

ESHRE Campus workshop

PREVENTION OF ENDOMETRIOSIS

Bordeaux, France

15 to 17 February 2007



Contents

Organisation	p. 3
Course description & objectives	p. 4
Program	p. 5
Speakers' take home messages	
1. Principles of preventive medicine J. de Mouzon (F)	p. 8
2. Can endometriosis be prevented? A provoking introduction covering an overview of the different fields to be covered during the course A. Bergqvist (S)	p. 9
3. What kind of disease: implantation or metaplasia? P. Vercellini (I)	p. 10
4. How big is the problem: data from the EAPPG survey L. Hummelshoj (UK)	p. 11
5. Heredity S. Kennedy (UK)	p. 12
6. The role of environmental/life style factors in developing endometriosis and degree of endometriosis K. Zondervan (UK)	p. 13
7. The role of endogenous hormones A. Prentice (UK)	p. 14
8. The role of OCs – do they increase or decrease risk? P. Vercellini (I)	p. 15
9. The role of infertility as a risk of endometriosis J. Garcia Velasco (E)	p. 16
10. The role of genital anomalies A. Audebert (F)	p. 17
11. Early diagnosis – are there non-invasive tests? R. Greb (D)	p. 18
12. Reduce retrograde menstruation? H. Fernandez (F)	p. 19
13. Reduce inflammation? A. Bergqvist (S)	p. 20

- 14. Reduce implantation potential of the endometrium?
P. Groothuis (NL) P. 21
- 15. How to prevent recurrence surgically
Ch. Chapron (F) p. 22
- 16. Managing the adolescent
A. Prentice (UK) p. 23
- 17. How to increase awareness in schools: the NZ experience
D. Bush (NZ) p. 24
- 18. How can diet impact the symptoms of the disease?
D. Shepperson Mills (UK) p. 25

Additional contributions

- 1. Environmental risk factors for endometriosis
K. Zondervan p. 26
- 2. Early diagnosis – Are there any non-invasive tests
R. Greb (D) p. 36
- 3. Reduce retrograde menstruation
H. Fernandez (F) p. 44
- 4. Reduce implantation potential of the human endometrium
P. Groothuis (NL) p. 48
- 5. How can diet impact the symptoms of the disease?
D. Shepperson Mills (UK) p. 60

NOTES p. 73

Organisation

Organising Committee

- Audebert (F)
- Bergqvist (S)
- L. Hummelshoj (UK)

Faculty

- A. Audebert (F)
- A. Bergqvist (S)
- D. Bush (NZ)
- Ch. Chapron (F)
- J. De Mouzon (F)
- H. Fernandez (F)
- R. Greb (D)
- P. Groothuis (NL)
- L. Hummelshoj (UK)
- S. Kennedy (UK)
- D. Mills (UK)
- A. Prentice (UK)
- P. Vercellini (I)
- K. Zondervan (UK)

Course description

Endometriosis is a highly prevalent disease, often diagnosed late, and often difficult to manage. This course will address the challenge of endometriosis, early signs and risk factors, and will provide a comprehensive review of the preventive mechanisms that are available today. The course is designed to facilitate interactive discussions in order to come up with a consensus to determine preventive measures, including:

- What are we trying to prevent?
- Why are we trying to prevent this?
- Who are we targeting for prevention?
- How are we going to do it?

Course objectives

At the end of this course, the participants to should be able to:

- Identify early signs of endometriosis
- Understand the challenges associated with endometriosis – from the physician's and the patient's point of view
- Identify risk factors associated with endometriosis
- Understand the principles of preventive medicine
- Apply the preventive mechanisms for endometriosis available today
- Consider preventive measures for the future

Target audience

Gynaecologists who treat women with endometriosis, and those in training. Scientists in the field of endometriosis. Nurses.

Thursday, 15 February

- 12.00-13.00 Registrations
- 13.00-13.15 Welcome. Practical information
Alain Audebert (F)
- 13.15-13.35 Principles of preventive medicine
Jacques De Mouzon (F)
- 13.35-14.00 Can endometriosis be prevented? A provoking introduction covering an overview of the different fields to be covered during the course
Agneta Bergqvist (S)

Issue: What is the problem?

- 14.00-14.45 What kind of disease: implantation or metaplasia?
Paolo Vercellini (I)
- 14.45-15.30 How big is the problem: Data from the EAPPG survey
Lone Hummelshøj (UK)
- 15.30-16.00 Break with refreshments

Issue: Risk factors

- 16.00-16.45 Heredity
Stephen Kennedy (UK)
- 16.45-17.30 The role of environmental/life style factors in developing endometriosis and degree of endometriosis
Krina Zondervan (UK)
- 17.30-18.00 General discussion
- 20.00 Dinner

Friday, 16 February

Issue: Risk factors

- 08.00-08.30 The role of endogenous hormones
Andrew Prentice (UK)
- 08.30-09.00 The role of OCs – do they increase or decrease the risk?
Paolo Vercellini (I)
- 09.00-09.30 The role of infertility as a risk of endometriosis
Juan Garcia Velasco (E)
- 09.30-10.00 Break with refreshments
- 10.00-10.30 The role of genital anomalies, retroflected uterus
Alain Audebert (FR)
- 10.30-11.00 Early diagnosis – are there any non-invasive tests?
Robert Greb (D)
- 11.00-12.15 Discussion on risk factors facilitated by
Stephen Kennedy (UK)
- 12.15-13.15 Lunch

Issue: What, why and who to prevent

- 13.15-14.45 Interactive discussions facilitated by
Lone Hummelshøj (UK) and Deborah Bush (NZ)
- What are we trying to prevent?**
- primary disease?
 - progression (and how do we evaluate this?)
 - recurrence (and what constitutes recurrence?)
- Why are we trying to prevent this?**
- pain/quality of life?
 - risk of infertility?
 - risk of malignancy?
 - risk of other concurrent diseases?
- Who are we targeting for prevention?**
- Teenagers?
 - Infertile women?
 - Women with a hereditary risk of endometriosis?
- 14.45-15.30 Summary of the session with refreshments

Friday, 16 February (continued)

Issue: How to prevent today

- 15.30-16.15 Reduce retrograde menstruation?
Hervè Fernandez (F)
- 16.15-16.35 Reduce inflammation?
Agneta Bergqvist (S)
- 16.35-16.55 Reduce implantation potential of the endometrium?
Patrick Groothuis (NL)
- 16.55-17.15 How to prevent recurrence surgically
Charles Chapron (F)
- 17.15-18.00 Summary of the session
- 20.00 Dinner

Saturday, 17 February

Issue: How to move forward

- 08.00-08.45 Managing the adolescent
Andrew Prentice (UK)
- 08.45-09.30 How to increase awareness in schools. The NZ experience
Deborah Bush (NZ)
- 09.30-10.00 How can diet impact the symptoms of the disease?
Dian Mills (UK)
- 10.00-10.30 Break with refreshments
- 10.30-11.30 Round table group discussions: How will I contribute to the prevention of endometriosis?
- 11.30-11.50 Summary: What is the take home message?
Lone Hummelshoj (UK)
- 11.50-12.00 Closing - **Alain Audebert (F)**

Principles of preventive medicine

Jacques de Mouzon
INSERM U 569, Paris, France
demouzon@vjf.inserm.fr

No input was received by the speaker.

Notes:

Can endometriosis be prevented? A provoking introduction covering an overview of the different fields to be covered during the course

Agneta Bergqvist
Pfizer, Sweden
agneta.bergqvist@pfizer.com

- Among the different pathogenetic factors involved in endometriosis there are some that we might regulate: Menstrual bleeding, oestrogen stimulation, progestagenic prevention.
- Continuous gestagens with (OC) or without oestrogen may prevent the development of endometriosis by inhibition of ovulations, reduction of menstrual bleedings, anti-inflammatory effect and growth inhibition of ectopic endometrial cells.
- Inhibition of retrograde menstruation hormonally or mechanically prevent the pelvic cavity from endometrial explants.
- Reduction of the inflammatory reactivity in the pelvis while preserving the cellular clearance of desquamated endometrial cells might prevent the establishment of endometriosis.
- Reduced exhibition to excess oestrogen-like substances in food and the environment might reduce the growth potential of endometrial explant.

Notes:

What kind of disease: implantation or metaplasia?

Paolo Vercellini
University of Milano, Italy
paolo.vercellini@unimi.it

No input was received by the speaker.

Notes:

How big is the problem: data from the EAPPG survey

Lone Hummelshoj
European Endometriosis Alliance and Endometriosis.org, United Kingdom
lone@endometriosis.org

- An average diagnostic delay of eight years was observed between presentation with symptoms suggestive of endometriosis and diagnosis, with less than 50% being taken serious, when they first consulted their GP. There was an additional delay of three years from the start of (pain) symptoms before women sought medical help.
- 65% were originally mis-diagnosed with another condition, and “hit and miss” treatments were largely in-effective in the surveyed population of 7,025 women with endometriosis.
- Would the outcome be improved if we were more problem orientated vs. lesion orientated, and if these women had been treated early within a multi-disciplinary context?
- Destruction of taboos and myths, increased awareness at primary level, and a more effective way to diagnose endometriosis is needed to prevent unnecessary hysterectomies, to preserve fertility, to improve quality of life in women with endometriosis, and to ensure that endometriosis does not interfere with their relationships, ability to finish an education or to maintain a career.
- Early intervention is the only prevention – Noah built his ark before it started to rain.

Notes:

Heredity

Stephen Kennedy
Oxford University, United Kingdom
stephen.kennedy@obs-gyn.ox.ac.uk

- Appraise clinical evidence suggesting that endometriosis has genetic basis.
- Understand principles underlying linkage analysis as method for identifying disease susceptibility genes in common genetic traits.
- Interpret results of first genome-wide screen in endometriosis.
- Appreciate the value of the rhesus monkey as a model to study the genetic epidemiology of endometriosis.
- Evaluate possible effects on clinical practice of finding genes that predispose women to develop endometriosis.

