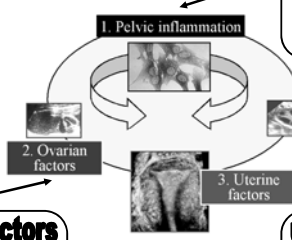
Global management

Effects of endometriosis on reporduction



pelvic factors

Pelvic inflammation: interferes w/ sperm-oocyte interaction.

Affects in-vivo fertility

ovarian factors

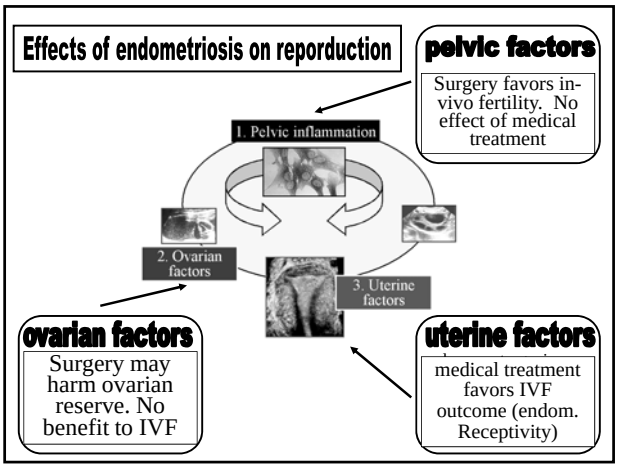
endometriomas and Sx. for endometriomas:

Affect ovarian reserve and (?) oocyte quality.

uterine factors

hyper estrogenism and P4 resistance interfere w/ embryo implantation.

Affects IVF outcome



Course 4: **Endometriosis and Infertility**
Ovarian and Endometrial Factors

Medical treatment and ART

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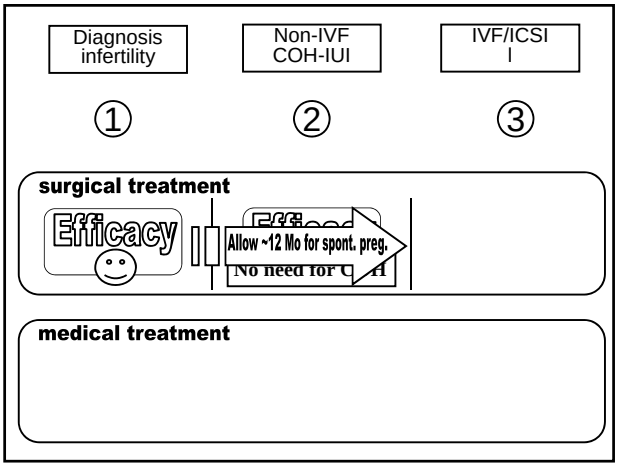


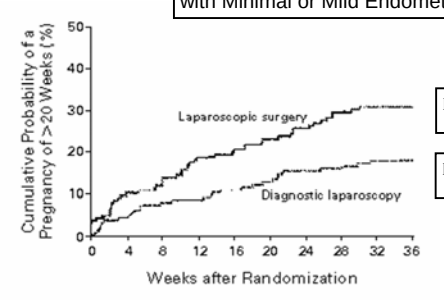
1 Consider Sx + 6-18 Mo in vivo

a
b
c

Marcoux S et al. *NEJM 1997;337:217-22.*

The NEW ENGLAND JOURNAL of MEDICINE

Laparoscopic Surgery in Infertile Women with Minimal or Mild Endometriosis.



preg x 8 Mo: 60.8%

preg x 8 Mo: 18.5%

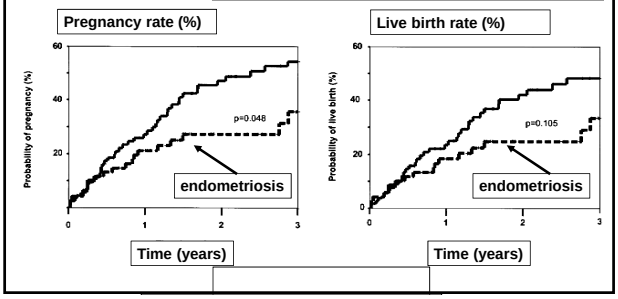
---

1 Consider Sx + 6-18 Mo in vivo

a
b
c

Akande VA, et al. *Hum Reprod. 2004;19:96-103.*

PR and Live birth rates in case of treated endometriosis.



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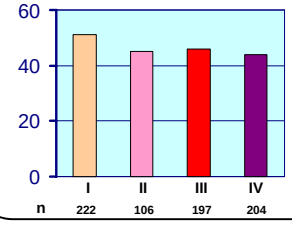
1 Consider Sx + 6-18 Mo in vivo

a
b
c

Vercellini P, et al. *Hum Reprod. 2006;21:2679-85.*

Classical staging of endometriosis fails to predict impact on reproductive outcome.

Cumulative pregnancy rates (3 years)



Conclusion

The current classification of endometriosis has an inadequate predictive value.

1 Consider Sx + 6-18 Mo in vivo

2 avoid COH-IUI

Does endometriosis alter the outcome of COH-IUI?

---

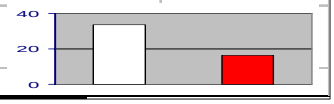
1 Consider Sx + 6-18 Mo in vivo

2 avoid COH-IUI

Does endometriosis alter the outcome of COH-IUI?

Yes

Omland AK et al. *Human Reprod 1998;13:2602-5.*

	Unexplained infertility	*St. I-II endometriosis*
number pts	119	49
1st COH (CC-hMG or FSH/hMG) PR (%)		

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1 Consider Sx + 6-18 Mo in vivo

2 avoid COH-IUI

Does endometriosis alter the outcome of COH-IUI?

No

Werbrouck E et al. *Fertil Steril 2006;86:566-71.*

In surgically treated minimal to mild endometriosis

n = 107
259 cycles

↗ Edom. n = 58, 137 cycles 21.0%

↘ 0 Endo n = 49, 122 cycles 20.5%

Conclusion

COH and IUI shortly after laparoscopic TT of endometriosis is as effective as in patients with unexplained subfertility.

1 **Consider Sx + 6-18 Mo in vivo**

2 avoid COH-IUI

Does endometriosis alter the outcome of COH-IUI?

Yes/No ???

Our approach at Cochin :

In principle, we do not offer COH-IUI in case of documented endometriosis.

- ⟶ Spontaneous PR is good.
- ⟶ Advantage of COH not proven.
- ⟶ May harm.
- ⟶ If no pregnancy, straight to IVF.

Diagnosis infertility | Non-IVF COH-IUI | IVF/ICSI I

① ② ③

surgical treatment

Efficacy

Allow ~12 Mo for spont. preg.

No need for C H

A **medical treatment**

No benefit
med treatment is contraceptive

Endometriosis: med TT and ART

medical treatment

In vivo

In vitro

Cochrane database 2008

Ovulation suppression for endometriosis (Review)

Hughes E., Brown J., Collins JJ., Farquhar C., Fedorkow DM., Vandekerckhove

Objective

Effects of ovarian suppression on in vivo conception

Endometriosis: med TT and ART

medical treatment

In vivo

Cochrane database 2008

Ovulation suppression for endometriosis (Review)

Hughes E, Brown J, Collins JJ, Farquhar C, Fedorkow DM, Vandekerckhove

Objective

Effects of ovarian suppression on in vivo conception

Conclusion

Ovarian suppression has no benefit in subfertile women with endometriosis who wish to conceive

In vitro

Course 4: **Endometriosis and Infertility**
Ovarian and Endometrial Factors

Medical treatment and ART

1. Endometriosis and reproduction
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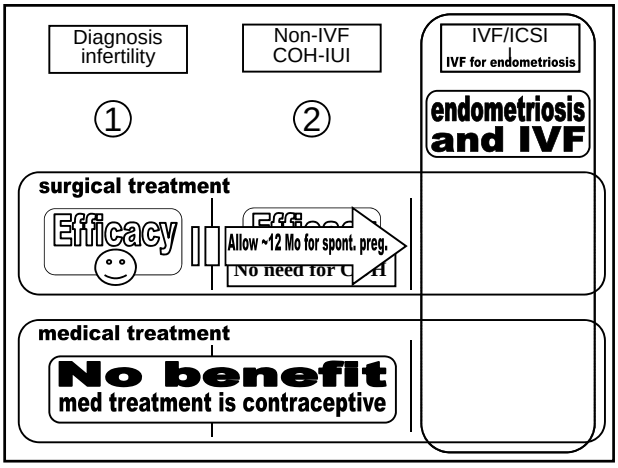
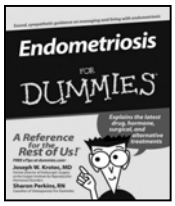


a Ovarian Endometriosis and IVF

b Medical treatment and IVF

c Surgical treatment and IVF



Ovarian Endometriosis and IVF

i) No impact

Geber S. et al. *Hum Reprod. 1995;10:1507-11.*
Olivennes F. et al. *Fertil Steril. 1995;64:392-8.*
Dmowski et al. *Fertil Steril 1995;63:555-62.*

ii) Dedreased ovarian response, but no impact on IVF outcome

Bergendal A. et al. *J Assist Reprod Genet. 1998;15:530-4.*
Pal L. et al. *J Assist Reprod Genet. 1998;15:27-31.*
Al-Azemi et al. *Human Reprod 2000;15:73-5.*
dos Reis RM. et al. *J Assist Reprod and Genet. 2004;21:311-4.*
Suzuki T. et al. *Fertil Steril 2005;83:908-13.*

iii) Dedreased IVF outcome ./· Severity of endometriosis

Matson and Yovich *Fertil Steril 1986;46:432-4.*
Oehninger et al. *J in Vitro Fert Embryo Transfer 1988;5:249-56.*
Benhart K. et al. *Fertil Steril 2002;77:1148-55.*

