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SCIENCE MOVING
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The impact of the reproductive tract environment on implantation success

Special Interest Group Endometriosis/Endometrium

6

3 July 2011
Stockholm, Sweden

The impact of the reproductive tract environment on implantation success

Stockholm, Sweden
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Organised by
Special Interest Group Endometriosis/Endometrium

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Course coordinators

Hilary Critchley United Kingdom, (SIGEE Coordinator) and Anneli Stavreus-Evers (Sweden, SIGEE Deputy Coordinator)

Course description

Multiple factors impact on implantation success. This course will address the potential systemic and local environmental influences on embryo implantation.

Target audience

This course will appeal to Obstetricians and Gynaecologists who manage patients with endometriosis and fibroids and in particular those practitioners with an interest in managing patients in early pregnancy/ problematic early pregnancy. Presentation content will also be of interest to basic scientists and paramedicals.

Scientific programme

Effects of the uterine environment

09.00 - 09.30	Endometrial gene pathways important for implantation - **Anneli Stavreus-Evers (Sweden)**
09.30 - 09.45	Discussion
09.45 - 10.15	Do uterine fibroids cause implantation failure? - **Peter Rogers (Australia)**
10.15 - 10.30	Discussion
10.30 - 11.00	Coffee break

Effects of the tubal environment

11.00 - 11.30	The expression of receptivity markers in the Fallopian tube epithelium – **Antonis Makrigiannakis (Greece)**
11.30 - 11.45	Discussion
11.45 - 12.15	Changes in the Fallopian tube microenvironment predisposing to tubal Implantation - **Andrew Horne (United Kingdom)**
12.15 - 12.30	Discussion
12.30 - 13.30	Lunch

Effects of the pelvic environment

13.30 - 14.00	Perturbations in endometrial gene expression in women with endometriosis: potential effects on embryo implantation **– Amelie Fassbender (Belgium)**
14.00 - 14.15	Discussion
14.15 - 14.45	Can pelvic infection explain implantation failure? - **Siladitya Bhattycharya (United Kingdom)**
14.45 - 15.00	Discussion
15.00 - 15.30	Coffee break

Effects of the systemic environment

15.30 - 16.00	Obesity and the endometrium: effects on implantation **– Jose Bellver (Spain)**
16.00 - 16.15	Discussion
16.15 - 16.45	The role of thrombophilia/haematological disorders and impact on endometrial function **– Lesley Regan (United Kingdom)**
16.45 - 17.00	Discussion

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ESHRE – European Society of Human Reproduction and Embryology

What is ESHRE?

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

- promote interest in, and understanding of, reproductive science
- facilitate research and dissemination of research findings in human reproduction and embryology to the general public, scientists, clinicians and patient associations.
- inform 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

Executive Committee 2009/2011

Chairman	• Luca Gianaroli	Italy
Chairman Elect	• Anna Veiga	Spain
Past Chairman	• Joep Geraedts	Netherlands
	• Jean François Guérin	France
	• Timur Gürgan	Turkey
	• Ursula Eichenlaub-Ritter	Germany
	• Antonis Makrigiannakis	Greece
	• Miodrag Stojkovic	Serbia
	• Anne-Maria Suikkari	Finland
	• Carlos Plancha	Portugal
	• Françoise Shenfield	United Kingdom
	• Etienne Van den Abbeel	Belgium
	• Jolieneke Schoonenberg-Pomper	Netherlands
	• Veljko Vlaisavljevic	Slovenia
	• Søren Ziebe	Denmark

ESHRE Organisation

- General Assembly of Members
  - Executive Committee
  - Committee of Nat. Representatives
  - Central Office
  - ESHRE Consortia
    - EIM Consortium
    - PGD Consortium
  - Sub-Committees
    - Finance Sub-Committee
    - Comm. Sub-Committee
    - Publ. Sub-Committee
      - Editorial Office
      - Publisher
      - Editors-in-Chief
    - Int'l Scientific Committee
    - SIG Sub-Committee
      - SIG Coordinators
  - Task Forces

ESHRE Journals

Human Reproduction with impact factor 3.859

Human Reproduction Update with impact factor 7.042

Molecular Human Reproduction with impact factor 3.005

Campus Activities and Data Collection

Campus / Workshops

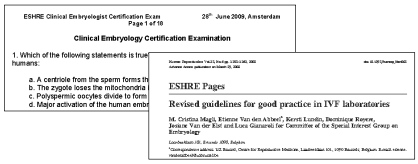
- Meetings are organised across Europe by Special Interest Groups and Task Forces
- Visit www.eshre.eu under CALENDAR

Data collection and monitoring

- European IVF Monitoring Group data collection
- PGD Consortium data collection

ESHRE Activities

- Embryology Certification
- Guidelines
- Position papers
- News magazine “Focus on Reproduction”


ESHRE Clinical Embryologist Certification Exam 20th June 2009, Amsterdam
Page 1 of 10

Clinical Embryology Certification Examination

1. Which of the following statements is true
humans:

a. A centriole from the sperm forms th
b. The zygote loses the mitochondria
c. Polyspermic oocytes divide to form
d. Major activation of the human embr

ESHRE Pages

Revised guidelines for good practice in IVF laboratories

M. Cristina Magli, Etienne Van den Abbeel, Kevrli Lundin, Dominique Royere, Jessica Van der Elst and Luca Gianaroli for Committee of the Special Interest Group on Embryology

ESHRE COMMUNITY

RSS feeds for news in reproductive medicine

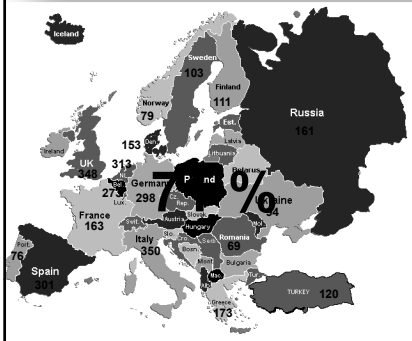
Since launch 12/2009: **1,360 Fans**

Since launch 12/2009: **190 followers** (journalists, scientific organisations, patient societies, governmental bodies)

Retweets to MHR

Find a member

ESHRE Membership (1/3)



4,017	Europe
476	Asia
372	North America
332	Middle East
221	Africa
142	Oceania
99	South America

TOTAL MEMBERSHIP*: 5 659 members

* as of July 2010

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.

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	(€ 250)

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

*workshop fees may vary

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

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

ESHRE – Annual Meeting

- One of the most important events in reproductive science
- Steady increase in terms of attendance and of scientific recognition

Track record:

ESHRE 2010 – Rome: 9,204 participants
ESHRE 2009 – Amsterdam: 8,055 participants
ESHRE 2008 – Barcelona: 7,559 participants

Future meetings:

ESHRE 2011 – Stockholm, 3-6 July 2011
ESHRE 2012 – Istanbul, 1-4 July 2012

ESHRE 2011, Stockholm, Sweden

When: **3 - 6 July 2011**
Where: **Stockholmsmässan, Mässvägen 1, Älvsjö, Sweden**
www.stockholmsmassan.se

Chair of conference: **Kersti Lundin**

Hotel and Travel:
MCI - Stockholm Office
Phone: +46 (0)8 54651500
E-mail: eshre@mci-group.com

For updates visit www.eshre.eu

ESHRE 2011, Stockholm

Keynote Lectures

Aneuploidy in humans: what we know and we wish we knew – Terry Hassold (USA)

Historical Lecture

A brave new world with a brave old humankind; quo vadimus – E. Diczfalusy (SE)

MHR Symposium – The paternal genome

Sperm chromatin packaging – B. Robaire (CDN)

The human sperm epigenome – B. Cairns (USA)

ESHRE 2011, Stockholm: Debates

This house believes that obese women should not receive treatment until they have lost weight

- **Yes: Mark Hamilton *(UK)***
- **No: Guido de Wert *(NL) - TBC***

Paramedical invited session: Should we pay donors?

- **Yes: Herman Tournaye (BE)**
- **No: Laura Witjens (UK)**

Annual Meeting – Pre-Congress Courses

- PCC 1: The challenges of embryo transfer (Paramedical Group)
- PCC 2: The blastocyst: perpetuating life (SIG Embryology and SIG Stem Cells)
- PCC 3: From genes to gestation
  (SIG Early Pregnancy and SIG Reproductive Genetics)
- PCC 4: Lifestyle and male reproduction (SIG Andrology)
- PCC 5: Ovarian ageing (SIG Reproductive Endocrinology)
- PCC 6: The impact of the reproductive tract environment on implantation success (SIG Endometriosis/Endometrium)
- PCC 7: Adhesion prevention in reproductive surgery
  (SIG Reproductive Surgery)

Annual Meeting – Pre-congress Courses

- PCC 8: Theory and practice update in third party reproduction
  (SIG Psychology and Counselling)
- PCC 9: Ethical aspects of non-invasive prenatal diagnosis
  (SIG Ethics & Law)
- PCC 10: Patient-centered fertility services
  (SIG SQUART)
- PCC 11: Clinical management planning for fertility preservation in female cancer patients
  (TF Basic Science and TF Preservation in Severe Disease in collaboration with the US OncoFertility Consortium)
- PCC 12: Opportunities for research in female germ cell biology
  (TF Basic Science)

Annual Meeting – Pre-congress courses

- PCC 13: Assisted reproduction in couples with HIV
  (TF Fertility and Viral Diseases)
- PCC 14: Prevention of infertility – from preconception to post-menopause
  (TF Reproduction and Society)
- PCC 15: Hot topics in male and female reproduction
  (ASRM exchange course)
- PCC 16: Academic Authorship programme
  (Associate Editors ESHRE journals)
- PCC 17: Science and the media, an introduction to effective communication with the media
  (Communications SubCommittee ESHRE)

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

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

Contact

ESHRE Central Office
Tel: +32 (0)2 269 09 69
info@eshre.eu / www.eshre.eu

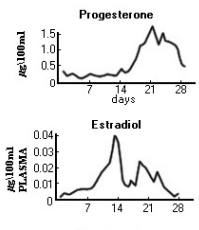
Endometrial gene pathways important for implantation

Anneli Stavreus-Evers PhD
Associate Professor
Department of Women's and Children's Health
Uppsala University

Learning objectives

- Understanding of hormonal regulation of endometrial receptivity
- Knowledge on genes involved in embryo-endometrial interactions

Estrogen and progesterone as main regulators of endometrial receptivity



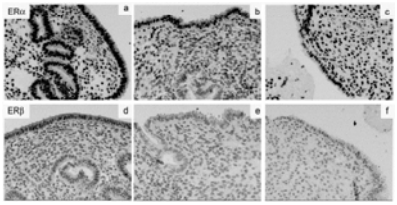
Estrogen and progesterone are important for:

- Endometrial development
- Fallopian tube function
- Maintenance of pregnancy

Estrogen and progesterone acts through their specific receptors ER and PR

Estrogen receptors

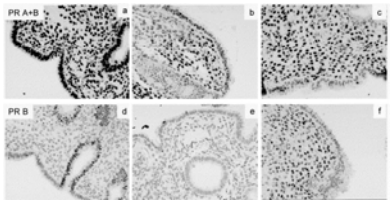
- ERα KO mice are infertile – hypoplastic endometrium
- ERβ KO mice – poor reproductive ability



Weihua et al 2001, PNAS, Stavreus-Evers Fertil-Steril 2001

Progesterone receptor

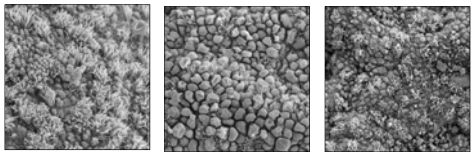
- PRA deficient mice – infertile
- PRB deficient mice – fertile



- Reduction of PRB at the time of implantation

Weihua et al 2001, PNAS, Stavreus-Evers Fertil-Steril 2001

Morphology and endometrial receptivity



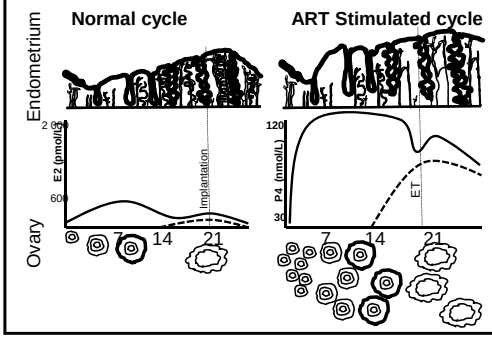
Before implantation Implantation window After implantation time

Gene expression and endometrial receptivity

Study	Samples	RNA pooled	First sample (day of cycle)	Second sample (day of cycle)	Fold-change	Up	Down
Kao *et al.* (2002)	11	No	Proliferative phase (8–10)	LH + (8–10) (21–23)	>2.0	156	377
Carson *et al.* (2002)	6	YES	LH + (2–4) (15–17)	LH + (7–9) (20–22)	>2.0	323	370
Borthwick *et al.* (2003)	10	Yes	Proliferative phase (9–11)	LH + (6–8) (19–21)	>2.0	90	46
Riesewijk *et al.* (2003)	10	No	LH + 2 (15)	LH + 7 (20)	>3.0	153	58
Mirkin *et al.* (2005)	8	No	Early-luteal (16)	Mid-luteal (21)	>2.0	49	58

Horcajadas et al Hum Rep Update 2007

Stimulated cycle vs. natural cycle



Stimulated cycle vs. natural cycle

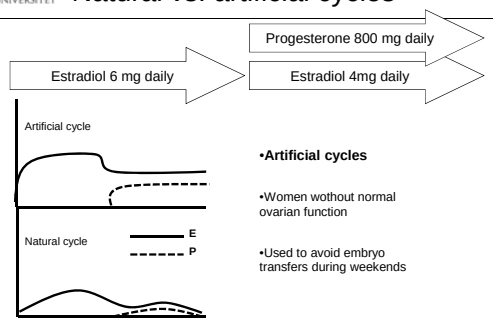
•Study	Days	Number of genes Up	Down
•Mirkin et al 2004	LH+8 vs hCG +9	6	6
•Horcajadas 2005	LH+7 vs hCG +9	281	277
•Simon et al 2005	LH+7 vs hCG +7	22	69
•Horcajadas et al 2008	LH+7 vs hCG +7	69	73
•Liu et al 2008	LH+7 vs hCG +7	244	159
•Macklon et al 2008	LH+5 vs hCG +4	142	98
•Haouzi et al 2009	LH+7 vs hCG +5	567	0

Stimulated cycle vs. natural cycle

Gonadotrophin treatment in controlled ovarian stimulation cycles lead to disruptions of the transcriptional activation of genes involved in normal endometrial receptivity

Haouzi et al Hum Rep 2009

Natural vs. artificial cycles

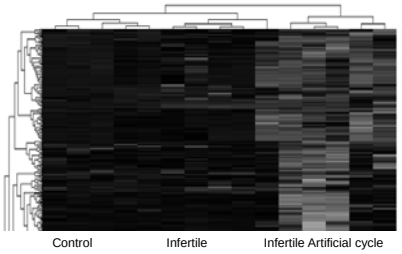


- **Artificial cycles**
- Women wothout normal ovarian function
- Used to avoid embryo transfers during weekends

Natural vs. artificial cycles

	Artificial cycle	Natural cycle	
Number of women	49	164	
Number of embryos	70	235	
Embryos/transfer	1.45	1.43	
Grade A embryos	73 %	62 %	
Positive hCG	28 %	25 %	
Biochemical pregnancies (early miscarriage)	**12.2 %**	**3.6 %**	**p=0.0331**
Clinical pregnancies	16.3 %	21.2 %	
Spontaneous abortions	2.0 %	4.2 %	
Delivery rate	14.3 %	18.9 %	

Natural vs. artificial cycles



Natural vs. artificial cycles

•Increased miscarriage rate was also seen in women undergoing embryo replacement in artificial cycles at the Centre of Reproduction in Uppsala (PO Karlström)

•This is due to disruptions of the transcriptional activation of genes involved in normal endometrial receptivity

•The normal balance between estrogen and progesterone and their cyclic release pattern is important for endometrial development to receptivity

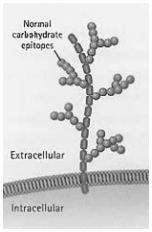
•Artificial menstrual cycles is not beneficial for women with normal menstrual cycles

Gene pathways of endometrial receptivity

- Adhesion and attachment (mucins and integrins
- Paracrine regulators (Interleukins, LIF, HB-EGF)

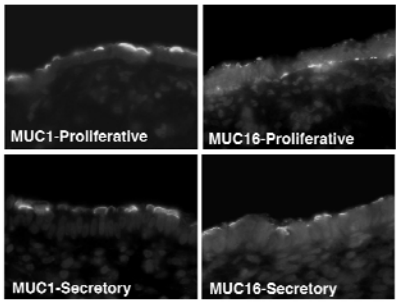
Gene pathways for adhesion and attachment
Mucin (MUC)

- Mucin 1 (Muc-1) is the major mucin on the endometrial surface
- Presented solely on the ciliated cells
- Increase in endometrium due to increased progesterone levels



Aplin RBM Online1999

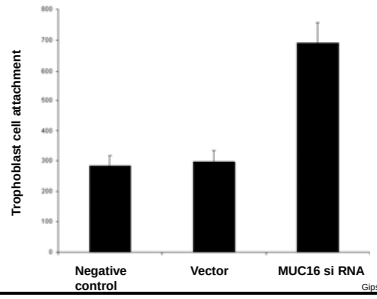
Gene pathways for adhesion and attachment
Mucin (MUC)



Gipson et al Biol Rep 2008

Gene pathways for adhesion and attachment
Mucin (MUC)

Silencing of MUC 16 using siRNA leads to increased trophoblast attachment

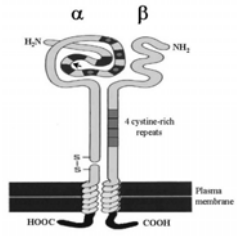


Gipson et al Biol Rep 2008

Gene pathways for adhesion and attachment Mucin1 and Mucin 16

- Disappear at the place of embryo attachment
- Embryo-endometrial paracrine action leads to loss of MUC1 and MUC 16 at the implantation site.

Gene pathways for adhesion and attachment Integrins

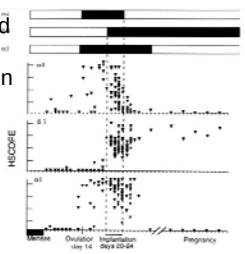


- Receptors for collagen, fibronectin and osteopontin
- Ligand for oncofoetal fibronectin present on the trophoblast cells
- Present on the endometrial surface and on the blastocyst
- Up-regulated by the blastocyst

Berman et al Membr Cell Biol 2000, Lessey et al Fertil Steril1995

Gene pathways for adhesion and attachment Integrins

- Integrins, b3, av, a1 and a3 are the most commonly ecpressed on at the time of implantation
- Progesterone and paracrine action is responsible for the regulation of integrins.