Notes:

The role of environmental/life style factors in developing endometriosis and degree of endometriosis

Krina Zondervan
Wellcome Trust Centre for Human Genetics, United Kingdom
krinaz@well.ox.ac.uk

- Epidemiological evidence generally supports Sampson's menstrual transplantation theory.
- It has been unable to shed much further light on other aetiological factors.
- Improved study design is important in increasing the potential of epidemiological studies, in particular:
 - using and reporting consistent and detailed endometriosis definitions and subtypes
 - paying attention to appropriate control selection using much larger sample sizes
- Future studies need to think more about cross-fertilization between basic science fields and epidemiology (translation of experimental and genetic findings); incorporating molecular epidemiology.

Notes:

The role of endogenous hormones

Andrew Prentice
University of Cambridge, United Kingdom
ap128@mole.bio.cam.ac.uk

- Sex steroids are the main trophic hormones for endometrium and endometriosis.
- Altered but physiological endocrine states influence symptoms in endometriosis.
- Pharmacological manipulation of the HPO axis influences endometriosis.
- Endometriosis may generate its own oestrogen.
- Altering the hormonal environment may have other adverse/beneficial effects.

Notes:

The role of OCs – do they increase or decrease risk?

Paolo Vercellini
University of Milano, Italy
paolo.vercellini@unimi.it

No input was received by the speaker.

Notes:

The role of infertility as a risk of endometriosis

Juan Garcia Velasco
IVI Madrid and Rey Juan Carlos University, Spain
jagarcia@sego.es

- The assumption that endometriosis and infertility are causally related is based on the higher prevalence of the disease in infertile women (20-55%) when compared to fertile women (2-5%). In baboons, monthly fecundity rate is also reduced when endometriosis is present.
- The concept that endometriosis is ONLY a hormonal disease has contributed to the lack of progress in the understanding of this disease, including not only treatment but also prevention.
- Hypo-oestrogenism (menopause) and pseudo-pregnancy have been advocated as two physiologic situations that protect against endometriosis. However, parous women are not protected against the disease, and menopausal women undergoing HRT may have a recurrence of the uncured disease, as symptoms regress but endometriosis is not cured.
- Pseudo-pregnancy to prevent or treat endometriosis is based in unproven facts, as there are no studies looking at endometriosis lesions before and after pregnancy.
- ART may have similar results in women with and without endometriosis, as large registries such as the Centre for Disease Control do not find any differences whereas meta analysis find poorer outcome in endometriosis patients. Folliculogenesis, egg quality, fertilisation, granulosa cell steroidogenesis, even tubal motility, embryo implantation and/or sperm phagocytosis may be hampered in women with the disease, but ART may by-pass these difficulties.

Notes:

The role of genital anomalies

Alain Audebert
Polyclinique de Bordeaux and Polyclinique Jean Villar de Bruges, France
audebert@alienor.fr

- Imperforate hymen should be detected during the neonatal period and treated surgically during infancy, before occurrence of menarche.
- Severe dysmenorrhoea in adolescent girls, failing to respond to medical treatments (including oral contraceptives), should be investigated by ultrasound examination in order to exclude a congenital anomaly of the genital and urinary tracts.
- Mullerian obstructive anomalies are most likely to favour the development of endometriosis. Endometriosis associated with genital anomalies usually regresses spontaneously after proper surgical correction, the only potential sequellae being adhesions when formed.
- Uterine cervix stenosis is a risk factor for endometriosis in menstruating women. When a surgical procedure needs to be performed on the uterine cervix, great care should be taken in order to avoid narrowing of the cervical canal.
- Primary mobile retroverted uterus tends to be more frequent in patients with endometriosis, but data are limited.

Notes:

Early diagnosis – are there non-invasive tests?

Robert Greb
Infertility Centre Dortmund, Germany
greb@ivf-dortmund.de

- The aim of early diagnosis is early treatment and prevention of the sequelae of chronic endometriosis: Thus, adequate treatment for symptom control could be applied early enough, including strategies to enhance or maintain patients' fertility.
- Reliable, early, non-invasive tests are lacking, but such tests could facilitate management at all stages of the disease, since laparoscopy is invasive and not always appropriate.
- Potential sources of early, non-invasive testing are blood (serum and cells), endometrium from menstrual flow or endometrial biopsies and imaging technologies. Useful marker molecules for such tests may be discovered by molecular screening technologies (e.g. genomics, proteomics). Molecular probes could also be applied in functional imaging technologies, e.g., fluorescence mediated tomography.
- Tests reflecting functional aspects of endometriosis lesions are conceivable: molecular markers may characterise biological features of lesions, e.g., invasiveness, inflammatory reaction or alterations of the normal eutopic endometrium of patients. Functional characterisation of the disease provides the basis for effective preventive and individualised therapeutic strategies.
- Inflammatory cyto- and chemokines (IL-6, CCR1) have shown promising potential to diagnose minimal-mild endometriosis, but their feasibility as early non-invasive tests has to be confirmed prospectively, in larger series of patients.

Notes:

Reduce retrograde menstruation?

Hervé Fernandez
Centre Hosp. Antoine Bécclère, Paris, France
herve.fernandez@abc.ap-hop-paris.fr

- Retrograde menstruation is a physiologic phenomenon.
- Retrograde menstruation influence the risk of endometriosis by short cycle length or heavy menstrual flow.
- Higher risk due to mullerian anomalies or stenosis of external ostium.
- Retrograde uterine contractions could play a role.
- Role of pills and/or progesterone to modify the risk factors?

Notes:

Reduce inflammation?

Agneta Bergqvist
Pfizer, Sweden
agneta.bergqvist@pfizer.com

- Reduction of the inflammatory reaction to desquamated endometrial cells might withdraw the cell growth stimulating potential that some of the cytokines have.
- The inflammatory potential might be down regulated by hormones or anti-inflammatory substances.
- Continuous treatment with NSAID or specific Cox-2-inhibitors might prevent the increase of prostaglandins driven by the inflammatory cascade and oestrogen.
- Vascular proliferation and support is a requirement for endometrial cell implantation, but the drugs available today are so far not suitable for preventive use.
- Specific inhibition of cytokines important for the inflammatory reaction are available but due to the general and severe side effects they are so far not suitable for preventive use.

Notes:

Reduce implantation potential of the endometrium?

Patrick Groothuis
Organon, The Netherlands
patrick.groothuis@organon.com

- The key to halting ectopic implantation and growth of endometrium tissue is to understand the mechanisms involved.
- The ectopic implantation process involves the attachment, invasion and survival of viable endometrial tissue.
- Ectopic endometrium survives because of gained resistance to apoptosis, by avoiding the immune system and enhanced local synthesis of oestradiol.
- Normal endometrium tissue is angiogenic, invasive, and a source of oestradiol.
- Ectopic implantation of endometrium can be reduced by inhibiting angiogenesis and matrix metalloproteinase activity, suppression of the immune system with NSAIDs, and suppression of oestrogen action by treatment with progestins or an ER β agonist.
- The peritoneal environment and host environment are important determinants in the ectopic implantation process.

Notes:

How to prevent recurrence surgically

Charles Chapron
Paris V University, France
charles.chapron@cch.ap-hop-paris.fr

- When surgical management is decided for endometriosis, excision must be complete in order to remove all the endometriotic lesions. Radical surgery is associated with lower recurrence rates.
- A full pre-operative work-up is absolutely necessary before surgery. The objective is to promote a one-step surgical procedure for the complete removal of the endometriotic lesions with the patient's fully informed agreement prior to surgery.
- For ovarian endometrioma, laparoscopic cystectomy is the gold standard surgical technique. With this surgical procedure the recurrence rate is significantly less than with drainage and ablation.
- If it is decided to remove the uterus, hysterectomy must be a total hysterectomy including bilateral salpingo-oophorectomy.
- Centres of excellence with a multidisciplinary approach are necessary for the surgical management of advanced deep endometriosis.

Notes:

Managing the adolescent

Andrew Prentice
University of Cambridge, United Kingdom
ap128@mole.bio.cam.ac.uk

- Endometriosis is an underestimated problem in the adolescent population.
- Endometriosis has been scantily studied in this population.
- Early onset disease may be progressive.
- Management may be modified but not fundamentally.
- Education and empowerment are very important in this age group.

Notes:

How to increase awareness in schools: the NZ experience

Deborah Bush
New Zealand Endometriosis Foundation, New Zealand
debush@xtra.co.nz

- It is becoming increasingly recognised that menstrual disorders in teenagers are extremely common, particularly endometriosis which can have a significant physical and emotional impact on a young woman's quality of life, education and future fertility.
- 'me' stands for Menstrual Health and Endometriosis and is a unique, fun, interactive and informative education programme developed in New Zealand for secondary schools with strong relationships forged with 70 schools since 1995.
- Early diagnosis following best practice treatment and management is essential for improved well being and the 'me' programme aims to:
 - address the global crisis of delayed diagnosis for endometriosis
 - reduce physical and emotional morbidity
 - reduce the likelihood of compromised fertility
 - give young women confidence and knowledge to seek and access appropriate help.
- 'me' has had a major impact showing an increasing trend of patients under 20 presenting with symptoms of endometriosis in the Canterbury region, where as of December 2006:
 - 35% patients presenting now under 20
 - 96% of those young women, confirmed laparoscopic diagnosis
 - 49% with stage 1 endometriosis
- It is essential to pitch an adolescent programme of this type age appropriately, so that it is based on a well model and dovetails into the school curriculum, in a non-discriminatory manner, encouraging a respectful friendly, open environment to ensure students recognise normal menstrual function and are empowered to seek help with knowledge and confidence.
- 'me' is highly accepted, well audited and evaluated, with established credibility and proven, measurable outcomes and is available to license anywhere in the world together with its specifically designed student/staff resources and complete training package.

Notes:

How can diet impact the symptoms of the disease?

Dian Shepperson Mills
The Endometriosis and Fertility Clinic, United Kingdom
dsm50@pavilion.co.uk

- Good health is conferred on the body by fresh food that is rich in essential vitamins, minerals and phyto-nutrients. These nutrients act as co-factors and co-enzymes in body cells.
- You are what you eat and can digest and absorb. Unless digestion is healthy you will not absorb the nutrients your body cells need to control pain pathways in the body.
- Healthy gut flora is essential to the way the body is able to degrade oestrogen, and it supports 80 per cent of the immunoglobulin production in the body.
- A healthy liver is essential to the way the body is able to biotransform and aid excretion of oestrogen from the body to stop it re-circulating and causing harm.
- One man's meat is another man's poison. Nutrigenomics is showing us that some genetic types should avoid certain foods as they lack the enzymes to digest them properly. Some people may have an immune reaction to certain phytochemicals in the foods.

