

Endometrial changes in endometriosis: do they affect implantation?



Antonis Makrigiannakis
School of Medicine
University of Crete
Greece

I declare no conflict of
interest

What is endometriosis?

- ☐ Estrogen-dependent inflammatory disease
- ☐ Affects 5-10% of women in reproductive age
- ☐ Symptoms: pelvic pain, chronic bleeding, inflammation and infertility

Endometrial tissue outside the uterine cavity
ECTOPIC ENDOMETRIUM

Pathogenesis of endometriosis

THE MOST ACCEPTED
retrograde menstruation

coelomic metaplasia theory

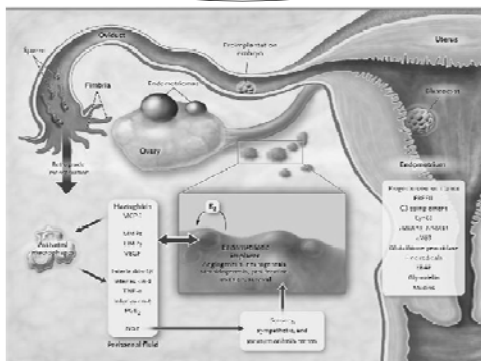
endometrial tissue
travelling
through veins or
lymphatic vessels

stem cells from
bone marrow differentiate
into endometriotic tissue
in different sites

inheritance

new concept
embryological origin
alterations in female genital
tract organogenesis

THE MOST ACCEPTED retrograde menstruation



Endometriosis and infertility

The role of oocyte

The role of the endometrium

Endometriosis

- Endometriosis is associated with affected implantation and clinical pregnancy rates.
 - Due to poor oocyte/embryonic quality
 - Due to endometrial receptivity problems

Human Rep Update 8: 95-103, 2002.

Ovarian quality and endometriosis

- Retrospective study: Oocytes from donors with endometriosis are associated with lower implantation but not clinical pregnancy rates compared to oocytes from normal donors

Hum Reprod. 1994 Apr;9(4):725-9

Ovarian quality and endometriosis Experience from donor programs

- Prospective study
 - Group 1: control
 - Group 2: donor with endometriosis/healthy recipient
 - Group 3: healthy donor/ recipient with endometriosis
- Pregnancy and implantation rates were significantly (2-fold) reduced in Group 2

Human Rep Update 8: 95-103, 2002.

Ovarian quality and endometriosis

■ Granulosa cells

- A decreased number of granulosa cells in the G2/M phase and an increase in both the S phase and apoptotic cells were documented in women with endometriosis

Gynecol Obstet Invest 2002;53(Suppl 1):46–51

Ovarian quality and endometriosis

■ Granulosa Cells

- Granulosa cell apoptosis increased proportionally with the severity of disease and resulted in poor oocyte quality and a reduction in fertilization and pregnancy rates
- A higher percentage of granulosa cell apoptosis was associated with significantly reduced pregnancy rates in patients with endometriosis or tubal factor infertility undergoing IVF

Fertil Steril 1997;67:302–8
Eur J Obstet Gynecol Reprod Biol 2002;103:150–3

Ovarian quality and endometriosis

■ Granulosa cells

- Oxidative stress markers were significantly elevated in granulosa cells of patients with endometriosis

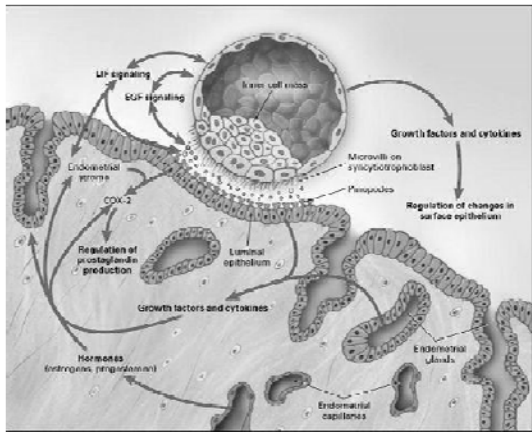
Gynecol Obstet Invest 2002;53(Suppl 1):46–51

**The new trend: endometrial
receptivity in infertile patients with
endometriosis**

**EUTOPIC VS ECTOPIC
ENDOMETRIUM**

- ☐ Steroid hormone responsiveness and receptor content
- ☐ Growth factor responsiveness and receptor content
- ☐ Protein production
- ☐ Expression of enzymes and their inhibitors

**Eutopic endometrium,
Endometriosis and
implantation**



Eutopic endometrium and implantation markers (1)

Summary of biomarker expression and statistical comparison of biomarker expression in endometrium from healthy control women and patients with endometriosis.