Ovarian Endometriosis and IVF

ii) Dedreased ovarian response, but no impact on IVF outcome

Pal L et al. *J Assist Reprod Genet. 1998;15:27-31.*

Retrospective analysis

	Group A (stage I-II)	Group B (stage III-IV)	P
Fertilization (%)	81.3	68	0.004
Cleavage (%)	93.3	88	NS
# emb transf	4	4	NS
IR (%)	19%	25.40%	NS
PR (%)	62.20%	54%	NS
Clin PR (%)	52.10%	38.50%	NS
Miscarriages (%)	13%	13%	NS

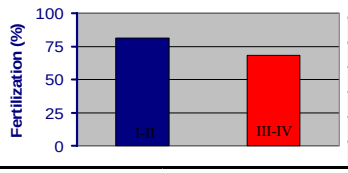
Ovarian Endometriosis and IVF

ii) Dedreased ovarian response, but no impact on IVF outcome

Pal L et al. *J Assist Reprod Genet. 1998;15:27-31.*

Retrospective analysis

	Group A (stage I-II)	Group B (stage III-IV)	P
Fertilization (%)			0.004
Cleavage (%)			NS
# emb transf			NS
IR (%)			NS
PR (%)			NS
Clin PR (%)			NS
Miscarriages (%)			NS



Ovarian Endometriosis and IVF

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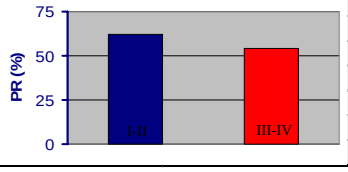
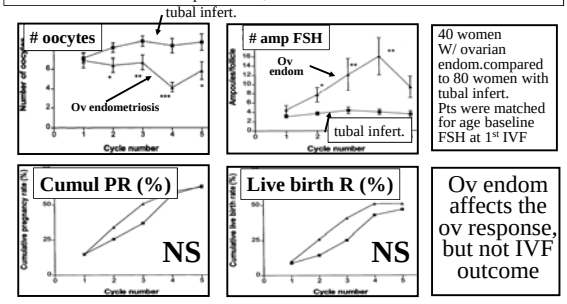


Ovarian Endometriosis and IVF

ii) Dedreased ovarian response, but no impact on IVF outcome

Al-Azemi et al. *Human Reprod 2000;15:73-5.*



40 women W/ ovarian endom.compared to 80 women with tubal infert. Pts were matched for age baseline FSH at 1st IVF

Ov endom affects the ov response, but not IVF outcome

Ovarian Endometriosis and IVF

Ovarian Endometriosis and IVF

iii) Decreased IVF outcome

Matson and Yovich *Fertil Steril 1986;46:432-4.*

Oehninger et al. *J in Vitro Fert Embryo Transfer 1988;5:249-56.*

Barnhart K et al. *Fertil Steril. 2002;77:1148-55.*

Meta-anlysis 22 published studies
Reduced chances of getting pregnant in case of endometriosis
Endo: Odd ratio (95%CI): → 0.56 (0.44, 0.7)
Severe endo: Odd ratio (95%CI): → 0.60 (0.42, 0.87)

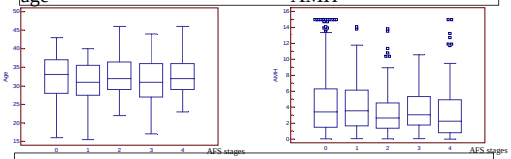
Possible adverse impact on:

1. Oocyte quality
2. Ovarian reserve
3. Implantation

Ovarian Endometriosis and IVF

AMH and ART

age AMH



Conclusion

AMH levels are lower in case of endometriosis but in proportion to decreased ovarian response

Course 4: **Endometriosis and Infertility**
Ovarian and Endometrial Factors

Medical treatment and ART

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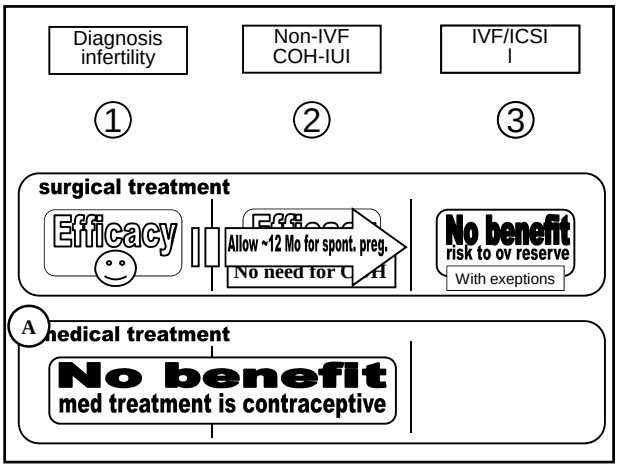


surgery before ART?

Dedreased IVF outcome

Aboulghar MA et al. The outcome of in vitro fertilization in advanced endometriosis with previous surgery: **a case-controlled study**. *Am J Obstet Gynecol. 2003;188:371-5.*

Case control study in pts w/ stage IV endometriosis

	Group A	Group B	P	OR
	(Stage IV endom)	(Tubal infert)		
nb patients	n = 85	n = 177		
Cx for poor resp.	29,70%	1,10%	<0.0001	26.03 (6.02, 112.45)
Clinical PR15.3%	15,30%	52,50%	<0.0001	0.29 (0.15, 0.55)

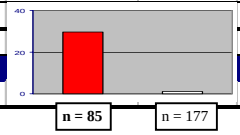
surgery before ART?

Dedreased IVF outcome

Aboulghar MA et al. The outcome of in vitro fertilization in advanced endometriosis with previous surgery: a case-controlled study. *Am J Obstet Gynecol. 2003;188:371-5.*

Case control study in pts w/ past Hx for stage IV endometriosis

	Group A	Group B	P	OR
nb patients				
Cx for poor resp.			<0.0001	26.03 (6.02, 112.45)
Clinical PR15.3%			<0.0001	0.29 (0.15, 0.55)



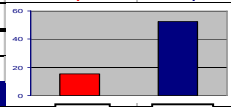
surgery before ART?

Dedreased IVF outcome

Aboulghar MA et al. The outcome of in vitro fertilization in advanced endometriosis with previous surgery: a case-controlled study. *Am J Obstet Gynecol. 2003;188:371-5.*

Case control study in pts w/ stage IV endometriosis

	Group A	Group B	P	OR
nb patients				
Cx for poor resp.			<0.0001	26.03 (6.02, 112.45)
Clin. PR			<0.0001	0.29 (0.15, 0.55)



Diagnosis infertility ① | Non-IVF COH-IUI ② | IVF/ICSI ③

surgical treatment

Efficacy

Allow ~12 Mo for spont. preg.