Notes:

Environmental risk-factors for endometriosis

Krina Zondervan
Wellcome Trust Centre for Human Genetics
University of Oxford



Bordeaux 2007

Overview

- Why study risk-factors?
- How to study risk-factors:
 - General study design
 - Specific problems for endometriosis
- Review of investigated 'environmental' (i.e. non-genetic) risk-factors for endometriosis

Why study risk-factors?

- Understanding disease mechanism (in conjunction with experimental studies)
- Drug/intervention targets
- *Prevention*

How to study risk-factors

Epidemiological study design

To ensure: Validity of results & reproducibility

- Disease/case definition
- Method: cohort / case-control / cross-sectional
- Sample size

Disease/case definition

Standard disease definition must be:

unambiguous
and
applicable to various populations



generalisability of results
comparison between studies

Disease/case definition

Standard definition of Endometriosis

.....a challenge

- 'Presence of endometrial-like glands and stroma in ectopic sites'too broad?
- 'Subtypes': e.g. deep infiltrating disease; ovarian vs. peritoneal disease?
- Definition depends on surgery: **not** applicable to all (general) populations

Disease/case definition

Holt & Weiss, 2000

'Definite disease':

- o Ovarian endometriomas of any size
- o Pelvic endometriotic implants, any size, >5 mm deep
- o Pelvic endometriotic implants, any size, adhesions not due to other cause

'Possible disease':

- o Pelvic endometriotic implants not meeting criteria **plus one or more of:** infertility; moderate/severe dysmenorrhoea, dyspareunia, or pelvic pain

Not applicable to general population

Disease/case definition

- o Changing hypotheses on what constitutes endometriosis as a disease or multiple diseases
- o Most importantly, investigators need to
 - Specify in detail in their publications how they defined endometriosis
 - Recruit sufficiently large sample sizes to be able to investigate sub-types that may have different aetiologies

Study method - general

Prospective cohort study ('gold standard')

- o Follow-up of unaffected population until disease develops
- o Risk-factor data collected before onset: unbiased
- o Expensive; unfeasible if disease is rare or follow-up long

Retrospective case-control study

- o Identification of incident cases and suitable control group
- o Risk-factor data pre-dating the onset of disease collected retrospectively: biased/incomplete?
- o Sometimes prevalent cases used

Study method - general

Cross-sectional study

- Prevalent cases compared with disease-free group from same population.
- Risk-factors that are involved in disease onset (incidence) may be different from those involved in disease maintenance (prevalence)
- Risk-factor data (environmental) truly predating onset of disease?
- Almost impossible to infer causal relationships with environmental factors from results

Case definition

- Incidence requires knowledge of time of (symptom) onset
- Difficult for many chronic diseases, in particular for endometriosis

Endometriosis :

- Continuum of biological changes considered 'clinically relevant' at a certain threshold
- Diagnosis only established through surgery: clinical rather than general population

Disease/case definition - Endometriosis

Cases

- Validated 'symptom complex' would allow case identification at population level
- Without this, cases will be identified in hospital at surgery
- 'New' endometriosis cases are inevitably **prevalent** cases (may have had symptoms for years)
- Best to identify cases at **first diagnosis**
- Risk-factor information preceding onset of symptoms

Disease/case definition - Endometriosis

Control selection

- Always requires careful consideration
- **Basic rule:** a control should have had the same opportunity to have been included in the study as a case, had she been affected
- **In practice:**
 - Controls should come from same population as cases
 - Controls should be similar (matched) to cases for factors not under study that may have influenced disease onset or the probability of being diagnosed
 - No one 'right' method, but decision has implications for interpretation of results

Disease/case definition - Endometriosis

Examples

- Cases attending infertility clinic vs. infertile controls from same clinic:
 - Risk-factor profiles of controls unlikely to be similar to those in general population
 - Overmatched?
- Cases attending clinic vs. controls undergoing laparoscopy for tubal ligation in same clinic:
 - Risk-factor profiles of fertile controls compared to general population?
- Best to use multiple control groups

Sample size

- Adequate size depends on research questions under study
- Essential (for any study) to:
 - Reduce probability of finding false effects by chance
 - Increase probability of finding true effect (power)
- Can easily be calculated

Endometriosis – Aetiology

A complex disease.....
(environmental + genetic factors involved)

Aetiological hypotheses

- I. Origin of endometrial-like tissue: 'retrograde menstruation'
- II. Differential adhesion and proliferation of endometriotic cells
- III. Toxins and detoxification mechanisms

Endometriosis aetiological factors

Environmental (broad sense)

- o Endogenous exposures (e.g. menstrual characteristics; oestrogen levels; behavioural characteristics)
- o Exogenous exposures (e.g. demographic 'exposures'; exogenous hormones; toxins)
Mangtani & Booth, *J Epi Comm Health* 1993; 47: 84-88
Eskenazi & Warner, *Obs Gyn Clin North Am* 1997; 24: 235-58

Genetic

- o Polymorphisms/candidate genes
Bisschoff & Simpson, *Hum Reprod Upd* 2000; 6: 37-44
Zondervan, Cardon & Kennedy, *Curr Opin Obs Gynae*, 2004

Gene-environment interplay (biological interaction)?

Endometriosis – 14 general studies of 'environmental' factors

Country	Study design	Endo stage (%)	Reference
UK	Cohort (N=2) 17,302 married, Caucasian, women, identified from family planning clinics; 313 cases	?	Vessey, 1993
USA	~100,000 female nurses; 1721 cases	III: 61; III/IV: 39	Missmer, 2004, 2006
USA	Case-control (N=12) 286 nulliparous, infertile cases; 3794 age-matched controls admitted for delivery	?	Cramer, 1986; Grodstein, 1993, 1994
Lebanon	170 cases, 170 age-matched controls (lap)	?	Obermeyer, 1986
Italy	376 cases, 522 controls admitted for acute conditions (no lap)	III: 54; III/IV: 46	Candiani, 1991; Parazzini, 1994, 1995, 2004
USA	104 cases; 100 'friend' controls / 98 clinic controls	?	Darrow, 1993, 1994; McCann, 1993
USA	126 multiparous cases, 504 controls (both lap sterilisation)	I (91)	Sagi-Haghpeykar & Poindexter, 1995
USA	50 infertile cases; 89 fertile/47 infertile controls	?	Signorello, 1997
USA	77 cases, 735 healthy controls	Endometrioma	Dhillon & Holt, 2003
Canada	890 cases, 1881 clinic controls (lap)	III: 78; III/IV: 22	Hemmings, 2004
Italy	366 cases, 248 clinic controls (lap)	III: 33; III/IV: 67	Ferrero, 2004
USA	32 cases, 52 clinic controls (lap)	III: 63; III/IV: 37	Hediger, 2005
Belgium	88 deep nodule/ 88 peritoneal cases, 88 clinic controls matched on age + symptom screened	Deep/Peritoneal	Heilier, 2007

Issues with published general risk-factor studies

- o Comparison of results impossible because of differences in:
 - o Case definition
 - o Control selection
 - o Little or no information on time point for risk-factor assessment (prior to symptoms/diagnosis?)

- o Sample sizes!

Demographics

Factor	Case-control studies: Endo risk ↑, =, or ↓ (n/N studies)	Cohort study evidence? <small>(Missmer, 2004/2006)</small>
Age	↑ with increasing age (5/10)	↓
SES	= (5/7)	
Education	↑ with higher education (2/4)	
Ethnic origin		
Asian	↑ compared to Caucasian (1/1)	=
Black african	↓ compared to Caucasian (1/1)	↓

Menstrual factors, childbirth, contraception

Factor	Case-control studies: Endo risk ↑, =, or ↓ (n/N studies)	Cohort study evidence? <small>(Missmer, 2004/2006)</small>
Cycle length	↑ with shorter cycle (4/8)	↑
Duration flow	↑ with longer duration (3/8)	↑
Age menarche	= (3/8)	↑ with younger age
Parity	↓ with more pregnancies (4/8)	↓
Duration lactation	↓ with longer lactation (1/1)	↓ with longer lactation
OC use	↓ with current/recent use (3/6) = with duration of use (5/5)	↑ with past use = with duration of use
IUCD use	↓ current/recent use (1/2)	↑
Barrier methods	= (3/3)	

Life-style & personal factors

Factor	Case-control studies: Endo risk ↑, =, or ↓ (n/N studies)	Cohort study evidence? (Missmer, 2004/2006)
Birth weight		↑ with low birth weight
BMI	↑ with low BMI (4/7)	↑ (only in infertile group)
Waist/hip ratio	= (2/2)	=
Height	↑ with taller height (2/3)	=
Smoking	↓ with current smoking (3/9)	↓ (only in infertile group)
Alcohol use	= (3/5)	↓ with moderate use
Exercise	↓ with increased exercise (2/4)	↓ with frequent vigorous exercise only
Diet	↓ with fruit/veg intake (1/1) ↑ with red meat intake (1/1) = with fatty foods (1/1)	

Dioxin & PCBs

Hypothesis generated because:

- Study of 24 rhesus macaques showed significant, dose-dependent, increase in risk (Rier, 1993)
[Critical re-analysis by Guo 2004: ns/equivocal]
- Action through: suppression immunological functions; oestrogenic effects?
- Ecological studies in Belgium: area with high dioxin levels also showed high endometriosis prevalence

