



# **Endometriosis: how new techniques may help**

Special Interest Group Endometriosis/Endometrium

**27 June 2010  
Rome, Italy**

# 6



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*Organised by the Special Interest Group Endometriosis/Endometrium*

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**ESHRE – European Society of Human Reproduction and Embryology**

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**What is ESHRE?**

ESHRE was founded in 1985 and its **Mission Statement** is to:

- promote interest in, and understanding of, reproductive science and medicine.
- facilitate research and dissemination of research findings in human reproduction and embryology to the general public, scientists, clinicians and patient associations.
- inform politicians and policy makers in Europe.
- promote improvements in clinical practice through educational activities
- develop and maintain data registries
- implement methods to improve safety and quality assurance




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**Executive Committee 2009/2011**

Chairman	• Luca Gianaroli	Italy
Chairman Elect	• Anna Veiga	Spain
Past Chairman	• Joep Geraedts	Netherlands
	• Jean François Guérin	France
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	• Miodrag Stojkovic	Serbia
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	• Veljko Vlaisavljevic	Slovenia
	• Søren Ziebe	Denmark




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### ESHRE Activities – Campus and Data Collection

- Educational Activities / Workshops
  - Meetings on dedicated topics are organised across Europe
  - Organised by the Special Interest Groups
  - Visit: [www.eshre.eu](http://www.eshre.eu) under CALENDAR
- Data collection and monitoring
  - EIM data collection
  - PGD data collection
  - Cross border reproductive care survey



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### ESHRE Activities - Other

- Embryology Certification
- Guidelines & position papers
- News magazine "Focus on Reproduction"
- Web services:
  - RSS feeds for news in reproductive medicine / science
  - Find a member
  - ESHRE Community



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### ESHRE Membership (1/3)

- ESHRE represents over 5,300 members (infertility specialists, embryologists, geneticists, stem cell scientists, developmental biologists, technicians and nurses)
- Overall, the membership is distributed over 114 different countries, with 50% of members from Europe (EU). 11% come from the US, India and Australia.



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### ESHRE Membership (2/3)

	1 yr	3 yrs
Ordinary Member	€ 60	€ 180
Paramedical Member*	€ 30	€ 90
Student Member**	€ 30	N.A.

\*Paramedical membership applies to support personnel working in a routine environment such as nurses and lab technicians.

\*\*Student membership applies to undergraduate, graduate and medical students, residents and post-doctoral research trainees.




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### ESHRE Membership – Benefits (3/3)

1) Reduced registration fees for all ESHRE activities:

Annual Meeting	Ordinary	€ 480	(€ 720)
	Students/Paramedicals	€ 240	(€ 360)
Workshops	All members	€ 150	(€ 200)

2) Reduced subscription fees to all ESHRE journals – e.g. for Human Reproduction €191 (€ 573!)

3) ESHRE monthly e-newsletter

4) News Magazine "Focus on Reproduction" (3 issues p. a.)

5) Active participation in the Society's policy-making




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### Special Interest Groups (SIGs)

The SIGs reflect the scientific interests of the Society's membership and bring together members of the Society in sub-fields of common interest

Andrology	Psychology & Counselling
Early Pregnancy	Reproductive Genetics
Embryology	Reproductive Surgery
Endometriosis / Endometrium	Stem Cells
Ethics & Law	Reproductive Endocrinology
Safety & Quality in ART	




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### Task Forces

A task force is a unit established to work on a single defined task / activity

- Fertility Preservation in Severe Diseases
- Developing Countries and Infertility
- Cross Border Reproductive Care
- Reproduction and Society
- Basic Reproductive Science
- Fertility and Viral Diseases
- Management of Infertility Units
- PGS
- EU Tissues and Cells Directive



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### Annual Meeting

Rome, Italy 27 June to 30 June 2010



Pre-congress courses (27 June):

- PCC 1: Cross-border reproductive care: information and reflection
- PCC 2: From gametes to embryo: genetics and developmental biology
- PCC 3: New developments in the diagnosis and management of early pregnancy complications
- PCC 4: Basic course on environment and human male reproduction
- PCC 5: The lost art of ovulation induction
- PCC 6: Endometriosis: How new technologies may help
- PCC 7: NOTES and single access surgery
- PCC 8: Stem cells in reproductive medicine
- PCC 9: Current developments and their impact on counselling
- PCC 10: Patient-centred fertility care
- PCC 11: Fertility preservation in cancer disease
- PCC 12: ESHRE journals course for authors



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### Annual Meeting – Scientific Programme (1/2)

Rome, Italy 27 June to 30 June 2010



- Molecular timing in reproduction
- Rise and decline of the male
- Pluripotency
- Preventing maternal death
- Use and abuse of sperm in ART
- Live surgery
- Emerging technologies in the ART laboratory
- Debate: *Multiple natural cycle IVF versus single stimulated cycle and freezing*



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## Annual Meeting – Scientific Programme (2/2)

- Fertility preservation
- Congenital malformations
- ESHRE guidelines
- Data from the PGD Consortium
- European IVF Monitoring 2007
- Debate: *Selection of male/female gametes*
- Third party reproduction in the United States
- Debate: *Alternative Medicine, patients feeling in control?*
- Historical lecture: "Catholicism and human reproduction"



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## Certificate of attendance

- 1/ Please fill out the evaluation form during the campus
- 2/ After the campus you can retrieve your certificate of attendance at [www.eshre.eu](http://www.eshre.eu)
- 3/ You need to enter the results of the evaluation form online
- 4/ Once the results are entered, you can print the certificate of attendance from the ESHRE website
- 5/ After the campus you will receive an email from ESHRE with the instructions
- 6/ You will have TWO WEEKS to print your certificate of attendance



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## Contact



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E-mail: [info@eshre.eu](mailto:info@eshre.eu)  
[www.eshre.eu](http://www.eshre.eu)



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# PRE-CONGRESS COURSE 6 - Programme

## Endometriosis: How new technologies may help

*Organised by the Special Interest Group Endometriosis/Endometrium*

Course coordinators: Juan A. Garcia-Velasco (Spain) and Paola Vigano (Italy)

Course description: Endometriosis diagnostic procedures as well as treatments seem to be intensely investigated by little progress made. During the present course, advances in both diagnostic and non-surgical treatments will be presented, as new technologies are opening new venues to correctly detect these patients and treat them in alternative ways as to what has been done for the last few years.

Target audience: Doctors, embryologists and nurses involved in infertility and pain management of patients with endometriosis

Scientific programme:

### Diagnosis

- 09:00 – 09:15 Introduction
- 09:15 – 09:45 Endometrial stem cells and endometriosis - **Carlos Simon (Spain)**
- 09:45 – 10:15 Research on serum markers of the disease: is it worthwhile - **Paola Vigano (Italy)**
- 10:15 – 10:30 Discussion
  
- 10:30 – 11:00 Coffee break
  
- 11:00 – 11:30 Diagnosis – Proteomics - **Juan A. Garcia–Velasco (Spain)**
- 11:30 – 12:00 Diagnosis – Genomics - **Stephen Kennedy (United Kingdom)**
- 12:00 – 12:15 Discussion
- 12:15 – 13:30 Lunch

### Non hormonal treatment

- 13:30 – 14:00 Dopamine agonists - **Antonio Pellicer (Spain)**
- 14:00 – 14:30 Statins - **Antoni J. Duleba (USA)**
- 14:30 – 15:00 Discussion
  
- 15:00 – 15:30 Coffee break
  
- 15:30 – 16:00 Modulation of the immune system - **Thomas D’Hooghe (Belgium)**
- 16:00 – 16:30 Adhesions prevention and surgical techniques - **Michel Canis (France)**
- 16:30 – 16:45 Discussion
- 16:45 – 17:00 Conclusions and adjourn



# ENDOMETRIAL STEM CELLS AND ENDOMETRIOSIS

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Scientific Director, Fundación IVI.

Scientific Director, Centro de Investigación Príncipe Felipe

The author declares no conflict of interest.



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CENTRO DE INVESTIGACION

VNIVERSITAT ID VALÈNCIA



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## LEARNING OBJECTIVES

- To acquire new concepts concerning the biology and origin of somatic stem cells, and their niche.
- To learn more about the existence of somatic stem cells (SSC) in murine and human endometrium.

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### Stem Cell

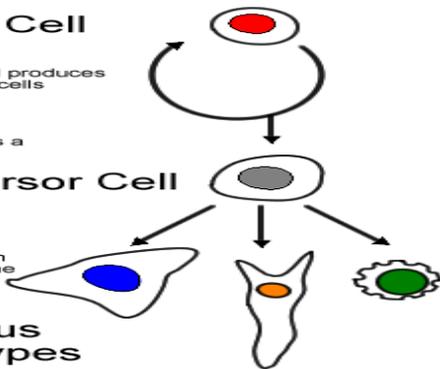
A stem cell produces more stem cells

or becomes a

### Precursor Cell

which, then can become

### Various cell types



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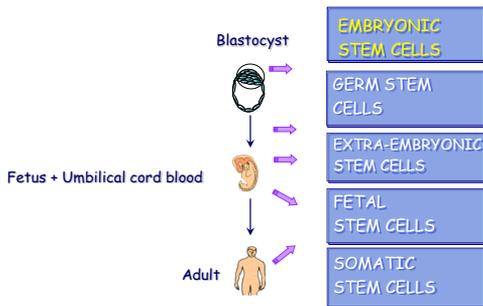
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## STEM CELLS. SOURCES AND TYPES




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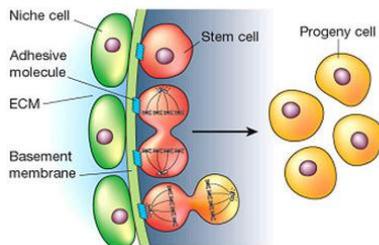
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## THE NICHE



The role of the niche is the support of the SSC population in an organ that suffers very frequently shedding of the functional layer.

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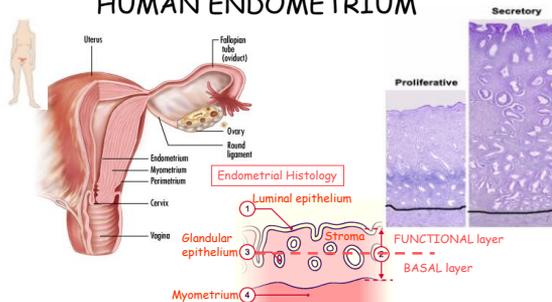
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## HUMAN ENDOMETRIUM



The endometrium is composed of luminal epithelium and epithelial-lined glands surrounded by a supportive stroma. It can be divided into a functional (removed layer) and basal (regenerating layer) compartments.

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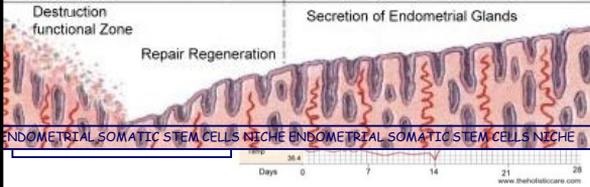
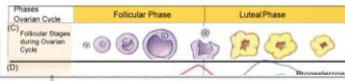
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# HUMAN ENDOMETRIUM

• Remarkable regenerative capacity during the menstrual cycle




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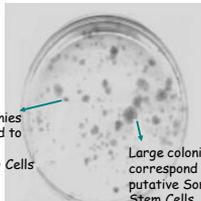
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## INDIRECT EVIDENCE FOR THE EXISTENCE OF ENDOMETRIAL SSC (ESSC)

CELL-CLONING STUDIES  
Capacity of forming colonies, one of the typical characteristics of stem cells.



Clones	Clonogenicity (%)	
	Epithelial	Stromal
Large	0.08 ± 0.03	0.02 ± 0.01**
Small	0.14 ± 0.04	1.23 ± 0.18**
Total	0.22 ± 0.07*	1.25 ± 0.18*

The cloning efficiency does not vary significantly across the menstrual cycle, or between cycling and inactive endometrium (Schwab et al, Fertil Steril 2005).

Chan et al., Biol Reprod 2004.

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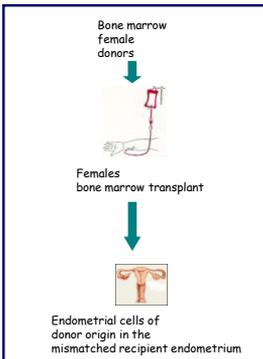
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## IMPLICATION OF BONE MARROW



- Percentage of donor-derived cells in endometrium increased with time:
  - 0.25% at 24 months,
  - 4% at 35 months,
  - 10.5% at 129 months,
  - 50% at 147 months.

Taylor., JAMA 2004.

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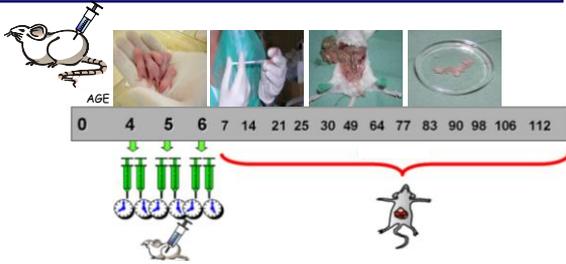
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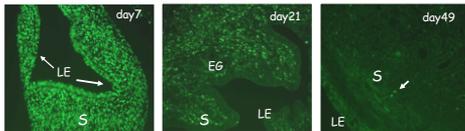
## ESSC IN MURINE ENDOMETRIUM

METHOD OF 5-BROME-2-DEOXYURIDINE (BrdU)  
 BrdU is incorporated into genomic DNA during the replication phase of the mitotic cycle labelling new cells. Identification of label retaining cells (LRC) in epithelial and stromal compartment of murine endometrium.



*Chan and Gargett., Stem cells 2006.*  
*Cervelló et al., Human Reprod 2007.*

## ESSC IN MURINE ENDOMETRIUM

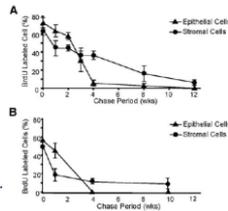


The BrdU retained cells in mice endometrium decreases with age.

*Cervelló et al., Human Reprod 2007.*

After labelling, the BrdU signal is progressively decreased in each division. The retention of the labelling in some populations means no division or very low rate of division, one of the main characteristic of somatic/progenitor stem cells.

*Chan and Gargett., Stem Cells 2006.*



## PUTATIVE ENDOMETRIAL STEM CELL MARKERS

Stem Cell Marker	Endometrial localization	Reference	
POU5F1	Embryonic stem cell	In humans, it co-localise with Vimentin and Cytokeratin. In murine populations, co-localization of BrdU- retaining cells.	Matthai <i>et al.</i> , 2006 Cervelló <i>et al.</i> , 2007
CD90	Cultured Mesenchymal stem cell	In humans, it differentiates the expression in the basalis and functionalis stroma.	Schwab and Gargett, 2008
CD146	Endothelial cell, perivascular cell and Mesenchymal stem cell	In humans, it co-expresses with PDGF-R $\beta$ .	Schwab and Gargett, 2007, 2008
c-Kit	Hematopoietic stem cell and mast stem cells	In humans, mainly in the stroma. In murine samples, co-localization of BrdU- retaining cells.	Cho <i>et al.</i> , 2004 Cervelló <i>et al.</i> , 2007 Goodell <i>et al.</i> , 2008
CD34	Hematopoietic stem cell and endothelial cells	In humans, mainly in the stroma.	Cho <i>et al.</i> , 2004
STRO-1	Mesenchymal Stem cells	In humans, is located on the perivascular regions of the endometrium	Schwab <i>et al.</i> , 2008.

*Cervelló et al., Expert Reviews 2009.*

- Existence of SSC in murine endometrium was demonstrated by BrdU method.  
(Chan and Gargett., *Stem cells*, 2006; Cervelló et al., *Human Rep.*, 2007)

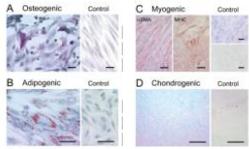
- Recent studies demonstrate that human endometrium contains a rare MSC-like population.  
(Chan et al., *Biol Rep.*, 2004; Schwab and Gargett., *Human Rep.*, 2007; Wolff et al., *Reprod.Sci.*, 2007.; Gargett et al., *Biol Rep.*, 2009)

- In the menstrual blood the existence of a stem cell-like population has been demonstrated.  
(Meng et al., *J Transl Med.*, 2007)

- SSC have differentiated into mesoderm-derived lineages *in vitro*.



Stromal cells



Schwab and Gargett., *Human Rep.*, 2007  
Wolff et al., *Reproductive Sciences*, 2007.

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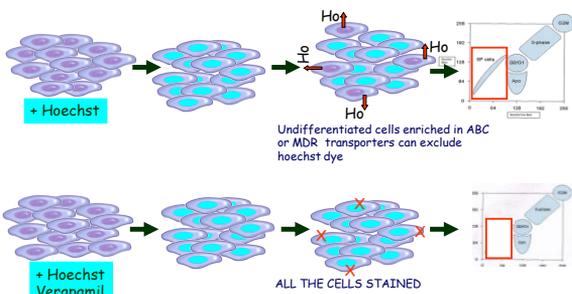
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## ESSC IN HUMAN ENDOMETRIUM

Side Population: Hoechst Method and Cell Sorting




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## SIDE POPULATION METHOD

- Side Population (SP) method was described for SSC isolation in bone marrow based on the ability to efflux Hoechst33342-fluorescence dye.  
(Goodell et al., *J Exp Med.*, 1996)

- This property is present in cells enriched in ABC transporters and has been documented in the detection of SSC in human myometrium, lung and dental pulp.  
(Ono et al., *PNAS*, 2007; Martin et al., *Cytotherapy*, 2008; Iohara et al., *Stem Cells*, 2008)

- It has also been proposed recently in the human endometrium although not functionally demonstrated yet.  
(Kato et al., *Human Rep.*, 2007; Tsuji et al., *Fertil Steril.*, 2008)

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## HYPOTHESIS

COULD THE SP REPRESENT THE SOMATIC STEM CELL POPULATION IN THE HUMAN ENDOMETRIUM?

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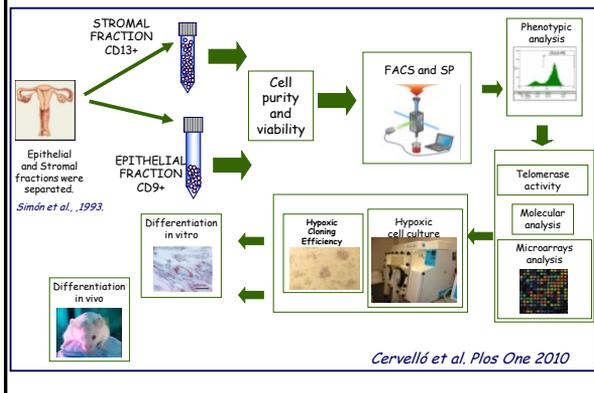
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## STUDY DESIGN



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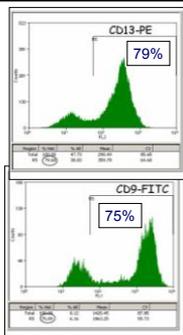
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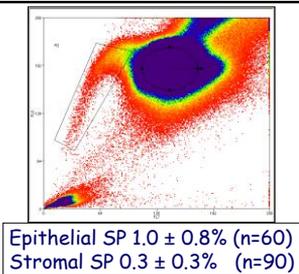
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### EPITHELIAL AND STROMAL CELL PURITY



### SP IN HUMAN ENDOMETRIUM



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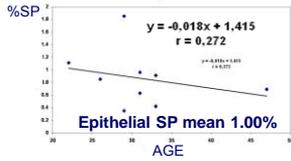
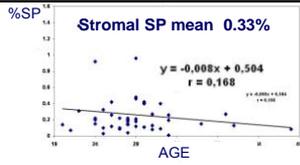
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### % SP during Reproductive Life




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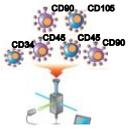
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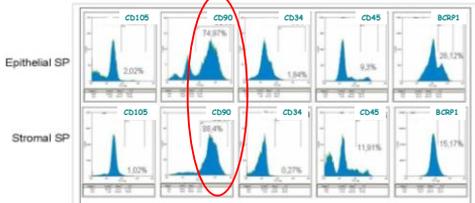
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### PHENOTYPIC ANALYSIS INTRODUCTION

✓ Phenotypic analysis of Side Population cells:



- The SP cells were labeled with:
- markers associated with Hematopoietic progenitor cells like CD45-FITC and CD34-PE.
  - Mesenchymal stem cells markers like CD90-PE and CD105-FITC.