Lessey et al. Fertil Steril 2001

Gene pathways for paracrine regulation Interleukin-1 (IL-1)

- Peptides IL-1a and IL-1b
- Antagonist IL-1ra
- Receptors IL1R type I and IL-1R type II

IL-1R

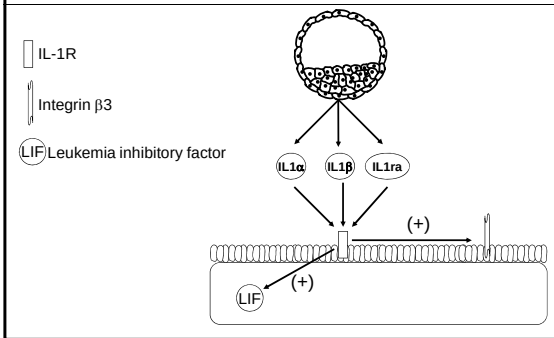
Kelly et al Reproduction 2001, Simon et al JCEM 1993

Gene pathways for paracrine regulation Interleukin-1 (IL-1)

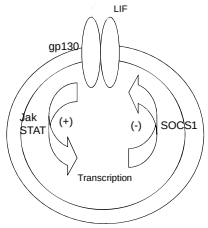
- High concentration of IL-1a in culture media is associated with successful implantation
- Block of IL-1R t1 by IL-1ra prevents implantation in mice
- IL-1a i localized on the maternal endometrium during implantation in the rhesus monkey

Illera et al Biol Rep 2000, Lessey et al Fertil Steril 1995, Simon et al JCEM 1999

Gene pathways for paracrine regulation Interleukin-1 (IL-1)



Gene pathways for paracrine regulation
Leukemia inhibitory factor (LIF)

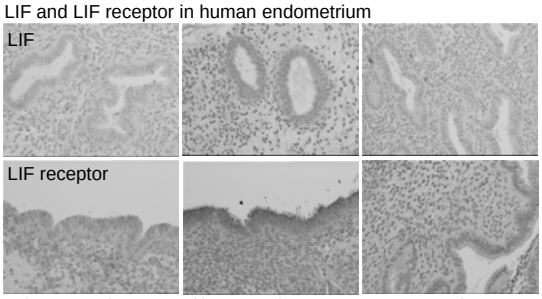


- Maternal LIF is nessecary for implantation in mice
- LIF is present in human embryo, endometrium and Fallopian tube
- Reduced levels of LIF in uterine secretion in infertile women

Stewart et al 1992, Li et al Mol Hum Rep 2004

Gene pathways for paracrine regulation
Leukemia inhibitory factor (LIF)

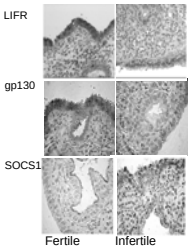
LIF and LIF receptor in human endometrium



Early secretory phase | Mid secretory phase | Late secretory phase

Aghajanova et al Fertil Steril 2003

Gene pathways for paracrine regulation
Leukemia inhibitory factor (LIF)



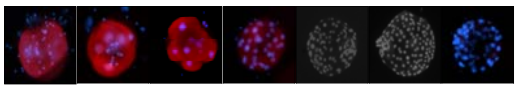
Endometrium from fertile compared with endometrium from infertile women

- Low LIFR
- Low gp130
- High SOCS1 (inhibitor of LIF action)

Aghajanova L *et al.* Fertil Steril 2008

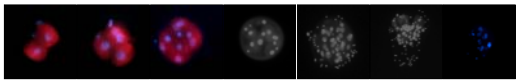
Gene pathways for paracrine regulation
Leukemia inhibitory factor (LIF)

LIFR



LIF receptor is present in all embryonic cells from 2- cell to blastocyst

Gp130



Gp130 is present in all embryonic cells from 2- cell to morula, thereafter only in the inner cell mass.

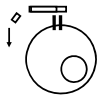
Wånggren et al

Gene pathways for paracrine regulation
HB-EGF

- Member of the EGF family
- Soluble HB-EGF: Proliferation, cell migration, inhibits apoptosis
- Trans-membrane HB-EGF: adhesion, pro-apoptotic
- Enhance human embryo development
- HB-EGF and its receptors HER1 and HER4 exists in the Fallopian tube and endometrium
- Facilitates adhesion of the embryo to the endometrial surface
- HB-EGF induce integrin avb3

Gene pathways for paracrine regulation
HB-EGF

•1. Endometrial maturation to receptivity

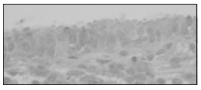


2. Signaling between the embryo and the endometrium

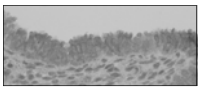
3. Attachment of the embryo

4. Re-epithelialization

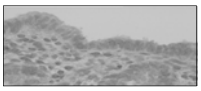
Gene pathways for paracrine regulation HB-EGF



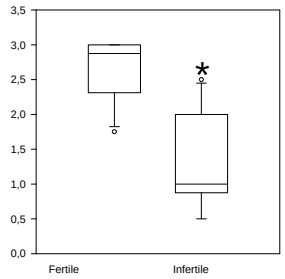
Early secretory phase



Mid secretory phase

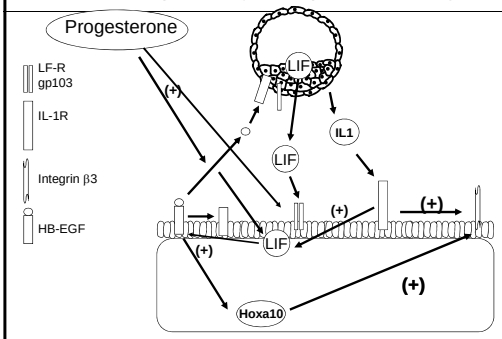


Late secretory phase



Stavreus-Evers et al 2002, Aghajanova et al 2007

Gene pathways for paracrine regulation



Unexplained infertility

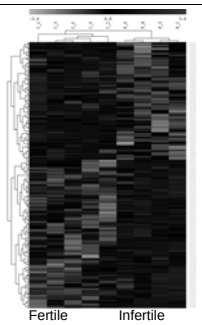
- Regular menstrual periods 25-35 days
- Normal ovulation
- Normal hormon levels FSH, LH, E, P, TSH, T3/T4, Prolactin
- Normal tubal function
- Inga klinical evidence of endometriosis
- Partner has normal sperm count

Unexplained infertility

- Heterogenous group

- Possible causes of unexplained infertility
- Subfertilitey
- Defekt endometrial development
- Genetic causes
- Fertilization problem
- Depression

Endometrial receptivity and unexplained infertility



Fertile Infertile

Altmäe et al Mol Hum rep 2010

Endometrial receptivity and unexplained infertility

Infertile/Fertile

- Endothelin (EDN3↑)
- Trefoil factor 3 (TFF3↑)
- Hemoglobins (HBA2↑, HBA1↑, HBGI↑, CYGB↑, HP↑)
- Mucins (MUC4↑, MUC-5 precursor↑)
- Wnt signalling pathway (WISP2↑, WNT3A↑, CXXC4↑, PRKCG↑)

- Metalloproteinases (MMP26↑, MMP10↓, MMP8↓)

- **Hyaluronan-binding protein 2 (HABP2 ↓)**
- Folate binding (SLC19A3↓)
- Estrogen receptor beta (ESR2↓)
- Insulin-like growth factor binding protein 1 (IGFBP1↓)
- Interleukin 21 (IL21↓)

Hyaluronanbinding protein 2 (HABP2)

- Binds hyaluronic acid and is involved in cell adhesion
- Activates the coagulation cascade by activation of factor VII
- Inhibts cell proliferation and migration of vascular smooth muscle cells

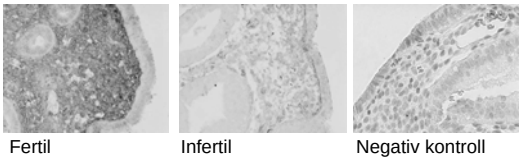
Embryo glue

- Embryo glue (hyaluronic acid) is present in some embryo transfer media
- Results in more implantation but also a higher risk of early miscarriage
- Number of deliveries does not increase when hyaluronic acid is present in the transfer medium

Hambiliki et al 2010

HABP2 and unexplained infertility

- Low mRNA and protein expression of HABP2 in endometria from women with unexplained infertility compared to endometria from fertile women



Fertil | Infertil | Negativ kontroll

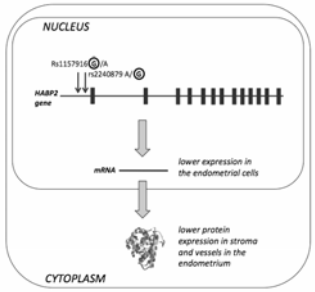
Altmäe et al 2011

HABP2 and unexplained infertility

		Infertile women n (%)	χ^2	Fertile women n (%)	χ^2	p-value
rs1157916	GG	44 (42.7)		54 (35.8)		
	GA	51 (49.5)		73 (48.3)		
	AA	8 (7.8)*		24 (15.9)*		
			1.690		0.007	>0.1
	p(G)	0.675		0.599		
	q(A)	0.325		0.401		
						0.051
rs2240879	AA	48 (46.2)*		88 (58.7)*		
	AG	43 (41.3)		50 (33.3)		
	GG	13 (12.5)		12 (8.0)		
			0.473		1.594	>0.1
	p(A)	0.668		0.753		
	q(G)	0.332		0.247		
						0.023

Altmäe et al 2011

HABP2 and unexplained infertility



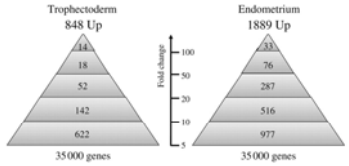
Altmäe et al 2011

Gene pathways in embryo-endometrial interactions

- Bioinformatic approach to study possible interactions between the blastocyst and the receptive endometrium
- Array from endometrium from fertile women(2 cycle phases, n=8)
- Array from 128 embryos

Number of genes that are over-expressed in the blastocyst trophoblast cells and in endometrium from infertile women after hormone stimulation.

A



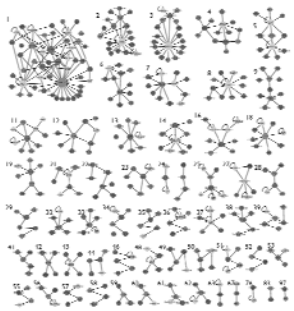
B

Gene name	Fold change	FDR
HSD3B1	529.6	0
ERVWE1	327.2	0
NALP7	313.1	0
C6orf148	302.5	0
LIN28	248.3	0
GAGE1	220.7	0
DNMT3L	205.1	0
LIN28B	202.2	0
GATA3	172.8	0
LAMA1	161.8	0

Gene name	Fold change	FDR
COL3A1	718.5	0
COL1A2	328.7	0
ESR1	328.5	0
EHF	310.7	0
SCGB2A1	294.9	0
ITGB8	277.8	0
IGFBP7	277.5	0
RXFP1	248.3	0
SCGB1D2	220.3	0
EVA1	219.8	0

Haouzi D et al. Hum. Reprod. 2011

Gene pathways in embryo-endometrial interactions



- Endometrium
- Embryo
- Both

Altmäe et al submitted

Conclusions

•Endometrial receptivity depends on hormonal regulation by estrogen and progesterone

•Endometrial receptivity is altered in stimulated and artificial cycles

• Several pathways of paracrine interactions is involved in embryo implantation

Acknowledgements

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- **Jose Martinez**
- **Andres Salumets** — **Tartu University, Estonia**

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- Altmäe et al Mol Hum rep 2010

Do uterine fibroids cause implantation failure?

Peter AW Rogers PhD
Professor of Women's Health research
University of Melbourne
Dept Obstetrics and Gynaecology
Royal Women's Hospital

Commercial relationships and potential conflicts of interest: Nil

Learning Objectives

Do uterine fibroids cause implantation failure?

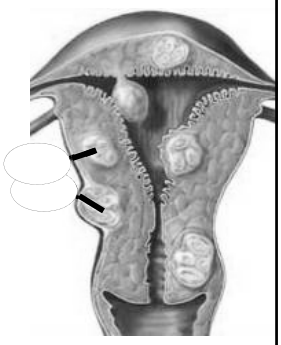
Racial differences in occurrence of fibroids and infertility

Fibroid heterogeneity: Symptomatic vs asymptomatic

Evidence for an effect of fibroids on fertility

Uterine Fibroids

- Benign tumours of myometrial smooth muscle cells
- Occur in up to 77% of women of reproductive age
  - ~ 25% symptomatic
- Cause of significant reproductive and gynecological problems
  - Abnormal uterine bleeding, pelvic pressure, pain and reproductive dysfunction
- The most common reason for hysterectomy
  - ~200,000 hysterectomies in US/year



High cumulative incidence of uterine leiomyoma in black and white women: Ultrasound evidence

Donna Day Baird, PhD,[a] David B. Dunson, PhD,[b] Michael C. Hill, MD,[c] Deborah Cousins, MSPH,[c] and Joel M. Schectman, MD[d]

(Am J Obstet Gynecol 2003;188:100-7.)

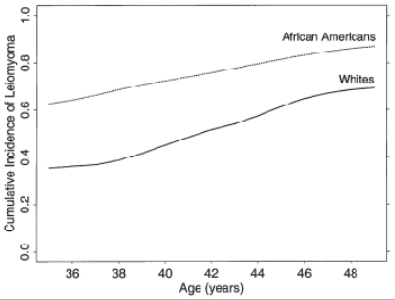


Fig 2. Estimated age-specific cumulative incidence of uterine leiomyoma for black women and white women, aged 35 to 49 years.

Infertility in married women aged 15-44 years (USA)

	1982	2002
White	8.5%	7.4%
Black	7.8%	11.6%

Stephen et al 2006

Declining estimates of infertility in the United States: 1982–2002

Elizabeth Hervey Stephen, Ph.D and Anjani Chandra, Ph.D

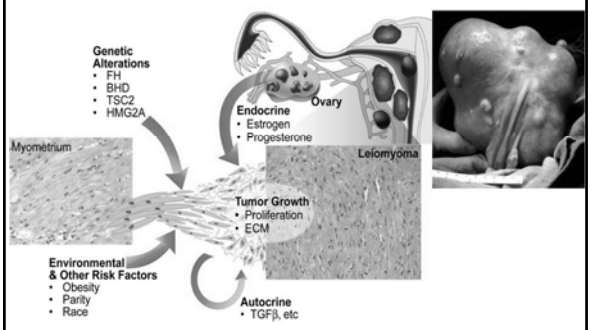
TABLE 3

Adjusted odds ratios (and 95% confidence intervals)[a] predicting 12-month infertility using multivariate logistic analysis of the combined 1982, 1988, 1995, and 2002 NSFG data (N = 15,303).

Characteristic	Bivariate	Multivariate
Year		
1982	1.0[b]	1.0[b]
1988	0.9 (0.8–1.1)	0.8 (0.7–1.0)
1995	0.8[a] (0.7–0.9)	0.7[a] (0.6–0.8)
2002	0.8 (0.7–1.0)	0.7[a] (0.6–0.9)

Fertility and Sterility® Vol. 86, No. 3, September 2006

Factors in Fibroid Growth



From: C. Walker and E. Stewart, Science, 2005

Structure of Talk

Fibroid heterogeneity: Symptomatic vs. asymptomatic fibroids

Evidence for an effect of fibroids on fertility

Fibroid heterogeneity: Symptomatic vs. asymptomatic fibroids

Very little is known about the mechanisms underlying clinical differences

Why do only some fibroids cause HMB?

No published studies have investigated vascular or cellular differences between symptomatic and asymptomatic fibroids

Human Reproduction Vol.19, No.10 pp. 2350–2355, 2004
Advance Access publication July 8, 2004
DOI: 10.1093/humrep/deh407

Uterine leiomyoma and menstrual cycle characteristics in a population-based cohort study

J.L.Marino[1,2], B.Eskenazi[1,*], M.Warner[1], S.Samuels[1], P.Vercellini[3], N.Gavoni[3] and D.Olive[4]

[1]School of Public Health, University of California, Berkeley, California, [2]School of Public Health and Community Medicine, University of Washington, Seattle, Washington, [3]Department of Obstetrics and Gynecology, University of Milan School of Medicine, Milan, Italy and [4]Department of Obstetrics and Gynecology, University of Wisconsin Medical School, Madison, Wisconsin, USA

[*]To whom correspondence should be addressed at: School of Public Health, University of California, 140 Warren Hall, Berkeley, CA 94720-7360, USA. E-mail: eskenazi@calmail.berkeley.edu

RESULTS:
Leiomyomata detected in 73/341 women (21.4%) not seeking gynaecological care.

Presence of a leiomyoma was not significantly related to menstrual cycle length, flow length or heaviness of flow.

Number, volume, tissue layer location (subserosal or intramural) and axial position (anterior or posterior) of the leiomyoma were not related to menstrual cycle characteristics.

Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Obstetrics & Gynecology and Reproductive Biology 115 (2004) 85–89
ELSEVIER
EUROPEAN JOURNAL OF OBSTETRICS & GYNECOLOGY AND REPRODUCTIVE BIOLOGY
www.elsevier.com/locate/ejogrb

Uterine fibroids—do size and location determine menstrual blood loss?

Suzanna Sulaiman[a], Aradhana Khaund[a], Nigel McMillan[b], Jon Moss[b], Mary Ann Lumsden[a,*]

[a]Division of Developmental Medicine, University of Glasgow, Glasgow, UK
[b]Department of Radiology, North Glasgow Hospitals University NHS Trust, Glasgow, Scotland, UK

Received 10 March 2003; received in revised form 14 May 2003; accepted 21 October 2003

Retrospective comparative study of 50 women with symptomatic fibroids

Uterine imaging and objective MBL measurement prior to uterine artery embolisation

33 (66%) women had objective menorrhagia with a MBL in excess of 80 ml per period

This study found that MBL correlated with neither fibroid size nor location.

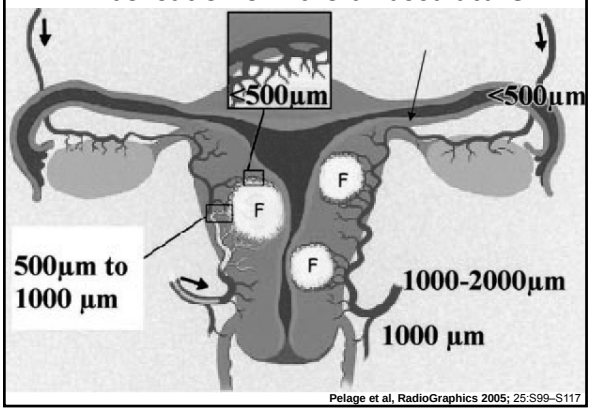
Self-Reported Heavy Bleeding Associated With Uterine Leiomyomata

Ganesa Wegienka, PhD, Donna Day Baird, PhD, MPH, Irva Hertz-Picciotto, PhD, Siobán D. Harlow, PhD, John F. Steege, MD, Michael C. Hill, MD, Joel M. Schectman, MD, and Katherine E. Hartmann, MD, PhD

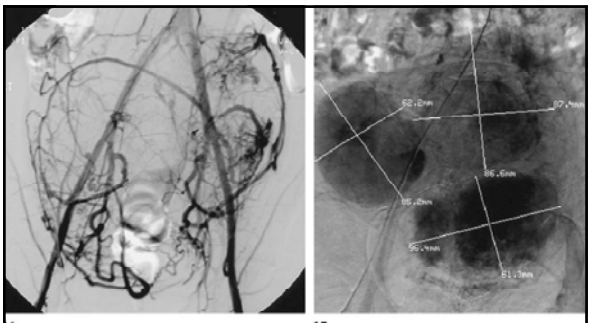
Obstet Gynecol 2003;101:431–7

Contrary to published articles, nonsubmucosal leiomyomata were associated with heavy bleeding to the same extent as submucosal leiomyomata.