Endometriosis – 7 studies of human dioxin/PCB exposure

Country	Study design	Endo stage (%)	Reference
Cohort (N=1)			
Italy	Serum dioxin in 601 women exposed to dioxin in Seveso chemical plant accident in 1976; 19 laparoscopy-confirmed cases	?	Eskenazi, 2002
Case-control (N=5)			
Israel	Dioxin in blood of 44 infertile cases and 35 age-matched infertile controls	I/II: 60, III/IV: 40	Mayani, 1997
Belgium	Serum dioxin and dioxin-like PCBs in 42 infertile cases and 27 infertile controls	I/II: 64, III/IV: 36	Pauwels, 2001
Belgium & Italy	Serum dioxin and dioxin-like PCBs in 12 Italian cases+ 10 Italian controls and 11 Belgian cases + 7 Belgian controls (pilot study)	?	De Felip, 2004
Japan	Serum concentrations of organochlorine compounds (PCBs, PCDDs, PCDFs) in 58 infertile cases and 81 infertile controls	I/II: 23, III/IV: 77	Tsukino, 2005
USA	Serum concentrations of 62 PCBs in 32 cases, 52 controls undergoing laparoscopy for tubal sterilisation or diagnosis	I/II: 63, III/IV: 37	Buck Louis, 2005
Belgium	Serum concentrations of 17 PCDDs/PCDFs and 12 dioxin-like PCBs in 50 cases and 21 healthy controls	Peritoneal/Deep	Heilier, 2005

Issues with published epidemiological studies into dioxin

- Assessment:
 - Type of assay
 - Serum vs fat
 - Relation to dietary intake?
- Sample size!!
- Many compounds tested: multiple testing problem

Dioxin & PCBs

Factor	Case-control studies: Endo risk ↑, =, or ↓ (n/N studies)	Cohort study evidence? <small>(Eskenazi, 2002)</small>
Dioxin	= (2/4) ↑ (2/4; Mayani; Heilier)	= (but OR 2.0; only 19 cases)
Dioxin-like PCBs	= (3/5) ↑ (2/5; Buck Louis; Heilier)	
Other organochlorines	= (1/1)	

↑

Where does this leave us: *Prevention?*

- Mechanism of disease (subtypes) unclear
- Prevention too early
- Need to:
 - Use and report consistent and detailed endometriosis definitions
 - Pay attention to appropriate control selection
 - Use **much** larger sample sizes
 - Think about cross-fertilization between other fields and epidemiological studies:
 - Experimental & genetic findings
 - Molecular epidemiology

Acknowledgements

Oxford University, UK

Nuffield Dept Obstet & Gynaecol:
Ann Lambert
Stephen Kennedy

*Wellcome Trust Centre for Human
Genetics:*
Lon Cardon

**Queensland Insitute for
Medical Research, Brisbane,
Australia**
Sue Treloar

**Dept. of Pediatrics, Medical
College of Wisconsin, USA**
Sun-Wei Guo

Early Diagnosis
- Are there any non-invasive tests ? -

Infertility Center Dortmund
Associates Dieterle, Neuer and Greb
www.ivf-dortmund.de



Robert Greb

ESHRE Campus workshop PREVENTION OF ENDOMETRIOSIS
Bordeaux, 16 February 2007

<p>Early Diagnosis <small>- Non-invasive tests ? -</small></p> <div style="border: 1px solid black; padding: 2px; margin: 5px;">WHY ?</div> <div style="border: 1px solid black; padding: 2px; margin: 5px;">WHEN testing ?</div> <div style="border: 1px solid black; padding: 2px; margin: 5px;">WHICH Tests ?</div> <p style="font-size: small; margin-top: 20px;">Bordeaux 16 Feb 2007</p>	<ul style="list-style-type: none"> ❑ WHY do we need early, non-invasive tests? ❑ WHEN / in which setting do early, non-invasive tests make sense? ❑ WHICH tests are available and conceivable ?
--	---

<p>Early Diagnosis <small>- Non-invasive tests ? -</small></p> <div style="border: 1px solid black; padding: 2px; margin: 5px;">WHY ?</div> <div style="border: 1px solid black; padding: 2px; margin: 5px;">WHEN testing ?</div> <div style="border: 1px solid black; padding: 2px; margin: 5px;">WHICH Tests ?</div> <p style="font-size: small; margin-top: 20px;">Bordeaux 16 Feb 2007</p>	<p style="text-align: center;">Goals of early diagnosis</p> <ul style="list-style-type: none"> ❑ Early treatment <ul style="list-style-type: none"> ▶ Prevention of progression ▶ Adequate treatment for symptom control ▶ Enhance/maintain fertility ❑ Coping with symptoms and potentially chronic disease
--	---

<p>Early Diagnosis - Non-invasive tests ?</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h3>Does early (aggressive?) treatment improve long-term outcome?</h3>
	<ul style="list-style-type: none"> ● Pro <ul style="list-style-type: none"> ▶ Recurrence rate higher in higher stages endometriosis ▶ Recurrence rate higher in posttherapeutically higher stages of endometriosis ▶ At earliest point (adolescent endometriosis) inverse relationship between stage and fecundability ▶ Recurrence rate higher in older patients ● Contra <ul style="list-style-type: none"> ▶ No level I evidence of beneficial effects of early treatment ▶ Stigmatization with having a chronic disease (without being able to predict the natural course of endometriotic lesions)

<p>Early Diagnosis - Non-invasive tests ?</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h3>Consequences of early diagnostic test</h3>
	<ul style="list-style-type: none"> ● Longer duration of asymptomatic life-span <p style="font-size: 2em; text-align: center; margin: 20px 0;">OR</p> <ul style="list-style-type: none"> ● More repetitive invasive treatments and associated morbidity

<p>Early Diagnosis - Non-invasive tests ?</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h3>PRO: Early treatment</h3>
	<p style="font-size: 0.8em;">Schindler AE et al. (2000) Early treatment of endometriosis with GnRH-agonists: impact on time of recurrence Eur J Obstet Gynecol Reprod Biol 93:123-5</p>

<p>Early Diagnosis</p> <p>- Non-invasive tests ?</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h2>PRO: Early treatment</h2>
<p>Parazzini F et al. (2005) Determinants of short term recurrence rate of endometriosis Eur J Obstet Gynecol Reprod Biol 121:216-9</p>	

<p>Early Diagnosis</p> <p>- Non-invasive tests ?</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h2>PRO: Early treatment</h2>
<p>Ventolini G et al. (2005) Endometriosis in adolescence: A long-term follow-up fecundability assessment Reprod Biol Endocrinol 3:14-7</p>	

<p>Early Diagnosis</p> <p>- Non-invasive tests ?</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h2>Delay of diagnosis to avoid laparoscopies</h2>
---	--

Early Diagnosis
- Non-invasive tests ?

Clinical settings of early, non-invasive testing

WHY ?

WHEN testing ?

WHICH Tests ?

Time →

Bordeaux
16 Feb 2007

Early Diagnosis
- Non-invasive tests ?

How early ?

TABLE 3
BMI and BMI percentile estimated from perceived body figure historically and currently by diagnosis for 84 women who underwent laparoscopy.

Age category	Endometriosis			No endometriosis		
	n	Percentile*	BMI (kg/m ²), mean ± SD	n	Percentile	BMI (kg/m ²), mean ± SD
15-19 y	32	22nd	20.6 ± 2.6	52	26th	21.0 ± 3.5
20-24 y	32	27th	21.1 ± 2.9	52	36th	22.1 ± 3.5
25-29 y	31	35th	21.7 ± 2.3	45	40th	22.8 ± 4.4
30-34 y	22	43rd	22.8 ± 3.6	32	50th	24.0 ± 4.1
35-39 y	12	50th	23.7 ± 4.1	12	51st	24.4 ± 4.8
40-45 y	—	—	—	2	86th	33.2 ± 9.6
Current	32	45th	23.1 ± 3.7	52	55th	25.0 ± 5.2

♀ In utero or early childhood origin for endometriosis?

Hediger ML et al. (2005)
Association of endometriosis with body size and figure
Fertil Steril 84:1366-74

Bordeaux
16 Feb 2007

Early Diagnosis
- Non-invasive tests ?

Infertility - Assisted reproduction

- ❑ Presence of (undetected) endometriosis?
 - ▶ Surgical intervention before IVF
 - ▶ Prognosis, number of embryos to transfer

Omland AK et al. (2005)
Pregnancy outcome after IVF and ICSI in unexplained, endometriosis-associated and tubal factor infertility
Hum Reprod 20:72-27

Bordeaux
16 Feb 2007

<p>Early Diagnosis</p> <p>- Non-invasive tests ? -</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h3>Empirical treatment in chronic pelvic pain</h3> <ul style="list-style-type: none"> ● Non-invasive test – a more solid rationale for endometriosis treatment w/o definitive diagnosis <p>Winkel CA (2001) Role of a symptom-based algorithmic approach to chronic pelvic pain. Int J Gynaecol Obstet 74(Suppl. 1):15-20</p>
---	---

<p>Early Diagnosis</p> <p>- Non-invasive tests ? -</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h3>Diagnostic tests</h3> <ul style="list-style-type: none"> ● Peripheral blood markers <ul style="list-style-type: none"> ▶ CA125 ▶ Leukocyte subsets ▶ CCR1-mRNA ▶ HpbetaE ▶ Thomsen-Friedenreich-Antigene ▶ Eosinophile Peroxidase (EPO) ● Imaging <ul style="list-style-type: none"> ▶ TVU ▶ TRU ▶ MRI ● Menstrual blood/endometrium <ul style="list-style-type: none"> ▶ Leukocyte subsets ▶ (Steroid receptors / aromatase)
---	---

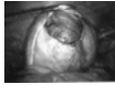
<p>Early Diagnosis</p> <p>- Non-invasive tests ? -</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h3>CA 125</h3> <ul style="list-style-type: none"> ● Significantly associated with all types of endometriosis lesions ● Sensitivity < 30 % <p>Somigliana E et al. (2004) Use of concomitant serum dosage of CA 125, CA 19-9 and interleukin-6 to detect the presence of endometriosis. Hum Reprod 19(8):1871-6</p>
---	---

Early Diagnosis
- Non-invasive tests ?

Imaging
not early, but non-invasive

WHY ?
WHEN testing ?
WHICH Tests ?

TVU (transvaginal ultrasound)
 TRU (transrectal ultrasound)
 MRI





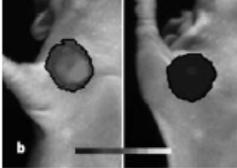
Bordeaux 16 Feb 2007
 Delpy et al. (2005)
 Value of Endorectal Ultrasonography for Diagnosing Rectovaginal Septal Endometriosis Infiltrating the Rectum
 Endoscopy 37(4):357-361

Early Diagnosis
- Non-invasive tests ?

Imaging
molecular imaging - future technologies

WHY ?
WHEN testing ?
WHICH Tests ?