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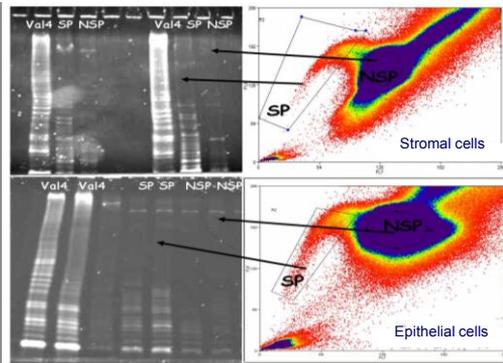
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### TELOMERASE ACTIVITY




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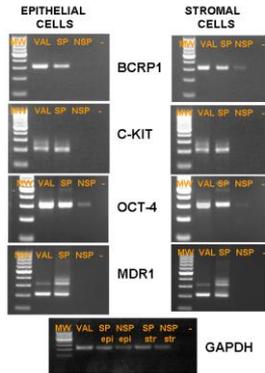
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## SP MOLECULAR ANALYSIS




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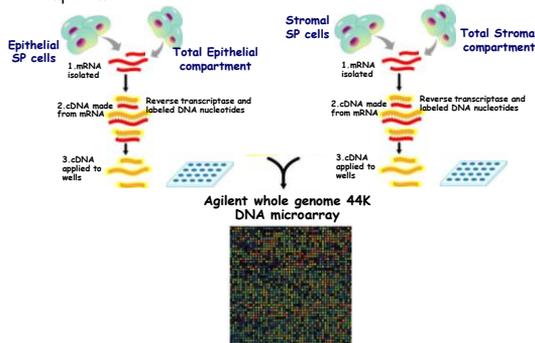
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## MICROARRAYS ANALYSIS

✓ Microarrays analysis of Epithelial and Stromal Side Population versus total compartments:




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## MICROARRAYS ANALYSIS

### TOP TEN MOLECULES SP stromal cells

Molecules	Exp. Value	Molecules	Exp. Value
MMP3	+6.532	VWF	+4.331
RND3	+6.412	SCGB1D2	+4.321
SERPINE2	+6.275	SERPINA3*	+4.185
SLC4A1	+6.026	ASRGL1*	+3.589
ANGPTL4	+5.820	SOX17	+3.567
INHBA	+5.674	SCGB2A1	+3.169
IER3	+5.578	HGD	+3.105
KRT34	+5.502	ACSL5	+3.105
GDF15	+5.367	TPD52L1	+3.085
ADM	+5.324	ST6GALNAC1	+3.083

SP stromal cells vs Stromal cells:  
121 genes up-regulated  
74 genes down-regulated

Molecular and cellular Functions:  
-Cell signalling and interaction  
-Cellular growth and proliferation  
-Cellular movement  
-Cell death  
-Cell cycle

### TOP TEN MOLECULES SP epithelial cells

Molecules	Exp. Value	Molecules	Exp. Value
IL1B	+19.623	FOXO1	+12.402
CXCL1	+19.069	PCSK1N	+11.151
HSPA6	+16.887	POU3F3	+9.126
TUBA4A	+13.661	NEUROG1	+8.531
CCL4	+13.402	SYNPO	+7.969
POLR2J2	+13.321	CACNA1E	+7.794
CACNG5	+13.281	CRHR2	+7.654
GDF15	+12.487	IER3*	+7.654
CD69	+11.971	NEUROG3	+7.593
RGS1	+11.604	SYN1	+7.002

SP epit cells vs Epithelial cells:  
196 genes up-regulated  
116 genes down-regulated

Molecular and cellular Functions:  
-Cell signalling and interaction  
-Cellular growth and proliferation  
-Cellular movement  
-Cell death

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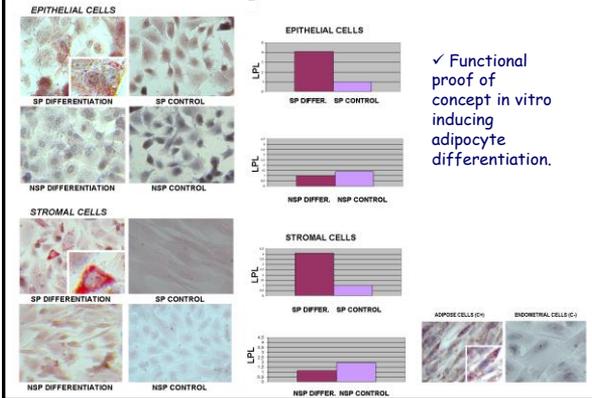
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## DIFFERENTIATION IN VITRO




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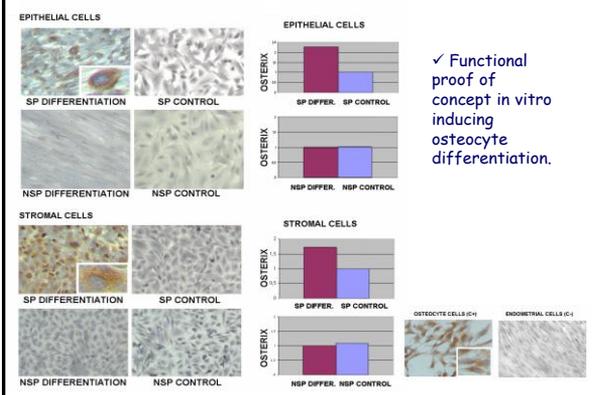
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## DIFFERENTIATION IN VITRO




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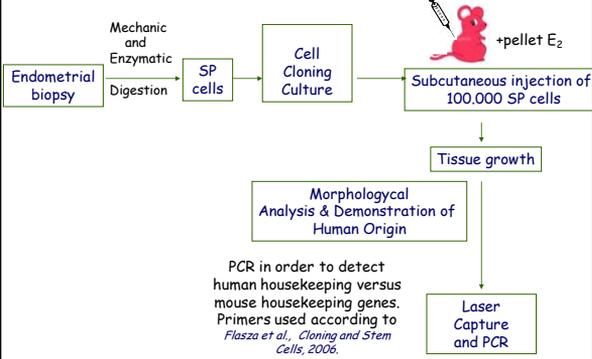
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## IN VIVO DIFFERENTIATION

✓ Functional proof of concept in vivo using NOD-SCID female mice:




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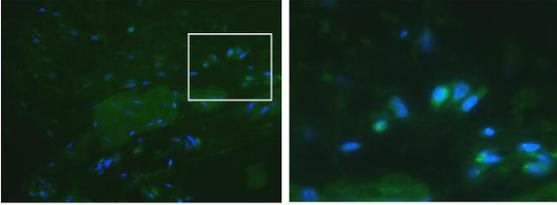
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## IN VIVO DIFFERENTIATION



Immunohistochemical analysis for Human Progesterone Receptor in endometrial like structures in mice subcutaneous tissue after stroma SP injection (40X). Right, Detail of green fluorescent signal due to Hu-PR co-localized with DAPI

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## CONCLUSIONS

- > SP account for 0.3% and 1% of the stromal and epithelial compartment respectively, remaining constant during reproductive life.
- > Phenotype of SP suggest a mesenchymal origin and they display an intermediate pattern of telomerase activity, being positive for c-Kit, Oct-4 and BCRP-1
- > Wide genome analysis demonstrated a differential gene expression profile of SP compared to its endometrial fraction. A common SP signature is suggested.
- > SP cells do not growth in normoxic conditions. In hypoxic conditions, SP cells display high cloning efficiency compared to NSP and total fraction.
- > Stromal and epithelial SP have been differentiated in vitro to adipocytes and osteocytes.
- > The functional proof of concept is given by the ability of SP cells to reconstruct the human endometrium in an animal model.

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**EVERY MONTH HOLDS A MIRACLE**  
C'ELLE IS AVAILABLE NOW! TO START COLLECTING AND PRESERVING YOUR BODY'S PRECIOUS STEM CELLS, PLEASE CLICK THE BUTTON BELOW.

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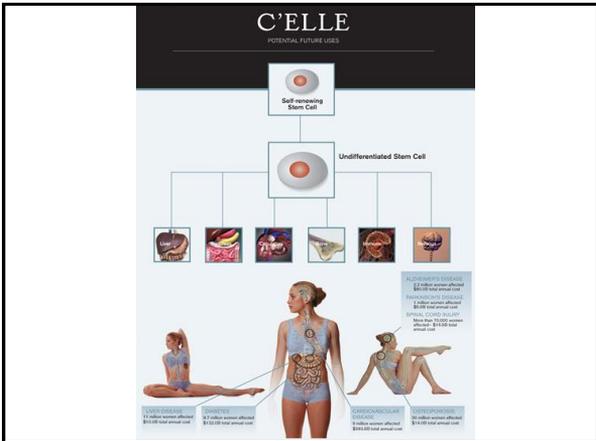
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CENTRO DE INVESTIGACION      VALÈNCIA

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**Research on serum markers of the disease:  
Is it worthwhile?**

Paola Vignani PhD

CROG (Center for Research in Obstetrics and Gynecology), Milano, Italy

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**LEARNING OBJECTIVES**

- >Accuracy of imaging for the diagnosis of endometriosis
- >Forms needing a biochemical marker
- >Elucidation of forms of prevention in endometriosis
- >Main criteria for an effective screening program
- >Satisfaction of the criteria in case of endometriosis

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**DIAGNOSIS**

*Ovarian cysts*

Presurgical findings	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	% correctly classified
Positive TV ultrasound	57	98	95	76	81

*(modified from Eskenazi et al. Fertil Steril 2001)*

*Uterosacral, Douglas and colorectal involvement*

Transvaginal findings	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
Uterosacral Involvement	75	83	95	45	77
Douglas Involvement	82	100	100	67	87
Colorectal Involvement	95	100	100	89	97

*(modified from Bazot et al. Hum Reprod 2003)*

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## NEED OF A MARKER

- peritoneal superficial forms
- adhesions
- deep infiltrating forms (ureter)

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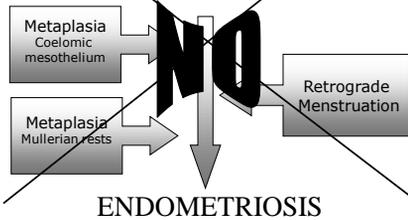
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## PRIMARY PREVENTION

involves the prevention of diseases and conditions before their biological onset



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## SECONDARY PREVENTION

consists in the identification and interdiction of diseases that are present in the body, but that have not progressed to the point of causing signs, symptoms, and dysfunctions

*Atti Indagine Conoscitiva del Senato svolta dalla 12ª Commissione Permanente del Senato (Igiene e Sanità), XIV legislatura. Fenomeno dell'endometriosi come malattia sociale. Roma: Senato della Repubblica, 2006.*

"By cumulating data from two American studies, it was found that the average time to diagnosis was 9.3 years (around 10 years still today according to Italian data), because it takes 4.7 years for the patient to see a doctor, and 4.6 years to identify and confirm the diagnosis, after having seen an average of about 5 physicians".

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## Menstrual Morbidities in teenagers

	N° responders	% (95%CI)
Sure there is something wrong with period	1039	10(8-12)
Two or more atypical symptoms	1051	15(13-17)
Three or more atypical symptoms	1051	6 (5-8)
Report severe pain and school absence	1016	10(9-12)
Report severe pain and have seen GP	1024	9 (8-11)
Report severe pain and low response to pain med.	657	5 (3-6)

modified from MEDOT study, BJOG, 2009

## Stage of the disease in adolescence

Table II. Surgical diagnosis of first surgery in adolescents

Authors	Year	Mild disease	Moderate/Severe disease
Goldstein et al	1980	30%	42%
Chattman & West	1982	30%	30%
Davis et al	1993	30%	30%
Rosse et al	1996	92%	3%
Laufer et al	1997	100%	0%
Wuolk-Bra et al	2002	54%	46%
Daglia et al	2009	74%	26%

Confarri et al, J Endom, 2010

## POTENTIAL CLASSES OF SERUM MARKERS

- **Growth Factors**  
(VEGF, IGF-I)
- **Cytokines**  
(IL-6, IL-8, TNF- $\alpha$ )
- **Chemokines**  
(MCP-1, CCR1)
- **Glycoproteins**  
(Ca-125, Ca19-9)
- **Adhesion molecules**  
(sICAM-1, sVCAM-1)
- **Molecules of apoptosis**  
(Fas ligand)
- **Others**  
(Leptin, CD163, CD44 sC5b-9)

**Priority: non invasive diagnostic test**

Diagnosis  Screening

**Screening rules: does endometriosis fit the model?**

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**Main criteria for an effective screening program**

Table I. Main desirable characteristics of a screening test and degree of satisfaction for endometriosis.

Items	Explanation
1. Health Relevance	Is the condition an important health problem (significant risk of mortality or morbidity)?
2. Acceptability of the disease	Is the disease acceptable in the population?
3. Natural course	Does the condition have a recognizable latent or early symptomatic phase? Is the natural history of the condition well understood?
4. Acceptability of the test	Is the test (and its consequences in terms of further diagnostic testing and subsequent treatment) acceptable to the population?
5. Effectiveness of treatment	Is early treatment of the condition effective? Does diagnosis of the disease before symptoms occur results in better outcome than waiting for symptoms?
6. Consensus	Does a consensus exist regarding proper management of abnormal test results?
7. Complication balance	Is the risk of complication from the test and subsequent evaluation and treatment lower than the risk of morbidity and mortality from the disease?
8. Cost-benefits balance	Are the costs of testing and treating asymptomatic disease acceptable? Do the objectives of the program justify the costs?

*Somigliana et al., submitted*

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**Health Relevance**

- (i) impact on the physical, mental, and social well being of a woman and can have a profound effect on her life
- (ii) the delay in diagnosis causes significant morbidity (physical and psychological)
- (iii) the annual costs of endometriosis is estimated at \$22 billion in 2002 in the United States
- (iv) association with cancer is plausible but the risk is low

**Acceptability of the disease**

- (i) condition of menstruation and infertility that are both commonly taboo topics in society. This disease is poorly recognized and therefore flies under the radar in terms of acceptability in society
- (ii) sexual dysfunction due to dyspareunia can disrupt relationships
- (iii) infertility may lead to social stigmatization

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## Natural course

Natural course of endometriosis is entirely unpredictable

### Evidence in favour of a progressive disease

- rarity of the diagnosis in the adolescent age
- marked tendency of the disease to relapse after surgical removal

### Evidence in favour of a non-progressive disease

- based on studies with second-look laparoscopies endometrial deposits resolved spontaneously in up to a third of women, deteriorated in nearly half and are unchanged in the remainder over 6-12 months (Sutton et al., 1997; Hottel et al., 2004).
- deep rectovaginal endometriotic nodules are progressive in less than 10% of cases

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## Acceptability of the test

- Acceptable in terms of pain, inconvenience and cost for blood samples; less acceptable for other human samples.

Presence of nerve fibers in endometrial biopsy in women with and without endometriosis

Sensitivity (%) 98

Positive Predictive Value (%) 91

Specificity (%) 83

Negative Predictive Value (%) 96

(modified from Al-Jefout et al., Hum Reprod, 2009)

- Less acceptable in terms of psychologically distress for the women and misuse of health care resources.

women's perceptions of illness based on the screening test

- clinically relevant?
- progression?
- infertility?

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## Effectiveness of treatment

➤ Surgery consents to remove the lesions but does not prevent recurrences. (Recurrence rate 20% at 2 years and 40-50% at 5 years) (Guo et al., 2009)

➤ Hormonal medical treatment effectively improves pain symptoms but its effect is limited to the period of assumption and pain typically resumes after discontinuation.

**And if diagnosed earlier or during the latent phase?**

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## Effectiveness of treatment

- ❖ Retrospective study
- ❖ Sequential cases of young women (ys 12-24) with chronic pelvic pain unresponsive to medical treatment for dysmenorrhea
- ❖ Initial laparoscopy for diagnosis and surgical destruction of the lesions
- ❖ All treated with standard continuous medical therapy
- ❖ Patients with exacerbation or recurrence of pain who elected a subsequent laparoscopy were eligible for the study (n=90).
- ❖ The median endometriosis stage was I.

Change in stage of endometriosis between 1st and 2nd surgery		
	N	%
Improved by two stages	1	1
Improved by one stage	17	19
Stage unchanged	63	70
Worsened by one stage	9	10
Total	90	100

modified from Doyle et al, J Pediatr Adolesc Gynecol, 2009

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## Consensus

**NO CONSENSUS**



*Surgical approach*



*The "pill"*



*In-depth Clinical Counselling*

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## Complication balance

➤ **From the test**

➤ Null

➤ **From subsequent evaluation and treatment**

➤

➤ the rate of major and minor complications associated with laparoscopy is 1.4% and 7.5%, respectively (*Chapron et al., 2002*)

➤ compliance derived from long-term administration of medical treatment using oral contraceptives or progestins

➤ the psychological consequences and the impact on the quality of life of being classified as "ill"

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## Cost-benefits balance

### **COSTS**

- > the costs of the test
- > the costs of the treatment (laparoscopy or medical drugs)
- > the necessity to develop facilities for confirming the diagnosis and for adequate treatments
- > the complications of the therapies
- > impact on the quality of life of women found to be positive

### **> BENEFITS**

- > ???

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## Main criteria for an effective screening program

Table 1. Main desirable characteristics of a screening test and degree of satisfaction for endometriosis.

Items	Explanation	Degree of satisfaction
1. Health relevance	Is the condition an important health problem?	++
2. Acceptability of the disease	Is the disease acceptable in the population?	-
3. Natural course	Does the condition have a recognizable latent or early symptomatic phase? Is the natural history of the condition well-understood?	-
4. Acceptability of the test	Is the test (and its consequences in terms of further diagnostic testing and subsequent treatment) acceptable in the population?	+
5. Effectiveness of treatment	Is early treatment of the condition effective?	+
6. Consensus	Does a consensus exist regarding proper management of abnormal test results?	..
7. Complication balance	Is the risk of complication from the test and subsequent treatment lower than the risk of morbidity and mortality from the disease?	+
8. Cost-benefits balance	Are the costs of testing and treating asymptomatic disease acceptable? Do the objectives of the program justify the costs?	+/-

*Somigliana et al., submitted*

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## CONCLUSIONS

At present, identifying non-invasive tools for the diagnosis of endometriosis is a priority for establish measures of prevention

> Only four out of eight characteristics for a screening program are satisfied, of whom only one is highly satisfied. Therefore, the identification of a non-invasive test for the diagnosis of endometriosis may be harmful if not properly used.

A prevention program requires a consensus regarding the proper management of an abnormal test

> Studies regarding the beneficial effects of surgery or medical treatment initiated during the latent phase of the disease should be performed.

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# Endometriosis: Proteomics

Juan A Garcia-Velasco, MD



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## Learning objectives

- To understand the limitations of the current diagnostic techniques in endometriosis
- To evaluate how proteomics may be useful in early diagnosis of the disease
- To review available evidence on the use of proteomics in endometriosis diagnosis

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## Disclosure

- No commercial or financial relationships or other activities that may be perceived as potential conflict of interest

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## Who are we facing?

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- Pain
- Infertile
- Asymptomatic?