Embolisation of fibroid vasculature



Pelage et al, RadioGraphics 2005; 25:S99–S117

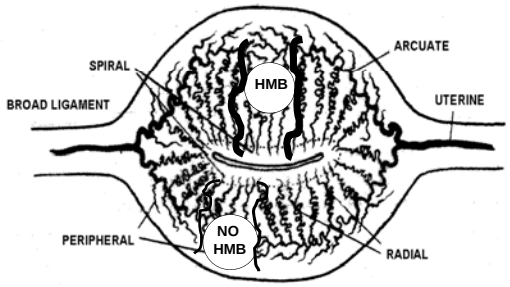


Figures 16, 17. (16) Flush pelvic aortogram shows diffuse uterine hypervascularity in a woman with a uterus enlarged (volume, 800 mL) by multiple fibroid tumors. **(17)** Late arterial phase flush pelvic aortogram in a woman with three intramural fibroid tumors shows three separate areas of localized hypervascularity with dimensions that correspond to those of the fibroid tumors.

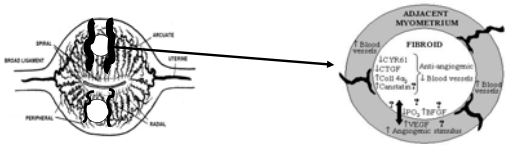
Pelage et al, RadioGraphics 2005; 25:S99–S117

How does fibroid disrupt menstruation?

- Blood flow to uterus…



Fibroids and Angiogenesis



- Fibroids display an anti-angiogenic profile →reduced vascular density and perfusion compared to myometrium.
- Peri-fibroid plexus has increased vascularity and blood flow
- Both the angiogenically compromised fibroid vasculature, and the increased peri-fibroid vasculature, represent targets for manipulation of angiogenesis.

Aims

OVERALL

Correlation between HMB and radiological and gene expression profiles

AIM 1

Identify radiological features of fibroids which may predict HMB

AIM 2

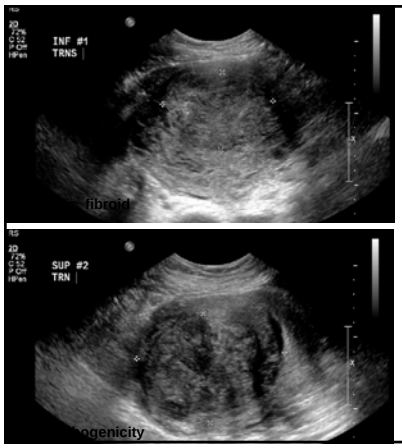
Identify molecular differences between fibroid, peri-fibroid myometrium and distant myometrium

PILOT STUDY

- 6 patients recruited
- Doppler Ultrasound of fibroids pre-hysterectomy to categorise blood flow by various measures
- Analyse genes - fibroid, peri-fibroid myometrium, distant myometrium - using quantitative RT-PCR and customised angiogenic arrays

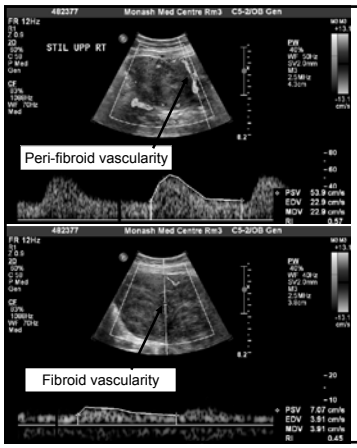
- Correlate patient symptoms with these findings

Picture used with patient's permission



RESULTS

Fibroid heterogeneity in same uterus by ultrasound



RESULTS

Higher mean peak systolic velocity in peri-fibroid region compared to within fibroid.

(p<0.001)

RESULTS

- Fibroids displayed variable echogenicity between and within patients
- 8 of 11 fibroids had increased blood flow in the peri-fibroid region
- Mean PSV was 11.6 ± 1.3 for fibroid compared to 30.2 ± 3.3 for the peri-fibroid region (p=0.001).

RT-PCR Array Results

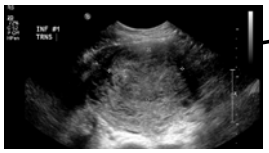
6 Patients

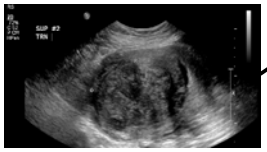
11 Fibroids, 11 Peri-fibroid myometrium (PM), 6 Distant myometrium (DM)

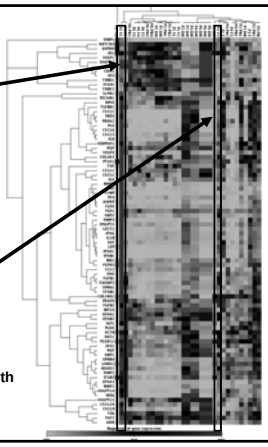
- 84 angiogenic genes on array (Human Angiogenesis RT2 Profiler™ PCR array. SA Biosciences, Qiagen GmbH, Hilden, Germany.

Generated gene lists of differences between groups

RESULTS

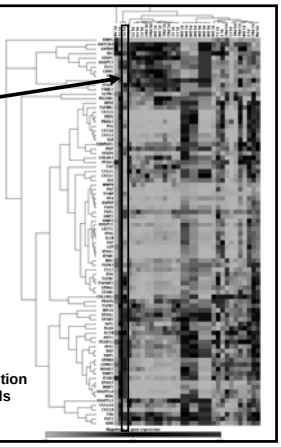






- **High levels of variability occur both with fibroid ultrasound appearance and differential gene expression**

RESULTS



- **Cystic by ultrasound, abnormal at dissection**
- **Different gene expression to other fibroids**
- **Evidence for correlation of clinico-pathological and molecular data**

Summary

- Fibroid heterogeneity – Symptoms (including HMB), ultrasound and gene profile
- Peri-fibroid region is different to distant myometrium
- Evidence for correlation of ultrasound with angiogenic gene expression and clinical symptoms
- More work required!

Structure of Talk

Fibroid heterogeneity: Symptomatic vs. asymptomatic fibroids

Evidence for an effect of fibroids on fertility

Gynecological Endocrinology, February 2006; 22(2): 106–109

Effects of the position of fibroids on fertility

MARIA LUISA CASINI[1], FEDERICA ROSSI[2], RICCARDO AGOSTINI[2], & VITTORIO UNFER[3]

Theories on how uterine fibroids may cause infertility:

- Pathological changes of the endometrium (atrophy, distortion, venule ectasia, ulceration).
  - Distortion of the uterine cavity.
  - Local inflammation leading to hostile intra-uterine environment.
  - Disrupted endometrial blood supply.
- Dysfunctional uterine contractility (sperm and ovum transport).
- Partial or complete tubal blockage.

Fig. 4. Submucosal leiomyoma. Due to compression and intracavitary friction, the endometrium has 'effaced' and is replaced by inflammatory granulation tissue with a new capillary network.

A. Ferenczy / Maturitas 45 (2003) 1/14

International Congress Series 1266 (2004) 191–196

Fibroids: effect on infertility and assisted reproduction

Cynthia M. Farquhar*

Table 1
The relationship between fibroids and infertility

Question	Explanation of question	Role of fibroids in infertility
Is there experimental evidence from human studies?	What happens to the pregnancy rate if fibroids are removed?	Only one comparative study available.

International Congress Series 1266 (2004) 191–196

Fibroids: effect on infertility and assisted reproduction

Cynthia M. Farquhar*

Obstetrics and Gynaecology, National Women's Hospital, University of Auckland, Claude Road, Auckland 1105, New Zealand

CONCLUSIONS:

- The case for fibroids causing infertility remains to be established.
- Conclude that fibroids probably do not cause infertility in most cases.
- Although the prevalence of fibroids in infertile women can be high, no causal relationship between fibroids and infertility has been demonstrated.
- Future studies, ideally, should be randomised controlled trials of myomectomy in women with fibroids and should report live birth outcomes.
- Such studies are long overdue.

Impact of subserosal and intramural uterine fibroids that do not distort the endometrial cavity on the outcome of in vitro fertilization–intracytoplasmic sperm injection

Flávio Garcia Oliveira, M.D.,[a] Vicente G. Abdelmassih, M.D.,[a] Michael P. Diamond, M.D.,[b] Dimitri Dozortsev, M.D.,[a] Nilson R. Melo, M.D.,[a] and Roger Abdelmassih, M.D.[a]

FERTILITY AND STERILITY®
VOL. 81, NO. 3, MARCH 2004

Design: Retrospective, matched-control study from January 2000 to October 2001.
Patient(s): Two hundred forty-five women with subserosal and/or intramural fibroids that did not compress the uterine cavity (fibroid group) and 245 women with no evidence of fibroids anywhere in the uterus (control group).

TABLE 2

Comparison of IVF-ICSI outcomes according to the number of pregnancies, abortions (first and second trimesters), and deliveries (with live birth) by week.

	Control group (n = 245)	Fibroid group* (n = 245)
Pregnancies	110 (45)	117 (48)
First trimester abortion[a]	31 (28)	37 (31)
Second trimester abortion[b]	1 (0.9)	2 (1.7)
22–26 weeks	2	2
26–30 weeks	3	5
30–34 weeks	6	5
34–37 weeks	6	4
>37 weeks	61	61
Total DR (live birth)	78/245 (32)	77/245 (31.5)
Preterm DR for singletons	4/49 (8)	5/50 (10)
Preterm DR for multiples	13/29 (45)	11/27 (41)

Note: Values are n or n (%). DR = delivery rate; NS = not significant. * P = NS (fibroid group vs. control group for all values, Fisher exact test).
[a] <14 weeks.
[b] 14–22 weeks.

TABLE 1

Description of the fibroid group.

	No. of patients	Pregnancy rate No. (%)	Abortion rate No. (%)
No. of fibroids			
1	152	75 (49)	20 (27)
2	66	31 (47)	15 (48)
3	18	7 (39)	0 (0)
4	9	4 (44)	1 (25)
Type of fibroid			
Subserosal (SS)	82	41 (50)	15 (35)
Intramural (IM)	130	63 (48)	17 (28)
IM-SS	33	13 (40)	4 (31)
Location (IM)			
Fundal	110	53 (48)	15 (28)
Corpus	53	23 (43)	6 (26)
Size of IM fibroid (cm)			
0.4–2.0	64	34 (53)*	9 (26)
2.1–4.0	58	30 (51)*	7 (23)
4.1–6.9	41	12 (29)	5 (41)

Note: * P= .025 (χ^2 for trend). There was a significant linear trend among the ordered categories. All other %s were not statistically different (χ^2 test, Fisher exact test).

Gynecological Endocrinology, February 2006; 22(2): 106–109

Effects of the position of fibroids on fertility

MARIA LUISA CASINI[1], FEDERICA ROSSI[2], RICCARDO AGOSTINI[2], & VITTORIO UNFER[3]

•181 infertile patients with uterine fibroids and no other identifiable causes of infertility.
•Inclusion criteria: age <36 years; infertility for at least 1 year; presence of one knot and/or fibroid of diameter <40 mm; and absence of other causes of infertility.
•Exclusion criteria: presence of two or more knots and/or fibroids of diameter >40 mm; body weight >20% of normal weight; and use of medication containing estrogens, progestins or androgens 8 weeks prior to study.
•Of 181 women, 68 became pregnant of whom 25 had a miscarriage.
•The percentage of miscarriages varied depending on both the location of the uterine fibroids and the treatment

Table II. Effect of fibroid location and treatment on pregnancy rate.

Group	Treatment	No. of patients	No. of pregnancies	Pregnancy rate (%)	p Value
SM (n = 52)	With surgery	30	13	43.3	<0.05
	Without surgery	22	6	27.2	
IM (n = 45)	With surgery	23	13	56.5	NS
	Without surgery	22	9	40.9	
SS (n = 11)	Without surgery	11	7	63.6	
IM-SS (n = 31)	With surgery	17	6	35.3	NS
	Without surgery	14	3	21.4	
SM-IM (n = 42)	With surgery	22	8	36.4	<0.05
	Without surgery	20	3	15.0	

SM, submucosal; IM, intramural; SS, subserosal; IM-SS, mixed intramural–subserosal; SM-IM, mixed submucosal–intramural; NS, not significant.

Fibroids and infertility: an updated systematic review of the evidence

Elizabeth A. Pritts, M.D.,[a] *William H. Parker, M.D.,*[b] *and David L. Olive, M.D.*[a]

(Fertil Steril 2009;91:1215–23).

Design: Systematic literature review and meta-analysis of existing controlled studies.

Only those studies with a control group were included.

Of 347 studies initially evaluated, 23 were included in the data analysis

Conclusion(s): Fertility outcomes are decreased in women with submucosal fibroids, and removal seems to confer benefit. Subserosal fibroids do not affect fertility outcomes, and removal does not confer benefit. Intramural fibroids appear to decrease fertility, but the results of therapy are unclear. More high-quality studies need to be directed toward the value of myomectomy for intramural fibroids, focusing on issues such as size, number, and proximity to the endometrium.

Leiomyoma Simultaneously Impair Endometrial BMP-2-Mediated Decidualization and Anticoagulant Expression through Secretion of TGF-β3

Donna C. Sinclair, Alex Mastroyannis, and Hugh S. Taylor

Yale University School of Medicine, Department of Obstetrics, Gynecology, and Reproductive Sciences, Division of Reproductive Endocrinology and Infertility, New Haven, Connecticut 06520

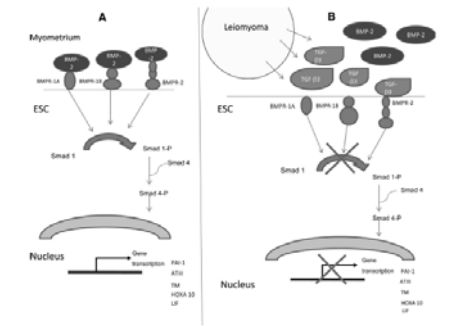


FIG. 9. Schematic representation demonstrating the effects of TGF-β3 on decidualization and hemostasis. A, In the non-leiomyomatous uterus, ESC respond to BMP-2 stimulation resulting in decidualization. To allow normal menstrual blood flow, ESC also make factors that contribute to efficient interruption of the coagulation cascade. These include ATIII, an inhibitor that neutralizes thrombin. ESC also express TM, a cellular receptor that catalyzes the activation of protein C by thrombin. u-PA is a fibrinolytic agent that is expressed by endometrial tissue and has been shown to increase in normal premenstrual and menstrual endometrial tissue. PAI-1 modulates the activity of u-PA, and higher levels are normally expressed in menstrual phase endometrium. B, In the presence of leiomyoma, excess TGF-β3 binds to BMPRs, resulting in their down-regulation, BMP-2 resistance, and thus, impaired decidualization. Excess TGF-β3 also impairs the production of PAI-1, ATIII, and TM in leiomyoma-associated ESC, resulting in defective hemostasis.

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Conclusions: Leiomyoma-secreted TGF-β3 induces BMP-2 resistance in endometrium by down-regulation of BMPR-2, likely causing defective endometrial decidualization. TGF-β3 also reduces expression of PAI-1, ATIII, and thrombomodulin in endometrium, likely contributing to menorrhagia. A single molecular signal targeting endometrium may mediate both leiomyoma-induced infertility and bleeding. ***J Clin Endocrinol Metab* 96: 412–421, 2011)**

Human Reproduction Update, Vol.17, No.2 pp. 242–253, 2011
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human reproduction update

Implantation failure: molecular mechanisms and clinical treatment

Hakan Cakmak and Hugh S. Taylor*

Leiomyoma

Uterine leiomyomas are the most common benign tumor in women of reproductive age, and their prevalence approaches to 70% in white women and more than 80% in black women by age 50 years (Day Baird et al., 2003). Leiomyomas are present in 5–10% of women with infertility and are the sole factor identified in 1–2.4% (Donnez and Jadoul, 2002). Intramural and submucosal leiomyomas can distort the uterine cavity or obstruct the tubal ostia or cervical canal and, thus, may affect fertility (Pritts, 2001).

Further studies are required to further delineate the molecular mechanisms of implantation failure in women with leiomyomas distorting the uterine cavity.

Is another meta-analysis on the effects of intramural fibroids on reproductive outcomes needed?

Mostafa Metwally, Cynthia M. Farquhar and Tin Chiu Li
a The Academic Unit of Reproductive and Developmental medicine, The University of Sheffield and Sheffield Teaching Hospitals, Sheffield, UK
b Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand
c Sheffield Teaching Hospitals, Sheffield, UK
Reproductive Biomedicine Online Received 26 May 2010

- Systematic review and meta-analysis of the currently available evidence – 10 studies.
- Showed no evidence of a significant effect for intramural fibroids on:
  - clinical pregnancy rate (OR 0.74, 95% CI 0.50–1.09)
  - live birth rate (OR 1.17, 95% CI 0.62–2.22)
  - miscarriage rate (OR 1.61, 95% CI 0.61–4.20)
- Findings highlight the current deficiency in the literature and suggest that evidence is insufficient to draw any conclusions regarding the effect of intramural fibroids on reproductive outcomes.

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The expression of receptivity markers in the Fallopian tube epithelium

Tubal pregnancy is unique in women, while in other mammals it occurs neither spontaneously nor experimentally

Corpa 2006, Tutton and Carr 1984; Pauerstein et al.1990

Tubal receptivity – Potential markers

- Pinopode formation
- Integrins
- Fibronectin
- Osteopontin
- Glycodelin A
- Leukemia Inhibitory Factor
- E-cadherin

Pinopodes

Pinopodes

Endometrium is receptive during a temporally and spatially restricted period that is defined as “window of implantation”.

A number of endometrial changes associated with receptivity have been described, including the transient fusion of the hairy-like epithelial cell microvilli to a single flower-like membrane projection called ‘pinopode’.

This plasma membrane transformation is a universal phenomenon

Nikas 2000, Murphy 2000

On an average, pinopodes occur on days 20–21 in natural cycles and earlier (days 19–20) in stimulated cycles.

Pinopodes

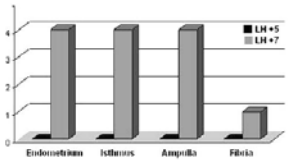
Pinopodes

Endometrium

On LH +7 day, the endometrium lacked microvilli and showed numerous developing or fully developed pinopodes protruding from the apices of the secretory cells, exceeding in length the cilia (pinopode score 4, mean from the 7 specimens)

Oviduct

On LH +7 day many secretory cells beared developing or fully developed pinopodes while ciliated cells remained unchanged (pinopode score 4, mean from the 7 specimens)



Pinopode score

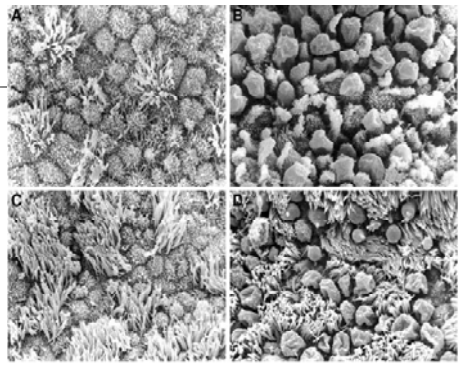
Makrigiannakis et al, 2009

Pinopodes

Pinopodes

Endometrium

Oviduct



Makrigiannakis et al, 2009

Integrins

Integrins

They are heterodimeric glycoproteins made up of a- and b-subunits

Indirect immunofluorescence on oocytes and preimplantation embryos demonstrates consistent expression of two a-integrin subunits (Av, A3) and four -subunits (B1, B3, B4, B5)

Campbell et al. 1995

In human endometrium, several integrin receptors have been identified

Lessey et al. 1992, 1994; Tabibzadeh 1992; Aplin et al. 1996; Franchi et al. 2008

A1B1, A4B1 and AvB3, which appear to be regulated during the luteal phase.