Fluorescence mediated tomography (FMT)
 Molecular probes permit functional analysis of lesions



Bordeaux 16 Feb 2007
 Bremer C et al. (2005)
 Optical imaging of spontaneous breast tumors using protease sensing 'smart' optical probes
 Invest Radiol 40(6):321-7

Early Diagnosis
- Non-invasive tests ?

What kind of test do we really need?

WHY ?
WHEN testing ?
WHICH Tests ?

	YES	NO
<input type="checkbox"/> Endometriosis (presence of lesions)	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> Presence of lesions with invasive potential or impairing fertility	YES	NO
	<input type="checkbox"/>	<input type="checkbox"/>

 **Functional information**

Bordeaux 16 Feb 2007

<p>Early Diagnosis - Non-invasive tests ?</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h3>Early, non-invasive test could revolutionize endometriosis therapy</h3> <ul style="list-style-type: none"> ● Surgical management ● Medical management ● Infertility treatment
---	--

<p>Early Diagnosis - Non-invasive tests ?</p> <p>WHY ?</p> <p>WHEN testing ?</p> <p>WHICH Tests ?</p> <p>Bordeaux 16 Feb 2007</p>	<h3>Key messages</h3> <ul style="list-style-type: none"> ● Reliable, early, non-invasive tests are lacking ● Tests could facilitate management at all stages of the disease ● Sources of early, non-invasive testing: <ul style="list-style-type: none"> ▶ Blood ▶ Menstrual shed endometrium or endometrial biopsy ▶ Imaging ● Tests reflecting functional aspects of endometriosis lesions are conceivable <ul style="list-style-type: none"> ▶ Molecular markers of e.g., invasiveness, inflammatory reaction, endometrial alteration ▶ Molecular imaging techniques
---	--

Reduce Retrograde Menstruation

E. Faivre MD, H. Fernandez MD.

For more than 70 years, various theories have been promulgated to explain the pathogenesis of endometriosis. The implantation theory, often referred to as Sampson's theory (1), proposes that endometrial tissue passes through the fallopian tubes, then attaches and proliferates at ectopic sites in the peritoneal cavity. It suggests that endometriotic implants result from retrograde menstrual flow through the fallopian tubes.

Recent studies have demonstrated that retrograde menstruation is a nearly universal phenomenon in women with patent fallopian tubes: Blumenkrantz et al (2), using a peritoneal dialysis catheter, described a prevalence of intraperitoneal blood cells of 82%. Halme et al (3) found bloody peritoneal fluid in 90 % of women having laparoscopy near the time of menses. If the fallopian tubes were occluded, then only 15% of the patients had evidence of blood in the pelvis. Liu and Hitchcock (4) reported retrograde spill of menstrual blood in 76% of women undergoing laparoscopy for sterilization during menstruation.

So, the question is: does retrograde menstruation occur more frequently in individuals with endometriosis?

Indirect data supporting this concept are available.

Firstly, epidemiological studies: they have clearly shown that there is an increased risk for endometriosis if there is a short cycle length (5, 6), or a longer or heavier menstrual flow (7). Cramer et al found (5) an Odd-Ratio equal to 1.8 for cycle length minor to 27 days. Vercellini et al (7) , using the pictorial blood loss assessment chart devised by Higham et al, showed that 53% of women with endometriosis had a menstrual chart score equal to or greater than 100 compared with 37% of those without. Menstrual flow duration was slightly longer in women with endometriosis (mean difference 0.33 days).

Secondly, some anatomic considerations among patients with endometriosis also support the concept of increasing retrograde flow: Ayers and Friedenstab demonstrated utero-tubal hypotonia associated with pelvic endometriosis (8). Measuring characteristics of contractility during menses in women with mild to moderate endometriosis, Bulletti et al (9) found that these patients, compared with controls, had uterine contractions with higher frequency, amplitude, and basal pressure tone. Retrograde bleeding was found in 73% of patients with endometriosis vs 9% of controls. Uterine contractions could play a role in retrograde menstruation.

In a model to examine the factors that control the directionality of menstrual flow (10), Barbieri et al show that the diameter of the external cervical os was the most important determinant of the directionality of the flow. As they reported in another study in 1998 (11), endometriosis was visually documented at surgery for 24 of 25 women with chronic pelvic pain and stenosis of the external cervical os.

It is now well known that patients with mullerian anomalies and obstructed menstrual flow through the vagina have an increased risk of endometriosis (12, 13). In a study including 64 women with mullerian anomalies and intra-abdominal surgery Olive and Henderson (12) found that 77% of women with obstructive anomalies had endometriosis whereas it could be identified in only 37% in women with no obstruction. Conversely, Fedele et al (14) found that no differences were observed in the frequency of endometriosis between infertile women with and without nonobstructive mullerian anomalies.

Some investigators have directly compared the prevalence of retrograde menstruation in patients with or without endometriosis: although some authors did not find a difference (3), others authors reported that the prevalence of retrograde menstruation was higher in women with endometriosis: 97% vs 60% (4). In baboons (15), the prevalence of retrograde menstruation has been reported in 62% of the investigated animals, and this prevalence was significantly higher in baboons with spontaneous endometriosis (83%) than in baboons without endometriosis (51%).

The problem with laparoscopic studies evaluating the prevalence of retrograde menstruation is due to the observation of the phenomenon only during one cycle. No data are available recording the recurrence of retrograde menstruation in the same women during consecutive cycles in women (16). Furthermore, the diagnosis of retrograde menstruation was made only qualitatively only, but not quantitatively.

What about preventing endometriosis by reducing retrograde menstruation?

No method that could exclusively influence retrograde menstruation as a preventive treatment is described in the literature. The only way to avoid retrograde flow would be to obture the fallopian tubes: this is not possible when considering young women or women with desire of future fertility. Medical treatments could reduce retrograde menstruation, by reducing the volume of the menses, or increasing the length of the cycle. Pills or progesterone could influence the risk of endometriosis this way. But no specific data are available.

There is only one situation where endometriosis can be prevented, and cured, by specific treatment that could reduce retrograde flow: patients with obstructive uterine anomalies, specially adolescent, may develop extensive endometriosis. The severity of the lesions

depends upon the delay in establish the diagnosis. Several studies (13, 17) have demonstrated that these lesions may regress spontaneously, sometimes completely, when the anomaly has been surgically treated.

References

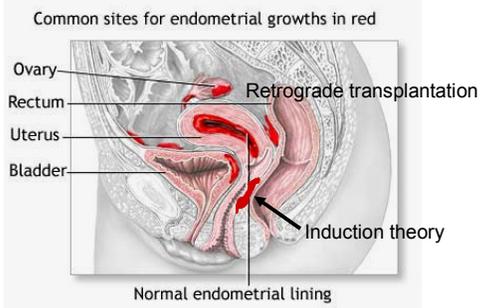
1. Sampson J. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol.* 1927;14:422-469
2. Blumenkrantz MJ, Gallagher N, Bashore RA, Tenckoff H. Retrograde menstruation in women undergoing chronic peritoneal dialysis. *Obstet Gynecol.* 1981;57(5):667-70
3. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet Gynecol.* 1984;64:151-154
4. Liu DT, Hitchcock A. Endometriosis: Its association with retrograde menstruation, dysmenorrhoea, and tubal pathology. *Br J Obstet Gynaecol.* 1986;93(8):859-862
5. Cramer DW, Wilson E, Stillman RJ, Berger MJ, Belisle S, Schiff I, Albrecht B, Gibson M, Stadel BV, Schoenbaum SC. The relation of endometriosis to menstrual characteristics, smoking, and exercise. *JAMA.* 1986;255:1904-1908
6. Arumugan K, Lim JM. Menstrual characteristics associated with endometriosis. *Br J Obstet Gynaecol.* 1997;104:948-950
7. Vercellini P, De Giorgi O, Aimi G, Panazza S, Uglietti A, Crosignani PG. Menstrual characteristics in women with and without endometriosis. *Obstet Gynecol.* 1997;90:264-268
8. Ayers JWT, Friedenstab AP. Utero-tubal hypotonia associated with pelvic endometriosis. 41 st annual meeting of the American Fertility Society. Chicago 1985. *Abst.* p131
9. Bulletti C, De Ziegler D, Polli V, Del Forro E, Palini S, Flamigni C. Characteristics of uterine contractions during menses in women with mild and moderate endometriosis. *Fertil Steril.* 2002;77:1156-1161
10. Barbieri RL, Callery M, Perez SE. Directionality of menstrual flow: cervical os diameter as determinant of retrograde menstruation. *Fertil Steril.* 1992;57:727-730
11. Barbieri RL. Stenosis of the external cervical os: an association with endometriosis in women with chronic pelvic pain. *Fertil Steril.* 1998;70:571-573
12. Olive DL, Henderson DY. Endometriosis and mullerian anomalies. *Obstet Gynecol.* 1987;69:412-425.