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## Introduction

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- Why do we need new a diagnostic approaches
- What is wrong with today's practice
- What new technologies may offer
- Where are we

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## Suspect Clinical records

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- Dysmenorrhea
- Dyspareunia
- Chronic pelvic pain
- Subfertility
  
- Asymptomatic 100%

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Suspect  
Inspection

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- Small external os diameter
- Cervical displacement
- Cervical or vaginal endometriosis

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Suspect  
Physical exam

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- Pelvic exam
  - Pain – lower abdomen
  - Adnexal pain
  - Uterosacral lig pain
  - Pain with cervical exam



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Suspect  
TV Ultrasound

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- Ovarian endometrioma
- Deep endometriosis nodules
  
- NO adhesions
- NO peritoneal lesions

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## How diagnose is made today

### Gold standard

- Surgical biopsy + pathology
  - Invasive
  - Painful
  - General anesthesia
  - Surgical risks
  - Cost
  - Late
  - Not 100% accurate



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## Early diagnosis

### To reduce disease progression

- School absentism
- Work absentism
- QoL (pelvic pain)
- Fertility

### Not indicated if:

- already pelvic pain/infertility
- endometriomas

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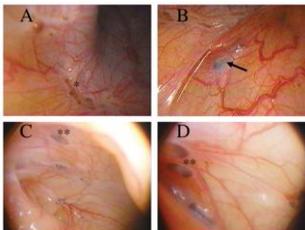
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## Early diagnosis

### Regular menses /sperm ok/ mild pain?



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## Early diagnosis

- Non invasive
  - serum or plasma
  - menstrual fluid
  
- Semi-invasive
  - peritoneal fluid (transvaginal puncture)
  - endometrial biopsy

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## Early diagnosis

- Detect all women with endo and other pelvic pathology in early stages
  - High sensibility (few FN)
    - Positive test and women w/endo
  
  - Specificity is less relevant
    - Positive test in healthy woman

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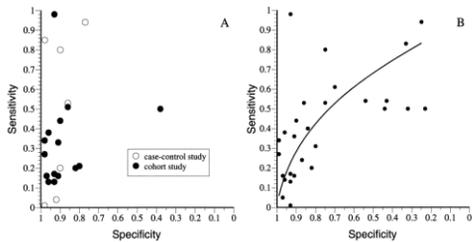
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## Early diagnosis

- CA 125



Mol et al. Fertil Steril 1998

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## Early diagnosis

### Combination of markers

- PCR
- TNF- $\alpha$
- MCP-1
- IL 6 y 8
- Amiloide A
- CA 19-9

D'Hooghe et al. Gynecol Obstet Invest 2006

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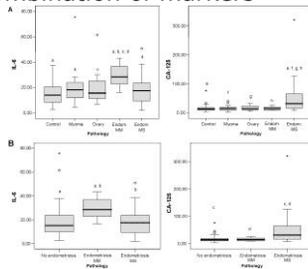
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## Early diagnosis

### Combination of markers



Martínez et al. Hum Reprod 2007

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## New technologies (...omics)

- Genomics
- Proteomics
- Metabolomics
- ..... translational medicine

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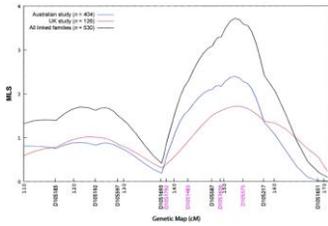
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## Early diagnosis

### Genetic predisposition

Genome-wide Linkage Study in 1,176 Affected Sister Pair Families Identifies a Significant Susceptibility Locus for Endometriosis on Chromosome 10q26



Treloar et al. Am J Hum Genet 2005

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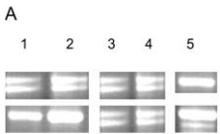
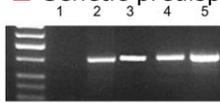
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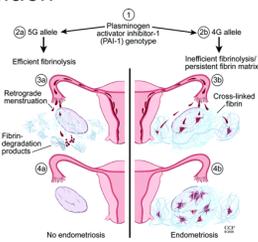
## Early diagnosis

### Genetic predisposition



A

B



Bedalwy et al. Obstet Gynecol 2006

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**Endometrial fluid is a specific and non-invasive biological sample for protein biomarker identification in endometriosis**

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## **Introduction - endometriosis**

### PROTEOMICS

- Allows comparison of protein expression of different scenarios (healthy vs pathologic)
- Similar work in serum, peritoneal fluid or endometrial tissue (eutopic and ectopic) in search of potential biomarkers – lack of further validation

Ferrero 2007; Casado-Vela et al 2009; Zhang 2006

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## **Objectives**

- Identify disease specific biomarkers for the development of a non-invasive diagnostic test for endometriosis
- Novel, non-invasive sample for research in endometriosis: uterine fluid aspirate
  - only previous experience in the study of endometrial receptivity for embryo implantation

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## **Material and Methods**

- Population studied
  - Age 18-45 years
  - Laparoscopic evidence of endometriosis (n=46) vs controls (n=32)
  - No previous hormonal treatment for at least 3 previous months
  - PCOS & systemic diseases excluded

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## Material and Methods

### □ Sample used

- Aspirate of **endometrial fluid** in the endometrial cavity
- Collected during the post-ovulatory secretory phase
- 2-3mm flexible cannula (Gynetics, Belgium) connected to the 5mL syringe followed by vacuum aspiration
- Aspirates (5 to 100uL) were frozen at -80°C until processed

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## Material and Methods

### □ Protein extraction and 2D electrophoresis

- Resuspended in PBS and purified to remove albumin (from blood) and class G immunoglobulins
- Precipitated with 15% w/v trichloroacetic acid (1h 4°C)
- Pellets washed in acetone, and resuspended.
- Bradford assay to quantify protein content
- 200 ug added to rehydration solution
  - 1st dimension pH 3—10, and isoelectric focussing
  - 2nd dimension SDS-PAGE 12.5%
  - silver stained

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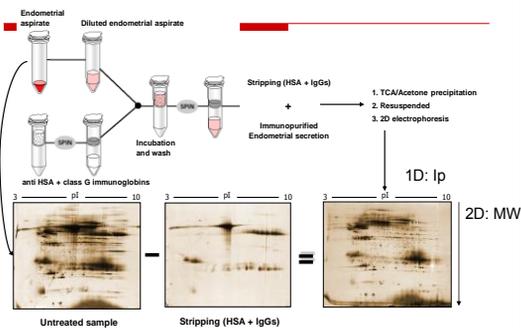
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### Sample processing




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## Material and Methods

### □ Protein pattern analysis

- Scanned images – automated spot detection (Progenesis software)
- Protein spots were manually matched to a virtual reference gel based on all the endometrial aspirate gels
- Image background subtraction

Data was analysed using SPSS. Non parametric statistics were used. Fold change of the expression levels of each protein was calculated for controls vs endometriosis I-II vs endometriosis III-IV

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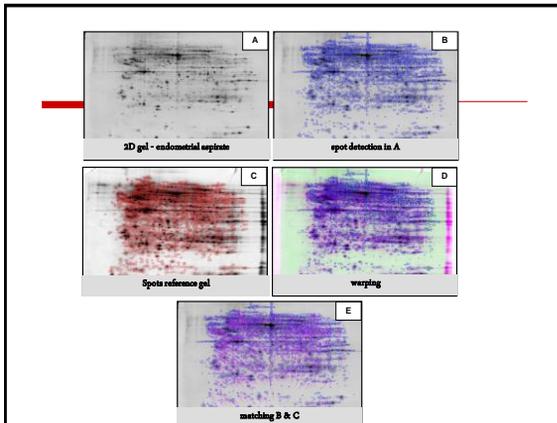
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## Material and Methods

### □ Protein identification by mass spectrometry

- Significant spots were excised from silver manually with pipette tip, digested with trypsin and subjected to peptide mass fingerprinting on a mass spectrometer (Bruker-Daltonics, Germany)
- Protein identification – non redundant protein database (NCBI)
- Functional analysis was carried out by Ingenuity pathways software

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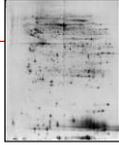
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## Results

□ Similar mean number of matched spots

- Control 494
- Endo I-II 548
- Endo III-IV 515 p=0.37



□ 378 spots were present in >70% of the cases

- 52 were significant
- 31 showed at least 2-fold discrepancy

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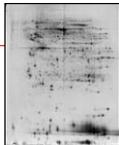
## Results

□ 21 proteins differentiate mild vs free

□ 10 proteins differentiate severe vs free

□ 23 proteins differentiate mild vs severe

- Cytoskeleton structure
- Cell motility
- Signal transduction
- Cell cycle regulation



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## Conclusions

□ Endometrial fluid aspirates offer specific and reliable data for endometriosis biomarker research

□ The identified proteins need to be validated to develop a non-invasive diagnostic test for endometriosis

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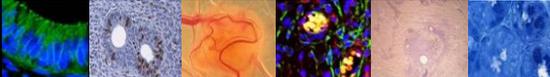
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Pre-congress Course 6  
Endometriosis: How new technologies may help

## Dopamine agonists

Prof. Antonio Pellicer


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### OBJECTIVES

At the end of this presentation, the student should:

- Understand the relevance of angiogenesis in the establishment of endometriosis lesions.
- Be familiar with the experimental work targeting angiogenesis to treat endometriosis.
- Know the experiments demonstrating the value of dopamine agonists in the treatment of endometriosis.
- Gain information about the clinical trials employing dopamine agonists in the treatment of endometriosis.

Prof. Antonio Pellicer

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### INTRODUCTION

Angiogenesis and Endometriosis

- Peritoneal endometriosis is believed to be the result of implantation of retrogradely shed endometrium during menstruation (Sampson, 1927).
- The endometrium has the capacity to adhere, attach, and implant ectopically (Maas et al, 2001).
- For the survival of endometrium in an ectopic location, the acquisition of an adequate blood supply is essential (Maas et al, 2001).

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**Angiogenesis and Endometriosis**

- The endometrium has angiogenic potential and endometriotic lesions grow larger in areas with a rich blood supply (Nisolle et al, 1993).
- Pro-angiogenic factors are increased and anti-angiogenic modulators decreased, in peritoneal fluid of women with endometriosis (Lasche and Menge, 2007).
- VEGF is released by peritoneal macrophages in increased amounts in women with endometriosis (McLaren et al, 1996).

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**Angiogenesis and Endometriosis**

- Positive correlation between the severity of the disease and secretion of VEGF in peritoneal fluid (Shifren et al, 1996; Mahnke et al, 2000; Bourlev et al, 2006).
- Increased expression of VEGF in active red lesions (Donnez et al, 1998).
- VEGF is particularly over-expressed in deep infiltrating endometriosis (Machado et al, 2007).

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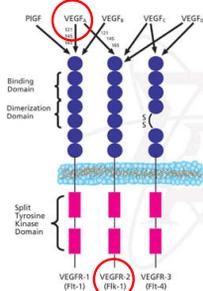
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**Vascular Endothelial Growth Factor Family**

**VEGF**



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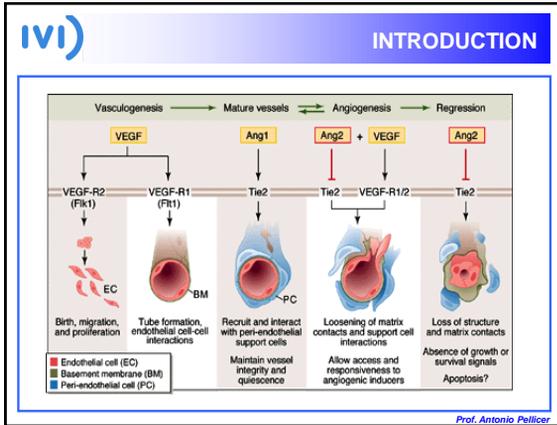
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**IVI) INTRODUCTION**

**Antiangiogenic Agents as a therapy for Endometriosis**

- **Antiangiogenic Agents are Effective Inhibitors of Endometriosis (Hull et al, 2003)**
  - soluble truncated receptor that antagonizes VEGF
  - anti-VEGF A antibody
- **Antiangiogenesis Therapy for Endometriosis (Nap et al, 2004)**
  - VEGF A inhibitor (avastin)
  - general efficient angiogenesis inhibitors (TNP-470, endostatin, anginex).

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**IVI) INTRODUCTION**

**Targeting VEGF system shows toxic effects**

Anti-VEGF drugs were first designed as antiangiogenic drugs for cancer treatment (*The oncologist*.2000;5 Suppl 1:51-4)

Although effective, they show toxic side effects, mainly:

- Vomiting (*J Clin Oncol*. 2002 Mar 15;20(6):1446-8)
- Headache (*Expert Opin Biol Ther*. 2003 Apr;3(2):263-76)
- Thromboembolic complications (*Clin Cancer Res*. 2003 May;9(5):1648-55)

**Toxic effects do not allow using this approach in ENDOMETRIOSIS**

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**Dopamine 2 Agonists (D2A) targeting VEGF**

- **D2A reduce angiogenesis and ascites in cancer models** (Basu et al, *Nature Med* 2001).
- **D2A successfully reduce vascular permeability and ascites in:**
  - Experimentally-induced OHSS model (Gómez et al, *Endocrinology* 2006).
  - Humans (Alvarez et al, *J Clin Endocrinol Metab* 2007).

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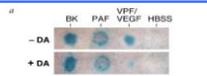
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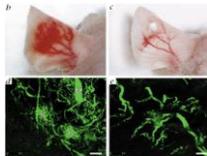
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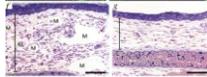
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**Which is the role of dopamine in angiogenesis ?**



**Selective inhibitory effects of dopamine on vascular permeabilizing and angiogenic effects of VPF/VEGF in a cancer mouse model**



**Dopamine decreases ascites and angiogenesis in cancer mouse models**

Basu et al. *Nature Medicine* 7, 569-74 (2001)

CONTROL DOPAMINE

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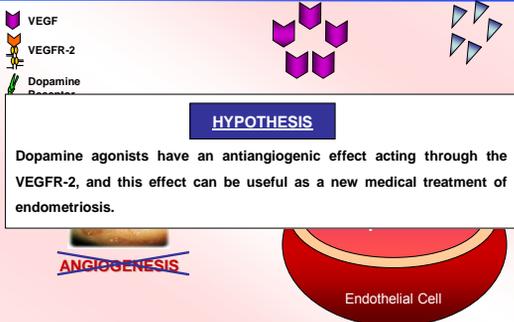
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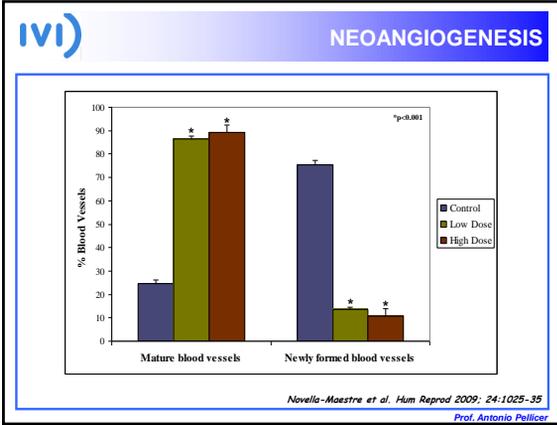
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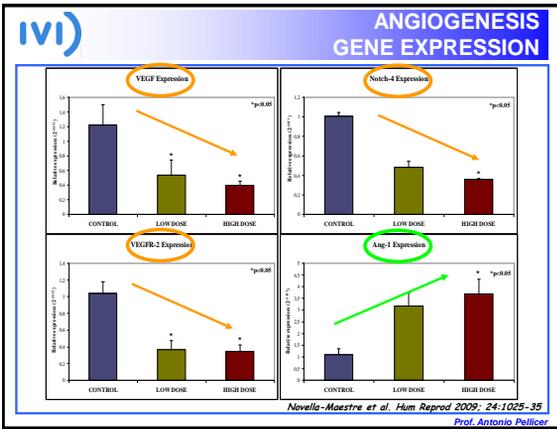
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**IVI) CONCLUSIONS**

Cb2 in experimental endometriosis act through D2 dopamine receptors to induce endocytosis of VEGFR-2, preventing VEGF binding, receptor phosphorylation:

new blood vessel formation  
 angiogenesis markers expression  
 cellular proliferation

Reduce active endometriotic lesions

These findings provide the rationale to test dopamine agonists as a novel therapeutic approach in human peritoneal endometriosis.

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**IVI) VALVULAR HEART DISEASE AND D2A**

		Adjusted incidence ratio	
Cabergoline	28.6%	dose < 3 mg/day	2.6
Non-ergot	0.0%	> 3 mg/day	50.3
Controls	5.6%	duration < 6 months	0.0
		> 6 months	7.8

*Zanettini et al. N Engl. J Med 2007; 356:39-46*  
*Schade et al. N Engl. J Med 2007; 356:29-38*

**Statement Endocrine Society: consider changing the treatment to QUINAGOLIDE (NORPROLAC)**

Prof. Antonio Pellicer

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**IVI) EXPERIMENTAL DESIGN**

Prof. Antonio Pellicer

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**IVI) CELLULAR PROLIFERATION**

**Ki-67 Cell proliferation antigen**

Group	Staining Area (approx.)
Control	5.5
Cabergoline	2.5*
Quinag. 50	2.5*
Quinag. 200	2.5*

**There is significant differences of expression of Ki67 in controls vs treated animals**  
 \*(Cabergoline p=0,020; Quinagolide 50 p=0,029; Quinagolide 200 p=0,039)

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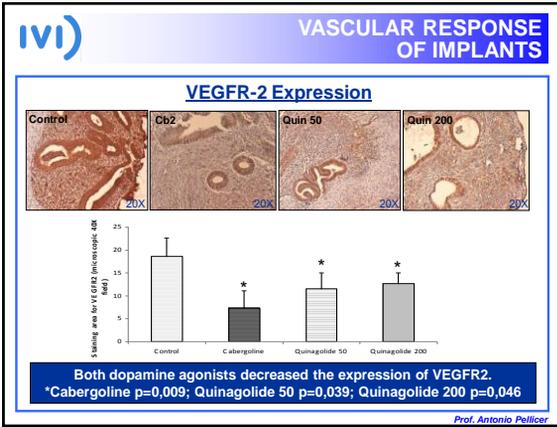
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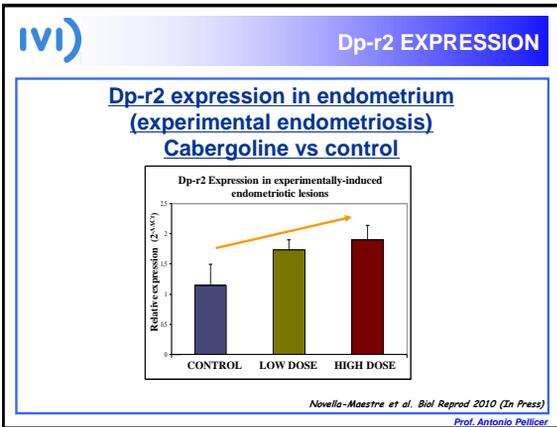
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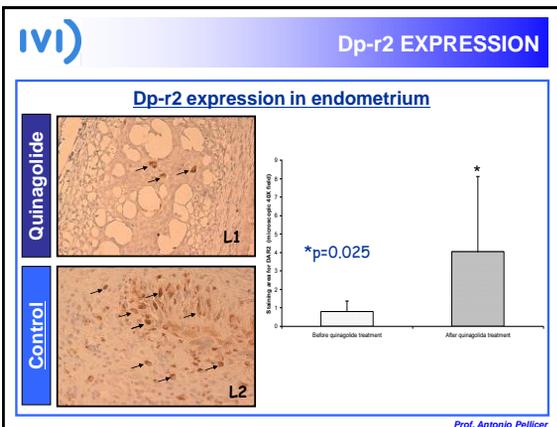
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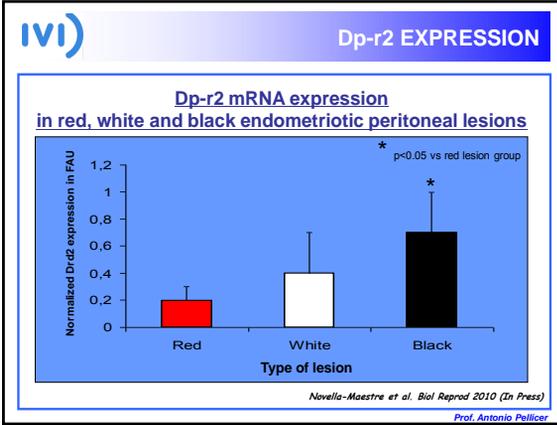
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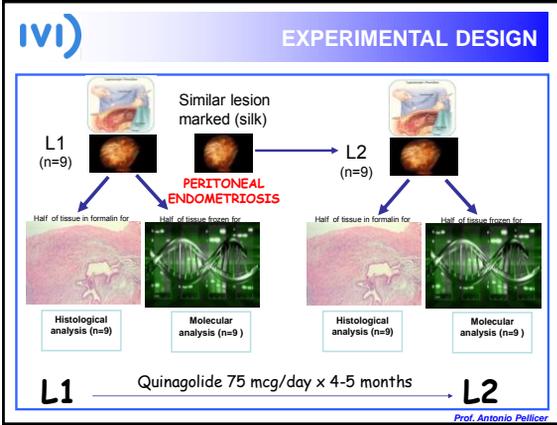
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**IVI) CASE 7**

**TITRIATED DOSE:**  
 First 15 days: 25mcg/day  
 Day 15 to 30: 50mcg/day  
 Day 30 onwards: 75 mcg/day

**TIME OF TREATMENT: 4 months**

**COMPLICATIONS:**  
 Any complication

**BIOCHEMISTRY:**

	Pre-treatment	Post-treatment
PRL (µg/ml)	91	15,1
Cat25 (U/ml)	31,5	15,5

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## CONCLUSIONS

**Cb2 & Quinagolide act through Dpr-2 and affect endometriosis lesions targeting angiogenesis**



This is a "proof of concept" to undergo further clinical trials employing Dopamine Agonists in the treatment of endometriosis

*Prof. Antonio Pellicer*

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## AKNOWLEDGEMENTS

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- Ferring Pharmaceuticals

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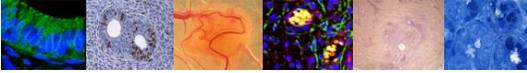
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IVI)

Thank you for your attention



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# STATINS: NOVEL TREATMENT OF ENDOMETRIOSIS ?