No need for C…H

No benefit
risk to ov reserve

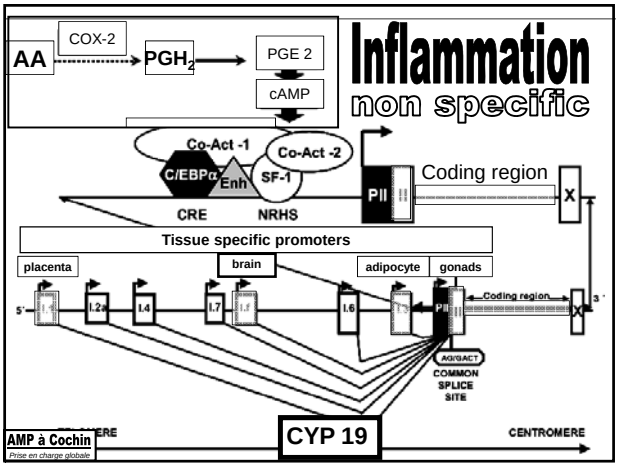
With exeptions

A…hedical treatment

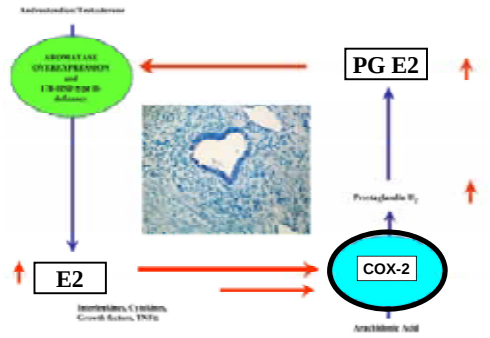
No benefit
med treatment is contraceptive

improves IVF outcome

Inflammation non specific



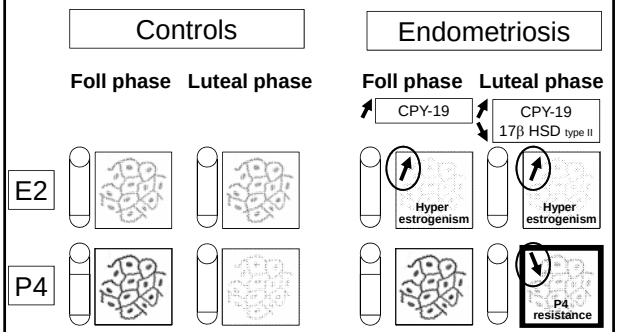
E2 and P4: Serum and tissue levels



AMP à Cochin
Prise en charge globale

Ebert et al, E J Obstet Gynecol 2005

E2 and P4: Serum and tissue levels

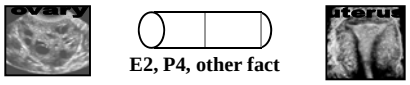


AMP à Cochin
Prise en charge globale

P4 resistance

In Endometriosis, altered E2 & P4 effects

Uterus in endometriosis



Endometriosis

in vivo

IVF

Pelvic cavity

Uterus

In Endometriosis, altered E2 & P4 effects

Uterus in endometriosis

E2, P4, other fact

Endometriosis

Ovarian impact

Uterus

in vivo

Fertilization:
intra pelvic disease
sperm ➡ oocyte

IVF

Ovarian function:
reduced quantity
preserved quality (?)

Adjust COH protocol as per ovarian reserve
(we use micro-flare protocol, as per Schoolcraft et al.)

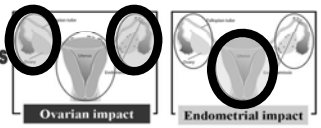
Retrievals of 2 oocytes may be successful

In Endometriosis, altered E2 & P4 effects

Uterus in endometriosis

E2, P4, other fact

Endometriosis



Ovarian impact

Endometrial impact

in vivo

Fertilization:
intra pelvic disease
sperm ➡ oocyte

Fertilization
is normal

IVF

Ovarian function:
reduced quantity
preserved quality (?)

Implantation:
altered in IVF
pre IVF treatment

In Endometriosis, altered E2 & P4 effects

Uterus in endometriosis

E2, P4, other fact

Endometriosis

Ovarian impact

Endometrial impact

in vivo

IVF

Ovarian function:
reduced quantity
preserved quality (?)

Implantation:
altered in IVF
pre IVF treatment

Inflammation

Effects of uterus on endometriosis:

- ***Invasive cells***
- ***Poor receptivity***

In Endometriosis, altered E2 & P4 effects

Uterus in endometriosis

Nerve fibers

Different types of small nerve fibers in eutopic endometrium and myometrium in women with endometriosis

(Fertil Steril® 2007;88:795–803.)

Natsuko Tokushige, Ph.D.,[a] Robert Markham, Ph.D.,[a] Peter Russell, M.D.,[b] and Ian S. Fraser, M.D.[a]

Aromatase

(*Endocrinology* 149: 1190–1204, 2008)

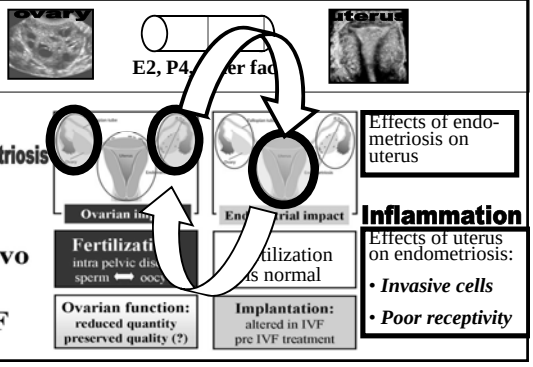
Inflammatory Status Influences Aromatase and Steroid Receptor Expression in Endometriosis

Orhan Bukulmez, Daniel B. Hardy, Bruce R. Carr, R. Ann Word, and Carole R. Mendelson

Departments of Obstetrics and Gynecology (O.B., B.R.C., M.A.W., C.R.M.) and Biochemistry (D.B.H., C.R.M.), The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390

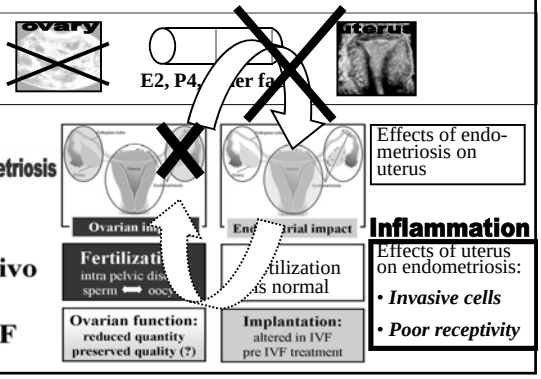
In Endometriosis, altered E2 & P4 effects

Uterus in endometriosis



In Endometriosis, altered E2 & P4 effects

Uterus in endometriosis



In Endometriosis, altered E2 & P4 effects

Uterus in endometriosis

Nerve fibers — Suppression of ovarian function

CLINICAL ARTICLE

Effects of hormonal treatment on nerve fibers in endometrium and myometrium in women with endometriosis

Fertil Steril® 2007

Natsuko Tokushige, Ph.D.,[a] Robert Markham, Ph.D.,[a] Peter Russell, M.D.,[b] and Ian S. Fraser, M.D.[a]

Aromatase — Suppression of ovarian function

ENDOMETRIOSIS

Gynecological Endocrinology, March 2008; 24(3): 123–128

The effect of oral contraceptives on aromatase expression in the eutopic endometrium of patients with endometriosis

HUGO MAIA JR[1], JULIO CASOY[1], TÂNIA CORREIA[2,3], LUIS A. FREITAS[3], KLEBER PIMENTEL[3], & CÉLIA ATHAYDE[1]

ART @ Cochin
Global management

Endometriosis: med TT and ART

medical treatment

In vivo

Cochrane database 2008

Ovulation suppression for endometriosis (Review)

Hughes E., Brown J., Collins JJ., Farquhar C., Fedorkow DM., Vandekerckhove

Objective

Effects of ovarian suppression on in vivo conception

Conclusion

Ovarian suppression has no benefit in subfertile women with endometriosis who wish to conceive

In vitro

Cochrane database 2006

Long-term pituitary down-regulation before in vitro fertilisation (IVF) for women with endometriosis (Revie

Sallam HN, García-Velasco JA, Dias S, Arici A

Objective

Effectiveness of administering GnRH-a for 3-6 months prior to IVF/ICSI with endometriosis.

Conclusion

In women with endometriosis, GnRH-a for x 3-6 Mo prior to IVF or ICSI increases PR x4.

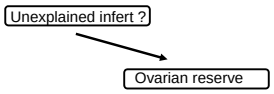
Course 4: **Endometriosis and Infertility**
Ovarian and Endometrial Factors

Medical treatment and ART

1. Endometriosis and reproduction
2. Medical vs. surgical treatment: Which comes first?
3. Endometriosis and ART
4. Medical treatment and/or surgery before ART?