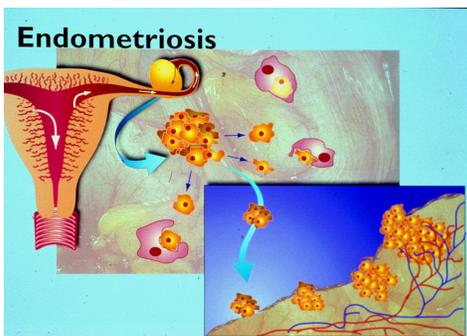
13. Sanfilippo JS, Wakim NG, Schikler KN Yussman MA. Endometriosis in association with uterine anomaly. *Am J Obstet Gynecol.* 1986;154:39-43
14. Fedele L, Bianchi S, Di Nola G, Franchi D, Candiani GB. Endometriosis and nonobstructive mullerian anomalies. *Obstet Gynecol.* 1992;79(4):515-517
15. D'Hooghe TM, Bambra CS, Raeymaeckers BM, Koninckx PR. Increased prevalence and recurrence of retrograde menstruation in baboons with spontaneous endometriosis. *Hum Reprod.* 1996;11:2022-2025
16. D'Hooghe TM, Debrock S. Endometriosis, retrograde menstruation and peritoneal inflammation in women and in baboons. *Hum Reprod Update.* 2002;8 (1):84-88
17. Audebert A. Characteristics of adolescent endometriosis: apropos of a series of 40 cases. *Gynecol Obstet Fertil.* 2000;28(6):450-454

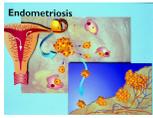
*Reduce implantation potential
of the human endometrium*

Patrick G. Groothuis, PhD
Department of Pharmacology, Organon N.V.
patrick.groothuis@organon.com

ESHRE Campus Course Endometriosis, Bordeaux 2007







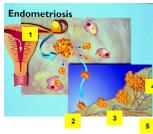
Retrograde transplantation Epidemiology

Endometriosis associated with the reproductive years

Higher prevalence in women with

- shorter menstrual cycles/longer menstrual periods
- heavier menstrual bleeding
- obstruction of antegrade shedding

Reviewed by D'Hooghe and DeBrock, 2002



Retrograde transplantation Key events

1. Retrograde transport of viable shed endometrial tissue
2. Adhesion of cells to the peritoneum
3. Invasion of cells and/or tissue into the peritoneum
4. Acquisition blood supply
5. Permissive host environment

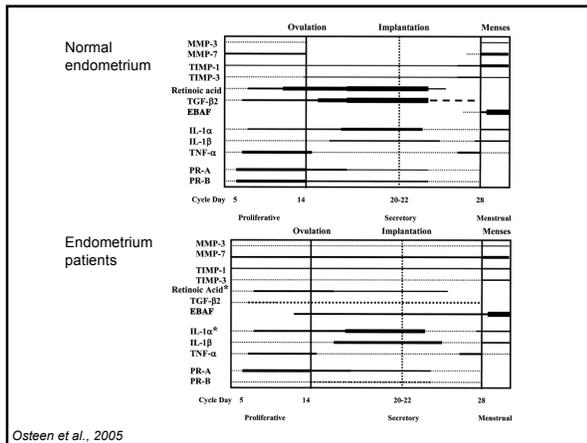


Retrograde transplantation Epidemiology

The 'seed' is different in women with endometriosis

- ↑ aromatase (estrogen synthesis)
- ↑ numbers of small nerve fibres (innervation/pain)
- ↑ kallikrein (invasion, angiogenesis)
- ↑ endoglin (angiogenesis)
- ↓ glycodelin A (immunosuppression)

Kao et al., 2004; Noble et al., 1996; Tokushige et al., 2006; Kim et al., 2001



Endometriosis

Retrograde transplantation
1. Retrograde shedding

Viable endometrial cells recovered from Fallopian tubes and peritoneal fluid

Bleeding from oviducts during surgery

Endometriosis

Retrograde transplantation
2. Adhesion to serosal surface

Integrin expression on shed menstrual endometrium

Koks et al., 2000

Endometriosis

Retrograde transplantation
2. Adhesion to serosal surface

Endometrium

Mesothelium

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

Adhesion of shed endometrial cells is largely mediated by the interaction between laminin and $\alpha_6\beta_1$

Control

4B4

Integrin	Control (%)	4B4 (%)
Col I	~100	~80
Col IV	~100	~80
Fibro	~100	~80
Lam	~100	~50

Koks et al., 2000

Endometriosis

Retrograde transplantation
2. Adhesion to serosal surface

Endometrium also adheres to mesothelial surface

EECs

Peritoneum

Endometrial Stroma

Endometrial Gland

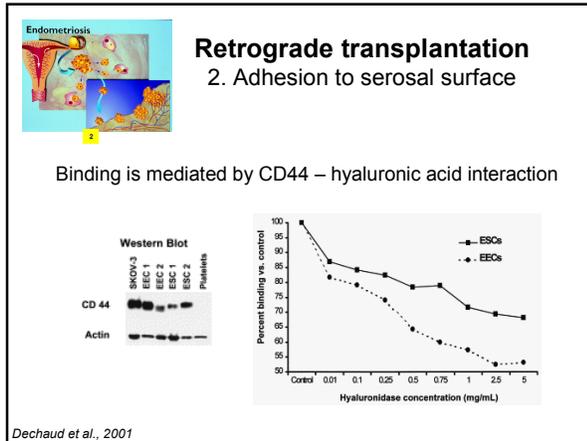
Peritoneum

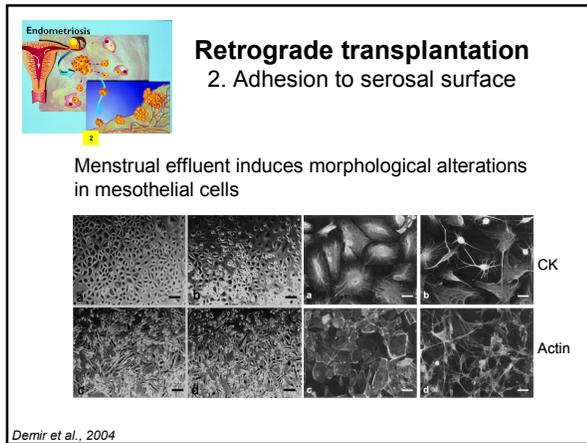
Endometrium

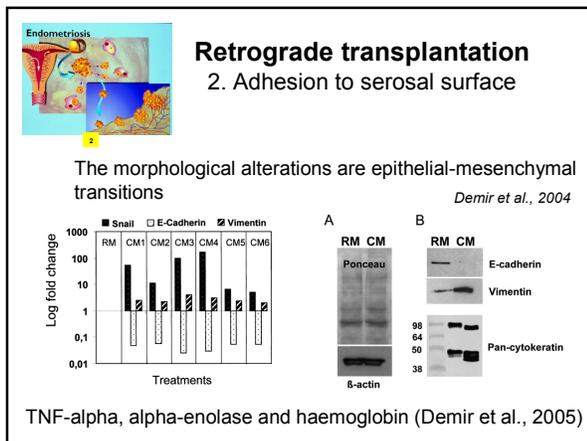
Mesothelial Cell

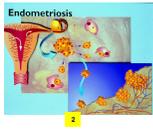
Binding is mediated by $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins

Witz et al., 2000; 2001





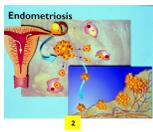




Retrograde transplantation
2. Adhesion to serosal surface

Inflammatory cytokines such as TNF α and TGF β promote peritoneal spread of tumor cells to the peritoneum

Nakashio et al., 1997; Mochizuki et al., 2004



Retrograde transplantation
2. Adhesion to serosal surface

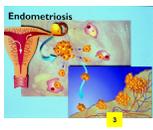
Administration of NSAIDs reduced lesion establishment in mice after transplantation of autologous endometrium

Not reproducible with nimesulide in immunodeficient mouse model after Transplantation of human endometrium

Treatment	Total burden
Ibuprofen	3.1 \pm 1.0 *
Indomethacin	1.4 \pm 1.4 **
Aspirin	5.9 \pm 1.3
Naproxen	2.7 \pm 1.2 **
Sulindac	3.1 \pm 1.5 *
Celecoxib	0.3 \pm 0.2 **
Rofecoxib	3.4 \pm 3.0
Controls	5.4 \pm 2.3

Efstathiou et al., 2005; Hull et al., 2005

* P<0.05
**P<0.01

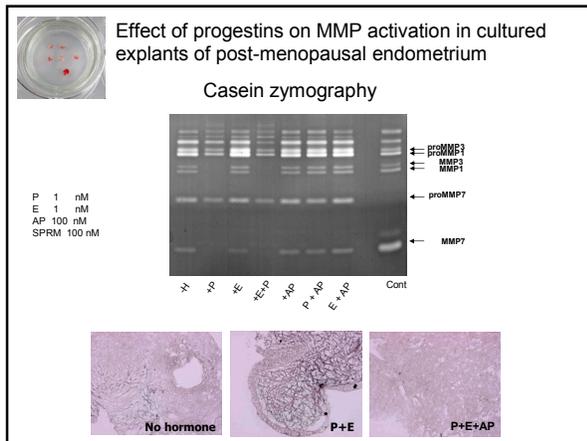


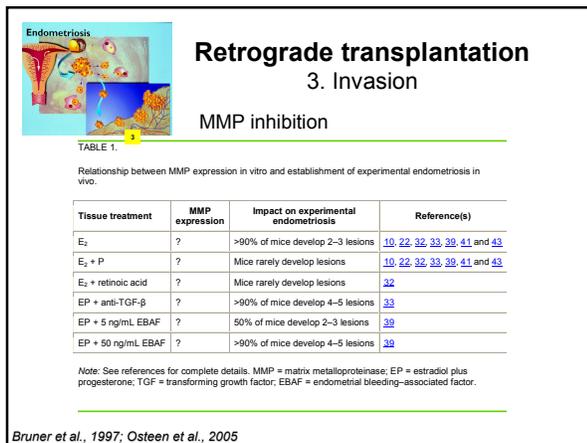
Retrograde transplantation
3. Invasion

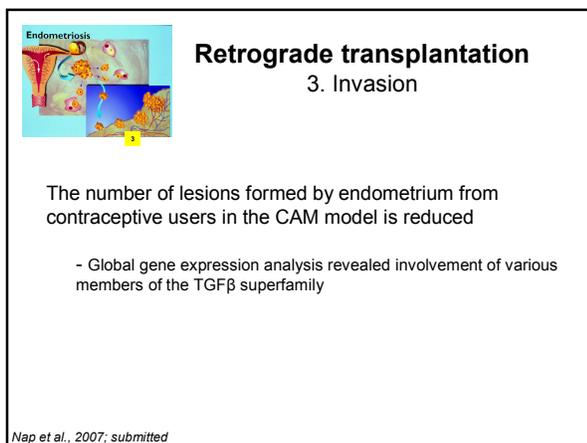
Matrix metalloproteinases are involved in the development of endometriosis