Antoni J. Duleba MD  
Professor of Ob/Gyn, University of California Davis

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## Disclosure

- The presenter is sponsored by NIH to carry out research on effects of statins on endometriosis
- No other conflict of interest



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## Learning objectives

- To review mechanism of action of statins
- To correlate key pathophysiological features of endometriosis with actions of statins
- To present *in vitro* and *in vivo* studies on effects of statins on endometrium and endometriosis



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## Endometriosis

- Benign condition: defined as the presence of ectopic endometrial glands and stroma
- Affects approximately 6-10% of women associated with:
  - pelvic pain (dysmenorrhea, intermenstrual pelvic pain, dyspareunia)
  - Infertility
  - and/or pelvic mass
  - bowel and bladder dysfunction



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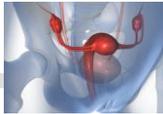
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## Endometriosis-pathogenesis



- Etiology poorly understood
- Dominant concepts are:
  - retrograde menstruation-induced implantation of endometrium
  - coelomic metaplasia
- Postulated predisposing factors include:
  - immune dysfunction
  - genetic predisposition
  - environmental pollutants

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## Limitations of current therapies of endometriosis

- Primary targets of established therapies: analgesic/anti-inflammatory and anti-estrogenic effects
- Typically address individual features of endometriosis
- Modest effectiveness
- Significant side-effects
- Upon discontinuation of these therapies, symptoms of endometriosis frequently return



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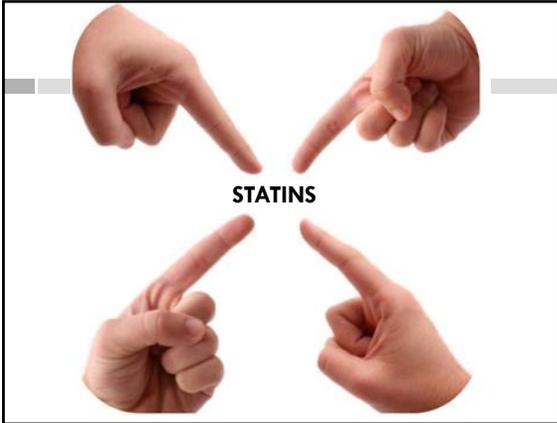
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## Effects of statins

- Statins are cholesterol-lowering agents effective in treatment of hypercholesterolemia and cardiovascular disorders
- Statins also possess cholesterol-independent actions: regulation of cell proliferation and apoptosis, antioxidant and anti-inflammatory properties



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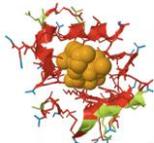
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## Statins: mechanism of action

- Competitive inhibition of the key enzyme regulating the mevalonate pathway: 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase
- Mevalonate pathway: series of reactions starting with acetyl-coenzyme A and involving the formation of farnesyl pyrophosphate (FPP), the substrate for:
  - cholesterol
  - isoprenylated proteins
  - ubiquinone
  - dolichol



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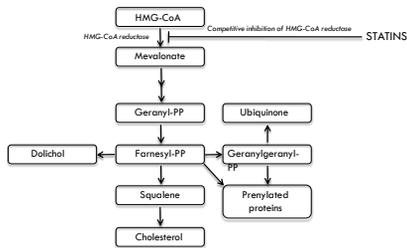
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## Mevalonate pathway




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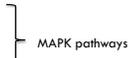
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## Statins: mechanism of action



- Key role of substrates of isoprenylation of proteins:
  - farnesyl-pyrophosphate (FPP)
  - geranylgeranyl-pyrophosphate (GGPP)
- Statins can impair geranylgeranylation and farnesylation by depletion of GGPP and FPP and hence affect signal transduction steps regulating
  - PROLIFERATION
    - Ras-Raf-Erk1/2
    - P38 kinase (p38K)
    - c-Jun N terminal protein kinase (JNK)
  - APOPTOSIS
    - phosphatidylinositol 3'-kinase/ protein kinase B (PI3 kinase/PKB) pathway
- p70S6 kinase (p70S6K) is important to proliferation and may be affected by both PI3K and MAPK




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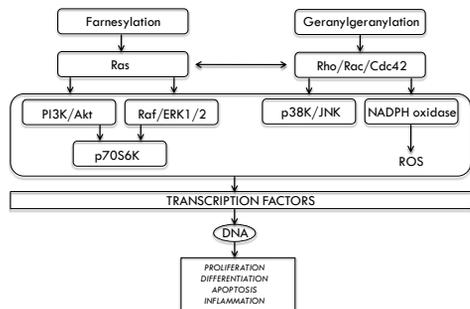
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## Effects of isoprenylation




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## Statins and oxidative stress

- Reduction of the synthesis of an antioxidant: Coenzyme Q (ubiquinone)
- Upregulation of expression and activity of catalase (CAT; an enzyme metabolizing  $H_2O_2$  into water and molecular oxygen)
- Isoprenylation-related inhibition of Rac1: a component of NADPH oxidase, a major source of ROS
- Intrinsic antioxidant activity
- Net effect of statins: reduction of OXIDATIVE STRESS



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## Statins: side-effects

- Pro-apoptotic and cytotoxic activity, including rhabdomyolysis and liver cytolysis
- Cytotoxic effects may be due to reduced activity of small GTPases and reduced Coenzyme Q
- Clinical trials evaluating Coenzyme Q supplementation during statin therapy: administration of Coenzyme Q does not fully prevent the toxicity of statins and is beneficial only to a small subgroup of patients



[Levy HS, et al. 2006]  
[Marcoff L, et al. 2007]  
[Anfossi G, et al. 2004]

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## Statins: side-effects

- Potential risk of teratogenicity (category X medications)
- The evidence for teratogenicity is based on theoretical considerations
- Conflicting findings from a small series of cases
- The use of statins should be avoided in sexually active women not using reliable contraception



[Edison RJ, et al. 2004]  
[Kazmin A, et al. 2007]  
[Taguchi M, et al. 2008]

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## The rationale for proposing statins as a treatment of endometriosis

1. The inhibition of HMG-CoA reductase depletes products of mevalonate pathway, especially isoprenyls and thus ↓ activity of small GTPases (Ras, Rho...) resulting in ↓ of signaling important to growth regulating pathways.

4. Statins possess antioxidant, anti-inflammatory and immunomodulatory properties: may reduce oxidative stress and inflammation associated with endometriosis



2. The inhibition of HMG-CoA reductase may reduce cholesterol, which is required for maturation of type I IGF-1 receptors, and hence may decrease the mitogenic effect of IGF-1 on endometrial stromal cells.

3. Statins may interfere with angiogenesis, which is necessary for the development of endometriotic implants.

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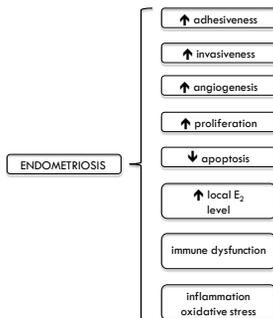
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## Features of endometriosis




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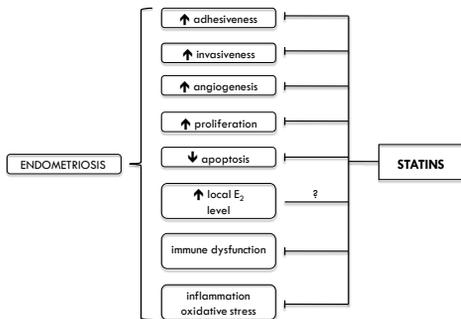
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## Proposed role of statins in treatment of endometriosis




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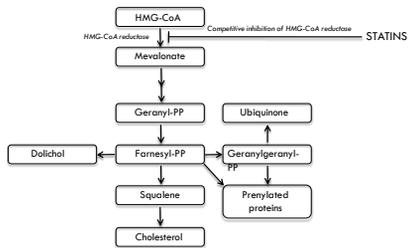
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### Sites of action of statins




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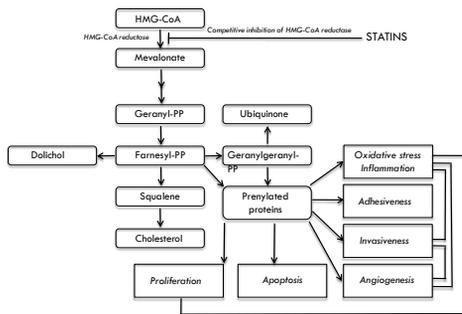
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### Sites of action of statins




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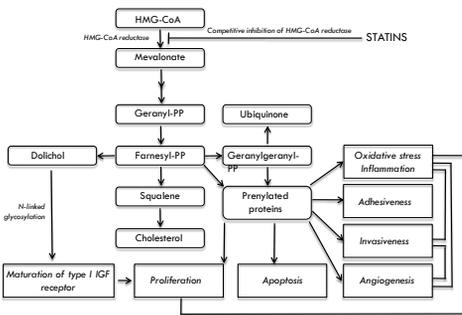
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### Sites of action of statins




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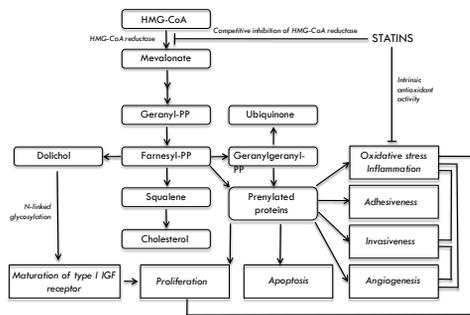
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## Sites of action of statins




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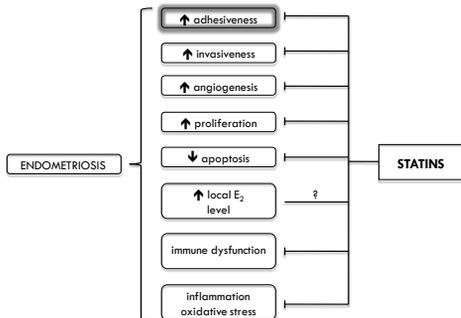
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## Proposed role of statins in treatment of endometriosis.




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## Increased adhesiveness of endometriotic cells

- Eutopic and ectopic endometrial stromal cells from women with endometriosis exhibit an aberrant integrin expression and increased adhesion capacity after exposure to several ECM components  
[Klement PA, et al. 2007]
- Menstrual phase endometrial mRNA levels of integrin  $\alpha v \beta 3$  are elevated in patients with endometriosis  
[Kyono CM, et al. 2008]
- Transcripts of integrins ( $\beta 1, \beta 3, \alpha v$ ) in xenografts in a nude mouse model of endometriosis  
[Hull ML, et al. 2008]
- Menstrual endometrial stromal cells derived from women with endometriosis: ↑ adherence to peritoneal mesothelium and ↑ expression of several isoforms of CD44 (v6, v7, v8, v9)
- Additional glycosylation sites on the variants of CD44 may contribute to increased adhesiveness of the endometrial cells  
[Griffith JS, et al. 2009]

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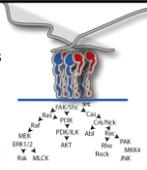
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Statins and endometrium: disruption of cell morphology and endometrial cells adhesiveness



Statins decrease endometrial stromal cell adhesiveness to collagen fibers in a 3-D matrix

- Untreated endometriotic stromal cells, isolated from endometrial cysts, cultured in 3-D collagen gels developed dendritic morphology, adhered to collagen fibers and formed tissue-like structures.
- Simvastatin treated cells did not adhere to collagen and cells became round or polygonal.

[Nasu K, et al. 2009]

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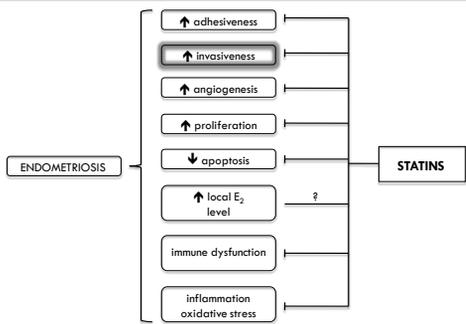
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Proposed role of statins in treatment of endometriosis




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Increased endometriotic cell invasiveness

- Several matrix metalloproteinases (MMPs) are inappropriately expressed in the endometrium of women with endometriosis and are upregulated by tumor necrosis factor alpha (TNF-α) and interleukin 1 (IL-1) [Chung HW, et al. 2002]
- The endometrium of women with endometriosis has ↑ mRNA levels of MMP-2 and MMP-3 and ↓ mRNA expression of tissue inhibitor of metalloproteinase 2 (TIMP-2) [Chung HW, et al. 2002]
- The continuous expression of several MMPs (MMP-2, -3, -7), and ↓ expression of TIMP-2 in endometriotic lesions plays a role in the establishment of endometriosis [Kyano CM, et al. 2006]
- Hemopexin domain expressed by MMPs, except MMP-7, can be recognized and bound by T-like autoantibodies in women with endometriosis leading to dysregulation of MMPs and TIMPs in ectopic lesions [Kim HO, et al. 2007]
- Women with endometriosis have reduced endometrial sensitivity of MMPs to progesterone [Chung HW, et al. 2002]
- Women with endometriosis have reduced endometrial sensitivity of MMPs to progesterone [Bruner-Tran K, et al. 2002]
- Women with endometriosis have reduced endometrial sensitivity of MMPs to progesterone [Siratandis C, et al. 2006]
- Women with endometriosis have reduced endometrial sensitivity of MMPs to progesterone [Osteen KG, et al. 2003]

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## Statins and endometrium: inhibition of MMPs

- MMP9 production may be affected by modulation of isoprenylation
  - Statins may decrease MMP9 production by monocytes via activation of the nuclear receptor transcription factor peroxisome-proliferator-activated receptor-γ (PPARγ) [Greenwood J, et al. 2004]
  - Simvastatin inhibits expression of MMP-3 in human endometrial stroma [Grip O, et al. 2002]
    - Endometrial stromal cells expressed abundant levels of MMP-3 following treatment with E<sub>2</sub>, but minimal levels in cultures also supplemented with simvastatin or MPA
    - IL-1α induced a profound increase in MMP-3 secretion from cells pretreated with E<sub>2</sub> alone; however, the addition of either simvastatin or MPA abrogated this effect
    - Cultures containing both simvastatin and MPA were the most resistant to MMP-3 induction by IL-1α
- Statins inhibit both basal and IL-1α-induced MMP levels by mechanisms independent of and complementary to MPA. [Bruner-Tran K, et al. 2009]

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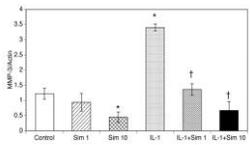
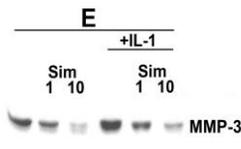
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## Statins and endometrium: inhibition of MMPs



Western analysis of expression and regulation of MMP-3 protein in human endometrial stromal cells isolated from proliferative phase endometrial biopsies. Cells were cultured for 48 h in the presence of estradiol (E, 1nM), followed by an additional 24 h with E alone or E plus simvastatin (Sim) at 1 or 10 μM. Some cultures were also exposed to IL-1α during the last 6 h of culture. [Bruner-Tran K, et al. 2009]

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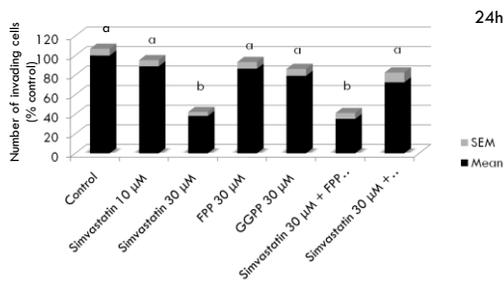
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## Statins and endometrium: Invasion assay




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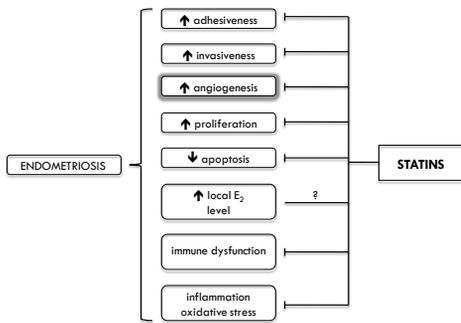
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## Proposed role of statins in treatment of endometriosis.




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## Endometriosis and angiogenesis

- Human endometrium is highly angiogenic [Growth PG, et al. 2005]
- Endometrial implants may send angiogenic signals to the murine vessels leading to their destabilization, migration of endothelial cells and induction of the growth of blood vessels into endometrial tissue [Gruner R, et al. 2001]
- Endometriosis is associated with an increased level of inducers of angiogenesis such as vascular endothelial growth factor (VEGF) and transforming growth factor  $\beta$  (TGF- $\beta$ ) [Sui HD, et al. 2007; Hoshida DE, et al. 2010]
- Activated peritoneal macrophages, T-cells, endometrium and endometriotic implants secrete VEGF; TGF- $\beta$  is predominantly produced by endometrial stroma, platelets, activated lymphocytes and macrophages [Savakidis C, et al. 2006; Okamoto CO, et al. 2010]
- VEGF promotes endothelial cell proliferation, migration, differentiation and capillary formation and it may play an important role in the progression of endometriosis [Domez J, et al. 1998]
- TGF- $\beta$  stimulates endometrial stromal cells to produce urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) playing the role in endothelial cells migration [Growth PG, et al. 2005]
- TGF- $\beta$  also stabilizes the vessel wall, by stimulating binding of the endothelial cells to the pericytes [Widke TE, et al. 2009]
- Expression of cyclooxygenase-2 (COX-2) is ↑ in endometriosis. COX-2 stimulates VEGF production by fibroblasts and, via prostaglandin-cAMP-PKA-dependent activation of small GTPase, promotes integrin  $\alpha$  $\beta$ 3-mediated adhesion and migration of endothelial cells [Fujiga C, et al. 2004]

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## Statins and endometrium: inhibition of angiogenesis

- Statins inhibit angiogenesis
  - Growth of human endometrial biopsy tissues in a three-dimensional culture in a fibrin matrix was observed during the first week of culture, while new vessel formation was noticed after 2-3 weeks
  - Lovastatin at 5-10  $\mu$ M induced a concentration-dependent inhibitory effect on endometrial cell growth and on angiogenesis
  - Lovastatin at 1  $\mu$ M concentration, inhibited only angiogenesis, with no demonstrable effect on cell proliferation

The proposed mechanism of diminished blood-vessel formation is related to statin-induced inhibition of expression of VEGF [Esfandiari N, et al. 2007]
- Atorvastatin inhibits both mRNA expression and protein level of COX-2 and VEGF in endometrial-endometriotic cell cultures [Shorita I, et al. 2009]
- The angiostatic effect of statins has been confirmed by *in vivo* studies using a nude mouse model of endometriosis [Bruner-Tran K, et al. 2009]




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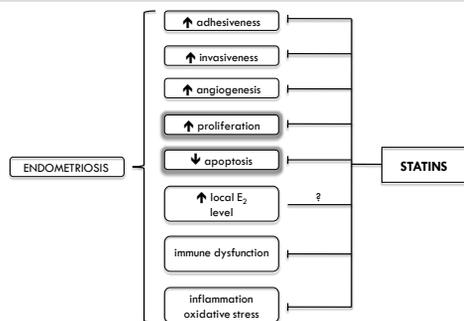
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## Proposed role of statins in treatment of endometriosis.