AvB3 appears on the luminal surface of the endometrium only during the receptive period, supporting the concept it is a potential biochemical marker of uterine receptivity

Lessey et al. 1992, 1994; Franchi et al. 2008

Integrin AvB3

Integrins

The AvB3 dimer binds to the tripeptide, arginine–glycine–aspartic acid (RGD) sequence found in:

- vitronectin
- Fibronectin
- Fibrinogen
- von Willebrand's factor
- Osteopontin (OPN)

Felding-Habermann and Cheresh 1993

Integrins in tubal epithelium

Integrins a1, a4, av, β3

	α1		α4		αv		β3	
Epithelium	nrp	rp	nrp	rp	nrp	rp	nrp	rp
Endometrial								
luminal	–	–	–	–	++	++	–	++
glandular	±	++	±	++	++	++	–	++
Tubal	–	–	–	–	++	++	±	++

nrp = non-receptive period; rp = receptive period.

The expression of the β3 subunit is under the same control as the endometrium and possibly the tubal epithelium has an implantation window

Sulz L et al, 1998

Integrins

Integrins in tubal epithelium

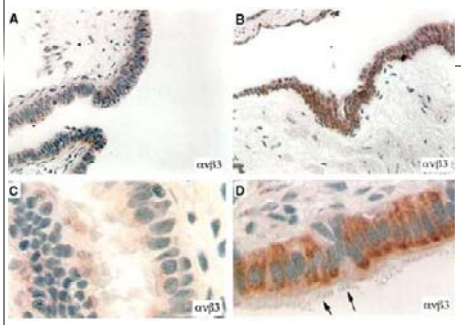
Integrin $\alpha_v\beta_3$

- Human tubal epithelium displays a cycle-dependent expression pattern of avb3 integrin.
- It is clearly upregulated during the luteal phase when strong immunostaining is observed.
- The expression of the molecule is limited to the ciliated epithelial cells.
- The intensity of immunostaining does not vary in the different segments of tubal epithelium (isthmic, ampulla and fimbriae).

Makrigiannakis et al, 2009

Integrins

IHC. Tubal $\alpha_v\beta_3$ in proliferative (a,c) and luteal phase (b, d)



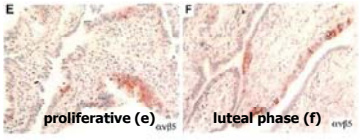
Makrigiannakis et al, 2009

Integrins

Integrins in tubal epithelium

Integrin $\alpha_v\beta_5$

- A cycle-independent expression pattern characterizes AvB5 integrin expression in human oviduct epithelium.
- The expression of AvB5 is also limited to the ciliated epithelial.
- The intensity of immunostaining does not vary between the different segments of tubal epithelium (isthmic, ampulla and fimbriae).



Makrigiannakis et al, 2009

Integrins

Integrins in tubal pregnancy

Integrins α3, αv, β1, α2β1

- In tubal decidualized epithelium, staining intensity of α3 and β1 integrins was strong in decidual cells, supporting tissue and placental villi compared to normal tubal epithelium
- No difference in αV and α2β1 integrin expression

Inan et al. 2004

Integrins α1, α5, α5β1

- Are expressed in trophoblast cells in tubal pregnancy

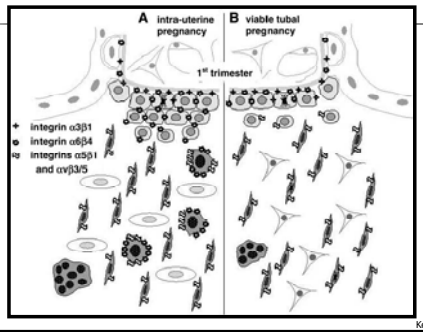
Qin et al. 2003

Integrin αvβ3

- Expressed in extra cellular matrix in viable tubal pregnancy

Kemp B, 2002

Integrins: Comparison of ECM patterns



Kemp B, 2002

Fibronectin

Fibronectin

- Fibronectin binds to integrins α5β1 and α3β1

Hynes 1992

- Human preimplantation blastocysts express fibronectin receptors

Campbell et al. 1995; Turpeenniemi-Hujanen et al. 1995

- Soluble fibronectin promotes the expression of its receptors in preimplantation blastocysts

Schultz and Armant, 1995

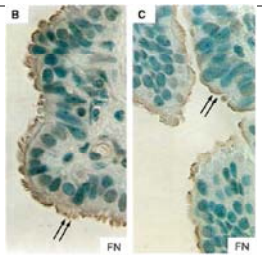
Fibronectin

Localization of fibronectin in the fallopian tube

•Localized to the luminal surface of ciliated cells with particularly intense staining on the apex of the cilia.

• There are no differences throughout the cycle.

• This distinct pattern is seen along the entire fallopian tube (isthmic, ampulla and fimbriae).



Makrigiannakis et al, 2009

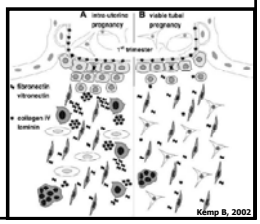
Fibronectin in tubal pregnancy

- Fibronectin IHC staining was strong in decidualized tubal epithelium in tubal pregnancy

Inan S et al, 2004

- Fibronectin is also detected in the extracellular matrix

Kemp B et al, 2002



Kemp B, 2002

Fibronectin

Fibronectin

- Fibronectin in the fallopian tube has long been advocated to promote the maturation of the transiting blastocyst

Hunter 1994

- Soluble Fibronectin within the oviduct may contribute to the maturation of adhesive and degradative properties of the embryo.

Makrigiannakis et al, 2009

Osteopontin

Osteopontin

- Osteopontin binds to the α_v integrins, some of which have been identified on preimplantation blastocysts and trophoblast
  Hu et al. 1995, Campbell et al. 1995, Damsky et al. 1993
- OPN may not only participate in adhesive events, but may also induce integrin mediated functional changes in the cell
  Denhardt and Guo 1993

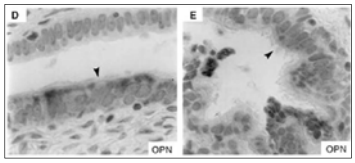
Osteopontin

Localization of Osteopontin in the fallopian tube

•Osteopontin is found to be expressed by ciliated cells, with no immunostaining seen in the dome-shaped secretory cells

•Immunolocalized to the apical cytoplasm but not on the luminal surface of the cells.

•There is no difference in OPN expression noted throughout the cycle or between the various segments of the fallopian tube (isthmic, ampulla and fimbriae).



Makrigiannakis et al, 2009

Osteopontin

Osteopontin in the fallopian tube

- OPN may also participate in the molecular mechanisms leading to the maturation of the blastocyst within the oviduct
- No study has shown OPN expression in tubal pregnancy

Glycodelin A

Glycodelin A

- Glycodelin is a member of the lipocalin family of proteins, is synthesized in the endometrium in response to progesterone and relaxin
- It has immunosuppressive properties

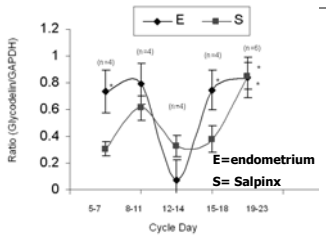
Halttunen M et al, 2000 - Alok A. & Karande AA, 2009.

- Glycodelin expression follows a cyclic pattern in the endometrium and maximizes during the "implantation window"
- It is suggested to assist in trophoblast harboring on the endometrial surface

Seppala et al, 2000

Glycodelin A

Glycodelin A in the fallopian tube



Amir A et al, 2009

Glycodelin A

Glycodelin A in the fallopian tube

Question:

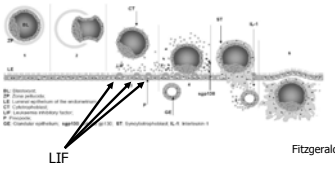
Can Glycodelin A participate in the harboring of the trophoblast in tubal pregnancies?

LIF

Leukemia Inhibitory Factor

LIF is assumed to facilitate implantation since LIF is present at high amounts in the maternal–fetal interface and because it is produced by both the human placenta and endometrium, where it is maximally expressed at the time of implantation

Bhatt et al, 1991
Cullinan et al, 1996
Kondera-Anasz et al,2004



Fitzgerald et al, 2008

LIF

LIF localization in the fallopian tube

- LIF seems to be increased in tubal epithelium in case of tubal pregnancy compared to normal pregnancy and non pregnant women.

Guney M et , 2007

Non-Pregnant *Intra-uterine Pregnancy* *Tubal Pregnancy*

- LIF is also found increased in the endometrium of women diagnosed with tubal pregnancy

McMinn MJ et al, 2004

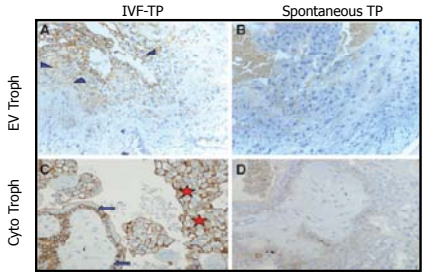
LIF

The role of LIF in tubal pregnancy

- It seems that LIF is regulated by trophoblast – endometrial and possibly tubal cross-talk.
- This regulation may be local (by contact) and remote.

E-Cadherin: Could it act as an IVF-specific marker of tubal pregnancy?

E-Cadherin



Revel A et al, 2008

Summary of evidence

Integrins expression / related to tubal implantation window

	α1	α3	α4	αv	β1	β3	αvβ3	avβ5	α1β2	α1β5
Tubal epithelium	-	*	-	+/-	*	+/+	+/+	+/-	*	-
Tubal Pregnancy	*	+	*	+	+	*	+	+	+	+

* : no data existing

Summary of evidence

Expression / related to tubal implantation window

	Fibronectin	Osteopontin	Glycodelin A	LIF	E-Cadherin
Tubal epithelium	+/-	+/-	+/+	-	*
Tubal Pregnancy	+	*	*	+	-
Tubal pregnancy post IVF	*	*	*	*	+

* : no data existing

Comments

The ***expression of pinopodes in human oviduct*** at the time of endometrial receptivity lightens a part of human tubal pregnancy physiology.

The expression of pinopodes in tubal epithelium synchronous to their corresponding endometrium underlies ***the hormonal dependence for their formation***

Pinopodes appearance is consistently associated with other receptivity changes, such as a loss of progesterone receptors and peak expression of αvβ3 integrin and OPN.

Integrins and fibronectin, which are needed in utero implantation, are expressed in tubal tissues during ectopic pregnancy and thus possibly involved in ectopic implantation

Not only β3 but also the intact dimer avb3 integrin is upregulated in the human oviduct during the luteal phase. ***Avβ5***, did not appear to be regulated in the same manner.

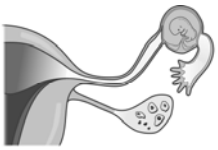
The fallopian tube epithelium secretes fibronectin and OPN

Leukemia inhibitory factor seems to play major role in TP pathophysiology

IVF triggers ***E-Cadherin*** expression in the tubal epithelium

However ***further studies are needed*** in order to clarify receptivity markers' expression during tubal pregnancy

Changes in the Fallopian tube microenvironment predisposing to tubal implantation



Andrew Horne

THE UNIVERSITY of EDINBURGH

MRC Centre for Reproductive Health

Learning objectives

- Understanding of potential mechanisms explaining tubal implantation and ectopic pregnancy
- Knowledge of the local changes in the normal tubal environment that encourage early implantation to occur
- Awareness of the data on the effects of risk factors for ectopic pregnancy on the Fallopian tube environment

Clinical problem

- Pregnancy implanted outside of the uterine cavity
- >98% implant in the Fallopian tube
- 1–2% of all pregnancies in Europe and the USA are ectopic
- In Western world most common cause of maternal mortality in the first trimester of pregnancy
- In developing world 1 in 10 women admitted with a diagnosis of ectopic pregnancy ultimately die from the condition
- Socioeconomic implications

Farquhar, Lancet 2005; Walker, Clin Obstet Gynecol 2007; Varma & Gupta, Clin Evid (Online)2009; Wedderburn et al, Hum Rep 2010

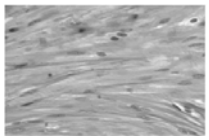
Hypothesis

Impaired tubal transport causing arrest of the embryo in the Fallopian tube

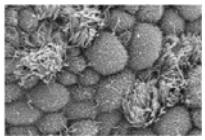
A change in the normal tubal environment encouraging early implantation to occur

Embryo-Tubal Transport

Smooth Muscle Contractility



Ciliary Beat Frequency

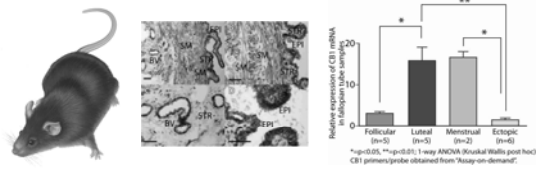


Endocannabinoid system (ECS)

- Endocannabinoids bind to G-protein-coupled cannabinoid receptors CB1 and CB2
- In animal studies, the ECS plays a pivotal role in reproduction
- Endocannabinoid signalling pathways are involved in fertilization, implantation, embryo development and maintenance of early pregnancy

Karasu et al. Hum Rep Update 2011

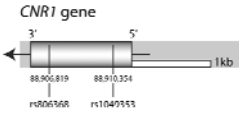
Endocannabinoid system (ECS)

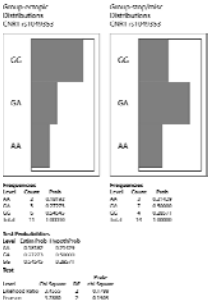


- CB1-knockout mice show retention of the embryo in the oviduct
- CB1 is expressed in human Fallopian tube, progesterone-regulated and increased in ectopic pregnancy

Wang et al. Nat Med 2004; Horne et al. PLoS One 2008

Endocannabinoid system (ECS)

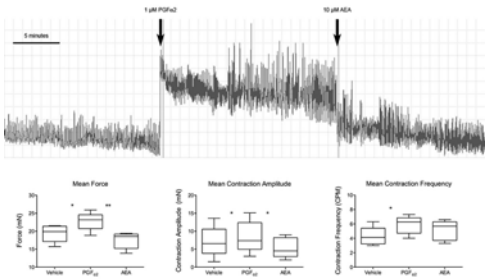




- There is a possible association between polymorphism genotypes of the CNR1 gene encoding CB1 and ectopic pregnancy

Wang et al. Nat Med 2004; Horne et al. PLoS One 2008

Endocannabinoid system (ECS)



- Endocannabinoids relax tubal smooth muscle contractility in-vitro

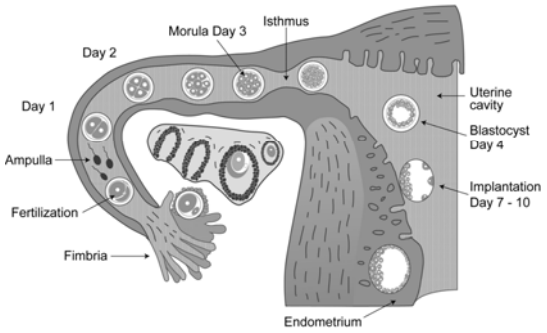
Brown et al. in preparation

Nitric oxide and nitric oxide synthase

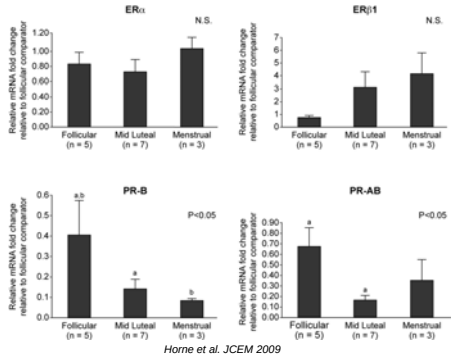
- Nitric oxide (NO) synthesized by iNOS increases ciliary beat frequency in epithelial cells of airway
- NO has a relaxing effect on tubal smooth muscle in-vitro
- Administration of iNOS inhibitors increases tubal smooth muscle contractility in rat
- iNOS greater in Fallopian tube of women with ectopic pregnancy compared with pseudo-pregnant women

Ekerhovd et al. Gynecol Endocrinol 1997; Perez et al. J Reprod Fertil 2000; Al-Azemi et al. Hum Reprod 2009

Tubal environment

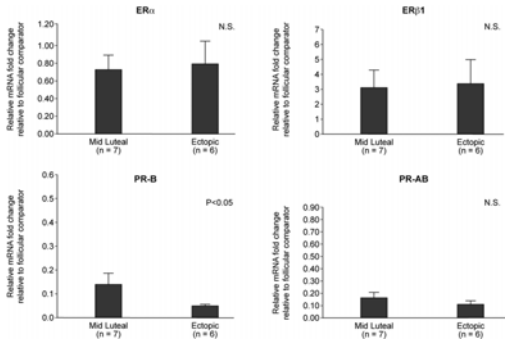


Sex steroid hormone receptors



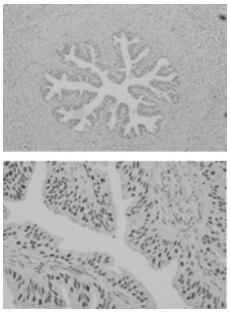
Horne et al. JCEM 2009

Sex steroid hormone receptors



Horne et al. JCEM 2009

Sex steroid hormone receptors



- ERα protein is expressed in the nuclei of the epithelium and stroma of the non-pregnant Fallopian tube at all stages of the menstrual cycle
- ERα protein is absent from Fallopian tube from women with ectopic pregnancy

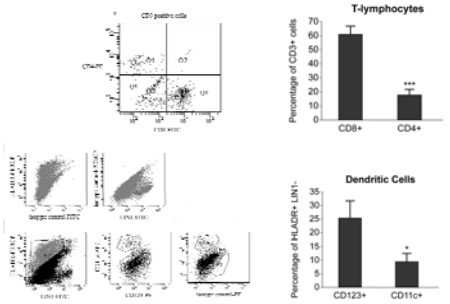
Horne et al. JCEM 2009

Immune cell populations

- Lymphoid and myeloid cell populations are well-documented in human endometrium and are known to play important roles in providing tolerance, controlling embryo invasion and mediating vascular remodeling
- Populations of leukocytes, including lymphocytes, macrophages, neutrophils, dendritic cells and uterine (u) NK cells have all been demonstrated in the endometrium

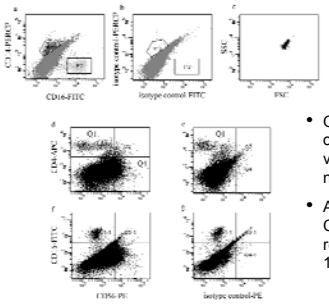
Critchley et al. 2001 Clin Endocrinol (Oxf); Laskarin et al. 2007 Am J Reprod Immunol; van Mourik et al. 2009 J Leukocyt Biol

Immune cell populations



Shaw et al, Am J Reprod Immunol 2011

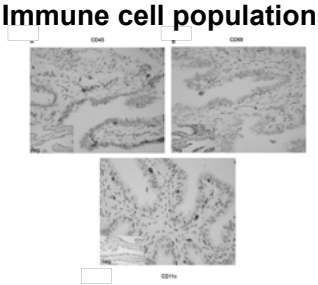
Immune cell populations



- Only a very small population of CD3-ve CD56^{bright} CD16-ve NK cells was found in the non-pregnant Fallopian tube
- A population of CD3-ve CD56^{dim} NK cells represented approximately 10% of all live cells