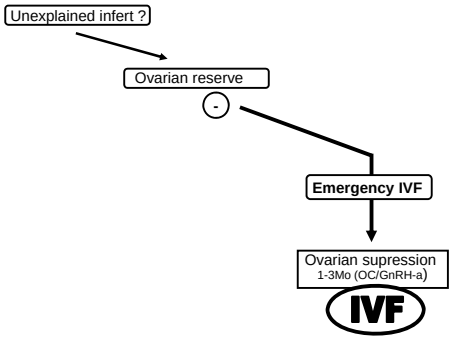
Conclusion

Endometriosis: med TT and ART



ART @ Cochin
Global management

Endometriosis: med TT and ART



ART @ Cochin
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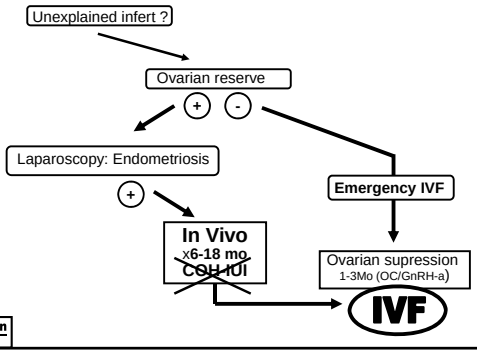
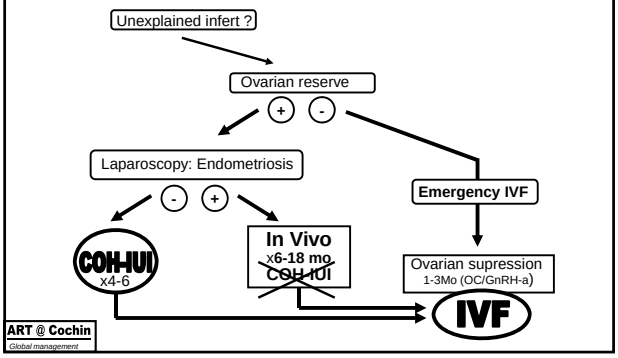
Endometriosis: med TT and ART

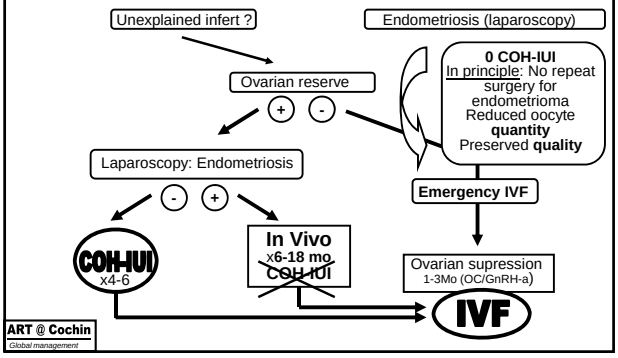


ART @ Cochin
Global management

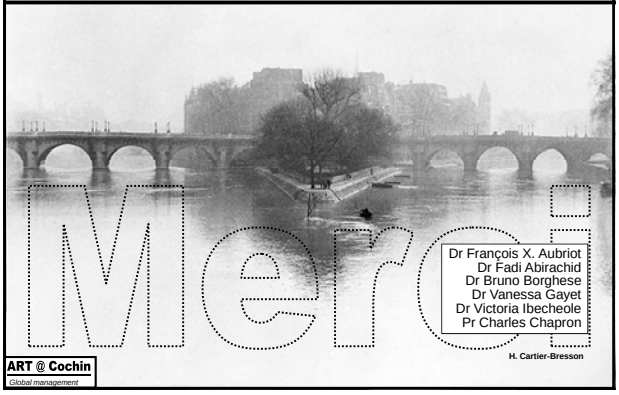
Endometriosis: med TT and ART



Endometriosis: med TT and ART

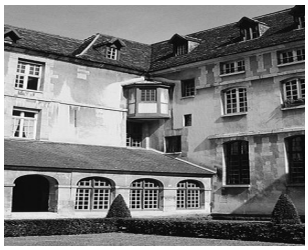


Endometriosis: med TT and ART



Endometriosis

Principles and results of surgical treatment



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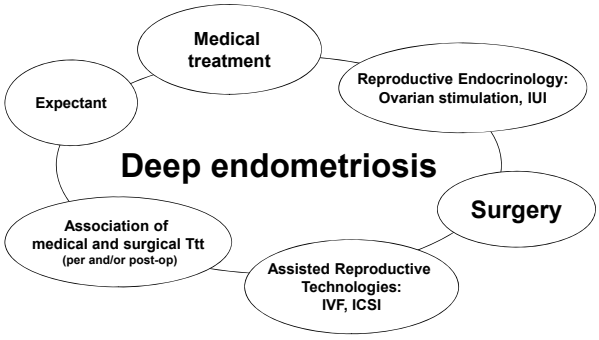
Disclosure

Charles Chapron, M.D.

No conflict of interest

Deep endometrisois: *Management options*



Deep endometrisois

Management options

- Medical treatment
- Assisted Reproductive Technology
- Surgery

Deep endometrisois

Management options

- Medical treatment
- Assisted Reproductive Technology
- Surgery

Endometriosis - associated pain Medical treatment

Human Reproduction Vol.20, No.10 pp. 2698–2704, 2005
Advance Access publication June 24, 2005.

Kennedy *et al.*, (2005)

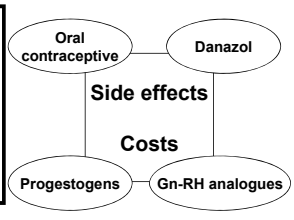
ESHRE guideline for the diagnosis and treatment of endometriosis

Suppression of ovarian function for 6 months reduces endometriosis-associated pain.

The hormonal drugs investigated (COC, danazol, gestrinone, medroxyprogesterone acetate and GnRH agonists) are equally effective

(Moer *et al.*, 2004; Prentice *et al.*, 2004; Selak *et al.*, 2004)

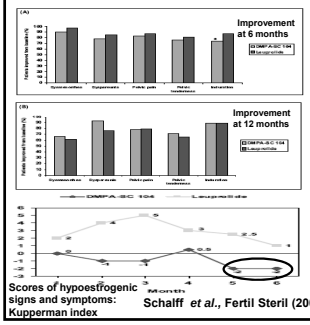
A Evidence: Level 1a



Endometriosis and pelvic pain:

Comparison sides effects of medical Tttt

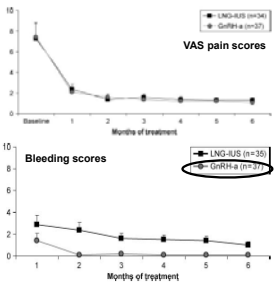
DMPA-sc versus GnRH-a



Scores of hypoestrogenic signs and symptoms: Kupperman index

Schalff *et al.*, Fertil Steril (2006)

LNG-IUD versus GnRH-a



Petta *et al.*, Hum Reprod (2005)

Deep endometriosis: Medical treatment

Authors	N	Route	Products
Igarashi *et al., (1998)*	56	Vaginal ring	Danazol
Fedele *et al., (2000)*	15	IM	GnRH analogs
Fedele *et al., (2001)*	11	IUD	Levonorgestrel
Hefler *et al., (2005)*	10	Vaginal suppository	Anastrozole (IA)

Deep endometriois: Medical treatment

Vaginal danazol ring

Novel vaginal danazol ring therapy for pelvic endometriosis, in particular deeply infiltrating endometriosis

DIE	N	Disappeared	Reduced	Unchanged
DIE volume	42	36 (86%)	6 (14%)	0 (0%)
DM	42	32 (76%)	9 (22%)	1 (2%)

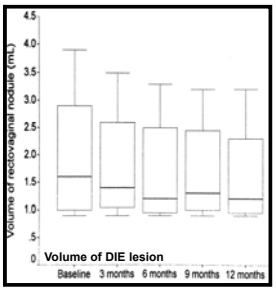
Igarashi *et al.*, Hum Reprod (1998)

Deep endometriois: Medical treatment

Levonorgestrel - releasing IUD (LNG – IUD):

Symptoms	Baseline	3 months	6 months	9 months	12 months
Dysmenorrhea					
Absent	0	8	10	11	11
Mild	0	3	1	0	0
Moderate	3	0	0	0	0
Severe	8	0	0	0	0
Pelvic pain					
Absent	6	9	11	11	11
Mild	1	2	0	0	0
Moderate	4	0	0	0	0
Severe	0	0	0	0	0
Dyspareunia					
Absent	1	3	3	5	5
Mild	2	5	7	6	6
Moderate	5	3	1	0	0
Severe	3	0	0	0	0

Changes in symptoms after LNG-IUD insertion



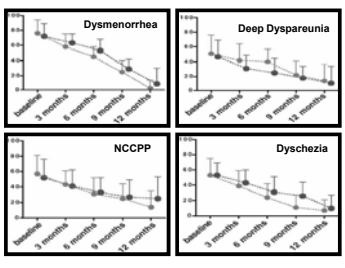
Fedele et al., Fertil Steril (2001)