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## Growth of endometrial tissue

- DNA synthesis in the endometrial stromal cells from patients with endometriosis is elevated in response to ECM components (fibronectin, laminin, vitronectin, tenascin-C), suggesting that excessive PROLIFERATION starts just after the endometrial stromal cells attach to ECM in the peritoneal cavity

[Klemm PA, et al. 2007]

- Proliferation is mediated by integrins, cell-to-cell and cell-to-ECM adhesion molecules functioning as signal-transducing receptors in mitogen-activated protein MAPK pathway

[Chan Q, et al. 1994; Meredith JE, et al. 1996; Lee JW, et al. 2004]

- Excessive proliferation may also be induced by a broad range of cytokines and growth factors secreted by immunocompetent cells:

- Hepatocyte growth factor (HGF), produced by peritoneal macrophages, increases *in vitro* growth of endometrial epithelial and stromal cells

[Khan KN, et al. 2005]

- Monocyte chemoattractant protein 1 (MCP-1) stimulates endometrial cell proliferation both directly and by stimulation of macrophages to secrete various growth factors (e.g. VEGF, TGF- $\beta$ , EGF) and cytokines (e.g. IL-1, IL-6, IL-8, IL-12, RANTES, TNF- $\alpha$ )

[Ariai A, et al. 1997]

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## Growth of endometrial tissue

- Endometrial stroma and glands express type I and type II IGF receptors
- IGF-I and IGF-II are mitogenic factors for endometrial stromal cells in culture while antibodies blocking IGF-I receptor induce partial inhibition of endometrial stromal cell proliferation
- The expression of these receptors may be stimulated by estrogen  
Estradiol also increases the sensitivity of cells to IGF by decreasing expression of IGF Binding Protein-3 (IGFBP-3)

[Giudice L, et al. 1993]

[Giudice L, et al. 1994]

[Kleinman D, et al. 1995]

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## Growth of endometrial tissue

- Eutopic and ectopic endometrium from women with endometriosis, independently of the cycle phase:

↓ APOPTOSIS

[Gebel HM, et al. 1998]

- Endometrial glandular cells from patients with endometriosis: ↓ apoptosis, especially during the late secretory and early proliferative phases



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## Growth of endometrial tissue

- Women with endometriosis have ↑ soluble form of Fas ligand (FasL) in peritoneal fluid, interfering with the scavenging activity of immune cells

[García-Velasco JA, et al. 2002]

- Stromal cells stimulated by TGF- $\beta$  and PDGF express Fas ligand (FasL) and induce apoptosis of Fas-bearing immune cells

[Lebovic DI, et al. 2001; García-Velasco JA, et al. 1999]

- Integrin-mediated endometrial cell attachment to the ECM components (laminin, fibronectin and collagen IV) up-regulates Fas ligand (FasL) expression, leading to immune cell apoptosis

[Sekim B, et al. 2002]

- B-cell lymphoma/leukemia-2 gene (Bcl-2), the proto-oncogene that blocks cell death without promoting cell proliferation, is over-expressed in the eutopic endometrium of women with endometriosis, leading to decreased apoptosis

[Merzaman GF, et al. 2000; Jones BK, et al. 1998]

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## Growth of endometrial tissue

- Molecular mechanisms of increased PROLIFERATION and reduced APOPTOSIS of endometrial cells from women with endometriosis invoke constitutive activation of the nuclear factor-kappa B (NF- $\kappa$ B) and MAPK pathway: extracellular signal-regulated kinase (ERK1/2)

[González-Ramos R, et al. 2007]

[González-Ramos R, et al. 2008]

[Munk W, et al. 2008]

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## Statins and endometrium: inhibition of endometrial cell growth

- Statins ↓ proliferation of endometrial stromal cells irrespective of the supply of cholesterol (*eutopic endometrium*)

This action of statins is related to decreased production of mevalonate and decreased activity of the MAPK pathway (? decreased isoprenylation of Ras)

[Piotrowski P, et al. 2006]

- Lovastatin inhibits in the concentration-dependent manner cell growth in an experimental model of endometriosis-like tissue (*eutopic endometrium*)

[Eftandari N, et al. 2007]

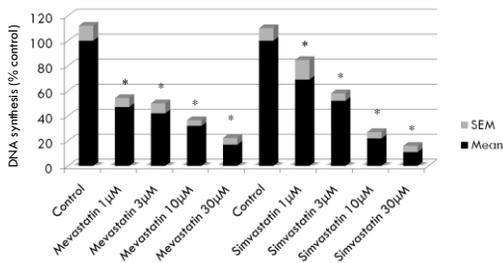
- Simvastatin inhibits proliferation of cells collected from endometriomas (*ectopic endometrium*)

[Nasu K, et al. 2009]

- Atorvastatin increased the level of IGFBP-1 in endometrial-endometriotic cell cultures treated with LPS. Increased IGFBP-1 level suggests reduced capacity of cells for proliferation and increased differentiation

[Sharma I, et al. 2009]

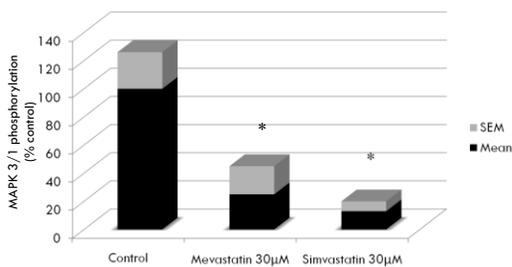
## Statins and endometrium: inhibition of endometrial cell growth



Piotrowski P, et al. 2006

## Statins and endometrium: inhibition of endometrial cell growth

Effect of statins on phosphorylation of MAPK3/1 by endometrial stromal cells



Piotrowski P, et al. 2006

## Statins and endometrium: induction of apoptosis

- Simvastatin induced significant time- and concentration-dependent apoptotic effects on human endometrial stromal cells as determined by:
  - increased activity of executioner caspases (Caspase-3/7 Assay)
  - DNA fragmentation (Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay)
- This effect was abrogated by GGPP, an important product of the mevalonate pathway.

[Sokalika A. et al., Society for Gynecologic Investigation 57<sup>th</sup> Annual Scientific Meeting, 2010, Orlando, Florida, USA]

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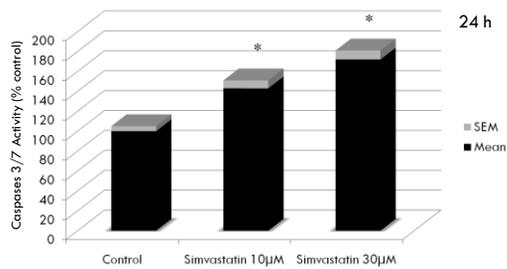
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## Statins and endometrium: induction of apoptosis



[Sokalika A. et al., Society for Gynecologic Investigation 57<sup>th</sup> Annual Scientific Meeting, 2010, Orlando, Florida, USA]

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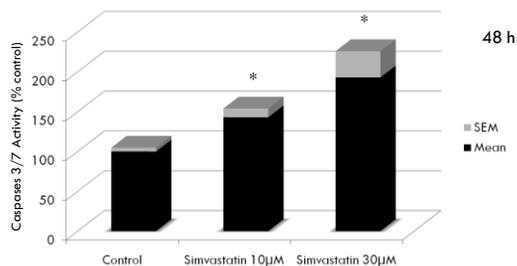
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## Statins and endometrium: induction of apoptosis



[Sokalika A. et al., Society for Gynecologic Investigation 57<sup>th</sup> Annual Scientific Meeting, 2010, Orlando, Florida, USA]

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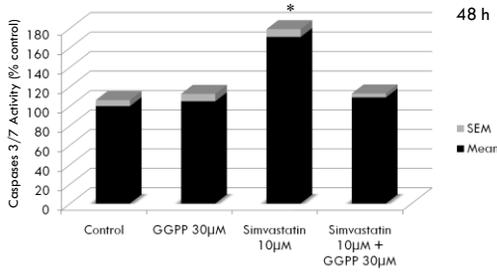
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### Statins and endometrium: induction of apoptosis



[Sakata A. et al., Society for Gynecologic Investigation 57<sup>th</sup> Annual Scientific Meeting, 2010, Orlando, Florida, USA]

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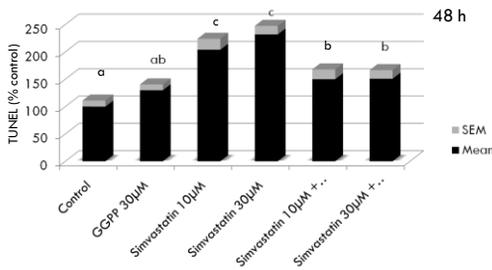
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### Statins and endometrium: induction of apoptosis



[Sakata A. et al., Society for Gynecologic Investigation 57<sup>th</sup> Annual Scientific Meeting, 2010, Orlando, Florida, USA]

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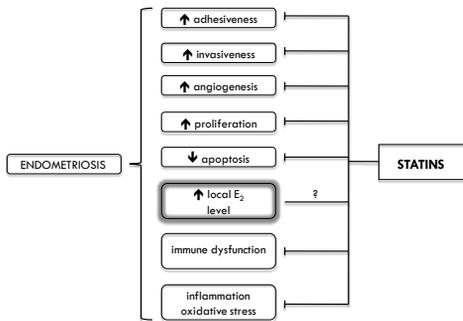
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### Proposed role of statins in treatment of endometriosis.




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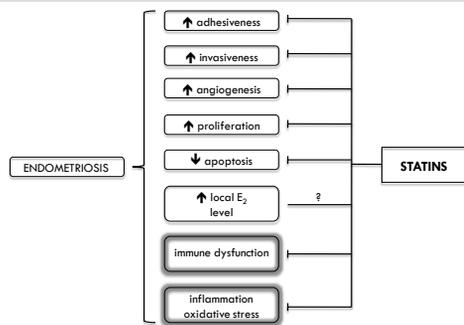
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## Proposed role of statins in treatment of endometriosis.




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## Endometriosis: inflammation, immune dysfunction and oxidative stress

- Increased concentrations of activated macrophages and changes in the cytokine network:
  - interleukin-8 (IL-8)
  - tumor necrosis factor  $\alpha$  (TNF- $\alpha$ )
  - monocyte chemoattractant protein 1 (MCP-1)
  - transforming growth factor  $\beta$  (TGF- $\beta$ )
  - regulated on activation normal T-cell expressed and secreted (RANTES)
  - macrophage colony-stimulating factor (M-CSF)
  - interferon- $\gamma$  (INF- $\gamma$ )
  - other pro-inflammatory chemoattractant cytokines (e.g. IL-1, IL-4, IL-5, IL-6, IL-10, IL-13, IL-15)

[Siristatidis C, et al. 2006]  
[Ariazi A, et al. 2002]  
[Fang CL, et al. 2009]
- Women with endometriosis have  $\uparrow$  mRNA levels of inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-8) in menstrual endometrium
 

[Kyama CM, et al. 2006]
- $\uparrow$  Interleukin-1 $\beta$  (IL-1 $\beta$ ) and RANTES mRNA levels in the luteal phase endometrium of subjects with endometriosis
 

[Kyama CM, et al. 2008]

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## Endometriosis: inflammation, immune dysfunction and oxidative stress

- Elevation of:
  - C-reactive protein (CRP)
  - serum amyloid A (SAA)
  - TNF- $\alpha$
  - IL-6
  - IL-8
  - MCP-1

in peripheral blood  $\rightarrow$  subclinical systemic inflammation

[Pizzo A, et al. 2002; Abrosio MS, et al. 1997]
- Impaired immune recognition and clearance of ectopic endometrial cells, suppressed cytotoxicity of natural killer (NK) cells, autoimmune activation of B-cells accompanied by increased production of antinuclear autoantibodies (ANA)
 

[Siristatidis C, et al. 2006]  
[Chishama F, et al. 2000]  
[Gupta S, et al. 2008]  
[Chuang PC, et al. 2009]
- Activated macrophages in patients with endometriosis, also promotes growth and vascularization of lesions
 

[Bacci M, et al. 2009]

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## Endometriosis: inflammation, immune dysfunction and oxidative stress



- ↑ ROS production in endometriotic cells  
[Ngo C, et al. 2009]
  - Evidence of ↑ in several enzymes involved in the generation ROS in endometrial tissues and endometrial implants from women with endometriosis  
[Ohta H, et al. 1999; Ohta H, et al. 2000; Ohta H, et al. 2001; Ohta H, et al. 2002]
  - Leukocytes attracted to the peritoneal cavity and endometriotic lesions and activated are also an important source of ROS
  - Endometriosis is associated with ↓ of antioxidant capacity. Intra-peritoneal levels of vitamin E are ↓, likely due to its consumption by oxidation reactions  
[Murphy AA, et al. 1998]
  - Proliferation of endometrial stroma is stimulated by moderate oxidative stress and inhibited by antioxidants  
[Fayouzi N, et al. 2004; Ngo C, et al. 2009]
- ROS at moderate levels may serve as a second messenger system modulating enzymes and intracellular signaling molecules; e.g. may stimulate MAP kinase ERK1/2 pathway  
[Adam O, et al. 2008; Ngo C, et al. 2009]

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## Statins and endometrium: increased expression of anti-inflammatory genes

- Anti-inflammatory and anti-oxidant properties of statins are well established in many biological systems
- Effects on endometrial/endometriotic tissues are not well known
- Atorvastatin in endometriotic cells exposed to lipopolysaccharide (LPS) in culture:
  - Decreased mRNA and protein expression of COX-2, a rate-limiting enzyme in prostaglandin synthesis
  - Increased mRNA and protein expression of the anti-inflammatory and anti-oxidative genes:
    - peroxisome proliferator activated receptor  $\gamma$  (PPAR- $\gamma$ )
    - liver X receptor- $\alpha$  (LXR- $\alpha$ )

[Sharma I, et al. 2009]

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## Statins: effects in vivo (rodent models of endometriosis)

### STUDY I [Oktem M, et al. 2007]

- **AIM:** to evaluate effects of atorvastatin on experimentally induced endometriosis in the rat model
- **MATERIALS AND METHODS:**
  - Wistar-Albino rats underwent laparotomy and endometrial tissue fragments were placed in the peritoneal cavity
  - 3 weeks later second laparotomy to evaluate the size of endometriotic implants
  - The rats randomly assigned into four groups:
    - **Low dose atorvastatin:** 0.5 mg/kg/day oral atorvastatin,
    - **High-dose atorvastatin:** 2.5 mg/kg/day oral atorvastatin,
    - **Control:** single dose of 1 mg/kg s.c. leuprolide acetate,
    - **Control**
  - After 21 days of treatment, the animals were euthanized and evaluated for:
    - implant size,
    - vascular endothelial growth factor (VEGF) level in peritoneal fluid
    - histopathological scores evaluating the presence of epithelial cells in the implants

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## Statins: effects in vivo (rodent models of endometriosis)

### RESULTS:

- High dose atorvastatin and GnRH agonist groups had smaller implants and lower VEGF levels in peritoneal fluid than low dose atorvastatin and control groups ( $P < 0.05$ )
  - The mean areas of implants
    - low dose atorvastatin:  $\uparrow$  from  $43.0 \pm 12.7$  to  $50.5 \pm 13.9$  mm<sup>2</sup>
    - high dose atorvastatin:  $\downarrow$  from  $41.2 \pm 13.9$  to  $22.7 \pm 13.9$  mm<sup>2</sup> ( $P < 0.05$ )
    - GnRH:  $\downarrow$   $41.2 \pm 18.1$  to  $13.1 \pm 13.8$  mm<sup>2</sup> ( $P < 0.05$ )
  - Histopathological scores of implants also decreased following atorvastatin treatment.
- **CONCLUSION:** high-dose atorvastatin caused a significant regression of endometriotic implants




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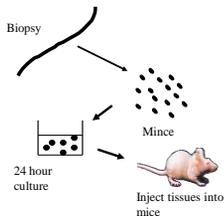
## Statins: effects in vivo (rodent models of endometriosis)

### STUDY II [Bruner-Tran KL, et al. 2009]

□ **AIM:** to evaluate the effects of simvastatin on a nude mouse model of endometriosis and the role of simvastatin in the modulation of MMP-3

### MATERIALS AND METHODS:

- Proliferative phase human endometrium established as organ cultures in 1 nM estradiol (E<sub>2</sub>) for 24 hours
- Endometrial tissues were injected intraperitoneally into ovariectomized nude mice
- All mice received E<sub>2</sub> (8 μg, silastic capsule implants)
- One day after injection of endometrium, the animals received gavage for 10 days:
  - Placebo (N=13)
  - Simvastatin (5 mg/kg/day; N=12)
  - Simvastatin (25 mg/kg/day; N=12)
- Mice were euthanized and endometrial implants were evaluated




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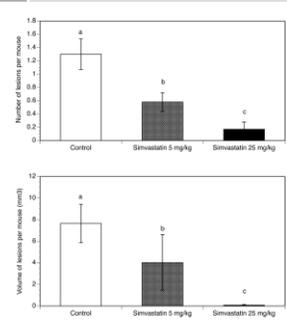
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## Statins: effects in vivo (rodent models of endometriosis)



**RESULTS:**

- Simvastatin induced a dose-dependent inhibition of the number and the volume of endometrial implants. At the highest dose:
  - 87% decrease in the number of lesions per mouse
  - 98% decrease in the volume of lesions per mouse

**CONCLUSION**

□ Simvastatin inhibits the development of endometriosis in the nude mouse

[Bruner-Tran KL, et al. 2009]

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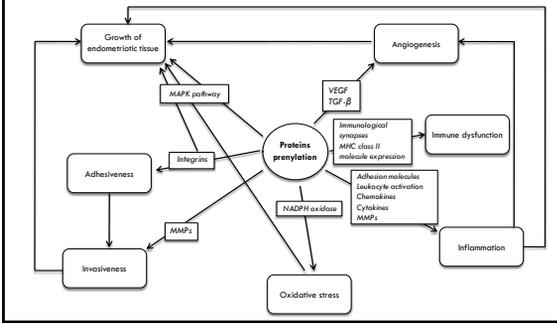
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### Proposed mechanisms of action of statins in relation to modification of isoprenylation




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### Other potential benefits of use of statins



- Activation of NF-κB (e.g. by TNF-α) which in turn, leads to expression of NF-κB-induced genes and release of cytokines in endometriosis  
[Gonzalez-Ramos R, et al. 2007; Gonzalez-Ramos R, et al. 2008]  
 [Huber AV, et al. 2007]  
 [Taniguchi F, et al. 2009]
- NF-κB underlie the complex regional pain syndrome, including inflammation, ischemia and sensitization, interact with substance P and modulate development of neuropathic pain  
[de Meis et al. 2009]  
 [Lübke K, et al. 1997]  
 [Tegeder I, et al. 2004]
- Statins ↓ expression of NF-κB in various tissues  
[Wang L, et al. 2010]  
 [Li J, et al. 2010]  
 [Ozbebek E, et al. 2009]  
 [Yin Z, et al. 2009]
- Atorvastatin ↓ nitroglycerin-induced activation of NF-κB in the trigeminal nucleus caudalis (TNC) and ↓ transmission of experimentally induced migraine-related pain in CNS  
[Yin Z, et al. 2009]

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### Other potential benefits of use of statins

- An observational study of the patients with coronary artery disease, long-term use of statins was shown to improve psychological well-being, as well as reduce risk of depression, anxiety and hostility  
[Young-Xu Y, et al. 2003]
- Possible role of statins in the inhibition of indolamine (2, 3)-dioxygenase (IDO). IDO degrades tryptophan, the precursor of the neurotransmitter serotonin (5-hydroxytryptamine), responsible for regulation of mood, appetite, sleep and cognitive functions including memory and learning  
[Weischenberg B, et al. 2004]




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## Other potential benefits of use of statins

- Statins stimulate bone formation and inhibit bone resorption  
[Mundy G, et al. 1999]  
[Stool A, et al. 2003]
- Clinical studies evaluating the risk of bone fracture among users and non-users of statins, have yielded conflicting results  
[Passo JA, et al. 2002]  
[von Skoo TP, et al. 2001]
- No information regarding the effect of statins on bone density in users of GnRH analogues




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## Summary

- Development of endometriosis involves adhesion, invasiveness, angiogenesis, growth and inflammation
- Statins may address all the above events
- Early in vitro and animal studies are promising
- Human trials urgently needed

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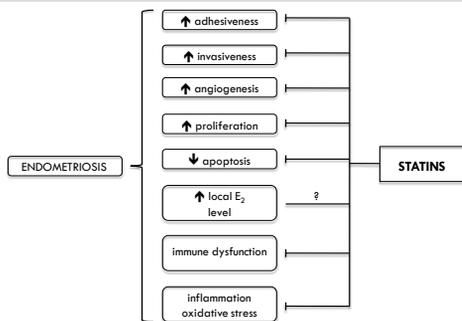
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## Proposed role of statins in treatment of endometriosis




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**Modulation of the immune system  
in endometriosis**