Shaw et al, Am J Reprod Immunol 2011

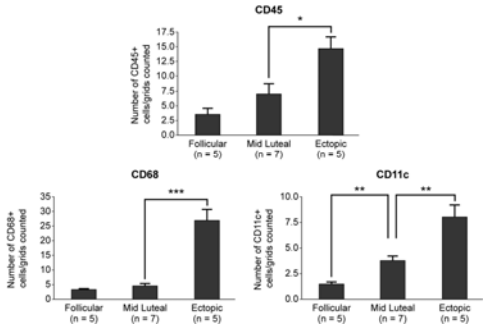
Immune cell populations



- CD45+ve leukocytes, CD68+ve macrophages and
- CD11c+ve dendritic cells localize to the epithelium of the non-pregnant human Fallopian tube

Shaw et al, Am J Reprod Immunol 2011

Immune cell populations



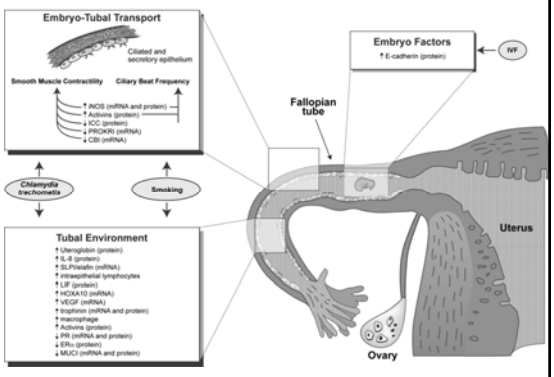
Shaw et al, Am J Reprod Immunol 2011

Immune cell populations

- Human Fallopian tube is populated by immune cells of myeloid and lymphoid lineages
- Dendritic cells and CD56dim CD16- NK cells are present in the non-pregnant Fallopian tube
- Immune cell populations are altered in the mid-luteal phase of the menstrual cycle
- Numbers of myeloid cells in Fallopian tube from women with ectopic pregnancy are different from the non-pregnant Fallopian tube

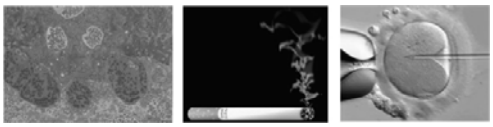
Shaw et al, Am J Reprod Immunol 2011

Gene	Ectopic pregnancy	Implantation site	Explanation	Refs
Uteroglobin	↑	↓	?regulated by embryo	*Quintar et al, 2008*
LIF	↑	↑	?chronic salpingitis	*Guney et al, 2008; Ji et al, 2009*
HOXA10	↑	↑	?regulated by embro	*Salih & Taylor, 2004*
VEGF	↑	↑	?regulated by hCG	*Lam et al, 2004*
MUC1	Glycosylation altered ↓	?	Increased receptivity	*Savaris et al, 2008; Al-Azemi et al, 2009*
Activins	↑	?	?due to chlamydia	*Refaat et al, 2008; Refaat et al, 2009*
Trophinin	↑	?	?regulated by hCG	*Nakayama et al, 2003*
Natural antimicrobials	SLPI and elafin ↑	?	?due to chlamydia	*Dalgetty et al, 2008*



Shaw et al. Hum Reprod Update 2010

Risk factors for ectopic pregnancy



Chlamydia trachomatis infection, smoking and IVF are most significant risk factors

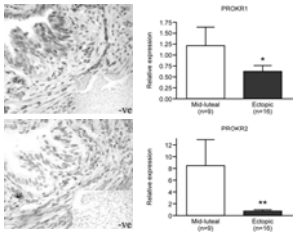
Farquhar Lancet 2005

Prokineticins

- Prokineticins are multifunctional secreted proteins originally identified as regulators of intestinal contraction but also shown to affect vascular function
- They are ligands for two G-protein coupled receptors, prokineticin receptor 1 (PROKR1) and PROKR2
- PROKs are also known for regulating genes that are important in implantation
- PROK1 has been shown to induce expression of LIF in human endometrium and LIF is known to play a crucial role in successful intrauterine implantation in mice

Maldonado-Pérez et al. Trends Endocrinol Metabol 2007

Prokineticins

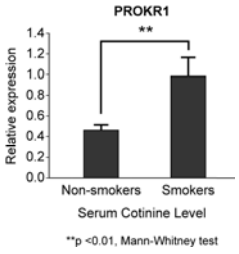


- PROKR1 and PROKR2 are expressed in the epithelium and smooth muscle of non-pregnant Fallopian tube
- They are down-regulated in Fallopian tube from women with ectopic pregnancy where implantation has occurred

Shaw et al. Fertil Steril 2010

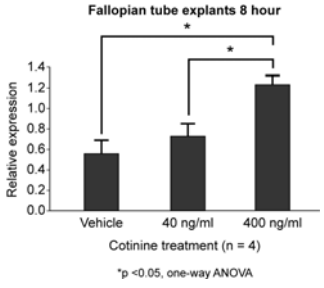
Prokineticins

Serum cotinine (ng/mL)	Self-reported smoking status
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
< 5	Non-smoker
7.4	Non-smoker
8.6	Non-smoker
11.3	Non-smoker
161	Smoker
> 500	Smoker
> 500	Smoker
> 500	Smoker
> 500	Smoker
> 500	Smoker



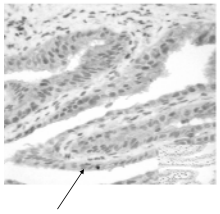
Shaw et al. Am J Path 2010

Prokineticins

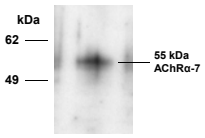


Shaw et al. Am J Path 2010

Nicotinic acetylcholine receptor α-7



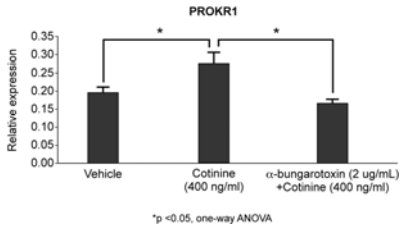
OE-E6/E7 cell lysates



nAChR⟨-7 expression in the Fallopian tube epithelium

Shaw et al. Am J Path 2010

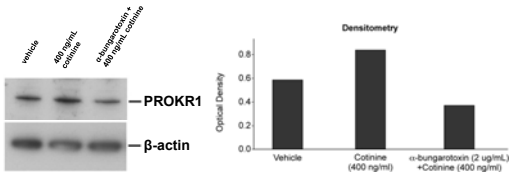
⟨-bungarotoxin (nAChRα-7 antagonist)



*p <0.05, one-way ANOVA

Shaw et al. Am J Path 2010

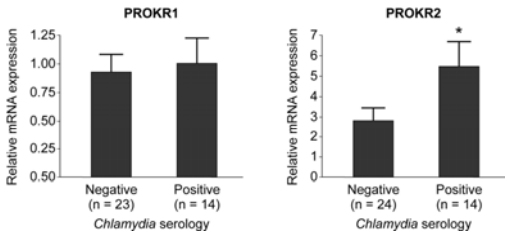
⟨-bungarotoxin (nAChRα-7 antagonist)



- Smoking targets the Fallopian tube via nAChRα-7 to increase PROKR1 expression resulting in a tubal microenvironment predisposing to ectopic pregnancy

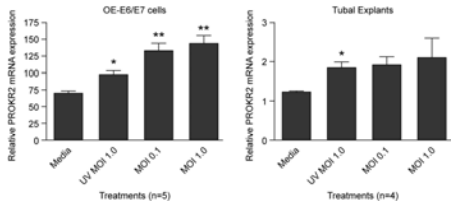
Shaw et al. Am J Path 2010

Prokineticins and *Chlamydia*



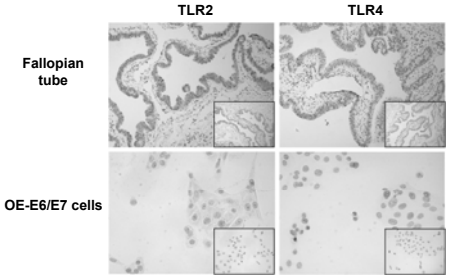
Shaw et al. Am J Path 2011

Prokineticins and *Chlamydia*



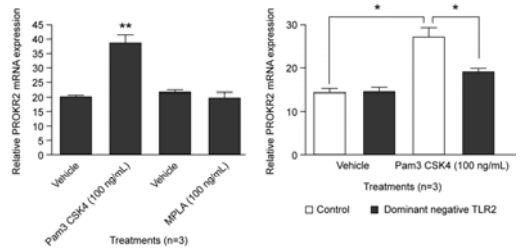
- PROKR2 mRNA expression levels were significantly increased in OE-E6/E7 cells treated with live *C. trachomatis* and UV-killed *C. trachomatis* after 8 hours of treatment

Shaw et al. Am J Path 2011



- Toll-like receptors are expressed in Fallopian tube epithelium and oviductal epithelial cells

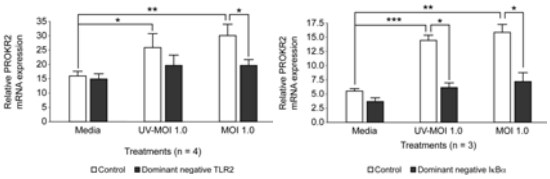
Shaw et al. Am J Path 2011



- PROKR2 mRNA expression in OE-E6/E7 cells can be induced by TLR2 activation

Shaw et al. Am J Path 2011

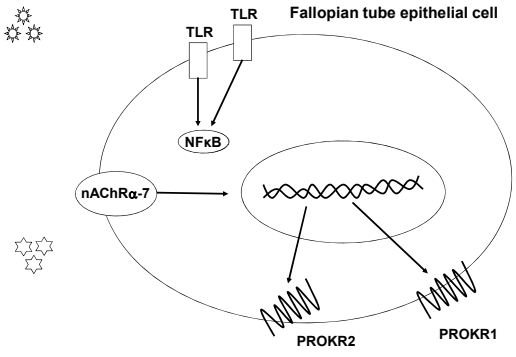
Prokineticins and *Chlamydia*



- *C. trachomatis* infection increases Fallopian tube PROKR2 via TLR2 and NFκB activation resulting in a microenvironment predisposed to ectopic pregnancy

Shaw et al. Am J Path 2011

Summary



Conclusions

- Ectopic pregnancy is caused by Fallopian tube dysfunction causing embryo arrest and changes in tubal environment
- Human studies of ectopic pregnancy comparing biopsies of Fallopian tube and implantation sites collected from women with ectopic pregnancy with Fallopian tubes collected from non-pregnant women and intrauterine implantation sites are providing exciting data that will help us understand the tubal microenvironment
- The precise sequence and details of the molecular interactions involved in tubal implantation are not fully defined
- Require more studies on functional consequences of smoking and infection on Fallopian tube pathophysiology

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Perturbations in endometrial gene and protein expression in women with endometriosis: potential effects on embryo implantation

Amelie Fassbender, K.U.Leuven

ESHRE 27th Annual meeting- endometriosis/endometrium, The impact of the reproductive tract environment on implantation success

3rd July 2011

Stockholm, Sweden.

No potential conflicts of interest to disclose

Learning Objectives

- Genes and proteins involved in endometriosis
- Potential effects on embryo implantation

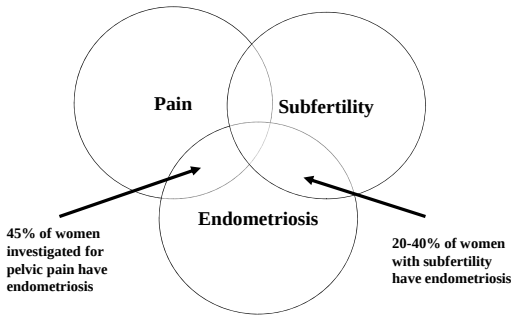
Outline

- Background
- Literature Review:
  - –Microarray
  - –Proteomics
- Conclusion
- Future direction

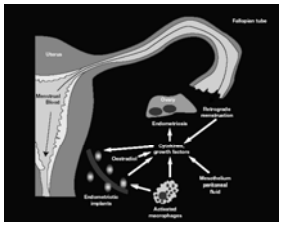
Background

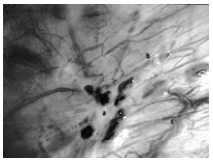
- Presence of endometrial-like tissue outside the uterine cavity
- Associated with pelvic pain and infertility
- Most common theory is “retrograde menstruation” (Sampson Hypothesis -1927)

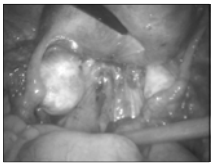
Endometriosis symptoms



What is endometriosis?







Origins of ectopic endometrium?
Retrograde menstruation
De novo origin (metaplasia)
Embryonic rests

The diagnosis of Endometriosis

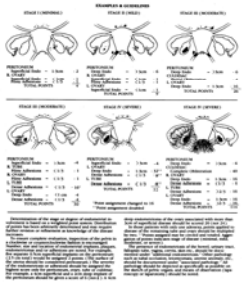
- Symptoms: pain and infertility
- Gold standard: laparoscopic inspection with histological confirmation
- Average diagnostic delay of 8/11 years (Arruda et al., 2003, Hadfield et al., 1996, Husby et al., 2003)

Prevalence of Endometriosis

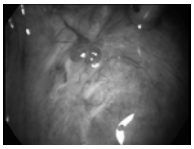
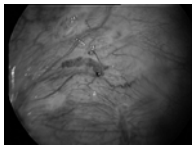
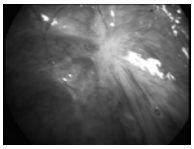
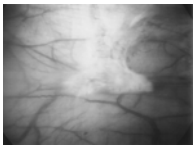
- More than 70 million women worldwide
- 10% women of reproductive age
- 30% and 60% in women with infertility and pelvic pain respectively
- Endo cost considerably higher than cost related to Crohn's disease or to migraine in the USA for 2002 (Simoens et al., 2007)

Endometriosis

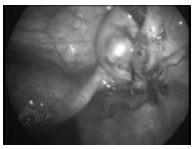
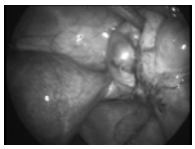
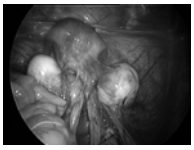
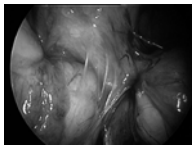
- rAFS-classification (revised American Fertility Society):
  scoresysteem
  - rAFS I: minimal
  - rAFS II: mild
  - rAFS III: moderate
  - rAFS IV: severe



What does mild endometriosis look like?

What does severe endometriosis look like?

Endometriosis and implantation failure

Endometriosis is more frequently diagnosed in patients with infertility than in a normal population

Tomasetti et al., 2006

Implantation failure

- Possible causes include
  - Embryonic aneuploidy
  - Abnormalities of the uterine cavity
  - Altered endometrial receptivity
  - Suboptimal embryo transfer techniques

Urman et al., 2005a &b

embryonic implantation and reproduction depends on the interaction of the embryo with the receptive endometrium

Borthwick et al., 2003

In preparation for embryo implantation

- responds to ovarian sex steroids by:
  - morphological,
  - biochemical
  - molecular changes

Sherwin et al., 2006

Pregnancy is dependent

- endometrium acquiring a receptive phenotype that facilitates:
  - apposition,
  - adhesion
  - invasion of a developmentally competent embryo

Brosens et al., 2010

Eutopic endometrium from fertile women

Micorarray

Reference	Sample number	Cycle phase	Microarray platform	Results
Rossi et al., 2005	Healthy volunteers (n=5) Stromal cells from eutopic endometrium treated with IL-1 beta	mid versus late (18–24 day) luteal phase	RNA Labchip Agilent	↑Through IL1beta 1. IL-8, 2. colony-stimulating factor 2 3. aldo-keto reductase family 1 member 1
Carson et al., 2002	Healthy volunteers ; eutopic endometrium (n=6)	Early versus Mid luteal phase	Human Genome U95A Array Affymetrix	693 >2 fold >4 fold change ↑Claudin 4 ↑Ostoepontin (OPN) ↓Betaig-H3

Reference	Sample number	Cycle phase	Microarray platform	Results
Riesewijk et al., 2003	Fertile women; eutopic endometrium (n=5)	Analysed paired samples Day 2 (n=5) and day 7 (n=5)	Affymetrix HG-U95A	Genes differentialy expressed: ↑153 ↓58 • glutathione peroxidase 3 • claudin 4 • solute carrier family 1 member 1
Kao et al., 2002	Normal cycling women; eutopic endometrium (28)	Follicular phase (10) Midluteal phase (n=18)	Affymetrix Genechip Hu95A	↑156, ↓377 **↑apolipoprotein (Apo)E (100 fold)** **↓intestinal trefoil factor (ITF)**

Reference	Sample number	Cycle phase	Microarray platform	Results
Borthwick et al., 2003	(no endometrisois) women; eutopic endometrium (n=10) Pooled samples • Sterilization, unexplained infertility, dyspareunia, fertile	Day 9 versus day 11	Affymetrix HG_U95 chip	100 genes as being differentially Expressed • remodelling factors TFF3 (52-fold greater level in follicular compared to luteal phase) • Glutathione peroxidase 3 (GPX3) highly expressed in luteal • metallothionein isoforms ↑ in luteal (MT-1G, MT-1E, MT-1H

Borthwick et al., 2003

- glutathione peroxidase and the metallothioneins are ↑ by progesterone in human endometrium to protect the implanting embryo from harmful reactive oxygen species and heavy metal ion toxicity

Carson et al., 2002 and Kao et al., 2002

- Members of Wnt family of signalling molecules
  - role in epithelial/stromal interactions during the receptive phase
  - in mice uterine Wnt signalling essential for implantation

Sherwin et al., 2006

Eutopic endometrium from patients with endometriosis

Micorarray

Reference	Sample number	Cycle phase	Microarray platform	Results
New data Fassbender et al., 2011	n=49 minimal-mild (n=16) moderate-severe (n=15) control (n=18)	Early luteal phase (n=27) and menstrual phase(n=22)	Affymetrix	No genes differentially expressed in women with endometriosis compared to controls
Sherwin et al. 2008	n=16 eutopic EM minimal-mild (n=5) moderate-severe (n=5) controls (n=6)	Late luteal phase	Agilent	8 genes upregulated >1.75 fold ($p<0.001$) and 1 gene down-regulated

Reference	Sample number	Cycle phase	Microarray platform	Results
Burney et al. 2007	n=37 moderate-severe (n=21) controls (n=16)	Follicular (n=6) Early Luteal (n=6) Mid luteal (n=9)	Affymetrix	87 transcripts were altered more than 4fold such as **FOXO1A**, MIG6, CYP26A1
Matsuzaki et al., 2005	n= 24 minimal-severe (n-12) controls (n=12)	Late follicular (n=6) Early, mid, late luteal (n=18)	Clontech Atlashuman Array 1.2 cDNA expression array	No gene was differentially expressed in a constant manner in eutopic endometrium

Reference	Sample number	Cycle phase	Microarray platform	Results
Absenger et al., 2004	Endometriosis (n=43) controlss=48	Follicular or luteal phase	Affymetrix	95>1.5 fold ↓64 ↑ 31 ↑Cyr61 in the luteal phase
Kao et al. 2003	n=20 mild-moderate (n=8) control (n=12)	Mid luteal phase (n=20)	Affymetrix	↑91 and ↓115 more than 2 fold

Kao et al., 2003

- Novel candidates genes:
- GlcNAc6ST, olfactomedin, C4BP, IL-15, Dickkopf-1, purine nucleoside phosphoyrlase, neuronal pentracxin II, glycodelin, S100E and BSEP

Kao et al., 2003

- GlcNAc6ST, olfactomedin may hinder the tethering and attachment mechanisms of human embryo implantation, resulting in implantation failure.