Recurrent deep endometriosis after surgery: Medical treatment

Authors	N	Route	Products
Vercellini *et al., (2005)*	45	Oral	Continuous Ethinyl E2 + Cyproterone Acetate
Vercellini *et al., (2005)*	45	Oral	Norethindrone acetate
Razzi *et al., (2007)*	21	Vaginal	Danazol

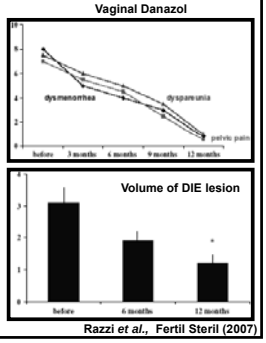
Recurrent deep endometriosis after surgery: Medical treatment

Continuous Ethinyl E2 + Cyproterone Acetate
versus
Norethindrone acetate



Vercellini *et al.*, Fertil Steril (2005)

Vaginal Danazol



Razzi *et al.*, Fertil Steril (2007)

Deep endometrisois

Management options

- Medical treatment

- Assisted Reproductive Technology

- Surgery

Deep endometriosis and Infertility:

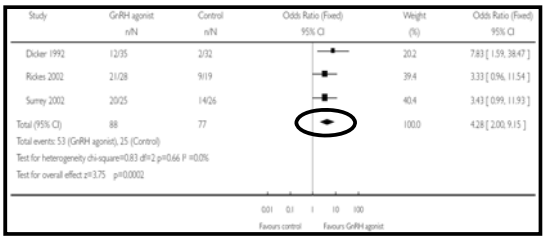
IVF results (n = 60)

	DIE	Endometriosis
- N punctions	122	593
- Mean no. of oocytes	9.8+/-6.8	10.6+/-7.3
- Fertilization rate	50.3 %	49.0 %
- Transfer rate	85.2 %	85.1 %
- Mean no. of embryo transfered	2.50+/-0.8	2.5+/-0.8
- Clinical P / punction	29.7 %	30.1 %
- Clinical P / transfer	34.6 %	35.4 %
- Delivery / punction	24.5 %	25.6 %
- Delivery / transfer	28.8 %	30.0 %

Pouly *et al.*, RHH (2000)

Endometriosis and infertility

Long term pituitary down-regulation before IVF for women with Osis



Sallam *et al.*, Cochrane (2006)

Deep endometrisois

Management options

- Medical treatment

- Assisted Reproductive Technology

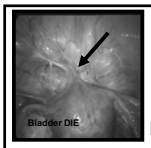
- Surgery

Surgery for DIE: Radical excision

Symptoms	Pre-op	Post-op	Delta
DM*	8.1 ± 1.8	2.8 ± 3.1	5.2 ± 3.5
DP*	6.5 ± 2.2	1.9 ± 2.6	4.6 ± 3.0
Painful defecation*	6.6 ± 2.4	2.1 ± 2.8	4.5 ± 3.5
Urinary tract S.*	6.1 ± 2.1	1.2 ± 2.6	4.9 ± 3.2
Gastrointestinal S.*	6.8 ± 2.2	2.7 ± 3.1	4.1 ± 3.5
CPP*	7.5 ± 1.6	2.8 ± 3.6	4.8 ± 3.4

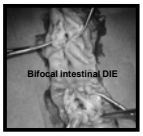
* : p < 0.001

Chopin – Chapron J Minim Invasive Gynecol (2005)



Surgery for deep endometrisois

Objective evaluation:
Pre versus postoperative pain score



		N	DM	DP	NCCPP
Anaf	2001	26	< 0.0001	< 0.001	< 0.001
Wright	2001	28	< 0.0001	< 0.0001	< 0.0001
Redwine	2001	67	< 0.0005	< 0.0005	< 0.0005
Abbott – Garry	2003	135	< 0.0001	< 0.0001	< 0.0001
Thomassin – Daraï	2004	27	< 0.0001	0.0002	0.001
Chopin – Chapron	2005	152	< 0.001	< 0.001	< 0.001

Endometriosis - associated pain

Post-operative treatment with GnRH

PRT

Authors (Year)	Type of study	Therapy group (Subjects)	Control group (Subjects)	Measurement parameters	Results (Therapy vs Control group)	Statistics
Parazzini et al. (1994)	RCT	36	39	Change in the 10-point pain scale value nine months after treatment	−7.0 ± 4.1 vs −6.9 ± 4.6	ns
Busacca et al. (2001)	RCT	44	45	Recurrence rate during follow up	23% vs 24%	ns
				Recurrence rate 18 months after treatment	23% vs 29%	ns
Hornstein et al. (1997)	RCT	56	53	Rate at which alternative therapy was required	31% vs 57%	sig.
				Time until alternative therapy was required (months)	> 24 vs 11.7	sig.
				Change in the three-point pain scale value post-therapy	−3.2 ± 2.7 vs −1.0 ± 2.3	nd
				Change in the three-point pain scale value six months after treatment	−1.5 ± 2.7 vs −1.1 ± 2.6	nd
Vercellini et al. (1999)	RCT	133	134	Recurrence rate one year after treatment	13.1% vs 21.4%	ns
				Recurrence rate two years after treatment	23.5% vs 36.5%	ns
				Time to recurrence according to survival analysis	$\chi^2 = 4.19$ (therapy > control)	sig.

Six-month post-operative administration of GnRH analogues significantly delayed the time to pain recurrence after conservative surgery.

Ozawa et al., Tohoku J Exp Med (2006)

Endometriosis III and IV - associated pain

Post-operative treatment after conservative surgery

PRT

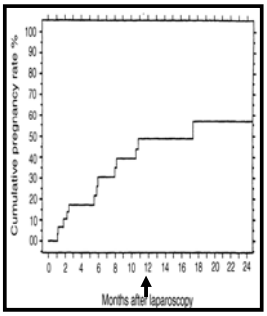
Pains scores at 12 months Symptom	Placebo (n = 110)	GnRH-a (n = 39)		Estroprogestin (n = 38)
Dysmenorrhoea				
Baseline value	7.9 ± 1.2	7.7 ± 1.0		8.2 ± 1.1
12-month value	6.4 ± 1.3	5.9 ± 0.9	=	5.5 ± 1.2
Nonmenstrual pelvic pain				
Baseline value	8.0 ± 1.4	8.4 ± 0.9		8.5 ± 0.8
12-month value	6.2 ± 0.9	5.0 ± 1.1	=	5.0 ± 0.8
Deep dyspareunia				
Baseline value	6.8 ± 1.2	6.9 ± 1.0		6.8 ± 1.2
12-month value	4.8 ± 1.2	4.3 ± 1.2	=	4.5 ± 1.3

Sesti *et al.*, Fertil Steril (2007)

Deep endometriosis infiltrating the USL:

Fertility results *(n = 30 patients)*

IUP	N	%
- Spontaneously	11	36.7
- Ovulation induction	3	10.0
	46.7% - 93.3%	
- IVF	1	3.3
TOTAL	15	50.0



Chapron *et al.*, Hum. Reprod. (1999)

Intestinal deep endometriosis :

Fertility results of surgical treatment by laparotomy

	IUP		
	N	n	%
Coronado, 1990	33	13	39.4
Bailey, 1992	49	24	48.9

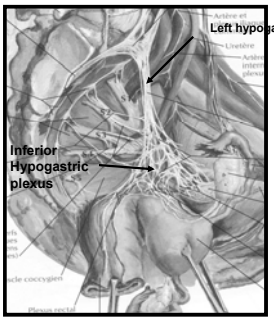
Fertility after laparoscopic colorectal resection for endometriosis

	Desire for P	Pregnant
	N	n
Nezhat *et al. (1992)*	8	1
Jerby *et al., (1999)*	7	3
Possover *et al. (2000)*	15	8
Redwine and Wright *(2001)*	28	12
Darai *et al. (2005)*	22	10
Lyons *et al. (2006)*	3	3
Total	83	37 (44.7%)

Major intestinal complications after laparoscopic colorectal resection for deep endometriosis

	Patients	Major complications	
	N	n	
Possover *et al. (2000)*	34	4	
Keckstein *et al. (2003)*	142	6	
Ribeiro *et al. (2006)*	125	2	
Darai *et al. (2007)*	71	9	
Mereu *et al. (2007)*	192	32	
Abrao *et al. (2007)*	110	3	
Total	674	56	8.3%

Laparoscopic Nerve sparing complete excision of DIE

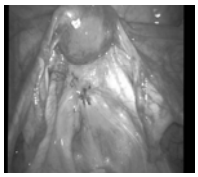


	Laparoscopic Nerve Sparing		
	No (n = 20)	Yes (n = 25)	
Mean time to resume the voiding function	12.5	3.0	0.01
Very satisfied	59.0%	87.7%	0.013

Landi *et al.*, Hum Reprod (2006)