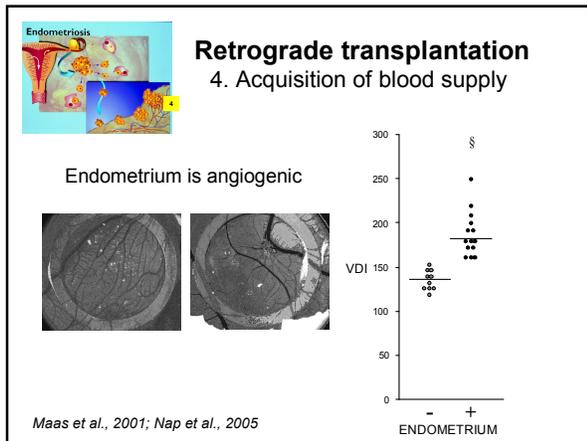
Aminoterminal propeptide of type III procollagen is increased in PF of women with early lesions of endometriosis (*Spuijbroek et al., 1992*)

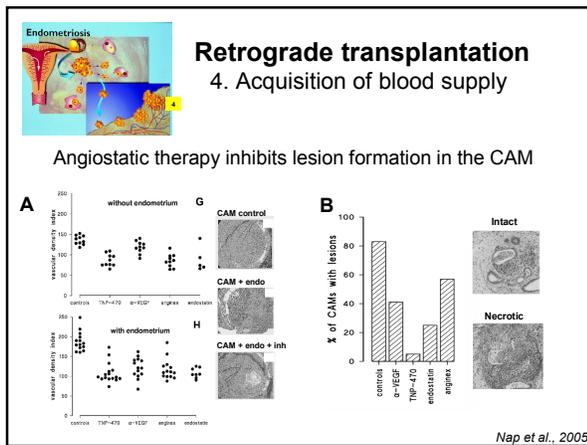
Suppression of matrix metalloproteinase activity inhibits the establishment of ectopic lesions by human endometrium in nude mice (*Bruner et al., 1997*)

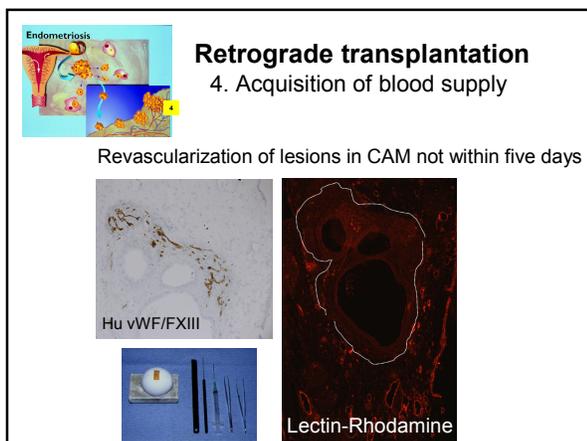








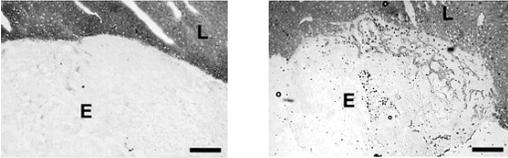






Retrograde transplantation
4. Acquisition of blood supply

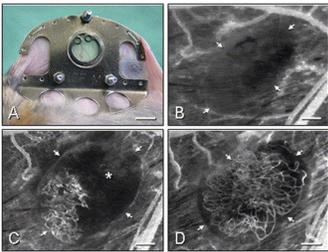
Human endometrium transplanted in immunodeficient mice



Revascularization initiated after four days

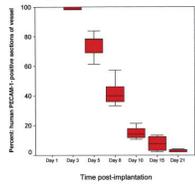
Grümmer et al., 2001

Dorsal skin fold chamber (hamster)

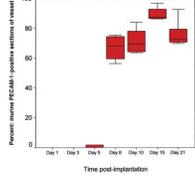


Laschke et al., 2005

A Nude mouse model



B

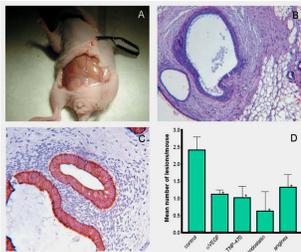


Eggemont et al., 2006



Retrograde transplantation
4. Acquisition of blood supply

Angiostatic therapy inhibits lesion formation in the mouse



Nap et al., 2004

Endometriosis

Retrograde transplantation
5. Role of the host environment

Factors produced by host influence implantation process

Wt mouse

Malignant keratinocytes

Bajou et al., 2001

Endometriosis

Retrograde transplantation
5. Role of the host environment

PAI-1 ^{-/-} mouse

Malignant keratinocytes

Endometriosis

Conclusions

How do we reduce implantation potential of endometrial tissue ?

- Minimize the activity of ECM degrading enzymes
- Minimize induction of intraperitoneal inflammation
- Inhibit angiogenesis/delay revascularization
- Suppress the host response



Aknowledgements

Departments of OB/GYN and Pathology



Hans Evers
Gerard Dunselman
Ton de Goeij
Chamindie Punyadeera
Annemiek Nap
Kim van Kaam
Jacques Maas
Ayse Demir
Carolien Koks
Helen Dassen

Christa de Veen
Rik Kamps
Bert Delvoux
Jacqueline Schouten
Lilian van Es



**Dian Shepperson Mills
The Endometriosis & Fertility
Clinic**

**Cert Ed., B.A., Dip ION, M.A.
Nutritional Therapist
0 (44) 1323 846888
dian@endometriosis.co.uk
www.endometriosis.co.uk
www.makingbabies.com**

©The Endometriosis and Fertility Clinic



**How can diet impact the
symptoms of the disease?**

Does what we eat have an effect on
endometriosis, pain and our fertility?

©The Endometriosis and Fertility Clinic



Prevention is better than cure

What is endometriosis?
What are we trying to cure?
What are the trigger factors?

©The Endometriosis and Fertility Clinic

What is endometriosis?



- An auto-immune disease?
- A disease which proliferates in presence of oestrogen?
- A disease of inflammation?
- A disease with extreme levels of pain and fatigue?
- A disease which compromises fertility?
- A disease with high level of prostaglandin PGE2?
- A disease which affects atopic/allergic women?
- A disease which affects skin and membranes?
- A disease which affects the digestive tract?

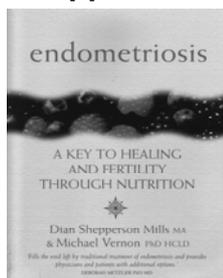
©The Endometriosis and Fertility Clinic

- The body is not a set of disparate organs that can be compartmentalised; body cells work together and have message receptors
- We have to work to correct health in the whole body



©The Endometriosis and Fertility Clinic

Healing With a Nutritional Approach



©The Endometriosis and Fertility Clinic

Dis-ease is a disturbance in body biochemistry



- Research shows that 35% of all cancers are diet related

Doll R, & Peto R **Causes of cancer**. Oxford, Oxford University Press, 1981

- 71% of adults questioned believe that the most important thing they do to protect health involves eating well

World Health Organisation 1994

©The Endometriosis and Fertility Clinic

Nutrition



- Nutrients are essential to life and health
- Eating is something we all do every day
- Sound food choice sustains us and keeps us healthy
- Poor food choice can make us unhealthy

©The Endometriosis and Fertility Clinic

Poor Nutrition



- Research into restricted calorie intake at the University of Pittsburgh discovered that “fasting for one day alone can change the suppression of luteinizing hormone”
- The implication for slimmers is that even short-term deficiency can have a profound effect on endocrine function
- If you are restricting nutrient intake in order to lose weight, you may be damaging your chances of becoming pregnant

©The Endometriosis and Fertility Clinic

A Comparison of Foods 1939 and 1991



Magnesium

Food	1939	1991	% change
Raw carrots	12	3	-75%
Celery	10	5	-50%
Potatoes	25	17	-32%
Tomatoes	11	7	-36%

McCance and Widdowson 1991
©The Endometriosis and Fertility Clinic

1936 American Senate Warning



- The alarming fact is that foods (fruits, vegetables, and grains) now being raised on millions of acres of land that no longer contains enough of certain minerals are starving us - no matter how much of them we eat
- No man of today can eat enough fruits and vegetables to supply his system with the minerals he requires for perfect health

©The Endometriosis and Fertility Clinic

Nutrients Known to Relieve Pain



- Vitamin C
- Vitamin E
- Vitamin K
- Zinc and selenium
- Essential Fatty Acids
- B1 + B6 + B12
- Magnesium
- DL Phenylalanine

©The Endometriosis and Fertility Clinic

Omega 3 and Omega 6 essential fatty acids and pain reduction



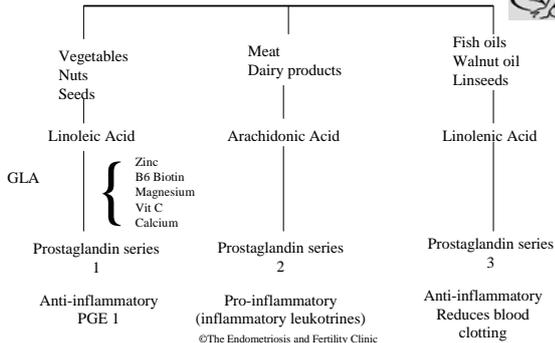
- In a group of Danish women, a higher intake of omega 3 fatty acids or a higher ratio of omega 3/omega 6 fatty acids was associated with reduced menstrual pain.

Deutch B. Menstrual pain in Danish women correlated with low omega 3 polyunsaturated fatty acid intake. *Eur J Clin Nutri* 1995;49: 500-16

- Women should reduce their saturated and trans fatty acid intake by half - then oestrogen levels will be around 20% lower
- Use oily fish, nuts and seeds, dark leafy vegetables, cold-pressed extra virgin olive and walnut oils

©The Endometriosis and Fertility Clinic

Fats and Oils



Bowel Health



- Every tissue in the body is fed by the bloodstream which is supplied by the bowel
- When the bowel is dirty, the blood is dirty and so are the organs and tissues
- It is the bowel that must be cared for first

Lindsay Duncan
Clinical nutritionist

©The Endometriosis and Fertility Clinic

Gut Flora



- 2-4 lbs of gut bacteria live in the intestines - 100 trillion organisms make up this flora
- 400-500 different species provide an active metabolic action equivalent to the liver
- 10^{10} immunoglobulin producing cells per metre of small bowel account for 80% of all immunoglobulin cells in the body
- They provide a protective atmosphere and are a critical factor in immune stimulation
- Antibiotics, oral contraceptives, hormone replacement therapy and steroids, non-steroidal anti-inflammatories and aspirin disrupt flora.