Kao et al., 2003

- The other gene candidates may promote an inhospitable endometrial milieu for embryonic implantation, due to embryo toxicity, immune dysfunction, inflammatory or apoptotic responses.

Kao et al., 2003

- S100E
  - Member of calcium-binding family
  - ↓ in eutopic endomtrium of endometriosis
  - Mice: a null mutation of S100A8 are infertile due to an implantation failure

Sherwin et al., 2006

Burney et al., 2007

- FOXOA1
  - ↓ endometriosis
  - progesterone-regulated transcription factor
  - cell cycle control
  - role in the incomplete transitioning of the endometrium from the proliferative-to early secretory phase.

Proteomics

The study of the protein library

Proteomic?

- Have a better understanding of the function of gene products in disease process
- Allows for the novel design of new therapies
- Has the potential for biomarker discovery as well as addressing the pathogenesis of endometriosis

Proteomic tools used in endometriosis

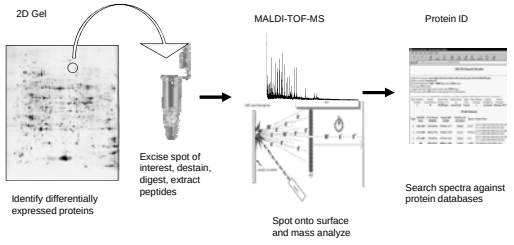
– 2DE → MALDI -TOF - MS

– SELDI-TO-MS

Protein profiling in women with endometriosis when compared with controls showed differentially expressed proteins/peptides [Chehna-Patel et al., 2010; Fowler *et al.*, 2007; Stephens et al., 2010; ten Have et al., 2007; Zhang *et al.*, 2006]

SELDI-TOF-MS profiling coupled to a learning algorithm has shown to offer diagnostic value in endometriosis [Ding et al., 2010, Fassbender et al., 2010; Kyama 2006 and 2011;Wang *et al.*, 2010]

Proteomics platform used in endometriosis



2DE/MADLI TOF MS results on endometrium

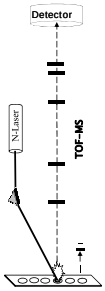
Reference	Sample Size	Technique	Results
Chehna-Petal et al., 2010	N=20 Paired endometriosis ectopic &eutopic endometrium (n=11) Controls (n=9)	2DE,western blotting, MALDI-TOF MS, immunohistochemistry	53 spots present in ectopic not in eutopic endometrium Validated proteins: 1. haptoglobin, 2. Rho-GDIα, 3. SM-22α, 4. Rab37
Stephens et al., 2010	N=8 eutopic endometrium Endometriosis (n=4) Controls (n=4)	2DE, western blotting, Immunohistochemistry , MALDI-TOF MS	20 differentially expressed proteins Validated proteins 1. Vimentin, 2. RNH1 **3. PRDX6** ↑2DE↓western blotting

Reference	Sample Size	Technique	Results kDa
Fowler et al., 2007	N=35 pooled eutopic endometrium samples Endometriosis (n=18) Controls (n=17)	2D PAGE, MALDI-TOF MS	**1. Apolipoprotein A1** 2. peroxiredoxin 2 3. heat shock protein 90 4. annexin A2 5. Proteins associated with DNA metabolism and catabolism
Ten Have et al., 2007	N=18 eutopic endometrium Endometriosis (n=6) Controls(n=12)	2D PAGE, MALDI-TOF MS	21 proteins only present in disease samples Apoptosis, immune reaction, glycolytic pathway, cell structure , transcription factor
Zhang et al., 2006	N=12 serum& eutopic endometrium Endometriosis(n=6) Controls(n=6)	2DE,western blotting, MALDI-TOF MS	13 differentially expressed proteins IDENTIFIED proteins (serum): 1. vimentin 2. beta-actin 3. ATP synthase beta subunit

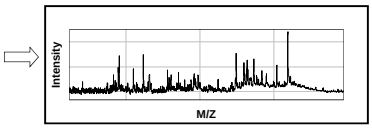
SELDI –TOF MS

- Surface Enhanced Laser Desorption Ionisation-Time of Flight Mass Spectrometry
  - Analysis of protein mixtures
  - In tissue samples, blood, urine, or other clinical samples
  - Comparison of protein levels between patients with and without the disease

Time-Of-Flight Mass Spectrometry



- Retained proteins are "eluted" from the ProteinChip Array by Laser Desorption/Ionization
- Ionized proteins are detected and their mass accurately determined by Time-of-Flight Mass Spectrometry



A mass spectrum

Literature eutopic endometrium

Reference	Sample Size	Surface	Results kDa	Sensitivity	Specificity
Ding et al., 2010 Mitochondrial protein expression	N=53 Stage I-II (n=19) Stage III-IV (n=5) Controls (n=29)	Cm10	1. 15.334 2. 15.128 3. 16.069	87.5%	86.2%
Wang et al., 2010	N=26 Stage I-II (n=8) Stage III-IV (n=5) Controls (n=13)	H4	1. 6898 2. 5891 3. 5385 4. 6448 5. 5425	91.7%	90%

Reference	Sample Size	Surface	Results	Sensitivity	Specificity
Fassbender et al., 2010	N=16 eutopic EM Stage I-II (n=5) Stage III-IV (n=5) Controls (n=6)	CM10 IMAC 30	32 peaks differentially expressed proteins in EM endo versus controls	-	-
Kyama et al., 2010	N=29 eutopic EM Stage I-II (n=9) Stage III-IV (n=10) Controls (n=10)	Q10 IMAC 30	•**T-Plastin** 90.675 •**Annexin 5** 39.956	100%	100%
Kyama et al., 2006	N=9 Stage II (n=3) Paired eutopic EM & peritoneum& peritoneal endometriotic lesions Controls (n=3) Eutopic EM	CM10 H50 IMAC 30 Q10	**Transgelin** 22-23kDa	-	-

SELDI-TOF MS in endometriosis Leuven group results :

Identified proteins:

- Transgelin
- Annexin 5, T-plastin

(100% specificity and 100% sensitivity)

Kyama et al., 2006 & 2010

Potential role in endometriosis

Transgelin endometriotic lesions

- 22-23kDa protein - a smooth muscle-actin binding protein
- Unknown in the development of endometriotic lesions
- Smooth muscle actin cells are present around the endometrioitic lesions but absent in unaffected peritoneal site and eutopic endometrium (Anaf et al., 2000)

Potential role in endometriosis

Annexin 5
(Secretory phase endometrium)

- In cancer may play a role in proliferation and/or cell mobility and have metastatic potential
- In endometriosis may play a role in the early invasion of endometrial cells into the mesothelium after initial attachment to the peritoneal wall

Potential role in endometriosis

T-Plastin
(Secretory phase endometrium)

- Plays a role in cellular motility, formation of actin bundles that are required for cell locomotion and maintenance of the cellular architecture

Proteomic analysis of endometrium of fertile and infertile patients

Reference	Sample Size	Surface	Cycle phase
Brosens et al., 2010	Fertile women (n=15) Infertile women (n=10)	Cm10 Q10	Luteal and follicular phase

apoA-1

- Speculate that apoA-1 may also serve
  - to restrict embryo implantation
  - abberant endometrial endometrial secretion contributes to implantation failure

Brosens et al., 2010

INTERESTING FACTS

α_3ß integrin

- α_3ß integrin chain is co-expressed with other specific integrin only during the implantation window and is a biomarker for endometrial receptivity
- α_3ß integrin expression is reduced in women with endometriosis

Lessey et al., 1992, 2002

HoxA-10 and HoxA-11

- are ↑ in the window of implantation of the human endometrium
- But not during this period in women with endometriosis

Taylor et al., 1999

Autoantibodies

- role in IVF success rates in endometirosis patients
- autoantibody +ve patients
  - interfere with implantation cause no difference in:
    - oocytes retrieved
    - fertilitzation rates
    - numbers of embryos transferred

Dmowski et al., 1995

Conclusion

- Proteomics helped in studying endometriosis and implantation failure
- apoA1 is suggested to have a role in embryo implantation

Conclusion

- to identify which of the genes perform an essential role in implantation and which do not.
- the molecular mechanisms by which endometrium becomes receptive remain unclear

Proteomics and endometriosis: future research needs

- Better understanding of relationship between proteomic results and mRNA/miRNA microarray in same samples
- Explore effects of menstrual cycle on proteomic results
- Sufficient N samples corrected for cycle phase

Future direction

- Identification of the protein findings
- Validation of the findings
- Adequate statistics used
- Collaboration for larger studies

Future studies

Assay improvement	Sample Population	Protein/peptide Identification
• Intra- and Interassay variability • Use and validation of depletion methods • Need for standardization of technique	• Large sample size • Control for cycle phase • Need for training and test set (validation in mono- and multicenter context) • Advanced bio-informatics	• MALDI-TOF/TOF MS • Confirmation tests using ELISA, IH,Western Blots,..

Future studies

Association: endometriosis & reproductive outcome

- prospective studies
- accurate laparoscopic description of the stage of endometriosis
- Date, number of procedures, and interval between surgical procedures

Future studies

Association: endometriosis & reproductive outcome

- Effectiveness of interim suppressive therapy
- Clear definition of implantation rate, pregnancy rate, abortion rate, and live-birth rate per started cycle, per oocyte aspiration, and per embryo transfer

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Questions?

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27. Urman B, Yakin K, Balaban B 2005a **Recurrent implantation failurein assisted reproduction: how to counsel and manage. A. Generalconsiderations and treatment options that may benefit the couple.** *Reproductive BioMedicine Online* **11**, 371–381.
28. Wang L, Zheng W, Ding XY, Yu JK, Jiang WZ, Zhang SZ: **Identification biomarkers of eutopic endometrium in endometriosis using artificial neural networks and protein fingerprinting**. *Fertil Steril* 2010, **93**(7):2460-2462.
29. Zhang H, Niu Y, Feng J, Guo H, Ye X, Cui H: **Use of proteomic analysis of endometriosis to identify different protein expression in patients with endometriosis versus normal controls**. *Fertil Steril* 2006, **86**(2):274-282.

Can pelvic infection explain implantation failure?

Siladitya Bhattacharya
University of Aberdeen

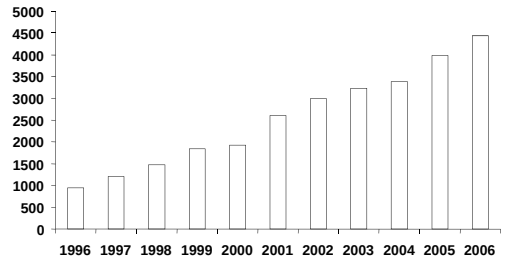
Outline of talk

- Epidemiological approach
- Previous pelvic infection
- Tubal disease, hydrosalpinx
- Bacterial vaginosis
- Chronic endometritis
- Non-infectious inflammation
- Tuberculosis
- Summary

Pelvic infection & implantation

- Impact of pelvic infection on conception
- Impact on outcome of IVF treatment
- Sequalae of pelvic infection and implantation
- Infection versus inflammation

New cases of Chlamydia in women in Scottish GUM clinics



http://www.isdscotland.org/isd/4907.html

Risk of infertility after Chlamydia

- **Systematic review**
- **Only one study in 496 adolescent women**
- **Follow up 1.5 to 4 yrs**

Chlamydia infection	Livebirth
Never (n = 319)	50%
Single episode (n = 109)	61.9%
Multiple episodes (n = 68)	52.6%

Wallace et al, Sex transm inf 2008 ; 84171-175

Tubal infertility: odds of livebirth

Cause of infertility	Unknown	1	1
	Tubal only	0.94 (0.90–0.97)	0.87 (0.83–0.90)
	Anovulatory only	0.93 (0.88–0.98)	0.95 (0.90–1.00)
	Endometriosis only	1.05 (0.98–1.13)	0.96 (0.89–1.03)
	Cervical only	0.41 (0.20–0.85)	0.39 (0.19–0.82)
	Male only	1.16 (1.13–1.20)	0.91 (0.87–0.95)
	Combination known causes	1.01 (0.96–1.06)	0.88 (0.83–0.92)

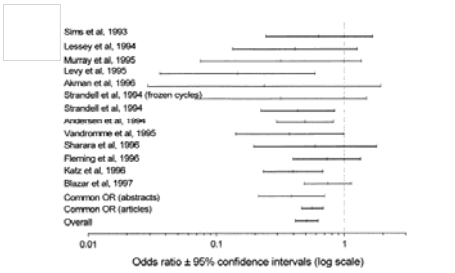
Nelson & Lawler, 2010

Tubal disease and hydrosalpinges

- **10% -13% by ultrasound**
- **30% by HSG**
- **Lower implantation pregnancy and livbirth**
- **Bathing of endometrium by inflammatory fluid *(Kodaman et al, 2004)***
- ***Decrease of specific factors ($a_v\beta_3$), LIF***

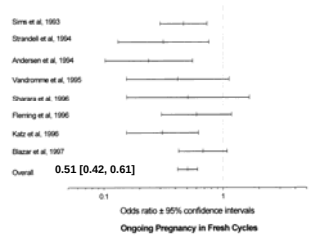
Anderson et al, 1994, Murray et al, 1997; Camus et al, 1999

Hydrosalpinx: pregnancy per cycle



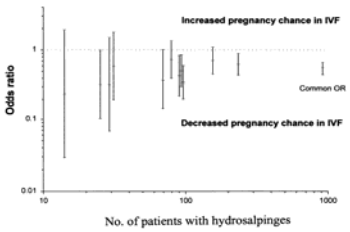
Zeyneloglu et al, 1998

Hydrosalpinx: ongoing pregnancy



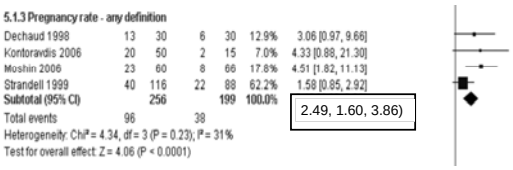
Zeyneloglu et al, 1998

Hydrosalpinx: pregnancy per ET



Zeyneloglu et al, 1998

Pregnancy rate after any surgery for hydrosalpinges



Johnson et al, Cochrane Library 2010

Chronic endometritis and IVF

- **Identified in 10/33 (30%) women with recurrent implantation failure**
- **Biopsy positive = 10**
- **Defined as plasma cells in endometrial stroma**
- **Confirmed by monoclonal plasma cell specific antibody CD 138**
- **Biopsy negative = 23**

Johnston-MacAnnany et al, 2009

Chronic endometritis and IVF

	Chronic Endometritis N= 10	Biopsy negative N = 23
Mean age (yrs)	34.5	34.7
Fertilisation rate	72%	63%
Good quality ET	2.1	1.7%
Clinical pregnancy rate	2/10 (20%)	12/23 (52%)

Johnston-MacAnnany et al, 2009

Bacterial vaginosis and IVF

- Cohort study
- 867 consecutive women
- 96 women excluded (No ET or slides broken)
- Gram stain
- Slides read by 2 observers
- Modified Spiegel's criteria (Hay et al, 1994)
- 190/771 (25%) women had BV

Ralf et al, 1999

Bacterial vaginosis: IVF outcome

	BV N = 190	No BV N = 581	Relative Risk 95% CI
Pregnancy	32%	30%	1.08 (0.85, 1.09)
Miscarriage	32%	19%	2.03 (1.09, 3.78)*

* Adjusted for: age, smoking, previous miscarriages, PCS

Ralf et al, 1999

Tuberculosis

- Prevalence variable
- 0.69% in Australia and 19% in India
- Infertility in 40% - 50%
- Tubal involvement
- Diagnosis by HSG. laparoscopy
- Endometrial involvement in 50% - 60%
- Endometrial biopsy for certain diagnosis
- Anti-tubercular chemotherapy

Gurgan & Demirol, 2004

TB and the endometrium

- Outcome data from case series
- Pregnancy rate 16% per cycle in only tubal involvement
- Poor prognosis in endometrial TB (10% pregnancy)
- Anti TB drugs
- Hysteroscopic adhesiolysis

Gurgan & Demirol, 2004

Endometrial TB: IVF outcome

Characteristic	Genital TB	Other tubal factor	*p* value
No. of patients	24	274	–
No. of cycles	44	366	–
No. of transfers	34	311	–
Mean age (years)	33.7	33.2	NS
Duration of stimulation (days)	10.3	7.5	<0.01
Ampules of HMG	27.3	18.4	<0.05
E_2 on the day of HCG (pg/ml)	852	1141	<0.05
No. of oocytes	5.8	7.7	<0.05
No. of embryos	2	2.9	<0.05
Clinical pregnancies (%)	4 (9.1)	78 (21.3)	<0.05
Implantation rate per embryo(%)	5.8	8.6	NS

Gurgan et al.

Gurgan & Demirol, 2004

Endometriosis

- **Poor folliculogenesis, oocyte quality, fertilisation, implantation**
- **Opinion divided about implantation rates**
- **Donor oocytes implant as well as in other recipients**
- **Reduced implantation in embryos from donors with endometriosis**
- **Implantation markers abnormal in endometriosis**

Cakmak & Taylor, 2011

IVF outcomes in Endometriosis Baseline characteristics

	Endometriosis $n=415$	Non-endometriosis $n=6871$	χ^2 test P value	Mann–Whitney test P value
Age	34 (21–46)	34 (19–47)		NS
FSH	6.4 (1.5–13.8)	6.4 (1.5–13.8)		NS
BMI	23.7 (21.9–6.1)	24.1 (21.7–27.2)		NS
Ethnicity				
White	319 (77%)	5359 (78%)		
Asian	75 (18%)	1099 (16%)		NS
Black	4 (1%)	68 (1%)		
Other	17 (4%)	345 (5%)		
Embryo:				
Single	147 (35.4%)	2441 (35.5%)		
Two	258 (62.2%)	4284 (62.3%)	NS	
Three	10 (2.4%)	146 (2.1%)		

Mohamed et al, 2010

IVF outcomes in Endometriosis

		Endometriosis	Non-endometriosis	χ^2 test P value
Frozen * $n=3523$		$n=148$	$n=3375$	
	LBR	25 (16.9%)	403 (11.9%)	0.07
	CPR	27 (18.2%)	428 (12.7%)	0.04
Fresh $n=3763$		$n=267$	$n=3496$	
	LBR	52 (19.5%)	675 (19.3%)	NS
	CPR	54 (20.2%)	730 (20.9%)	NS

*** Down regulated cycles**

Mohamed et al, 2010

Colorectal endometriosis: IVF outcome

	Colorectal N = 29	Tubal N = 157	Male N = 340
Mean age	32.4	34.2	33.7
BMI	23.4	24.3	23.4
Pregnancy	41%	26%	34%
Live birth	27%	23%	27%

D'Argennt et al, 2010

Pelvic Infection & Implantation: Summary

- Impact of past pelvic infection
- Hydrosalpinges affect outcome of IVF
- Role of tubal excision/occlusion before IVF
- IVF: Chronic endometritis may affect implantation
- IVF: Bacterial vaginosis increases risk of miscarriage
- Endometrial TB may compromise pregnancy rates
- Endometriosis: opinion divided

Obesity and the endometrium: effects on implantation

José Bellver-Pradas, MD, PhD

Instituto Valenciano de Infertilidad (I.V.I.). Valencia. Associate Professor. Department of Pediatrics, Obstetrics and Gynecology. Faculty of Medicine. University of Valencia.