**TM D’Hooghe, MD, PhD**  
 Director, Center of Reproductive Medicine,  
 Leuven University Hospitals (Belgium) and  
 Research Associate/Chair International  
 Advisory Board, Institute of Primate  
 Research, Nairobi (Kenya)  
 ESHRE PCC Endometriosis: how new  
 technologies may help, Roma, Italia, 2010

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**Endometriosis**

- Defined as the presence of endometrial tissue (glands/stroma) outside the uterus
- 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
- Most common theory is “retrograde menstruation” (Sampson Hypothesis -1927)

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**Prevalence of Endometriosis**

- More than 70 million women worldwide
- 10% women of reproductive age
- 30% and 60% in women with infertility and pelvic pain respectively
- Endo cost considerably higher than cost related to Crohn’s disease or to migrane in the USA for 2002 (Simoens et al., 2007)

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### Ideal non-invasive test for endometriosis

- 100% sensitivity, even if specificity only 50%
- Identify patients who might benefit from a laparoscopy (endometriosis/other fertility problems)  
– D’Hooghe et al, 2006
- Do not miss patients with endometriosis, since surgery may double their MFR

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### Overview of potential biomarkers

- Glycoprotein markers: CA-125, CA-19-9
- Cytokine markers: IL-6, TNF-alpha, MCP-1;MIF
- Adhesion molecules: sICAM-1
- Angiogenic factors: VEGF, leptin
- Anti-endometrial antibodies
- CCR1
- Novel candidates of biomarkers:  
HSP-90-beta; annexin A2, A5;  
glycodelin; Apo A1; transgelin

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## Novel non-hormonal medical treatment for endometriosis

**TM D’Hooghe, MD, PhD**  
Leuven (Belgium) and Nairobi (Kenya)  
14<sup>th</sup> Seoul International Symposium,  
Seoul , South Korea,  
November 24<sup>th</sup>, 2009

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## Endometriosis management

### RATIONALE

- Pain
- Infertility
- ? Spontaneous progression

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## ESHRE guideline for diagnosis and treatment of endometriosis



Human Reproduction, Vol 20, No 10 pp 2698-2704, 2005

S. Kennedy, A. Bergqvist, C. Chapron, T.D'Hooghe, G.Dunselman, R. Greb, L.Hummelshoj, A. Prentice, E. Saridogan

on behalf of the ESHRE Special Interest Group for Endometriosis and Endometrium Guideline Development Group

University of Oxford, Oxford, UK, Karolinska Institutet, Stockholm, Sweden, Clinique Universitaire Baudelocque, Paris, France, Leuven University, Leuven, Belgium, Maastricht University, Maastricht, The Netherlands, Muenster University Hospital, Muenster, Germany, Endometriose Foreningen, Denmark, University of Cambridge, Cambridge, UK and University College Hospital, London, UK

ALL AUTHORS CONTRIBUTED EQUALLY TO THE MANUSCRIPT

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## Hormonal suppression

Progestins, oral contraceptives, danazol, gestrinone, LHRH agonist: equivalent (ESHRE Guidelines, 2005)

- hypo-estrogenism -> suppression of lesion size → suppression of symptoms
- direct effect on lesions (GnRH receptors in endometriosis tissue)
- Immunological effects?

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### **Ideal anti-endometriosis drug**

1. Cures existing endometriosis and prevents development of endometriosis during treatment
2. Prevents recurrence of endometriosis after cessation of treatment
3. Improves endo-related pain and subfertility, equal to or better than currently available drugs
4. No interference with menstrual cycle (ovulation, menstruation)
5. Safe during early pregnancy
6. Favorable side-effect profile
7. Low cost/convenient administration

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### **Menstruation = Pelvic inflammation**

Pelvic inflammation (WBC X 3 increased) during menses compared to nonmenstrual phases in women (Debrock et al, 2000) and baboons (D'Hooghe et al, 2001)

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### **Endometriosis = Pelvic inflammation**

- Patients have chronic pelvic inflammation
  - ↑ PF volume and PF WBC concentration
  - ↑ activation of PF macrophages
  - ↑ PF inflammatory cytokines/growth factors
- ↑ pelvic inflammation in baboons after intrapelvic injection of endometrium (D'Hooghe et al, 2001)

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**Endometriosis = Pelvic inflammation with active endometrial and PERITONEAL contribution**

- Endo versus controls:
  1. RT PCR endometrium (Kyama et al, 2005, FS)  
Menstrual EM: increased expression of TNF-alpha, IL-8 and MMP-3  
Luteal EM: increased expression of IL-1beta and RANTES
  2. RT PCR peritoneum (Kyama et al, 2005)  
Menstrual peritoneum: increased expression of ICAM-1, TGFbeta, IL-6 and IL-1beta

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**Biomarkers of systemic inflammation**

- Glycoprotein markers: CA-125, CA-19-9
- Cytokine markers: IL-6, TNF-alpha, MCP-1;MIF
- Adhesion molecules: sICAM-1
- Angiogenic factors: VEGF, leptin
- Anti-endometrial antibodies
- CCR1
- Novel candidates of biomarkers:  
HSP-90-beta; annexin A2, A5;  
glycodelin; Apo A1; transgelin

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**Anti-inflammation = local intralesional hypo-estrogenism**

IL1beta → COX-2 → PG-E2 → aromatase → E2 → VEGF → VEGF

COX-2 elevated in eutopic/ ectopic EM and PF macrophages from women with endometriosis vs controls (Ota et al, 2001; Chishima et al, 2002; Wu et al, 2002)

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### Immunological effects of standard medical therapy

- Danazol: immunosuppression, i.e., inhibition of IL-1 and TNF-alpha production by monocytes (Mori et al, 1990) and of macrophage-mediated cytotoxicity (Braun et al, 1992)
- Progesterone: immunosuppressive
- GnRH agonist: restoration of PF TIMP-1 (Sharpe-Timms et al, 1998), modulation of IL-2 receptor mRNA in vitro (Chen et al, 1999)

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### Immunological effects of new endocrine treatment

- ERB 041 (selective ERbeta agonist)  
PF macrophages Erbeta Positive: Endo > Controls  
Effect of ERB 041 on PF Macrophages:  
Inhibition of
- LPS-induced iNOS expression and
- NFkappaB activation by preventing its nuclear translocation

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### Non-hormonal action of anti-endometriosis drugs

Reduction of:

1. Endometriosis-associated peritoneal **inflammation** (e.g., anti-inflammatory, anti-TNF-alpha)
2. Endometrial-peritoneal adhesion (anti-adhesion molecules)
3. Endometrial-peritoneal **angiogenesis** (anti-angiogenesis)
4. Endometrial-peritoneal **invasion** (anti-proteases)

? Alternative strategy: promote immune surveillance:  
increase macrophage activity/NK cell activity

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## Anti-inflammatory agents

1. NSAIDs  
block both COX-1 and COX-2:  
first line treatment for dysmenorrhea
2. COX-2 inhibitors
  - Rofecoxib: regression of endo in rats (Dogan, 2004) and effective in reduction of endo-associated pain (Cobellis et al, 2004)
  - Celecoxib: regression of endo in mice (Efstathiou, 2005)
  - Nimesulid: no effect in nude mice model (Hull et al, 2005)

! Safety profile! No NHP data !

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## Anti-TNF-alpha treatment

### PREVENTION INDUCED ENDOMETRIOSIS

- **Recombinant human TNF-binding protein (r-hTBP-1) inhibits the development of endometriosis in baboons: a prospective, randomized, placebo- and drug-controlled study**  
D'Hooghe et al, Biol Reprod 2005  
(ASRM General Program Prize Winning paper 2001)

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## Result

- In baboons, r-hTBP-1 inhibited
  - the development of endometriotic lesions
  - the development of endometriosis-associated adhesions

as effectively as Antide (GnRH antagonist)  
but *without causing hypo-estrogenism*

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## Anti-TNF-alpha treatment in baboons

### Treatment of spontaneous peritoneal endo

Barrier et al, Fertil Steril 2004

- Placebo-controlled RCT: etanercept (TNFR (p75)-IgG (Fc) fusion protein)
- Significant reduction in number of lesions, especially red polypoid type

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## Anti-TNFalpha treatment in baboons

### Baboons with induced peritoneal endo

Falconer et al, Hum Reprod 2006

- Placebo controlled trial with c5N (Centocor)
- c5N reduces surface area + volume of endo
- Mainly reduction of SA and N red lesions (considered most active type of lesions)
- No effects on the menstrual cycle
- ! ? Side effects?

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### All 3 baboon studies:

#### Inhibitory effect of TNF-alpha antagonists, mainly on active red peritoneal lesions

If confirmed in women, anti-TNF- $\alpha$ :

- first effective medical treatment of peritoneal endometriosis allowing ovulation (and conception if safe?)
- preventing progression to severe/deep disease ?
- preventing recurrence and onset of new disease?
- ? SIDE EFFECTS

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### **TNFalpha inhibitors in women with endometriosis**

- Only studied in 21 women with severe deep endometriosis-associated pain and rectovaginal nodule of at least 1 cm diameter awaiting surgery (Koninckx et al, 2008)
- Placebo-controlled RCT 2:1 (14 infliximab; 7 placebo)
- 12 week treatment period followed by surgery
- Outcome: 25-30% decrease in pain in both groups; no difference between both groups
- Comment: ? Inappropriate patient selection (TNFalpha inhibitors do not work well in fibrotic IBD)

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### **Anti-TNF therapy: pentoxifylline**

- Pentoxifylline:  
Methylxanthine acting as a phosphodiesterase inhibitor
- Used in conditions involving a defective regional microcirculation but also in RA and IBD
- Via increase in cAMP in PB cells:
  - decreased potential for Pt aggregation and thrombus formation
  - Downregulation of TNF-alpha, IL-1 production

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### **Anti-TNF therapy: pentoxifylline**

- Rats: reduction of endometriotic implant growth (Nothnick et al, 1994)
- Hamster: reduction of endometriosis-associated infertility (Steinleitner et al, 1991)
- Women
  - Placebo-controlled RCT, 800 mg/day PO, 12 months: life table analysis: overall pregnancy rate 31% versus 18% (NS, but..) (Balasch et al, 1997)
  - Placebo-controlled RCT during 6 months after surgery: similar PR/recurrence rate in both groups

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## TZDs activating PPAR-gamma

PPAR-gamma = peroxisome proliferator activated receptor – gamma

- Inhibits TNF-alpha, IL6, IL1beta and MMP9 expression in macrophages
- TZDs (thiazolidinediones):
  - activate PPAR-gamma
  - strong inhibitors of cell growth and angiogenesis, RANTES expression in EM
  - inducers of apoptosis

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## TZDs activating PPAR-gamma

### Rat model:

ciglitazone: reduction induced endo  
(Lebovic et al, 2003)

### Baboon model:

pioglitazone:  
prevention + reduction of induced endo  
(Lebovic et al, 2007; Lebovic et al, 2009)

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## Anti-angiogenesis

- Endostatin
  - 20kDa carboxy-terminal fragment of Collagen XVIII
  - Induces apoptosis of endothelial cells
  - Suppresses EM induced angiogenesis in CAM model (Nap et al, 2005)
  - Inhibits growth of newly implanted lesions (autotransplantation mouse model; Becker et al, 2005)
  - Inhibits growth of established lesions (murine model of xenotransplanted human EM; Nap et al, 2004)
  - No negative effects on reproduction/offspring (rodents)
  - NO studies in NHPs or women with endometriosis

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### **Anti-angiogenesis**

- Angiostatin: effective in rodents with induced endo (Dabrosin et al, 2002), but reproductive SAEs
- Anginex: effective in rodents with induced endo (Nap et al, 2004), but no information on reproductive function
- Caplostatin: inhibits endometriotic growth in novel mouse model of endometriosis (transplantation of transgenic, luciferase-expressing EM; Becker et al, 2006)
- Atorvastatin (Oktem et al, 2007): in rat model  
High dose (2.5 mg/kg per day-21 days): as effective as GnRH agonist in decreasing endo implant size  
Equal to high dose of 80 mg/day in humans...

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### **Anti-angiogenesis via direct VEGF inhibition**

- Antihuman VEGF or sflt-1 (Hull et al, 2003): inhibition of EM growth in nude mouse model with implanted human EM
- Anti-human VEGF and Rapamycin: inhibition of EM growth in dorsal skin fold chamber model in hamsters (Laschke et al, 2006)
- No studies in NHPS or in women (angiogenesis key factor in ovulation, menstruation, implantation)

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### **Anti-angiogenesis via gene therapy (Rein et al, 2009)**

- VEGF-targeted conditionally replicative adenovirus
- Efficient viral replication and induction of apoptosis in endometriotic cells in vitro
- ? Potential toxicity to normal cells: Ad5VEGF1 lower targeting to liver and uterus in a mouse model

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### **Kinase inhibitors**

MAPKinase: major role in signaling pathway between extracellular signals (ie inflammatory cytokines) and vital cellular processes (apoptosis/survival)

- FR167653 (p38 MAPK inhibitor):  
decreased IP inflammation  
decrease EM lesions  
in murine model (Yoshino et al, 2006)

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### **Kinase inhibitors**

JNK (c-Jun NH2 terminal kinase) inhibitors

- Baboon model induced endo
- Reduction of endo surface area/volume, similar to GnRh antagonist
- No effect on cycle

(Hussein et al, 2009 ASRM oral presentation)

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### **NfkappaB and protease inhibitors**

Nfkappa B: transcription factor inducing expression of genes participating in inflammatory/immune responses

- Nuclear factor KappaB inhibitors (rats, nude mice) and Protease inhibitor bortezomib (rats):  
-reduced endo lesion volume,  
cell proliferation, ICAM-1 expression  
-increased apoptosis  
(Gonzalez-Ramos et al, 2008; Celik et al, 2008)

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## Immunostimulation

- Concept of immunostimulation of native immune system to prevent escape of EM cells from immunosurveillance
- Promising Rodent studies with IFN-alpha, IL-2, IL-12, Loxorubin, Imiquimod, leflunomide
- Important side effects
- Disappointing results with human IFN-alpha (more recurrence) and human IL-2 studies (intracystic injection)

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## Summary nonhormonal medical treatment

- Potential:
  - really NEW vs hormonal suppression:
  - direct inhibition of endo lesions
  - without inhibition of reproduction (Ov, Me, Im)
- Issues at stake:
  - Safety (general, reproductive)
  - Efficiency
  - Need for more NHP studies
  - Target: inflammatory peritoneal endometriosis,

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### Leuven University Fertility Center

<b>Gynaecology</b> T D'Hooghe C Meuleman L Meeuwis K Peeraer C Tomassetti S Pelckmans P De Loecker L Segal A Spaepen I Thijs Ph Albertyn V. Vloeberghs Gastro enterological surgery A. D'Hoore	<b>Psychology and Counseling</b> K Demyttenaere P. Enzlin U. Vandenbroeck M Vervaeke Center for Medical Genetics JP Fryns E Legius T de Ravel de L'Argentière Andrology D Vanderschueren Ph Marcq Urology D Deridder G Bogaert	<b>Paramedical staff</b> E Bakelants H De Bie K Dhondt J Gevaerts V Gilissen S Kurstjens K Lerut L Magis L Rijkers S Schildermans H Verbiest S Verschuere A Verlinden C Craenen W Leus G Roels M Toetenel Research coordinator M Welckenhuysen	<b>Fertility Lab</b> C Spiessens S Debrock G Bertin D Willemen H Devroe H Afschrift K Lerut O De Maeght L. Hollanders A Velaers F Vynckier P Bols E Vergison K Bullens B Quintens
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**Leuven Endometriosis Research Group/Network:  
6 PhD students**

<p><b>Clinical Leuven</b></p> <p><b>GYN</b></p> <p>T D'Hooghe C Meuleman L Meeuwis K Peeraer C Tomassetti S Pelckmans P De Loecker V Vloeberghs</p> <p><b>URO</b></p> <p>B. VCleynenbreugel</p> <p><b>GE surgery</b></p> <p>A D'Hoore</p> <p><b>Clinical Nairobi</b></p> <p>D Chai</p>	<p><b>Postdocs Leuven</b></p> <p>A Mihalyi; S. Debrock</p> <p><b>PhD Students Leuven-Nairobi</b></p> <p>C Kyama A Atunga</p> <p><b>PhD Students Leuven</b></p> <p>A Vodolazkaia A Fassbender C Meuleman</p> <p><b>PhD Students Leuven – int'l</b></p> <p>P Simsa (Budapest) A Bokor (Budapest) H Falconer (Karolinska)</p>	<p><b>Research Nairobi</b></p> <p>J Mwenda D Chai N Kulla E Omolo Veterinary staff Animal attendants</p> <p><b>Leuven Research coördinator</b></p> <p>M Welckenhuysen</p>	<p><b>International collaborators</b></p> <p>D. Lebovic (Ann Arbor, USA) G. Fried (Karolinska, Stockholm, SE) G. Dunselman (Maastricht, NL) A. Sharkey (Cambridge, UK) F. Vilmos (Budapest, HUN) K. Coleman (Oregon Primate Center, USA) EU Network for Endometriosis (ENE)</p>
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**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

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**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

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# Adhesion Prevention

M Canis  
Clermont-Ferrand

What is the evidence ?

What is the future ?




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27/05/2010

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## Objectives

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- Teach the rules of microsurgical techniques
- Demonstrate the potential of the peritoneal surgical environment
- Review the literature on adhesion prevention
  - Which product should be used ?
  - When should we use it ?
  - Do we have evidence in favour of one of them ?

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27/05/2010

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## Conflict of interest

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- Consultant for Ethicon
- Consultant for Covidien
- Consultant and investigator (geneva study) for Baxter
- Consultant for Nicomed
- Design of a clinical study for prevadh (covidien, sofradim)
- Animal research on adhesion and tumor dissemination partly funded by Karl Storz
- Organisation in 2008 in Clermont Ferrand of the PAX meeting funded by all the companies involved in adhesion prevention

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27/05/2010

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## Surgical Technique ??

Winston  
Gomel  
Swolin  
Brossens

## Principles of Microsurgery

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## Principles of Microsurgery

- Magnification
- Atraumatic handling of tissues
- Meticulous hemostasis
- Avoid intraperitoneal foreign material
- Avoid unnecessary ischemia of the tissues
- Complete excision of abnormal tissues
- Precise alignment and approximation of tissue planes
- Careful identification of the cleavage plane
- Irrigation et humidification of the tissues

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## Surgical technique is essential !!

### Commentary Prevention of intra-abdominal adhesions in gynaecological surgery

Gene S diZerega<sup>1</sup>, Toggas Tulandi<sup>2</sup>

« Good surgical techniques and perhaps the use of approved devices for adhesion reduction would give patients the best chance to benefit from reproductive and gynaecological surgery »

Reprod Biomed Online 2008

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## Good Surgical technique ??

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## Excision of adhesion ??

- Two situations
  - Dense adhesions
    - No distance between the organs so that excision is impossible
  - Filmy adhesions
    - Should we excise the band which is evidenced when exposing the plane ??

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## Infertility Surgery Results: Schippert et al Fertil Steril 2010

TABLE 1

Results in (%) of reconstructive tubal surgery due to acquired tubal damages (group 1) and reinfertilization after prior tubal sterilization (group 2).

Group	Number in group	Pregnancy rate	Abortion	Ectopic pregnancy	Birth rate
<b>Group 1:</b>					
microsurgery due to acquired tubal damages					
Adhesiolysis (12.6%)	116	49 (42.2%)	3 (2.6%)	9 (7.8%)	37 (31.9%)
Fimbrioplasty (17.2%)	55	30 (54.6%)	6 (10.9%)	3 (5.5%)	21 (38.2%)
Salpingostomy (49.7%)	103	53 (51.5%)	7 (6.8%)	12 (11.7%)	34 (32.9%)
Anastomosis (20.2%)	68	38 (55.9%)	9 (13.2%)	7 (10.3%)	23 (33.8%)
Total (100%)	342 (287 patients)	170 (49.7%) to total number of interventions	25 (7.3%)	31 (9.1%)	114 (33.3%) to total number of interventions
<b>Group 2:</b>					
microsurgery					
Reinfertilization after previous sterilization (all types of anastomosis and length of fallopian tubes)	69 (100%)	65 (73.9%)	14 (15.7%)	6 (6.7%)	45 (60.6%)

Note: Group 1, acquired tubal damages, 426 patients contacted, 267 patients answered; median age 31.0 years (21 to 42 years); multiple methods of surgeries during one intervention possible; total rates are related to total number of interventions. Group 2, reinfertilization, 127 patients contacted, 89 patients answered; median age 35.4 years (28 to 42 years); Medical School of Hannover, Germany, 1998-2001; analysis in 2004.