Surgical management of DIE

n = 135 Follow-up 3.2 years *(range 2 - 5)*



Complete pouch of Douglas obliteration

Rate of further surgery at 60 months **36 %**

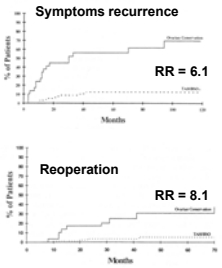
Risk factors (multivariate analysis)

rAFS score > 70 **p < 0.03**

Abott – Garry *et al.*, Hum. Reprod. (2003)

Cumulative incidence of symptom recurrence and reoperation after HT for endometriosis

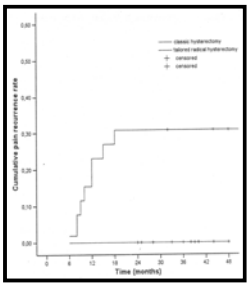
	Hysterectomy (n = 138)			
	Ovarian preservation (n = 29)		BSO (n = 109)	
Recurrent pain	18	62%	11	10%
Required reoperation	9	31%	4	3.7%



Namnoum *et al.*, Fertil Steril (1995)

Deeply infiltrative endometriosis :
Modalities for non conservative surgery

Hysterectomy	Pain recurrence N	n	%
Standard extrafascial	26	8	31
Tailored radical	12	0	0



Fedele *et al.*, AJOG (2005)

Deep endometriosis:
Surgical treatment and risk of recurrence
(Multivariate analysis)

Recurrence		OR	95%CI	p
Pain	Age	0.9	0.81 – 0.99	< .05
Clinical signs	Obliteration of pouch of Douglas	1.46	1.16 – 16.2	< .05
Reoperation for DIE	Incompleteness of 1st surgery	21.9	3.2 – 146.5	< .001

Vignali *et al.*, J Min Inv Gynecol (2005)

Deep endometriosis:
Surgical treatment and risk of recurrence
(Multivariate analysis)

Reoperation for DIE

n	%
12	10.4

In those women who underwent a second operation, the recurrence of deep endometriosis was observed in the same aera of the pelvis involved in the first operation

Vignali *et al.*, J Min Inv Gynecol (2005)

Deep endometriosis
Questions on the surgical treatment

All these arguments are in favor of radical treatment if surgery is decided.

Surgical management for DIE
Risk factors for recurrences

AGE *(years)*	36 months actuarial rate of recurrence *(reoperation or medical Ttt)*	P
< 25	54	
26 - 30	23	0.007
31 - 35	13	
> 35	0	

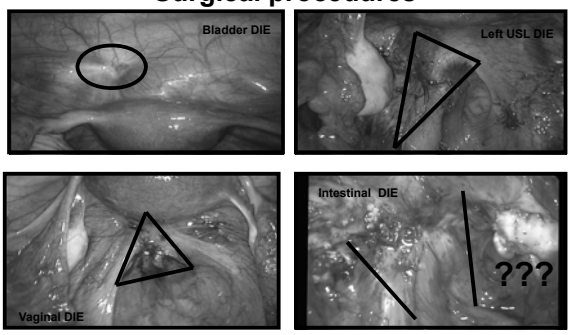
Fedele *et al.*, (2004)

Importance of the patient's age:
MAJOR risk factors for recurrences

Three hypothesis:

- **Higher duration of exposition to retrograde menstruation**
- **More aggressive endometriosis: Heterogeneity**
- **Inadequate previous surgical management**

Surgery for deep endometriosis
Surgical procedures

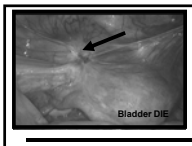


Laparoscopic colorectal surgery for deep endometriosis
Surgical procedures

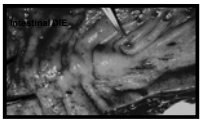
	Segmental resection	Full-thickness disc excision	Superficial thickness excision
Nezhat *et al. (1992)*	10	5	0
Redwine and Wright, *(2001)*	6	21	23
Jerby *et al., (1999)*	7	5	18
Possover *et al. (2000)*	34	0	0
Duepree *et al. (2006)*	18	5	26
Darai *et al. (2005)*	40	0	0
Campagnaci *et al. (2005)*	3	4	0
Ribeiro *et al. (2006)*	115	2	8
Panel *et al. (2006)*	18	3	0
Jatan *et al. (2006)*	14	20	61
Lyons *et al. (2006)*	7	0	0
Total	272 57%	65	201 = 43% 136

!!!

Adaptated from Daraï *et al.*, Curr Opin Obstet Gynecol (2007)



Surgical management of Deep Osis

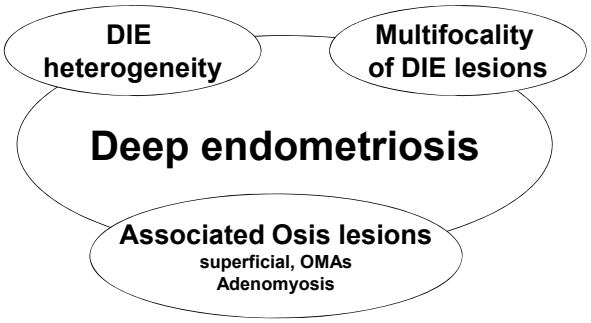


OBJECTIVE EVALUATION:
Pre versus postoperative pain score according to the surgical classification

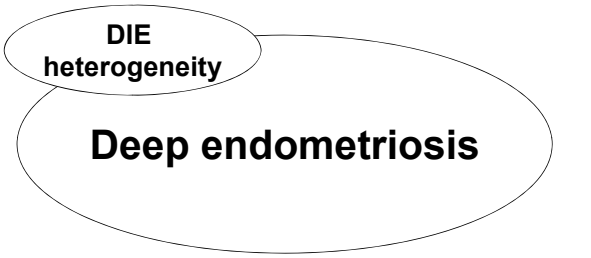
	USL	Vagina	Bladder	Intestine
DM	< 0.0001	0.0001	0.0022	0.0004
DP	< 0.0001	0.0001	0.0117	0.0015
P Defecation	0.0001	0.0007	0.0679	0.0033
Lower urinary TS	0.0011	0.0679	0.022	0.0679
NCCPP	< 0.0001	0.0171	0.01	0.0277

Chopin – Chapron *et al.*, J Min Inv Gynecol (2005)

Deep endometrisois: *Characteristics*



Deep endometrisois: *Characteristics*

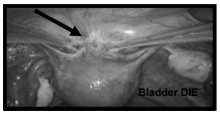


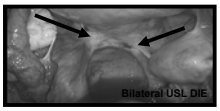
Deep endometriosis: Location heterogeneity

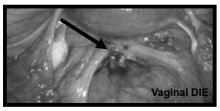
Main DIE lesion	N	rAFS stage I	II	III	IV
USL	279	70	108	59	42
Vagina	93	9	42	19	23
Bladder	51	9	20	8	14
Intestine	184	0	28	19	137
Ureter	29	0	2	0	27
Total	636	88	200	105	243
		45%		55%	

Chapron *et al.*, (2008)

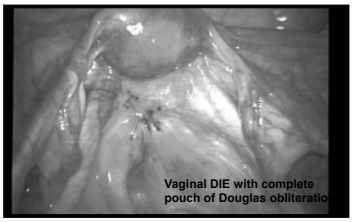
Deep endometriosis: Location heterogeneity







rAFS Stages I and II DIE



Deep endometriosis is not synonymous of rAFS stage IV endometriosis ++++

Deep endometriosis: Painful heterogeneity

Is rectovaginal endometriosis a progressive disease?