Jose.Bellver@ivi.es

* No commercial and/or financial relationships with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices

Learning objectives

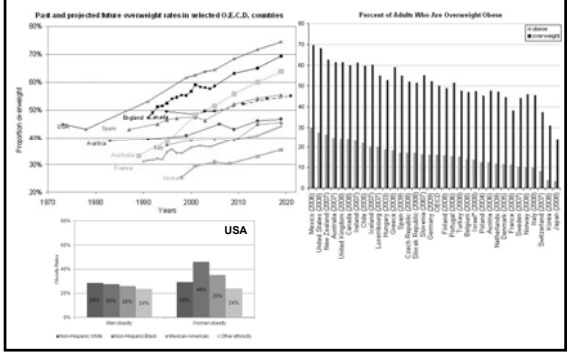
* Brief introduction
- Epidemiology of obesity
- Pathophysiology of obesity

* Reproductive performance in obese women
- Infertility
- Poor assisted conception outcome
- Pregnancy complications

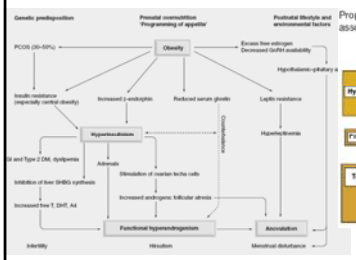
* Origin of fertility impairment
- Sperm
- Oocyte/embryo
- Endometrium

* Obesity, endometrium and implantation

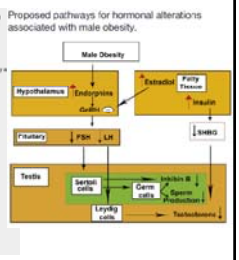
Epidemiology



Pathophysiology

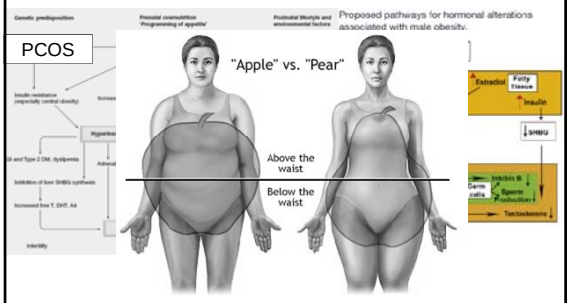


Bellver, 2008

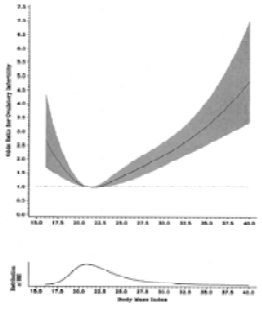


Hammoud et al, 2008

Pathophysiology



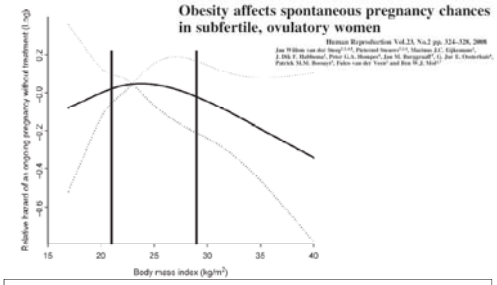
Fertility impairment



Multivariate odds ratios and 95% confidence intervals (shaded areas) of ovulatory disorder infertility by body mass index (BMI, kg/m^2)

Rich-Edwards et al, 2002

Obesity and spontaneous conception



The effect of obesity on fecundity persists for women with regular menstrual cycles

Jensen et al, 1999; Bolumar et al, 2000; Hassan and Killick, 2004; Gesink Law, 2007

Ovarian response

GONADOTROPINS- OI & IVF / CLOMIPHENE- OI

*** Lower ovarian response**
*** Lower E2 levels**
*** Larger doses**
*** More days of stimulation**
*** Increased follicular asynchrony**
*** Less ovulation**
*** More abandoned and cancelled cycles**
*** In long and short COH protocols**
*** ↓ periovulatory intrafollicular hCG concentration**
*** Lower bioavailability of injected (sc or im) hCG**

Ovarian response to gonadotropins and clomiphene citrate is negatively correlated with the BMI

IVF/ICSI: Pregnancy rates

IMPLANTATION RATES

- Reduced: *Loveland et al, 2001; Nichols et al, 2003; Martinuzzi et al, 2008*

- Not affected: *Fedorcsák et al, 2004; Dechaud et al, 2006; Dokras et al, 2006*

PREGNANCY RATES

- Reduced: *Halme et al, 1986; Loveland et al, 2001; Carrell et al, 2001; Ku et al, 2006*

*** OR: 0.44-0.53** *Nichols et al, 2003; Jungheim et al, 2008*

*** Each ↑1 BMI unit, OR ↓ by 0.84** *Ferlitsch et al, 2004*

*** When WHR > 0.80** *Wass et al, 1997*

*** From 25 (OR: 0.81) to ≥ 35 kg/m² (OR: 0.50)** *Wang et al, 2000*

- Not affected: *Lashen et al, 1999; Wittemer et al, 2000; Loh et al, 2002; Spandorfer et al, 2004; Dokras et al, 2006; van Swieten, 2005; Dechaud et al, 2006; Martinuzzi, 2008*

Effect of overweight and obesity on assisted reproductive technology—a systematic review

Human Reproduction Update, pp. 1–12, 2007

A. Maheshwari[1,4], Lawrize Stofberg[2] and S. Bhattacharya[3]

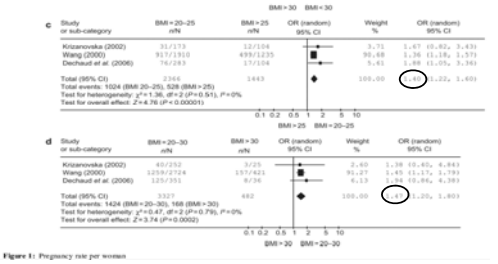
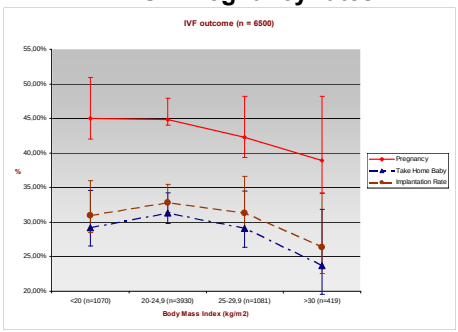


Figure 1: Pregnancy rate per woman

IVF/ICSI: Pregnancy rates



Bellver et al, F & S 2010

IVF/ICSI: Pregnancy rate

Female obesity adversely affects assisted reproductive technology (ART) pregnancy and live birth rates

Human Reproduction, Vol.26, No.1 pp. 245–252, 2011

45,163 ET

Barbara Luke[1,5,*], Morton B. Brown[2], Judy E. Stern[4], Stacey A. Missmer[3], Victor Y. Fujimoto[4], and Richard Leach[1]
A SART Writing Group

Table II ART therapy and pregnancy outcome by women's BMI group.

	All groups	Underweight 16.0–18.5	Normal Wt 18.5–24.9	Over Wt 25.0–29.9	Obese Class I 30.0–34.9	Obese Class II 35.0–39.9	Obese Class III 40.0–46.0
(n embryo transfers)	(45,163)	(1255)	(25,860)	(10,581)	(4599)	(2005)	(863)
Clinical Intrauterine Gestation	43.7	44.7	44.5	43.4	42.1	39.2	37.4
Pregnancy Outcome (n)	(19,642)	(559)	(11,468)	(4580)	(1931)	(782)	(322)
(%) Fetal Loss or Stillbirth	16.9	14.1	15.4	18.3	20.3	21.3	21.8
Live Birth	83.1	85.9	84.6	81.7	79.7	78.6	78.2

Woman's BMI		
Underweight	1.01	0.89–1.15
Normal weight	1.00	Reference
Overweight	1.03	0.98–1.09
Obese Class I	1.13	1.05–1.21
Obese Class II	1.30	1.17–1.44
Obese Class III	1.54	1.32–1.80

Effect of overweight and obesity on assisted reproductive technology—a systematic review

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A. Maheshwari[1,4], Lawrize Stofberg[2] and S. Bhattacharya[3]

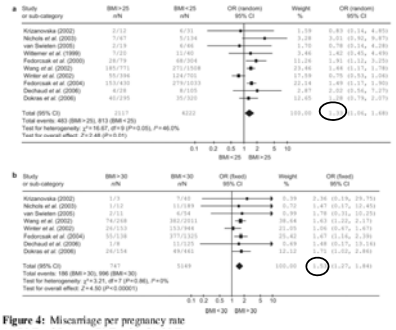


Figure 4: Miscarriage per pregnancy rate

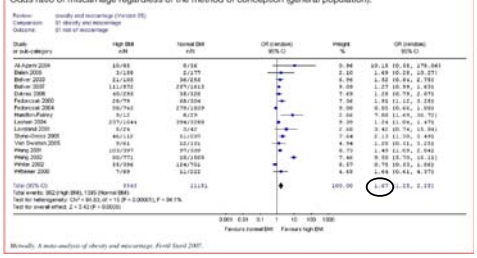
Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? A meta-analysis of the evidence

Mostafa Metwally, M.R.C.O.G., Kee J. Ong, F.R.A.N.Z.C.O.G., William L. Ledger, D.Phil. and Tin Chiu Li, Ph.D.

Fertil Steril 2008; 90:714-26

FIGURE 2

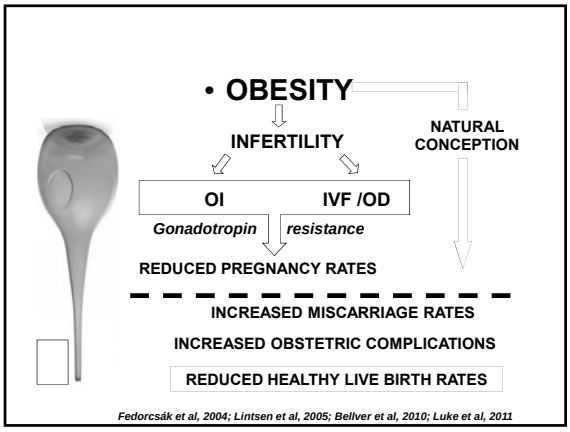
Odds ratio of miscarriage regardless of the method of conception (general population).

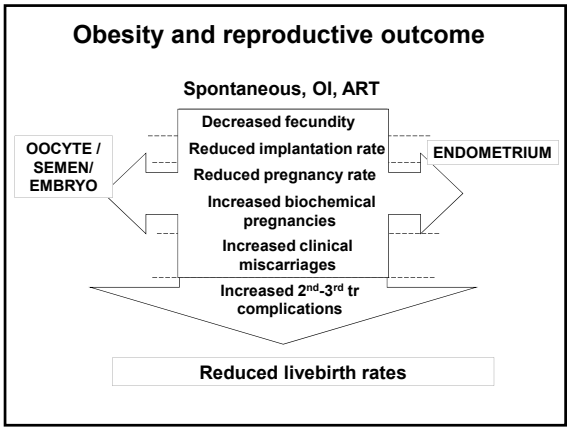


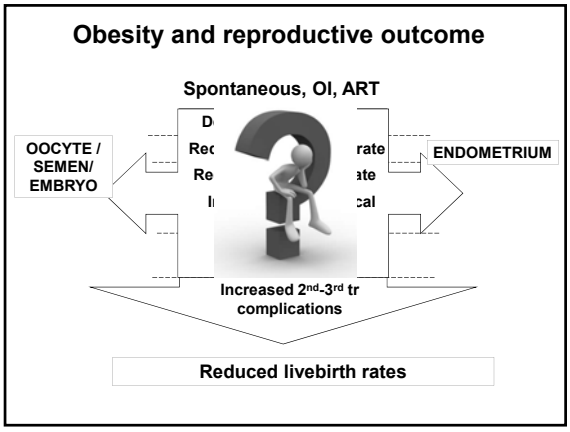
Pregnancy complications

Maternal complications	Fetal complications
- Hypertension & preeclampsia	- Congenital malformations (x 2-3)
- Gestational diabetes	- Preterm delivery (& postterm)
- Urinary infections	- Sudden intrauterine death
- Induction of labor	- Perinatal death
- Assisted vaginal delivery	- Macrosomy
- Cesarean section	- Hydramnios
- Wound infection	- Shoulder dystocia
- Postpartum bleeding	- NICU admission
- Thromboembolism	
- Anaesthetic problems	
- Longer hospitalization	
- Death	

Kabiru et al, 2004; Andreasen et al, 2004; Linné, 2004; Hall- Neubert, 2005; Nelson & Fleming, 2006; Yu et al, 2006; Catalano & Ehrenberg, 2006; Ramsay et al, 2006; Stotland, 2009


• OBESITY
INFERTILITY
NATURAL CONCEPTION
OI
IVF /OD
Gonadotropin
resistance
REDUCED PREGNANCY RATES
INCREASED MISCARRIAGE RATES
INCREASED OBSTETRIC COMPLICATIONS
REDUCED HEALTHY LIVE BIRTH RATES
Fedorcsák et al, 2004; Lintsen et al, 2005; Bellver et al, 2010; Luke et al, 2011


Obesity and reproductive outcome
Spontaneous, OI, ART
OOCYTE / SEMEN/ EMBRYO
Decreased fecundity
Reduced implantation rate
Reduced pregnancy rate
Increased biochemical pregnancies
Increased clinical miscarriages
ENDOMETRIUM
Increased 2nd-3rd tr complications
Reduced livebirth rates


Obesity and reproductive outcome
Spontaneous, OI, ART
OOCYTE / SEMEN/ EMBRYO
ENDOMETRIUM
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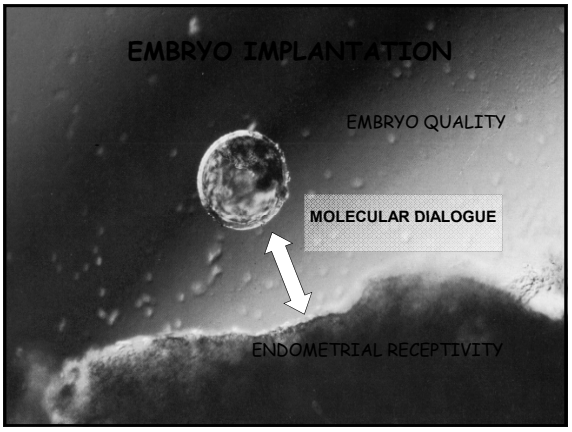
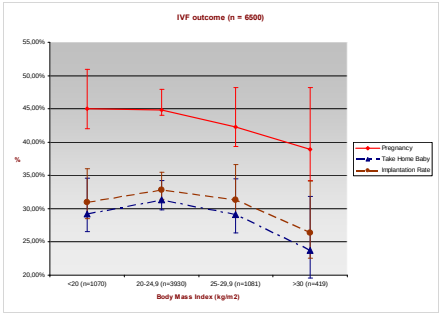


Obesity & oocyte/embryo quality after IVF

	Obesity affects	Obesity does not affect
No collected oocytes	*Lewis et al, 1990; Crosignani et al, 1994; Wittemer et al, 2000; Fedorcsák et al, 2000, 2001 & 2004; Spandorfer et al, 2004; Maheshwari et al, 2007; Matalliotakis, 2008; Jungheim et al, 2009; Zhang et al, 2010*	*Lashen et al, 1999; Loveland et al, 2001; Nichols et al, 2003; van Swieten, 2005; Dechaud et al, 2006; Martinuzzi et al, 2008; Orvieto et al, 2009; Farhi et al, 2010; Vilarino et al, 2010*
No mature oocytes	*Wittemer et al, 2000; Carrell et al, 2001; Dokras et al, 2006; Esinler et al, 2008*	*Marquard et al, 2010*
Oocyte quality	*Dokras et al, 2006; Esinler et al, 2008; Marquard et al, 2010*	*Wang et al, 2000; Nichols et al, 2003; Metwally et al, 2007; Sneed et al,2008 (>35 y); Matalliotakis, 2008; Robker et al, 2009; Sathya et al, 2010*
Fertilization rate	*Salha et al, 2001; Krizanovská et al, 2002; van Swieten, 2005; Matalliotakis, 2008; Orvieto et al, 2009; Jungheim et al, 2009; Zhu et al, 2010*	*Lewis et al, 1990; Lashen et al, 1999; Nichols et al, 2003; Fedorcsak et al, 2000 & 2004; Dokras et al, 2006;Dechaud et al, 2006; Martinuzzi et al, 2008; Esinler et al, 2008; Robker et al, 2009; Farhi et al, 2010; Sathya et al, 2010*
Embryo quality	*Carrell et al, 2001; Metwally et al, 2007 (< 35y); Jungheim et al, 2009; Zhang et al, 2010; Zhu et al, 2010*	*Wang et al, 2000; Fedorcsak et al, 2000 & 2004; Nichols et al, 2003; Dechaud et al, 2006; Metwally et al, 2007 (> 35 y); Sneed et al, 2008 (>35 y); Matalliotakis, 2008; Robker et al, 2009; Sathya et al, 2010; Vilarino et al, 2010*
Incidence of embryo transfer	*Dokras et al, 2006; Esinler et al, 2008 Fedorcsák et al, 2004; Robker et al, 2009*	*Lashen et al, 1999; Nichols et al, 2003; Matalliotakis, 2008; Esinler et al, 2008; Vilarino et al, 2010*
No total or transferred embryos	*Fedorcsák et al, 2004; Robker et al, 2009*	*Lashen et al, 1999; Loveland et al, 2001; Nichols et al, 2003; Dokras et al, 2006; Dechaud et al, 2006; Matalliotakis, 2008; Esinler et al, 2008; Orvieto et al, 2009; Jungheim et al, 2009; Farhi et al, 2010; Sathya et al, 2010; Vilarino et al, 2010*

Obesity & oocyte/embryo quality after IVF



Bellver et al F & S, 2010

Obesity & oocyte/embryo quality after IVF

81581 oocytes

Obese group: ↑ Oligo-anovulation, years of infertility, FSH dose

TABLE 2

In vitro fertilization laboratory parameters.

	< 20 kg/m² (n = 1,070)	20–24.9 kg/m² (n = 3,930)	25–29.9 kg/m² (n = 1,081)	≥ 30 kg/m² (n = 419)
Retrieved oocytes (n)	12.6 ± 7.7	12.6 ± 8.0	12.3 ± 7.9	13.0 ± 8.8
Metaphase II oocytes (n)	8.8 ± 5.5	8.9 ± 5.9	8.5 ± 5.6	9.1 ± 6.2
Insemination procedure (%)				
IVF	6.1	5.1	5.4	6.3
ICSI	17.8	16.0	16.5	14.4
IVF-ICSI	76.1	78.9	78.1	79.3
Fertilization rate (%)	72.4 ± 24.4	73.0 ± 24.2	72.5 ± 24.2	74.0 ± 24.1
Day of ET	3.4 ± 1.3	3.5 ± 1.3	3.5 ± 1.3	3.6 ± 1.3
Embryos transferred (n)	2.0 ± 0.7	2.0 ± 0.7	2.0 ± 0.7	2.0 ± 0.6
Cryopreserved embryos (n)	1.4 ± 2.6	1.4 ± 2.7	1.5 ± 2.9	1.4 ± 2.7
Blastocyst transfer (%)	29.3	31.7	34.3	35.3

Note: Unless otherwise indicated, values are means ± SD.
P—not significant for all comparisons.