©The Endometriosis and Fertility Clinic

Stool Findings



- 73% Gluten sensitivity
- 78% Decreased beneficial bacteria
- 26% Yeast overgrowth
- 46% Resistant yeast
- 68% Pathogenic bacteria
- 73% Antibiotic resistance

©The Endometriosis and Fertility Clinic

The Immune/Digestive System Link



Healthy gut flora is the key to good health



Immuno-globulins needed for immune system to fight infection

Gut flora produces B vitamins for gut membrane



Gut membrane produces 80% of body's immuno-globulins



SHBG binds to cell receptors to aid oestrogen excretion

©The Endometriosis and Fertility Clinic

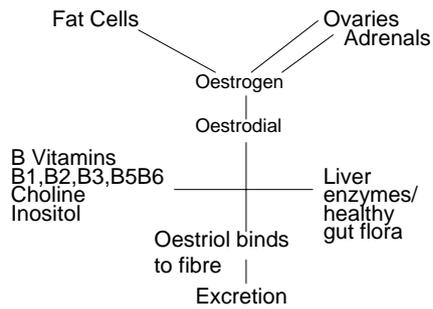
Gastrointestinal Flora



- The liver detoxifies oestrogen via attachment of glucuronic acid to oestrogen and excreting it in the bile
- Beta-glucuronidase a bacterial enzyme - it uncouples the bond between the excreted toxin and glucuronic acid - can increase cancer risk
- The activity of this enzyme can be reduced by establishing proper bacterial flora using a probiotic of 4 billion viable L acidophilus and B bifidum
- Eat foods such as onions, asparagus, bananas, maple syrup to encourage good gut flora

©The Endometriosis and Fertility Clinic

Oestrogen Breakdown



©The Endometriosis and Fertility Clinic

Phase I Liver Detoxification



- To neutralise unwanted compounds e.g. hormones, histamines, which could build up to become harmful
- Cytochrome P450 enzymes convert some chemicals to neutralize them, others are processed to an intermediate form ready for Phase II. They can be toxic if they hang around if Phase II is sluggish
- Eat brassica vegetables, caraway and dill, avoid grapefruit

©The Endometriosis and Fertility Clinic

Phase II Liver Detoxification



- Methylation - SAM S-adenosylmethionine prevents oestrogen induced cholestasis, promotes oestrogen excretion, increases membrane fluidity (which oestrogen decreases)
- Sulfation - to detoxify and eliminate steroid hormones to prevent them building to dangerous levels, requires molybdenum to function, is damaged by NSAIDS, aspirin, E102 tartrazine
- Magnesium deficiency leads to build up of toxic intermediates

©The Endometriosis and Fertility Clinic

Factors which Raise Oestrogen Levels



- Excess wheat intake
- Excess soya intake
- Excess citrus fruit intake
- Excess vitamin C intake
- Excess folic acid intake
- Excess Korean ginseng intake
- Environmental levels of PCB's, dioxins, hormones in meat and dairy foods
- Too little fibre intake
- Too little protein intake
- Too little B vitamin intake

©The Endometriosis and Fertility Clinic

Control of oestrogen is a nutritional process disturbed by:



- Lack of vitamin B complex, B1 B2 B3 B5 B6 Choline Inositol (Eat Green Vegetables)
- Too little protein - eat 30gm per day - legumes, nuts, seeds, white meat, fish, wholegrain cereals, non-bovine dairy food, organic eggs
- Too much refined sugar = deficiency of B3 zinc and chromium = malfunction of blood sugar levels = Hyperinsulinism PCO?Endo?

Fredericks C. *Guide to Women's Nutrition* Perigree, New York 1989

©The Endometriosis and Fertility Clinic

Sweet Nothings



- The average American eats 145 pounds of sugar per year - 28 teaspoons per day
- We should have about 2 teaspoons in our blood and 300 calories stored as glucogen in the liver
- Insulin resistance - the body struggles to acquire resistance to the hormone insulin
- Insulin receptors are found in the ovary, skin, brain, kidney, and blood vessels

©The Endometriosis and Fertility Clinic

Thyroid disease and endometriosis



- Auto-immune diseases such as those involving the thyroid, are thought to be involved in fertility
- "44% of women who miscarried were seen to have antibodies implicated with anti-cardiolipin, it has also been speculated that thyroid auto-antibodies are involved with reproductive failure
- Oestrogen and thyroxine are antagonistic hormones
- Thyroid auto-antibodies are used to predict women at risk for miscarriage

Singh A, Dantas ZN, Stone SC and Asch RH, 'Presence of thyroid anti-bodies in early reproductive failure: biochemical versus clinical pregnancies' in *Fert & Steril*, 63 (2): 277-81, 1995

©The Endometriosis and Fertility Clinic

Hypothyroid



- Symptoms low thyroid, weight gain, low energy, hair loss, insomnia, thickening skin, constipation
- Reduce goitergenic foods - turnip, cabbage, soya, wheat, tapioca, peanuts, pine nuts, millet, broccoli, carrots, mint, horseradish, cauliflower, spinach, pear (eat cooked not raw)

©The Endometriosis and Fertility Clinic

Findings in Women with Endo Hormone Abnormalities



- 54% Thyroid
- 13% GnRH Deficiency
- 29% Cortisol
- 73% Insulin resistance

©The Endometriosis and Fertility Clinic

96% Food allergies and intolerances in women with endo



- One man's meat is another man's poison
- Try a 1 month exclusion diet
- Avoid wheat - eat oats, rye, barley, corn, rice, buckwheat, millet, quinoa, tapioca, arrowroot
- Avoid bovine dairy - eat goat, sheep, buffalo, soya, rice milk, oat milk, almond and hazelnut milk
- Avoid coffee and citrus as major gut irritants
- Avoid aspartame, mono-sodium glutamate

©The Endometriosis and Fertility Clinic

Health Strategies



1. Breakfast like a King, lunch like a Prince, dinner like a pauper
2. Regular 3 unhurried meals, 2 snacks a day
3. Shopping frequently for fruits and veggies
4. Daylight walk for zinc absorption to aid ovaries
5. Unrefined, cold-pressed unhydrogenated oils e.g. extra-virgin olive
6. Drinking plenty of fresh filtered or mineral water.
7. Organic, as fresh as our Hunter-Gatherer ancestors ate.

©The Endometriosis and Fertility Clinic

Foods to Enjoy



- Vegetables of all kinds: dark green leafy, legumes (peas, beans, lentils), red colored veg, roots and tubers
- Raw fresh fruits in moderation, especially berries
- White and oily deep sea fish, and organic lean meat
- Vary dairy foods - goats, sheep, buffalo, soy, rice, oat, almond
- Vary carbs - rye, barley, oats, rice, corn, buckwheat, quinoa, millet, tapioca, arrowroot
- Snack on nuts, seeds, sugar-free bars, corn tacos, popadoms, fruits, crudites

©The Endometriosis and Fertility Clinic

Foods to Reduce or Avoid



- Chocolate, sweets, candies, ice-cream
- Wheat - bread, cakes, cookies, pasta
- Bovine dairy - cheese, cream, milk
- Coffee, alcohol, fizzy drinks, aspartame, MSG
- Red meat, pork, lamb, beef - use organic only
- Hidden trans-fats in refined foods

©The Endometriosis and Fertility Clinic

Nutritional Supplements



3-6 months - whilst correcting the diet.
Nutrients must be yeast, wheat, gluten, dairy and sugar free and natural

1. Multi-vitamin/mineral, (low vitamin A 2000iu)
2. Evening primrose and fish oils 1-2000mg
3. Magnesium 300mg (to relax smooth muscle, promote sleep, calm nerves)
4. Acidophilus 4 billion (to rebalance gut flora)

©The Endometriosis and Fertility Clinic

Supplements to aid digestion



- Slippery elm tablets to heal the gut membrane
- Vegan digestive enzyme with dinner
- Candistatin, Pau D'Arco, Biocidin
- Vitamin C with bioflavonoids 1000mg
- Vitamin E 300iu
- Proanthocyanandins to reduce inflammation

©The Endometriosis and Fertility Clinic

Nutrition Consultations



- 1st One Hour - Nutrition Questionnaire, 15 page patient pack, Patient History, MYMOP - Measure Yourself Medical Outcome Profile from MRC UK
- 2nd Half Hour - Review of Diet and Supplements, Making changes according to progress, MYMOP
- Tests - Sweat analysis, Lactose and gluten intolerance, Hormone profile

©The Endometriosis and Fertility Clinic

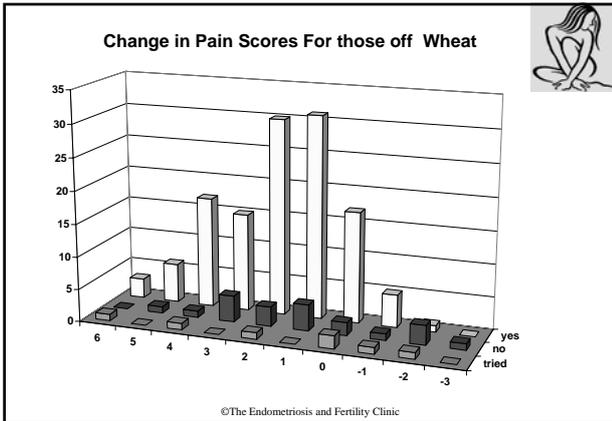
MYMOP

Measure Yourself Medical Outcome Profile



- MRC Clinical Audit
- Symptom 1
- Symptom 2
- Activity
- Well-being
- Symptom 3 (from 2nd appointment)
- Medications
- Nutritional Supplements
- Dietary changes and adverse events

©The Endometriosis and Fertility Clinic



Results 198 Women

Measure Yourself Medical Outcome Profile

- Over 6 months the women reported an average 50% reduction in their pain scores
- 34% reported infertility as a problem
- 52% of the sub-fertile group fell pregnant
- 82% reported adverse reactions from wheat
- 55% reported adverse reactions from bovine dairy food

©The Endometriosis and Fertility Clinic



From Lynne in Scotland

- My message is quite simple, healing through nutrition works, thanks to Dian I have what everyone wants. I have my life back and so much hope for my future
- dian@endometriosis.co.uk

©The Endometriosis and Fertility Clinic