Schippert, Contraception, April 2010.

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## Two informations

- Pregnancy rates are similar to those obtained in the early years of laparoscopic surgery and during these early years adhesion were cut not excised
- Infertility surgery is still very effective when adequately performed by qualified surgeons not by IVF physicians who are preparing future IVF cycles performing salpingectomies !!

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## CORRESPONDENCE

### Infertility surgery is dead: only the obituary remains?

Despite the multiple advantages of assisted reproductive technology compared with surgery, there remain several diagnoses for which surgery is still widely performed: distal tubal occlusion, repair of permanent sterilization, and endometriosis. Assisted reproductive technology is superior to surgery and should be offered as first-line treatment. (Fertil Steril® 2007; ■■■. ©2007 by American Society for Reproductive Medicine.)

Eve C. Feinberg, M.D.<sup>a,b,c,d</sup>  
Eric D. Levens, M.D.<sup>a,b,c,d</sup>  
Alan H. DeCherney, M.D.<sup>a,b,c,d</sup>

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## But ?

- Patients who do not want to undergo IVF
- Patients who wish to stop IVF after the failure of the 1<sup>st</sup> cycle
- Patients who cannot pay for it
- Prevention of infertility
  - Myomas
  - Endometriomas
- Management of infertility in developing countries
- Patients with previous failed IVF

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## Excision is important and even essential when adhesion is explained by infiltrative endometriosis

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## The surgical environment

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## Effects of supplemental perioperative oxygen on post-operative abdominal wound adhesions in a mouse laparotomy model with controlled respiratory support<sup>®</sup>

Sachiko Matsuzaki<sup>1,2,5</sup>, Michel Camis<sup>1,2</sup>, Jean-Etienne Bazin<sup>1,3</sup>, Claude Darcha<sup>4</sup>, Jean-Luc Pouly<sup>1,2</sup> and Gérard Mage<sup>1,2</sup>

*<sup>1</sup>Université d'Auvergne - Clermont 1, Faculté de Médecine, Centre d'Endoscopie et des Nouvelles Techniques Interventives (CENTI), Clermont-Ferrand, France; <sup>2</sup>CHU Clermont-Ferrand, Polyclinique-Hôtel-Dieu, Gynécologie Obstétrique et Médecine de la Reproduction, Boulevard Léon Maltejean, 63000 Clermont-Ferrand CéDEX, France; <sup>3</sup>CHU Clermont-Ferrand, Hôtel Dieu, Service d'Anesthésie Réanimation, Clermont-Ferrand, France; and <sup>4</sup>CHU Clermont-Ferrand, Hôtel-Dieu, Service d'Anatomie et cytologie pathologiques, Clermont-Ferrand, France*

<sup>5</sup>Correspondence address: Tel: +33-4-73-75-01-38; Fax: +33-4-73-93-17-06; E-mail: sachikoma@uclm.com

**BACKGROUND:** Post-operative adhesion formation is a major clinical problem. Tissue oxygenation is one of the most important determinants in adhesion formation. The objective of this study was to investigate whether supplemental perioperative oxygen could reduce post-operative adhesion formation through increasing the peritoneal tissue oxygen tension (PtiO<sub>2</sub>) in a mouse model. **METHODS:** Adult C57BL/6 mice were randomly assigned to two groups: Group 1 (n = 20), Fraction of Inspired Oxygen (FIO<sub>2</sub>): 0.21; Group 2 (n = 20), FIO<sub>2</sub>: 0.80. On day 0, over the course of the 90 min procedure including the 60 min of laparotomy, PtiO<sub>2</sub> was continuously monitored. On day 7, a second laparotomy was performed to assess abdominal wound adhesions. Real-time RT-PCR was performed to measure expression levels of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) mRNA in peritoneal tissues. **RESULTS:** The PtiO<sub>2</sub> levels in Group 2 were significantly higher compared to Group 1 (P < 0.001) and controls (P < 0.003). There was no significant difference in the incidence of abdominal wound adhesions; however, the severity of adhesions was significantly reduced in Group 2 compared to Group 1 (P < 0.02 and P < 0.002, respectively). **CONCLUSIONS:** Supplemental perioperative oxygen may help to reduce post-operative adhesion formation.

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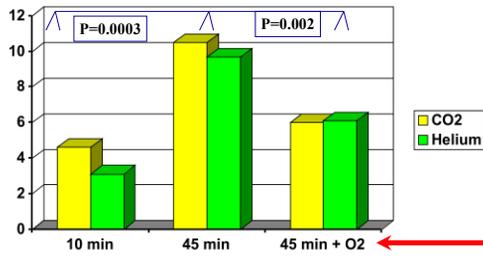
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## Adhesions ; Hypoxemia ; Duration

Rabbit Model ; Standard laparoscopic Trauma

Adhesion Scores Molinas et al Hum reprod 2000



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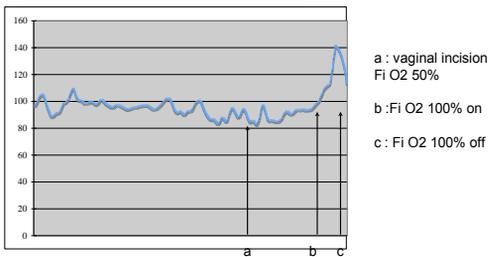
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## Peritoneal tissue oxygen pressure



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## Material and Methods

- General anesthesia
- Endotracheal intubation
- Vertical midline laparotomy from the xyphoid to the pubis
- Peritoneal tissue oxygen pressure measurement
- 60 minutes of laparotomy
- Day 7
  - Adhesions were assessed using two lateral laparotomies so as to be able to visualize adhesions to the midline incision

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## Material and Methods

- Control : Anesthesia alone n = 5
- Group 1 : FiO2 = 0.21
- Group 2 : FiO2 = 0.80



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## Assessment of postoperative adhesions

- Incidence
  - Extent
  - Type
- 0 = no adhesion
  - 1 = 1-25%
  - 2 = 26-50%
  - 3 = 51-75%
  - 4 = 76-100%
- 1 = filmy
  - 2 = blunt dissection possible
  - 3 = sharp dissection possible
  - 4 = damage of organs likely

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## Results: PitO2

	PitO2 in non injured peritoneum (mmHg)
□ Controls	35.8 ± 3.2
□ Group 1 (FiO2 = 0.21)	38.1 ± 10.3
□ Group 2 (FiO2 = 0.80)	95.4 ± 14.2

P < 0.001

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## Results adhesions

	Group 1 (FIO2 = 0.21)	Group 2 (FIO2= 0.80)
Incidence	50 %	20 %
Severity	2.87 ± 0.74 <sup>*</sup>	0.25 ± 0.55 <sup>*</sup>

P < 0.03

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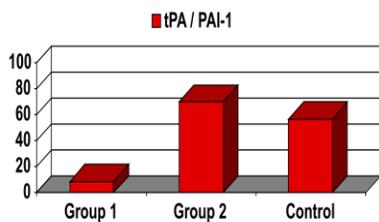
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## Results tPA/PAI-1



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## Conclusion

- Supplemental perioperative oxygen may help to reduce postoperative adhesion formation

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## Adhesion prevention products

- Hydroflotation
- Site specific products
- Pharmacological agents

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## Pharmacological agents

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## Pharmacological agents

- Systemic steroids are probably useful
  - Small molecules are quickly removed from the peritoneal cavity so that a systemic administration should be preferred to a local administration
  - If a local administration was used, the steroids would have to be combined with a slowly degraded matrix so as to allow a treatment during several days.

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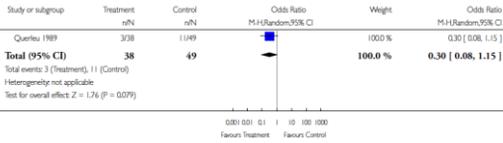
## Systemic steroids

### Analysis 1.4. Comparison 1 Steroids (any route) versus no steroids, Outcome 4 deterioration of adhesion score.

Review: Fluid and pharmacological agents for adhesion prevention after gynaecological surgery

Comparison: 1 Steroids (any route) versus no steroids

Outcome: 4 deterioration of adhesion score



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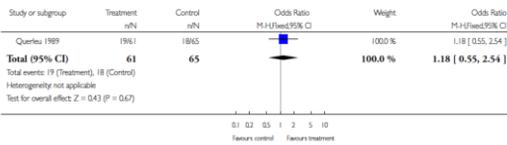
## Systemic steroids

### Analysis 2.1. Comparison 2 Systemic steroids versus no steroids, Outcome 1 clinical pregnancy rate.

Review: Fluid and pharmacological agents for adhesion prevention after gynaecological surgery

Comparison: 2 Systemic steroids versus no steroids

Outcome: 1 clinical pregnancy rate



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## But in the abstract and in the paper

- Adjuvant treatment of tubal surgery. Randomized prospective study of systemically administered corticoids and noxythiolin
- [Querleu D, Vankeerberghen-Deffense F, Boutville C.](#)
- ..... 40% of patients (13 out of 32) in the corticosteroid group, versus 26% (5 out of 29) in the noxythiolin and corticosteroid group and 19% (6 out of 31) in the control group (p less than 0.02) became pregnant.
- No adverse effect has been noted.

**These results support the use of corticosteroids in infertility surgery.**

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## Hydroflotation

### Adept (icodextrin 4% solution) reduces adhesions after laparoscopic surgery for adhesiolysis: a double-blind, randomized, controlled study

Colin B. Brown, M.D., F.R.C.P.<sup>a</sup> Anthony A. Luciano, M.D.<sup>b,c</sup> Dan Martin, M.D.<sup>d</sup> Elizabeth Peers, Ph.D.<sup>e</sup> Alison Scrimgeour, M.Sc.<sup>f</sup> and Gere S. diZerega, M.D.<sup>g</sup> on behalf of the Adept Adhesion Reduction Study Group



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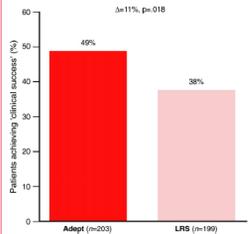
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## Pamela study

FIGURE 2

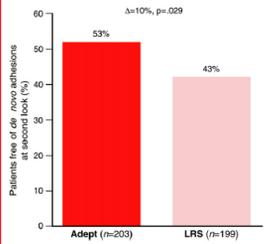
Clinical success. The percentage of patients in whom the number of sites with adhesions decreased by at least three or 30% of the number of sites lysed (PP population;  $P= .018$  between groups).



Brown. Adept reduces adhesions in laparoscopy. Fertil Steril 2007.

FIGURE 4

Percentage of patients free of de novo adhesions in the Adept and LRS treatment groups at second laparoscopy (PP population;  $P=.029$ ).



Brown. Adept reduces adhesions in laparoscopy. Fertil Steril 2007.

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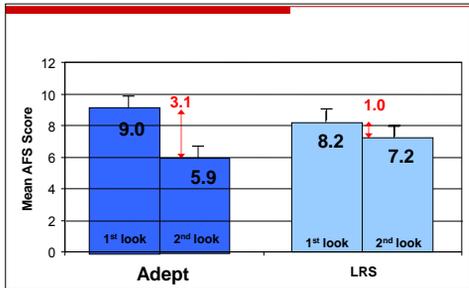
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## Improvement in AFS score - Infertility Patients



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## Pamela study

- AFS score—all patients:
- Forty-three percent of all Adept patients had a reduction in AFS adnexal adhesion score at follow-up compared with 35% of all LRS patients (P1/4.065).
- The mean reductions in AFS (SD) score per patient between baseline and follow-up were  $2.70 \pm 6.18$  for Adept and  $1.19 \pm 5.98$  for LRS.

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## Pamela study

Statistically Significant	Yes
Clinically relevant	??

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## Barriers / Site specific products

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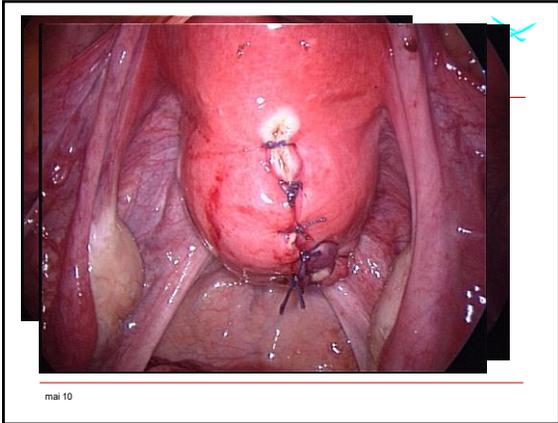
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### Interceed®

- Interceed® (oxidised regenerated cellulose)
- forms a viscous gel when it comes into contact with fluids
- completely resorbed after 4 weeks.
- Meticulous haemostasis is important, as the efficacy of the product is reduced in the presence of blood

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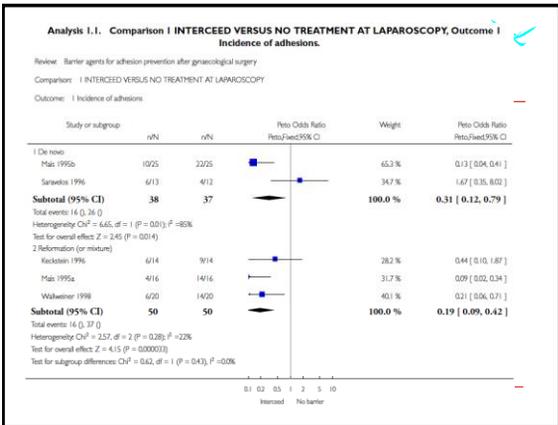
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## Interceed

Sawada T, Nishizawa H, Nishio E, Kadowaki M (2000)  
 Postoperative adhesion prevention with an oxidized regenerated cellulose adhesion barrier in infertile women.  
 J Reprod Med 45:387–389

This work with Interceed® indicated that its effect on reducing adhesions results in improved pregnancy outcomes in infertile patients.

While the number of patients in this study was limited, the use of Interceed® resulted in a significant increase in the pregnancy rate compared to surgical controls.

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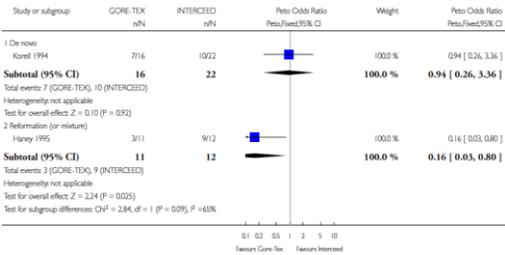
## Preclude® (Gore-tex—expanded polytetrafluoroethylene,PTFE)

### Analysis 5.1. Comparison 5 GORE-TEX VERSUS INTERCEED, Outcome 1 Incidence of adhesions.

Review: Barrier agents for adhesion prevention after gynaecological surgery

Comparison: 5 GORE-TEX VERSUS INTERCEED

Outcome: 1 Incidence of adhesions



## Interceed versus Goretex

- However, the major disadvantage of Gore-Tex is the additional surgery required for its removal after peritoneal healing is complete. In addition, it is not known whether adhesion formation could occur after removal. This perceived need for removal has been questioned by some (Haney 1995) since Gore-Tex has been left in place as pericardial or vascular grafts for many years without any adverse effects.

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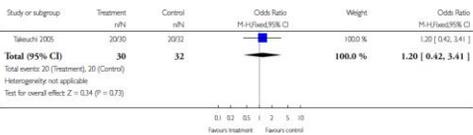
## Fibrin sheet

### Analysis 3.1. Comparison 3 FIBRIN SHEET VERSUS NO TREATMENT AT LAPAROSCOPIC MYOMECTOMY, Outcome 1 Incidence of adhesions.

Review: Barrier agents for adhesion prevention after gynaecological surgery

Comparison: 3 FIBRIN SHEET VERSUS NO TREATMENT AT LAPAROSCOPIC MYOMECTOMY

Outcome: 1 Incidence of adhesions



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## Technique of Oxiplex/AP Gel Application

For patients who received Oxiplex/AP Gel, the following procedures were followed.

At the end of surgery, subjects were placed in a reverse Trendelenburg position to facilitate collection of residual fluid from the cul-de-sac. Thereafter, residual fluid was aspirated until 10 mL of fluid remained in the cul-de-sac.

A single layer of gel was applied with a 30.5 cm x 5 mm canula applicator in sufficient volume to completely coat the surgical site with a viscous layer of gel.

The gel treatment sites included the anterior and posterior surfaces of the ovary, fallopian tube including mesosalpinx, the surfaces between the fallopian tube and the ovarian surface, adjacent pelvic sidewall including the ovarian fossa, and the lateral aspect of the uterus that could come in contact with the adnexa.

The amount of gel required to coat the adnexa and adjacent tissues on a single side did not exceed 30 mL. Thereafter, the surgical instruments were removed, and the pneumoperitoneum evacuated.

Young et al Fertil Steril 2005

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## Application of the product



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## Patients included

Young et al Fertil Steril 2005

- Laparoscopic adhesiolysis
- Endometriosis
- Excision of lesions of the ovarian fossa
- Treatment of dermoid cysts
  
- 18 patients received the gel
- 10 controls
  
- 2 to 1 randomization
- Blind reviewer of first and second look laparoscopy

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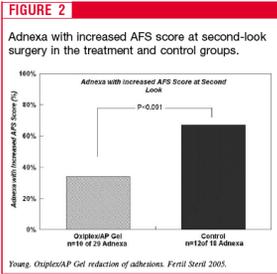
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Young et al Fertil Steril 2005



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Young et al Fertil Steril 2005



In conclusion, Oxiplex/AP Gel was found to be safe and easy to use during laparoscopic surgery. There was a marked reduction in the number of adnexa that had an increase in adhesions when treated with Oxiplex/AP Gel, compared with surgery-only controls.

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Lundorff et al / Hum Reprod 2005



Human Reproduction Vol.20, No.2, pp. 514-520, 2005  
 Advance Access publication December 9, 2004  
 doi:10.1093/humrep/dh6651

**Clinical evaluation of a viscoelastic gel for reduction of adhesions following gynaecological surgery by laparoscopy in Europe**

P.Lundorff<sup>1</sup>, J.Donnez<sup>2</sup>, M.Korell<sup>3</sup>, A.J.M.Audebert<sup>4</sup>, K.Block<sup>5</sup> and G.S.dizZerega<sup>6,7</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Viborg Hospital, Viborg, Denmark, <sup>2</sup>Université Catholique de Louvain, Cliniques Universitaires Saint-Luc, Brussels, Belgium, <sup>3</sup>Department of Obstetrics and Gynecology, Klinikum Duisburg, Zaden Rehwiesen 9, Duisburg, Germany, <sup>4</sup>Institut Gynecol France, Bouleaux, France, <sup>5</sup>FrioMod Inc, 231 Bonetti Drive, San Luis Obispo, California, and <sup>6</sup>Obstetrics and Gynecology, Livingston Reproductive Biology Laboratories, Keck-USC School of Medicine, Los Angeles, California, USA

<sup>7</sup>To whom correspondence should be sent at: University of Southern California Keck School of Medicine, Obstetrics and Gynecology, Livingston Laboratories, 1321 N. Mission Road, Los Angeles, CA 90033, USA. E-mail: GSD1270@aol.com

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## Lundorff et al / Hum Reprod 2005

A total of 49 female patients, aged 18–46 years, received treatment at four centres.

Of the 25 treatment patients, surgery was performed on 45 adnexa followed by coverage of those adnexal sites by Oxiplex/AP Gel.

Of the 24 control patients, surgery alone was performed on 41 adnexa.

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## Lundorff et al / Hum Reprod 2005

	Treatment	Control
Adhesiolysis	12	8
Endometrioma	6	3
Peritoneal Endometriosis	33	33
Stage IV	6	6

All patients returned for second-look laparoscopy within 6–10 weeks.  
As a result, efficacy analyses are presented for all 86 adnexa.

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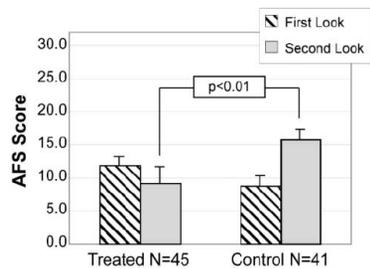
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## Lundorff et al / Hum Reprod 2005



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## Lundorff et al / Hum Reprod 2005



Table II. Outcome of clinical trials using the adnexal adhesion score of the American Fertility Society (AFS) as established in 1988

	Individual AFS scores			AFS category		
	Improved or unchanged	Worsened	Total	Improved or unchanged	Worsened	Total
Oxiplex	87% (39)	13% (6)	45	93% (42)	6% (3)	45
Control	32% (13)	68% (28)	41	56% (23)	44% (18)	41

The significant benefit of Oxiplex/AP Gel in reducing adhesions was shown by both a reduction in average AFS score as well as reduction in AFS prognostic category as a result of treatment ( $P < 0.01$  for both).