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Obesity & oocyte/embryo quality after IVF

81581 oocytes

Correlations between fertilization rate, average embryo fragmentation, average number of cells 48 and 72 hours after fertilization and BMI

BMI	IVF/ICSI	
	r	p
Blastomeres number 48h	0.012	0.477
Blastomeres number 72h	0.011	0.505
Embryo fragmentation 48h	0.006	0.737
Embryo fragmentation 72h	0.007	0.693
Fertilization Rate	0.015	0.160

Bellver et al F & S, 2010

Obesity & transgenerational effects

PREGRAVID & GRAVID OBESITY

- Embryo genome?

"Nutritional programming"

Pregnancy:
- infertility (poorer implantation)
- miscarriage
- congenital malformations
- intrauterine death

Childhood and adulthood (offspring):
- obesity (OR: 2- 6)
- type II diabetes
- cardiovascular disease
- metabolic syndrome (OR: 1.8)

Gillman et al, 2003; Whitaker, 2004; Li et al, 2005; Boney et al, 2005; Lawlor et al, 2007; Chang et al, 2008; Catalano et al, 2009; Nohr et al, 2009; Symonds et al, 2009

Male obesity: sperm

Reduction in semen quality

- **Significant reduction in sperm concentration (by 22%) & total sperm count (by 24%)**
- **Significant reduction in motility and rapid motility**
  - **Trend to impaired sperm morphology**
  - **Increased DNA fragmentation**
- **Significant ↓ testosterone, inhibin B & SHBG ↑ FAI & estradiol**

Jensen et al, 2004; Magnusdottir et al, 2005; Koloszár et al, 2005; Kort et al, 2006; Hammond et al, 2008; Chavarro et al, 2009; Stewart et al, 2009; Paasch et al, 2010; Martini et al, 2010

Associated male obesity

OR for infertility: 1.20 (♂ overweight)
1.36 (♂ obesity)

26,303 planned pregnancies

Nguyen et al, 2007

Subfecundity in overweight and obese couples

C.H.Kamphuis-Hansen[1,2,*], A.M.Thulstrup[2], E.A.Nohr[2], J.P.Bonde[2], T.I.A.Sørensen[1] and J.Olsen[1]

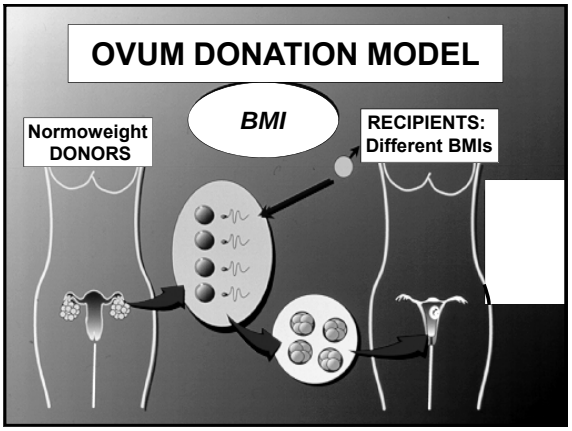
64,167 pregnancies

TTP > 12 months

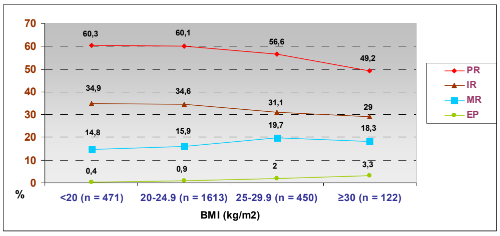
Table I. Odds ratios (ORs) for subfecundity (time to pregnancy of > 12 months) according to categories of men's and women's BMI

Women's BMI (kg/m²)	Men's BMI (kg/m²)				
	<18.50 OR (95% CI)	18.50–24.99 OR (95% CI)	25.00–29.99 OR (95% CI)	≥30 OR (95% CI)	Women[a] OR (95% CI)
<18.50	N/A	1.00 (0.82–1.22)	1.20 (0.94–1.53)	1.95 (1.06–3.59)	1.02 (0.88 to 1.18)
18.50–24.99	0.69 (0.34–1.38)	1.00 (Reference group)	1.18 (1.10–1.27)	1.53 (1.32–1.77)	1.00
25.00–29.99	1.63 (0.67–4.01)	1.36 (1.23–1.50)	1.41 (1.28–1.56)	1.72 (1.49–2.14)	1.27 (1.18–1.36)
≥30	3.79 (1.48–9.74)	1.74 (1.51–2.02)	2.07 (1.82–2.36)	1.98 (1.27–3.30)	1.78 (1.63–1.95)
Men[b]	0.97 (0.61–1.54)	1.00	1.15 (1.00–1.22)	1.50 (1.34–1.66)	—

OVUM DONATION MODEL



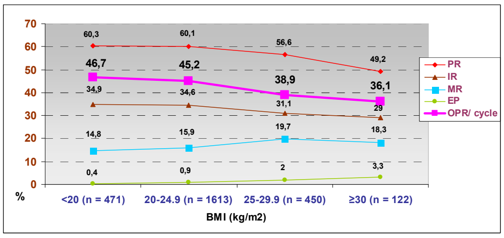
Endometrium: Ovum donation



n = 2656

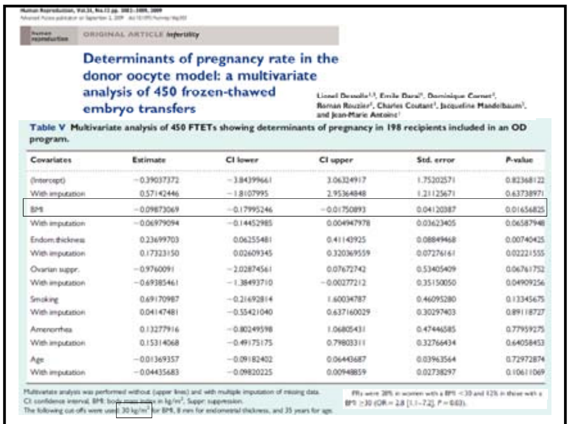
Bellver J et al. Fertil Steril 2007, 88:446-51

Endometrium: Ovum donation



n = 2656

Bellver J et al. Fertil Steril 2007, 88:446-51



ORIGINAL ARTICLE Infertility

Determinants of pregnancy rate in the donor oocyte model: a multivariate analysis of 450 frozen-thawed embryo transfers

Table V Multivariate analysis of 450 FTETs showing determinants of pregnancy in 198 recipients included in an OD program.

Covariates	Estimate	CI lower	CI upper	Std. error	P-value
(Intercept)	−0.39037372	−3.84399661	3.06324917	1.75202571	0.82368122
With imputation	0.57142446	−1.8107995	2.95364848	1.21125671	0.63738971
BMI	−0.09873069	−0.17995246	−0.01750893	0.04120387	0.01656825
With imputation	−0.06979094	−0.14452985	0.004947978	0.03623405	0.06587948
Endom.thickness	0.23699703	0.06255481	0.41143925	0.08849468	0.00740425
With imputation	0.17323150	0.02609345	0.320369559	0.07276161	0.02221555
Ovarian suppr.	−0.9760091	−2.02874561	0.07672742	0.53405409	0.06761752
With imputation	−0.69385461	−1.38493710	−0.00277212	0.35150050	0.04909256
Smoking	0.69170987	−0.21692814	1.60034787	0.46095280	0.13345675
With imputation	0.04147481	−0.55421040	0.637160029	0.30297403	0.89118727
Amenorrhea	0.13277916	−0.80249598	1.06805431	0.47446585	0.77959275
With imputation	0.15314068	−0.49175175	0.79803311	0.32766434	0.64058453
Age	−0.01369357	−0.09182402	0.06443687	0.03963564	0.72972874
With imputation	−0.04435683	−0.09820225	0.00948859	0.02738297	0.10611069

Surrogate obesity negatively impacts pregnancy rates in third-party reproduction

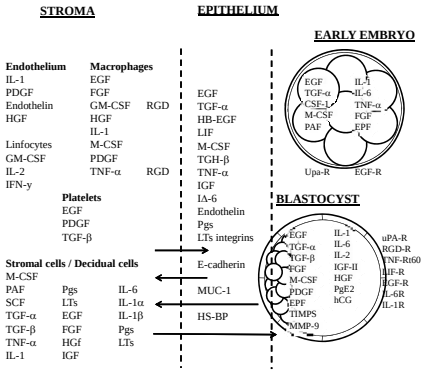
DeUgarte et al. Fertil Steril 2010; 93:1008-10

TABLE 1

Unadjusted and model-adjusted pregnancy outcome rates for all cycles and first-cycle only.

	Unadjusted (%)			Adjusted (%)		
	BMI < 35	BMI ≥ 35	*P* value	BMI < 35	BMI ≥ 35	*P* value
All cycles (n = 551)						
Live birth	48%	27%	.015	49%	28%	.051
Clinical pregnancy	59%	32%	.003	61%	36%	.019
Clinical miscarriage	16%	17%	.963	16%	17%	.901
Mean % implantation	35%	15%	.001	34%	16%	.003
First-cycle only (n = 349)						
Live birth	50%	24%	.028	49%	25%	.041
Clinical pregnancy	61%	33%	.017	61%	35%	.030
Mean % implantation	38%	16%	.006	38%	18%	.021

N = 349 women; 551 cycles of OD in surrogate mothers
No severe male factor



What do we know?

*** Overweight vs normoweight women with unexplained RM**

- Negative correlation: BMI and endometrial glandular LIF
- No difference: endometrial steroid receptors, leukocyte populations, endometrial morphology *Metwally et al, 2007*

*** Reduced endometrial expression of SLC2A4 & SLC2A4 mRNA in obese PCOS women** *Mioni et al, 2004*

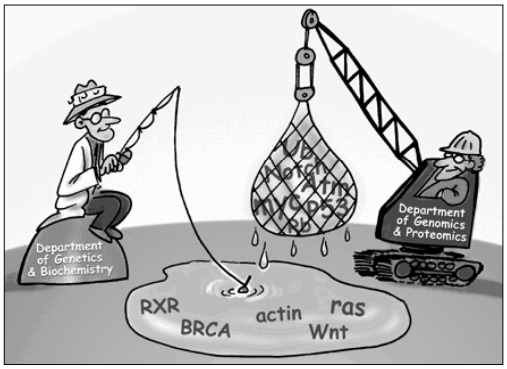
Theories about the effect of obesity on endometrial receptivity:

*** State of relative hyperestrogenemia** *Erel & Senturk, 2009*

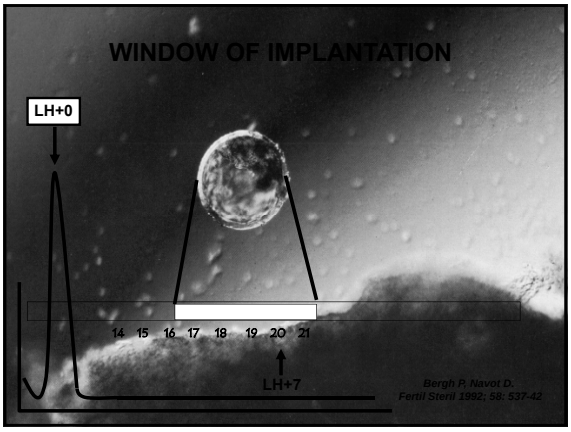
*** Insulin resistance → Hyperinsulinemia → ↓ glycodelin & IGFBP1** *Carrington et al, 2005; Levens & Skarulis, 2008*
→ altered receptivity *Strowitzki et al, 1993*

*** Elevated inflammatory markers** *Eisnler et al, 2008; Levens & Skarulis, 2008*

Traditional versus genomic approach



WINDOW OF IMPLANTATION



Bergh P, Navot D. Fertil Steril 1992; 58: 537-42

Endometrial gene expression

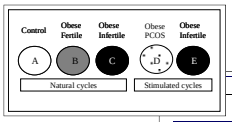


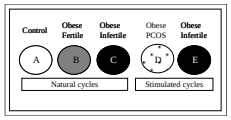
Table 1. Characteristics of the study groups

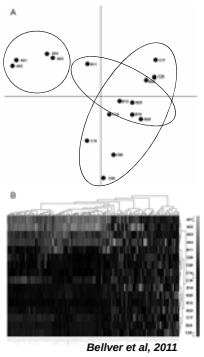
	Group A (n = 4)	Group B (n = 6)	Group C (n = 6)	Group D (n = 6)	Group E (n = 6)
Age (years)	31.00 (2.94)	29.67 (3.67)	33.17 (1.60)	31.13 (3.83)	31.50 (4.59)
BMI (kg/m^2)	22.18 (1.67)	34.03 (3.09)	38.83 (2.35)	38.33 (6.16)	33.13 (3.26)
Waist (cm)		96.33 (4.68)	103.83 (5.91)	109.5 (11.98)	96.17 (8.64)
Hip (cm)		122.17 (7.83)	122.33 (6.95)	124.25 (12.07)	117.17 (12.04)
WHR		0.82 (0.05)	0.85 (0.03)	0.87 (0.06)	0.83 (0.11)
Basal FSH (mIU/ml)	5.82 (1.80)	6.20 (1.94)	6.76 (2.42)	4.67 (1.15)	7.20 (1.35)
Basal LH (mIU/ml)	3.10 (1.10)	3.22 (1.30)	3.42 (1.41)	8.00 (1.44)	3.60 (0.98)
Basal E_2 (pg/ml)	41.03 (4.82)	43.40 (9.54)	39.80 (5.40)	57.00 (12.92)	44.83 (9.93)
E_2 (pg/ml)*	123.00 (31.72)	118.67 (22.06)	134.00 (34.75)	330.00 (279.96)	360.6 (20.42)
P_4 (ng/ml)†	15.20 (5.49)	16.17 (3.45)	10.76 (2.35)	13.43 (10.72)	19.8 (11.7)

*Values are expressed as mean (standard deviation) BMI: Body mass index; WHR: Waist-to-hip ratio; E_2: Estradiol; *Estradiol serum concentration on the day of endometrial biopsy; †P_4: Progesterone serum concentration on day of endometrial biopsy.*

Bellver et al, 2011

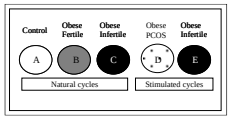
Endometrial gene expression

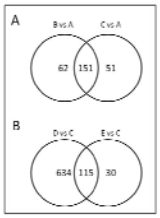


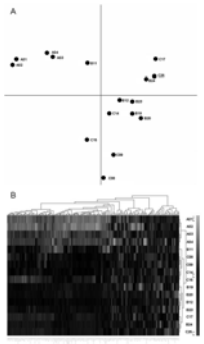


Bellver et al, 2011

Endometrial gene expression

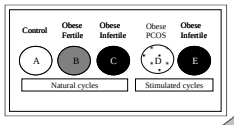


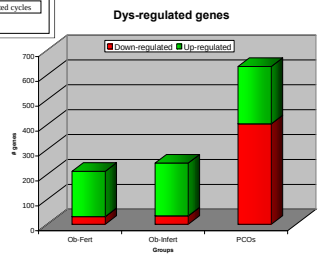




Bellver et al, 2011

Endometrial gene expression





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Endometrial gene expression

ACCN	Name	Description	NC	COS	IUD	PCOS	pfp	
NM_002084	GPX3	Glutathione peroxidase 3 (plasma)	25.9	-11.8	-13.4	-22.3	0.0042	←
NM_002571	PAEP	Placental protein 14	81.6	-9.8	-10.2	-10.7	0.0274	←
NM_021603	FXYD2	FXYD domain containing ion transport regulator 2	4.1	-4.5	-9.4	-3.3	0.0082	
NM_001935	DPP4	Dipeptidylpeptidase 4	31.4	-37.1	-8.5			←
NM_002309	LIF	Leukaemia inhibitory factor	36.6	-23.0	-4.6	-4.3	0.0133	
NM_001013398	IGFBP3	Insulin-like growth factor-binding protein 3	4.0	-4.3	-3.8			
NM_001924	GADD45A	Growth arrest and DNA-damage-inducible, alpha	8.0	-3.0	-3.5	-3.3	0.0367	←
NM_004132	HABP2	Hyaluronan-binding protein 2	5.9	-6.4	-3.4	-12.4	0.0105	
NM_003991	EDNRB	Endothelin receptor type B	8.2	-3.2	-3.4			
NM_012134	LMOD1	Leiomodin 1 (smooth muscle)	29.7	-4.1	-3.1			
NM_001299	CNN1	Calponin 1, basic, smooth muscle	10.3	-9.3	-2.9			
NM_203339	CLU	Clusterin	28.8	-7.1	-2.8	-6.2	0.0020	
NM_001001522	TAGLN	Transgelin	8.4	-3.7	-2.8			
NM_000771	CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9	2.5	-4.7	-2.5			
NM_014214	IMPA2	Inositol(myo)-1(or 4)-monophosphatase 2	5.2	-5.9	-2.2			
NM_014289	CAPN6	Calpain 6	-4.6	10.3	11.2	2.1	0.0302	←
NM_006528	TFPI2	Tissue factor pathway inhibitor 2	-3.5	5.4	7.3	2.5	0.0110	
NM_002758	MAPK6	Mitogen-activated protein kinase 6	-4.9	8.6	6.6	2.6	0.0193	
NM_004389	CTNNA2	Catenin (cadherin-associated protein), alpha 2	-8.4	7.3	6.4			
NM_003104	SORD	Sorbitol dehydrogenase	-2.4	11.6	5.9	3.0	0.0134	
NM_002120	HLA-DOB	Major histocompatibility complex, class II, DO beta	-16.5	12.2	4.9	2.8	0.0043	
NM_183050	BCKDHB	Branched chain keto acid dehydrogenase E1, beta polypeptide	-3.3	10.3	4.1			
NM_000695	ALDH3B2	Aldehyde dehydrogenase 3 family, member B2	-3.1	4.3	3.5			
NM_201535	NDRG2	NDRG family member 2	-6.0	5.9	3.3			
NM_016725	FOLR1	Folate receptor 1	-10.5	9.3	2.5			

Horcajadas et al, 2007

Altmae et al, 2010

Bellver et al, 2011

Conclusions

- **Obesity impairs reproductive and obstetric outcome regardless the method of conception**
- **Gametes, embryos and uterus seem to be affected by obesity**
- **Obese women present a different endometrial gene expression than normoweight controls during the WOI which is more pronounced when infertility or polycystic ovary syndrome are associated**
- **Some of the dys-regulated genes have been previously related to embryo implantation**

- Best advice: weight reduction before conception

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The role of thrombophilia/haematological disorders and impact on endometrial function

Lesley Regan

Contribution not submitted by speaker

Mark your calendar for the upcoming ESHRE campus workshops!

- Early pregnancy disorders: integrating clinical, immunological and epidemiological aspects
  23-26 August 2011 - Copenhagen, Denmark
- The management of infertility – training workshop for junior doctors, paramedicals and embryologists
  7-8 September 2011 - St. Petersburg, Russia
- Basic genetics for ART practitioners
  9 September 2011 - Bucharest, Romania
- The whole man
  22-23 September 2011 - Sevilla, Spain
- Accreditation of a Preimplantation Genetic Diagnosis Laboratory
  3-4 October 2011 - Athens, Greece
- Human reproductive tissues, gametes and embryos: Innovations by science-driven culture and preservation systems
  9 October 2011 - Cairns, Australia
- Comprehensive preimplantation screening: dynamics and ethics
  13-14 October 2011 - Maastricht, The Netherlands
- Endometriosis and IVF
  28-29 October 2011 - Rome, Italy
- Endoscopy in reproductive medicine
  23-25 November 2011 - Leuven, Belgium
- What you always wanted to know about polycystic ovary syndrome
  8-10 December 2011 - Sofia, Bulgaria

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