Luigi Fedele, MD,[a,*] Stefano Bianchi, MD,[b] Giovanni Zanconato, MD,[c] Ricciarda Raffaelli, MD,[c] Nicola Berlanda, MD[a]

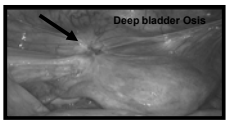
Prospective observational study
88 patients with untreated asymptomatic DIE
Median follow-up time: 5.7 years (1 – 9)
No DIE treatment during laparoscopy
Peritoneal and ovarian lesions fully treated
DIE lesions biopsied

Progression of disease and/or appaerance of pain symptoms attributable to DIE:
6 patients; 6.8% 95% CI: 1.9% - 11.7%

Estimated cumulative proportion of patients with progression of disease and/or appearanceof pain symptoms attributable to DIE after 6 years: 9.7%

Fedele *et al*, AJOG (2004)

Deep endometriosis
Painful heterogeneity



Bladder Osis

N = 74

Follow-up: 59.2 ± 44 months
(range 4 – 180)

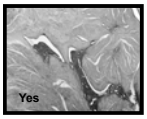
	N	%	Reoperation for recurrence %
Isolated	28	37.8	0
Associated posterior DIE	47	66.2	
Symptomatic (Surgical exeresis)	33	44.6	0
No symptoms (NO Surgical exeresis)	13	17.6	0

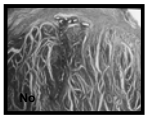
Chapron - Bourret *et al.*, (2008)

Deep endometriosis: Histological heterogeneity

Anatomopathological lesions of bladder endometriosis are heterogeneous

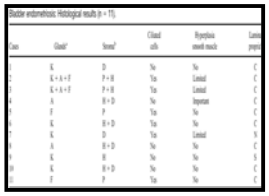
Charles Chapron, M.D.,[a] Eric Boucher, M.D.,[b] Arnaud Fauconnier, M.D.,[a] Marco Vieira, M.D.,[a] Jean-Bernard Dubuisson, M.D.,[a] and Marie-Cécile Vacher-Lavenu, Ph.D.[b]





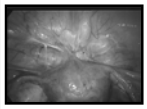
Bladder DIE: Adenomyotic nodule

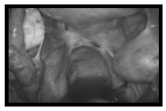
Bladder muscularis propria	%
Hyperplasia of the fibromuscular tissu	36.4
Focal	27.4
Considerable	9.0
Simple dissociation of the smooth muscle bunches with no true « disorganization »	36.4
Simple densification of the interstitial collagen structure or sclerosis	27.2



Chapron *et al.*, Fertil Steril (2002)

Deep endometriosis

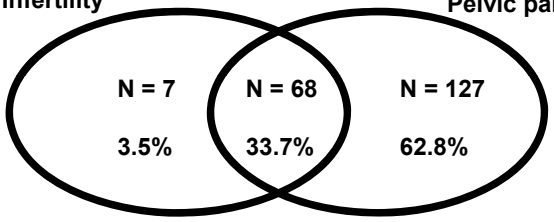




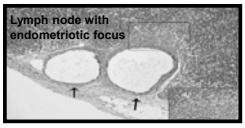
Clinical heterogeneity

CHU Cochin'experience
January 2005 – February 2008
Prospective study: n = 202 DIE

Infertility — **Pelvic pain**



Chapron *et al.*, (2008)



Deep endometriosis:

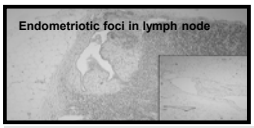
Lymphatic spread theory ?
Pathogenesis heterogeneity

Distribution of the patients according to lymph node involvement and the histological characteristics of the lesion.

Characteristic	Lymph node positive	Lymph node negative	*P*
Patients (n, %)	5 (26.5%)	14 (73.8)	
Age (yrs)	36.2 ± 3.7	35.5 ± 1.0	ns
Length (cm)	5.50 ± 3.221	2.98 ± 1.327	.095
Thickness (cm)	1.380 ± 0.3962	0.923 ± 0.3345	.026
% of circumference affected	92.850 ± 14.3	50.818 ± 14.5	.003

Surgical procedure: Bowel resection

Abrao *et al.*, Fertil Steril (2006)



Deep endometriosis:

Lymphatic spread theory ?
Pathogenesis heterogeneity

Clinicopathologic findings of patients with and without lymph node involvement by endometriosis.

	Lymph node positive (n = 11)	Lymph node negative (n = 15)	P
Age, years	31 ± 6.3	31.5 ± 5.5	NS
Size, mm	31.27 ± 8.6	25.5 ± 8.7	<.05
Lymph nodes retrieved	8 ± 3	5 ± 3	<.05
Length of resected bowel, cm	15.09 ± 8.08	14.33 ± 5.72	NS
Lymphovascular invasion	4 (36.3%)	2 (13.3%)	<.05

Risk of recurrences?:
Associated medical treatments?
Pseudo chemotherapy +++

Noel – Chapron *et al.*, Fertil Steril 2008 (in press)

Deep endometriosis:

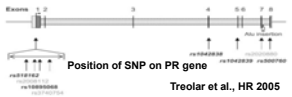
Hormonal heterogeneity

1995: Complex of the PR gene polymorphisms were designated as PROGINS

Advances in Brief

Ovarian Carcinoma-associated *Taq*I Restriction Fragment Length Polymorphism in Intron G of the Progesterone Receptor Gene Is Due to an Ala Sequence Insertion[1]

Susan M. Rowe, Sorcha J. Coughlan, Neil J. McKenna, Elaine Garrett, Dirk G. Kieback, Desmond N. Carney, and Denis R. Hanahoe[2]

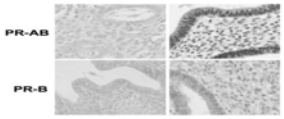


Position of SNP on PR gene

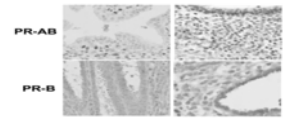
Treolar et al., HR 2005

Relationship Progins and Osis:
- Weiser *et al.*, Fertil Steril 2002
- Lattuada *et al.*, Clin Endocrinol 2004
- Treolar *et al.*, Mol Hum Reprod 2005
- van Kaam *et al.*, Hum Reprod 2006

Heterogeneous expression of Progesterone Receptor in Osis lesions



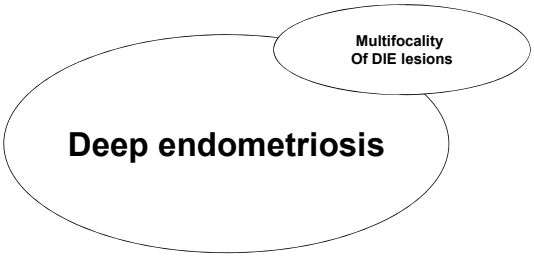
Patients with no polymorphism



Patients with PROGINS polymorphism

van Kaam *et al.*, Hum Reprod 2006

Deep endometrisois: *Characteristics*



Deep endometriosis: Anatomic distribution

(n = 426 patients)

Main lesion	N	Associated lesions							Total
		USL			Va	Bl	Ur	In	
		R	L	B					
BLADDER	37	2	1	3	3	37			49
USL	222	57	109	56					278
VAGINA	61	5	6	11	61				94
URETER	15	2	4	3	9	3	16	17	57
INTESTINE	91	12	12	22	50	8		155	281
	426	78	132	95	123	48	16	172	759

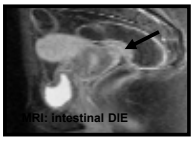
Multifocality +++

Chapron *et al.*, Hum Reprod (2006)

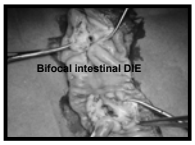
Intestinal endometriosis

Anatomic distribution

(n = 212 patients)



MRI: intestinal DIE



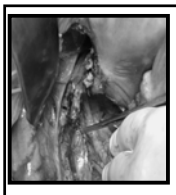
Main characteristics	N	%
- Isolated without other DIE lesions	23	10.8
- Multifocal intestinal DIE lesions	102	48.1
- Associated left/ right lesions	35	17.3

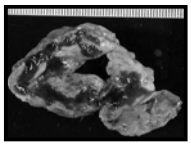
Chapron - Dousset (2008)

Ureteral endometriosis

Associated DIE lesions

(n = 29 patients)





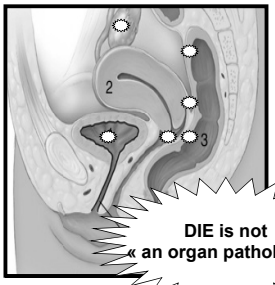
	Patients		DIE lesions
	N	%	N
USL	20	68.9	32
VAGINA	20	68.9	20
BLADDER	7	24.1	7
INTESTINE	28	96.5	46
URETER	29	100.0	34
Total	29		139

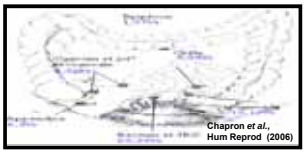
4.8 ± 1.9 (range 2 to 9)

Ureteral DIE is ALWAYS associated to other DIE lesions

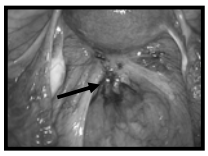
Chapron *et al.*, (2008)

Deep endometriosis
Global approach

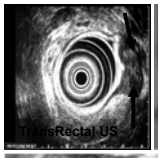


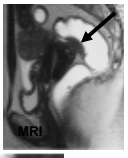


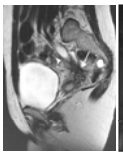
DIE is not
« an organ pathology »