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## Lundorff et al / Hum Reprod 2005



In summary, the use of Oxiplex/AP Gel in this multicentre evaluation showed a significant reduction in the number of adnexa that developed adhesions following surgery.

In the Oxiplex/AP Gel-treated group, 93% of the adnexa did not have a worse adhesion category in contrast to 56% of the control adnexa at the time of second look.

These differences are highly significant and demonstrate the overall benefit of Oxiplex/AP Gel when used together with good surgical technique to enhance the likelihood of a good response to surgical therapy.

Patients with severe adhesion scores at the initial laparoscopy and concurrent stage IV endometriosis did not have a reduction in adhesion score even with the use of Oxiplex/AP Gel.

The gel was safe and no complications or adverse events were observed in the treatment group.

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## Conclusion: Intercoat



- Easy to apply
  - Easy to introduce through the trocar
  - Stay in place after application
  - Ability to cover large areas
  - Visible
  - Does not require an increase in intraperitoneal pressure
  - Safe
  
- Limited number of patients
  - Pb of the control group
  - Technique used in the application (drying ??)
  - Limited efficacy in advanced disease

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## Spraygel conclusion

- Easy to use at laparoscopy and at laparotomy
- But increased intra peritoneal pressure with air
- Good coverage of the areas
  
- Effective but limited evidence

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## Sprayshield

- Surg Technol Int. 2009;18:137-43. Pre-clinical evaluation of a next-generation spray adhesion barrier for multiple site adhesion protection. Ferland R, Campbell PK, Brown
- The SprayShield Adhesion Barrier System (Covidien, Waltham, MA) is initially sprayed as a liquid. SprayShield solidifies within 2 seconds of contact with tissue through a polyethylene glycol (PEG) ester-Trilysine reaction to form an adherent, internal tissue barrier that protects the underlying tissues for several days after surgery.
- It is absorbed within 7 days. Safety testing has shown the product to be nongenotoxic, noncytotoxic, nonsensitizing, and nonirritating.
- SprayShield has been shown to adhere well to tissue, with the mechanism of adherence believed to be mainly due to tissue surface mechanical interlocking.
- Compared to Controls, SprayShield demonstrated a statistically significant reduction in the number of adhesions (46%, p=0.04) and in the area of adhesions (83%, p=0.012) to injured sites.
- With its ease of application, biocompatibility and adhesion prevention efficacy, SprayShield may be an effective next-generation adhesion prevention product.

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## Sprayshield



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## Hyalobarrier

- A highly viscous gel of HA (hyaluronic acid) derivatives obtained through an auto-crosslinking process that does not introduce foreign bridge molecules, namely Hyalobarrier®, has recently been developed.

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## Hyalobarrier

- Laparoscopic Myomectomy

### Reduction of postoperative adhesions with an auto-crosslinked hyaluronan gel in gynaecological laparoscopic surgery: a blinded, controlled, randomized, multicentre study

V.Mais<sup>1,2</sup>, G.L.Bracco<sup>2</sup>, P.Litta<sup>3</sup>, T.Gargiulo<sup>4</sup> and G.B.Melis<sup>1</sup>

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## Hyalobarrier

Assessed for Eligibility (n=60)

Table III. Incidence and severity of adhesions at second-look in a subgroup of patients who underwent myomectomy without concomitant surgery (n = 23)

	Hyalobarrier (n = 12)	Control (n = 11)	P
Adhesion-free patients	8/12 (67%)	4/11 (36%)	NS
Patients with adhesions	4/12 (33%)	7/11 (64%)	
Total score at second-look			
Mean ± SD	1.5 ± 3.2	2.7 ± 2.4	0.04
Median	0	2	0.03
Uterine score at second-look			
Mean ± SD	0.5 ± 0.9	1.4 ± 1.1	0.02
Median	0	1	0.02
Uterine score second-look versus baseline			
Mean ± SD	0.5 ± 0.9	1.2 ± 1.0	0.03
Median	0	1	0.03

NS, not significant.

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## Hyalobarrier



- Fertil Steril. 2003 Aug;80(2):441-4. Effectiveness of autocrosslinked hyaluronic acid gel after laparoscopic myomectomy in infertile patients: a prospective, randomized, controlled study. Pellicano M, Bramante S, Cirillo D, Palomba S, Bifulco G, Zullo F, Nappi
- OBJECTIVE: To assess the efficacy of autocrosslinked hyaluronic gel in postsurgical adhesion prevention after laparoscopic myomectomy. DESIGN: Prospective, randomized, controlled study. SETTING: University of Naples "Federico II". Thirty-six infertile women with symptomatic myomas were randomly divided into two groups of 18 patients each. INTERVENTION(S): Laparoscopic myomectomy with subserous sutures or interrupted figure 8 sutures, with (group A) or without (group B) application of autocrosslinked hyaluronic acid (HA) gel. MAIN OUTCOME MEASURE(S): Rate of postsurgical adhesions at 60-90 days of follow-up. The rate of subjects who developed postoperative adhesions was significantly lower in group A in comparison with group B (27.8% vs. 77.8%). In both groups, the rate of adhesions was significantly higher in patients treated with interrupted figure 8 sutures than with subserous sutures. CONCLUSION(S): Autocrosslinked HA gel is a promising resorbable agent barrier for the reduction of postoperative adhesions after laparoscopic myomectomy

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## Hyalobarrier



- Easy to apply
- Used at laparoscopy, at laparotomy, at hysteroscopy
- Safe
- No side effect
  
- Effective
- Limited data published

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## PREVADH™ film (Covidien)



- Adhesion barrier device
- Hydrophilic and resorbable collagen membrane/fleece composite film (Porcine atelocollagen type I, PEG, glycerol)
- PREVADH™ adhesion barrier is fully resorbed in 3 weeks
- PREVADH™ was found to reduce adhesions frequency and severity to parietal and visceral peritoneal surfaces<sup>1, 2</sup> as well as in gynecologic model<sup>3</sup>



<sup>1</sup>Baulieu J. and al *Annals of Surgery*, 2004;129 (3): 9-12  
<sup>2</sup>Mabrut JY. And al *Hepato-gastroenterology*, 2008; 55: 517-521  
<sup>3</sup>Wiseman DM. and al *Fertil Steril*. 2001; 76: 175-80

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## OBJECTIVES



Clinically evaluate the efficacy of the Prevadh™ film in adhesion prevention and study post-operative complications and pregnancy rate

### Population:

Patients having immediate or differed pregnancy desire and suffering from symptomatic or asymptomatic fibroma interfering with fertility.

### Evaluation of adhesions:

Laparoscopic 2nd look (10 to 20 weeks)  
Post-operative complications

Annual clinical follow-up during 3 years

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## STUDY DESIGN



### Methodology:

Prospective comparative randomized study  
80 patients 13 centers  
Prevadh™ film vs Ringer® lactate directly applied to the uterine scars  
Indication: myomectomies via open surgery  
Ethics and competent authority approvals  
Signed informed consent

### Primary endpoint:

Assessment of adhesion rate to the uterine scars during a laparoscopic second look.

### Secondary endpoint:

Pelvic adhesion according to AFS scoring  
Adverse events related to adhesions

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## Results



- From May 2006 to February 2008: 52 patients included (25 PREVADH™, 27 Ringer®)
- Age: 34 years ± 5 years
- 34 second-looks collected at 105.1 days ± 47.5 (18 patients PREVADH™, 16 patients Ringer®)
- No serious adverse event related to PREVADH™ or control

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## Results

➤ Scars adhesions  
Prevadh™: 12/36 (33.3 %)  $p = 0,0001^*$

Ringer®: 22/27 (81.5 %)

➤ Patients adhesions  
Prevadh™: 9/18 (50.0 %)  $p = 0,005^*$

Ringer®: 15/16 (93.7 %)

	Mean adhesion severity scores	Mean adhesion extent scores
➤ Mean adhesion severity/ extent scores Prevadh™	1.2 ± 0.4**	1.6 ± 0.8***
Ringer®	1.6 ± 0.5**	2.3 ± 0.8***

\*Chi<sup>2</sup> test

\*\* p = 0.03 (Mann Whitney test)

\*\*\* p = 0.02 (Mann Whitney test)

## Conclusion

- PREVADH™ film significantly reduces post-operative adhesions after myomectomy by laparotomy.
- PREVADH™ acts first as a barrier, its resorption restores the plan of a natural split between the structures previously separated by this barrier.
- Tolerance of PREVADH™ film is excellent.
- PREVADH™ is effective in reducing adhesion incidence, severity and extent of uterine adhesions after myomectomy, demonstrating the need for adhesion prevention in gynaecology.

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## Other products

- Seprapray
- Coseal (hydrogel .....

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Which one should we use ??

- Most of them are clinically effective in reducing adhesion formation
- Very few studies of pregnancy rates have been performed
- How should we choose ?
- Their efficacy roughly appear similar

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Comparative Studies

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### A Direct Comparison of Seprafilm, Adept, Intercoat, and Spraygel for Adhesion Prophylaxis<sup>1</sup>

Taufiek Konrad Rajab, M.B., B.Chir.,<sup>\*,2</sup> Markus Wallwiener, M.D.,<sup>\*</sup>  
Constanze Planck,<sup>\*,†</sup> Christoph Brochhausen, M.D.,<sup>‡</sup> Bernhard Kraemer, M.D.,<sup>\*,1</sup>  
and Christian Wilhelm Wallwiener<sup>\*,†,1</sup>

TABLE 1  
Adhesion Quantity

Treatment group	Fraction of the traumatized area covered by adhesions (Mean ± Standard deviation)	Adhesion-free incidence
Control	0.77 ± 0.19	0% (0/30)
Seprafilm	0.46 ± 0.32 *	20% (6/30) <sup>†</sup>
Adept	0.54 ± 0.19 *	0% (0/30)
Intercoat	0.55 ± 0.37 *	20% (6/30) <sup>†</sup>
Spraygel	0.63 ± 0.32	3% (1/30)

\*Indicates a statistically significant difference versus control (P < 0.05).

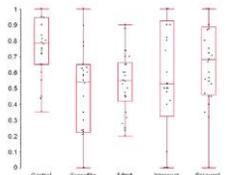


FIG. 2. Fraction of the traumatized area covered by adhesions. The boxplots show minimum observation, lower quartile, median, upper quartile, and maximum observation.

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## Comparative Studies

### Influencing factors of adhesion development and the efficacy of adhesion-preventing agents in patients undergoing laparoscopic myomectomy as evaluated by a second-look laparoscopy

Hiroyuki Takeuchi, M.D., Mari Kitade, M.D., Iwaho Kikuchi, M.D., Hiroto Shimanuki, M.D., Jun Kumakiri, M.D., and Satoru Takeda, M.D.

Department of Obstetrics and Gynecology, Juntendo University School of Medicine, Tokyo, Japan

**Objective:** To examine the factors influencing the development of adhesions after laparoscopic myomectomy (LM) and the efficacy of adhesion-preventing agents.

**Design:** Prospective nonrandomized study.

**Setting:** University-affiliated hospital.

**Patient(s):** Three hundred seventy-two patients who underwent LM alone by the same surgeon between 2000 and 2005 were included for the analysis in this study.

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## Factors Influencing the Development of Adhesions at the Site of the Uterine Surgical Wound (Table 1)

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TABLE 1

Risk factors of wound adhesion calculated with logistic regression.

	Exp(B)	95% CI	P value
Logistic regression step 1			
Age	1.006	0.992-1.062	.844
Size of largest myoma	1.014	1.002-1.026	.027
Total no. of myomas	1.125	1.059-1.194	.000
Preoperative GrRIs	1.208	0.622-2.347	.578
Adhesion-preventing agent*			
Fibrin glue	0.317	0.143-0.703	.005
Fibrin sheath	1.175	0.561-2.462	.669
Seprafilm	0.214	0.105-0.433	.000
Interced	0.314	0.144-0.684	.004
Logistic regression step 2			
Size of largest myoma	1.014	1.002-1.026	.026
Total no. of myomas	1.126	1.062-1.194	.000
Preoperative GrRIs	1.208	0.621-2.348	.578
Adhesion-preventing agent*			
Fibrin glue	0.318	0.144-0.704	.005
Fibrin sheath	1.162	0.566-2.470	.656
Seprafilm	0.213	0.105-0.432	.000
Interced	0.314	0.144-0.685	.004
Logistic regression step 3			
Size of largest myoma	1.015	1.002-1.027	.019
Total no. of myomas	1.128	1.064-1.196	.000
Adhesion-preventing agent*			
Fibrin glue	0.313	0.142-0.693	.004
Fibrin sheath	1.151	0.554-2.391	.706
Seprafilm	0.212	0.105-0.429	.000
Interced	0.310	0.142-0.674	.003

Abb: CI = confidence interval; GrRIs = GrRIs agonist.

\*The frequency ratio relative to that in the group in which no adhesion-preventing agent had been used was calculated.

Table 1. Adhesion after laparoscopic myomectomy. *BMC Surg* 2006.

## Factors Influencing the Development of de Novo Adhesions of the Uterine Adnexa (Table 2)

27/05/2010

TABLE 2

Risk factors of adnexal de novo adhesion calculated with logistic regression.

	Exp(B)	95% CI	P value
<b>Logistic regression step 1</b>			
Age	0.951	0.874-1.035	0.247
Size of largest myoma	1.025	1.008-1.043	0.005
Total no. of myomas	1.073	0.994-1.158	0.070
Preoperative GnRHs	1.416	0.464-4.318	0.541
<b>Adhesion-preventing agent*</b>			
Fibrin glue	1.339	0.378-4.749	0.651
Fibrin sheath	2.046	0.657-6.367	0.217
Seprafilm	0.658	0.197-2.204	0.498
Interceed	0.837	0.230-3.051	0.788
<b>Logistic regression step 2</b>			
Age	0.951	0.874-1.036	0.250
Size of largest myoma	1.026	1.008-1.044	0.003
Total no. of myomas	1.076	0.997-1.161	0.061
<b>Prevent adhesion agent*</b>			
Fibrin glue	1.335	0.376-4.373	0.655
Fibrin sheath	1.995	0.644-6.183	0.231
Seprafilm	0.655	0.199-2.226	0.508
Interceed	0.824	0.226-3.001	0.769
<b>Logistic regression step 3</b>			
Age	0.951	0.874-1.033	0.247
Size of largest myoma	1.025	1.008-1.043	0.003
Total no. of myomas	1.063	0.988-1.145	0.103
<b>Prevent adhesion agent*</b>			
Fibrin glue	1.275	0.361-4.505	0.706
Fibrin sheath	1.851	0.603-5.677	0.282
Seprafilm	0.659	0.197-2.200	0.498
Interceed	0.805	0.222-2.923	0.742

Note: CI = confidence interval.  
\*The frequency ratio relative to that in the group in which no adhesion-preventing agent had been used was calculated.

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## Takeuchi et al

In conclusion, SLL in patients in whom relatively large myomas were enucleated by LM by the same surgeon at the same medical institution revealed that factors influencing the development of postoperative adhesions at the site of the uterine surgical wound were found to include the diameter of the largest myoma, the number of myomas, and the nonuse/type of adhesion-preventing agent used.

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## Prevention of adhesion formation in a laparoscopic mouse model should combine local treatment with peritoneal cavity conditioning†

M.M. Binda<sup>1</sup> and P.R. Koninckx

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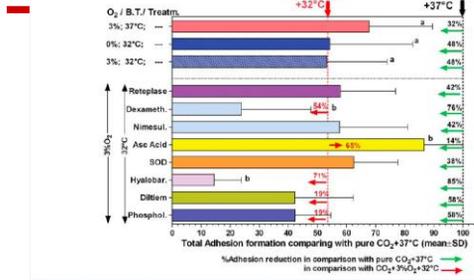


Figure 1 Adhesion prevention in a laparoscopic mouse model.

27/05/2010

## Prevention of adhesion formation in a laparoscopic mouse model should combine local treatment with peritoneal cavity conditioning†

M.M. Binda<sup>1</sup> and P.R. Koninckx

**Results:** The addition of 3% O<sub>2</sub> to the pneumoperitoneum or a lower BT decreased adhesions by 32% or 48%, respectively (P, 0.05, Wilcoxon), but were without additional effects when combined. In addition, if dexamethasone or Hyalobarriew gel were administrated, the total reduction was 76% (P 1/4 0.04) or 85% (P, 0.02), respectively.

**Conclusions:** Combining pneumoperitoneum conditioning together with dexamethasone or a barrier resulted in significant adhesion reduction in a laparoscopic mouse model.

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Which pressure is acceptable in a mouse model ?

CO<sub>2</sub> pneumoperitoneum, intraperitoneal pressure and peritoneal tissue hypoxia: A mouse study with controlled respiratory support

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CONCLUSIONS

- Surgical technique is essential !!
- Infertility surgery is not dead! It should not die !! We have to teach it again and again !!!
- Adhesion prevention and prevention of reformation are different stories which should be studied in different studies
- Corticosteroids were effective in microsurgical studies, they are effective in animal studies, it seems reasonable to recommend them when there are no risk of bowel complications

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Conclusions

- Animal studies demonstrated the potential improvement of surgical results using the pneumoperitoneum and the parameters of ventilation
- These approaches have to be further evaluated in clinical practice
- The models used are controversial and discussed

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## Conclusions

- Clinical studies confirmed that the prevention of adhesion is possible using one of the commercially available devices
- Clinical comparative studies did not show significant different results when comparing these devices
- In contrast clinical results reported are quite surprisingly similar, showing effective prevention but not allowing to prevent all post operative adhesions
- It should be emphasized that the clinical data are limited with a small number of patients and almost no clinical outcome
- Safety, Cost, Ease of use are essential to choose the product which should be used

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Mark your calendar for the upcoming ESHRE campus workshops!

- **Basic Genetics for ART Practitioners**  
*organised by the SIG Reproductive Genetics*  
16 April 2010 - Porto, Portugal
- **Array technologies to apprehend developmental competence and endometrial receptivity: limits and possibilities**  
*organised by the Task Force Basic Science in Reproduction*  
22 April 2010 - Brussels, Belgium
- **The management of infertility – training workshop for junior doctors, paramedicals and embryologists**  
*organised by the SIG Reproductive Endocrinology, SIG Embryology and the Paramedical Group*  
26-27 May 2010 - Kiev, Ukraine
- **Preimplantation genetic diagnosis: a celebration of 20 years**  
*organised by the SIG Reproductive Genetics*  
1 July 2010 - Rome, Italy
- **EIM 10 years' celebration meeting**  
*organised by the European IVF Monitoring Consortium*  
11 September 2010 - Munich, Germany
- **The determinants of a successful pregnancy**  
*organised by the SIGS Reproductive Surgery, Early Pregnancy and Reproductive Endocrinology*  
24-25 September 2010 - Dubrovnik, Croatia
- **Basic training workshop for paramedics working in reproductive health**  
*organised by the Paramedical Group*  
6-8 October 2010 - Valencia, Spain
- **Forgotten knowledge about gamete physiology and its impact on embryo quality**  
*organised by the SIG Embryology*  
9-10 October 2010 - Lisbon, Portugal

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## Upcoming events

- **Female and male surgery in human reproductive medicine**  
8-9 October 2010 - Treviso, Italy
- **Promoting excellence in clinical research: from idea to publication**  
5-6 November 2010 - Thessaloniki, Greece
- **“Update on pluripotent stem cells (hESC and iPS)” and hands on course on “Derivation and culture of pluripotent stem cells”**  
8-12 November 2010 - Valencia, Spain
- **Women’s health aspects of PCOS (excluding infertility)**  
18 November 2010 - Amsterdam, The Netherlands
- **Endoscopy in reproductive medicine**  
24-26 November 2010 - Leuven, Belgium
- **Fertility and Cancer**  
25-26 November 2010 - Bologna, Italy
- **The maternal-embryonic interface**  
2-3 December 2010 - Valencia, Spain
- **GnHR agonist for triggering of final oocyte maturation – time for a paradigm shift**  
3 December 2010 - Madrid, Spain
- **Raising competence in psychosocial care**  
3-4 December 2010 - Amsterdam, The Netherlands

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# NOTES

# NOTES