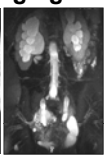


Deep endometriosis:
Preoperative work-up importance of imaging

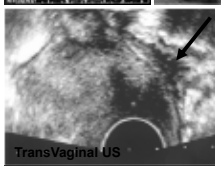


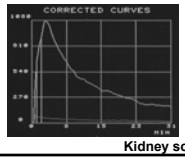


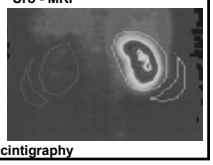




Uro - MRI



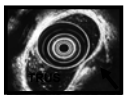


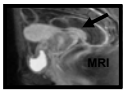


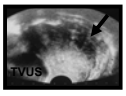
Kidney scintigraphy

Deep endometriosis: Rectal wall infiltration

	N	Se	Sp	PPV	NPV
TRUS					
Chapron *et al., (2004)*	81	97	89	87	98
Bazot *et al., (2007)*	81	89	93	96	81
Chapron *et al. (2008)*	134	96	100	100	95
MRI					
Chapron *et al., (2004)*	81	76	98	96	85
Abrao *et al., (2007)*	104	83	98	97	84
Bazot *et al. (2007)*	88	83	93	96	79
TVUS					
Abrao *et al. (2007)*	104	98	100	100	98
Bazot *et al., (2007)*	81	93	100	100	87
Chapron *et al. (2008)*	134	90	96	97	89

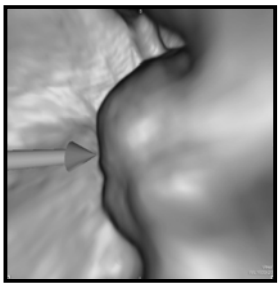


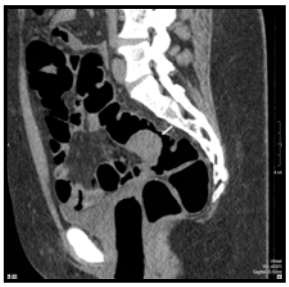




Deep endometriosis:

Future preoperative work-up: Virtual Colonoscopy





Van der Wat *et al.*, JMIG (2007)

Deep endometrisois: *Characteristics*

Deep endometriosis

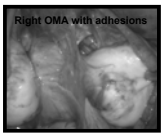
Associated Osis lesions
superficial, OMAs
Adenomyosis

Deep endometriosis:

Frequency of associated other OSIS forms

Forms of the disease	n	%	95%CI
Superficial peritoneal	57	61.3	51.4-71.2
Ovarian endometriomas	47	50.5	40.3-60.7
Pelvic adhesions	69	74.2	65.3-83.1
Overall	87	93.5	87.7-97.2

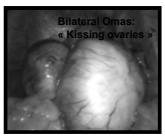
Somigliana *et al.*, Hum Reprod (2004)



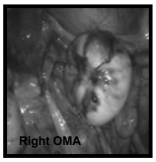
Deep endometriosis:

Frequency of associated ovarian endometriomas

(n = 636 patients)



Main lesion	N	Associated OMAs n	%
BLADDER	51	8	15.7
USL	279	49	17.6
VAGINA	93	19	20.4
URETER	29	13	44.8
INTESTINE	184	86	46.7
Total	636	175	27.5



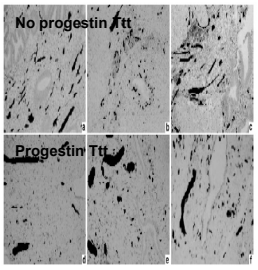
Chapron *et al.*, 2008

Heterogeneity of endometriotic lesions

Different susceptibility to progestin Ttt

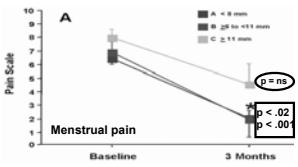
Number of vessels/mm²	No treatment	Progestin treatment	
Endometrium biopsies	211 (64-526)	129 (15-360)	< .0001
Superficial Endometriotic lesions	216 (62-365)	149 (30-280)	= .0002
Deep endometriotic lesions	225 (74-424)	181 (47-400)	= .0004

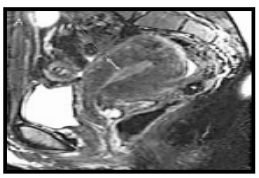
Vessel are (µm²)	No treatment	Progestin treatment	
Endometrium biopsies	270 (95-1047)	369 (128-2704)	= .0001
Superficial Endometriotic lesions	141 (77-389)	474 (136-2467)	<. 0001
Deep endometriotic lesions	194 (53-581)	284 (36-1811)	< .0001

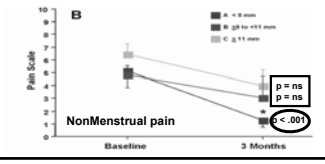


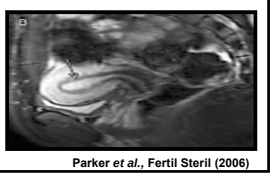
Jondet and Chapron, Angiogenesis (2006)

Pelvic pain after surgery for endometriosis









Parker *et al.*, Fertil Steril (2006)

Take home messages

Strategy: Global approach **Multifocal pelvic pathology**

Management:

2 efficient options

- **Surgical Ttt: Radical surgery**
  - Exeresis of all Osis lesions
  - If HT indicated: Total HT + BSO
- **Medical Ttt: Cost, side effects …**

Referral center with multidisciplinary approach:

Ability to perform a satisfactory preoperative management and a complete operation ++++

Deep endometriosis: *Criteria for decision*

Patients' criteria

- Age
- Associated infertility factor
- Ovarian reserve:
  - Hormonal level
  - Antral folicule count

Infertility or Desire for pregnancy

Importance of painful symptoms

Desire of the patient

Deep endometriosis

DIE criteria

Multifocality of DIE lesions

DIE heterogeneity

Associated Osis lesions
superficial, OMAs
Adenomyosis

Modern management of Deep Osis

Multidisciplinary global approach

Molecular biology research

Genetics research

Surgery

ART
FIV, ICSI

Medical treatment

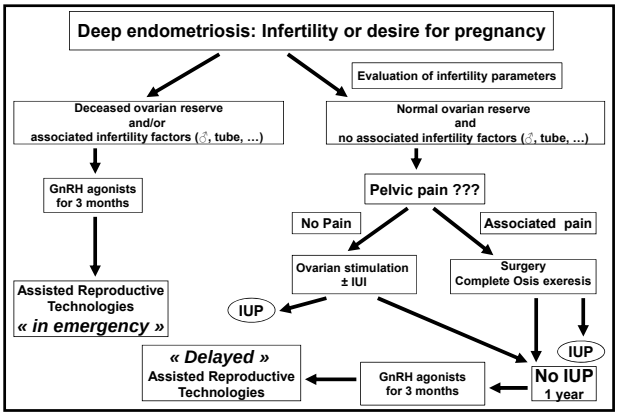
Immunology research

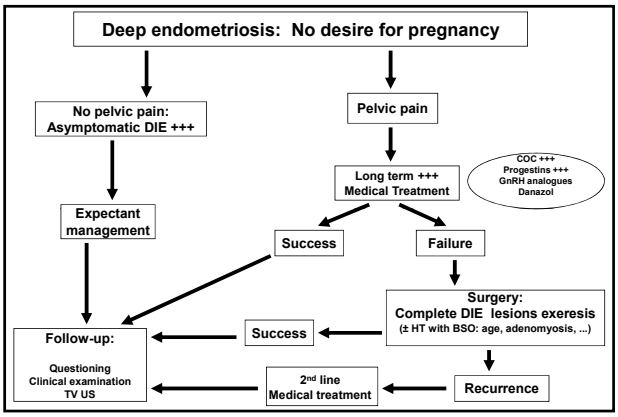
infertility

Pelvic pain

Ovarian reserve

Reproductive endocrinology
ovarian stimulation, IUI


Deep endometriosis: Infertility or desire for pregnancy
Evaluation of infertility parameters
Deceased ovarian reserve and/or associated infertility factors (♂, tube, …)
Normal ovarian reserve and no associated infertility factors (♂, tube, …)
GnRH agonists for 3 months
Pelvic pain ???
No Pain
Associated pain
Ovarian stimulation ± IUI
Surgery Complete Osis exeresis
Assisted Reproductive Technologies « in emergency »
IUP
IUP
« Delayed » Assisted Reproductive Technologies
GnRH agonists for 3 months
No IUP 1 year


Deep endometriosis: No desire for pregnancy
No pelvic pain: Asymptomatic DIE +++
Pelvic pain
Long term +++ Medical Treatment
COC +++ Progestins +++ GnRH analogues Danazol
Expectant management
Success
Failure
Surgery: Complete DIE lesions exeresis (± HT with BSO: age, adenomyosis, ...)
Follow-up: Questioning Clinical examination TV US
Success
2nd line Medical treatment
Recurrence

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