Biomarker	Histologic secretory phase	Mean HSCORE \pm SD		P value*
		Normal	Endometriosis	
GdA	Middle	2.3 \pm 1.1 (n = 9)	2.0 \pm 0.7 (n = 6)	.761
	Late	3.1 \pm 0.4 (n = 6)	2.2 \pm 0.9 (n = 9)	.049
OPN	Middle	3.6 \pm 0.3 (n = 9)	3.4 \pm 0.4 (n = 6)	.127
	Late	3.6 \pm 0.2 (n = 6)	2.8 \pm 0.4 (n = 9)	.0001
LPAS	Early	3.7 \pm 0.3 (n = 7)	3.1 \pm 0.5 (n = 7)	.061
	Middle	3.3 \pm 0.4 (n = 9)	2.4 \pm 0.4 (n = 6)	.006
HOXA10 stroma	Early	3.5 \pm 0.4 (n = 8)	2.6 \pm 0.5 (n = 9)	.002
	Middle	3.1 \pm 0.4 (n = 7)	2.8 \pm 0.4 (n = 7)	.225
HOXA10 gland	Middle	3.2 \pm 0.3 (n = 9)	2.5 \pm 0.3 (n = 8)	.0006
	Late	3.2 \pm 0.2 (n = 8)	2.4 \pm 0.3 (n = 9)	.0001
HOXA10	Early	1.6 \pm 0.6 (n = 7)	1.4 \pm 0.4 (n = 7)	.591
	Middle	2.2 \pm 0.7 (n = 9)	1.1 \pm 0.1 (n = 6)	.006
	Late	2.8 \pm 0.3 (n = 8)	2.0 \pm 0.6 (n = 9)	.002

Note: n = number of subjects.

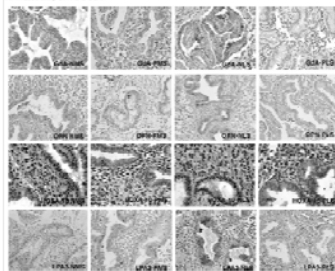
*P value determined by Wilcoxon test comparing HSCOREs values between patients with endometriosis and healthy control subjects and computing exact P values.

Not significant in comparison to endometriosis (P > 0.05).

Fertility and Sterility® Vol. 91, No. 5, May 2009

Eutopic endometrium and implantation markers (2)

Immunohistochemical localization of GdA, OPN, LPAS, and HOXA10 in the endometrium of healthy women and women with endometriosis during the early secretory, midsecretory, and late secretory phases. LS = healthy women; P = women with endometriosis; ES = early secretory; MS = midsecretory; LS = late secretory. Expression was significantly reduced in endometriosis in all paired patient samples, except for midsecretory OPN and LS.



Not significant in comparison to endometriosis (P > 0.05).

Fertility and Sterility® Vol. 91, No. 5, May 2009

**Eutopic endometrium and implantation
markers (3)**

- RT-PCR for LIF and IL-11
- Endometrial flushings
- There is no receptivity defect with regard to LIF and IL-11 secretions by eutopic endometrium in infertile women with endometriosis.

Human Reproduction Vol.21, No.12 pp. 3054–3058, 2006

**Eutopic endometrium and implantation
markers (4)**

- Integrin AvB3 is down-regulated in eutopic endometrium of patients with endometriosis

Fertil Steril 1994;62:497–506
J Clin Endocrinol Metab 1994;79:643–9.

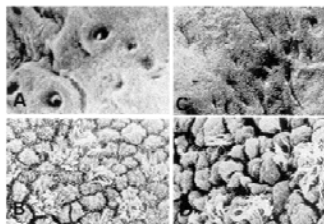
**EUTOPIC ENDOMETRIUM
CHANGES IN WOMEN WITH
ENDOMETRIOSIS**

Changes in eutopic endometrium of women with endometriosis

- Structure and histology
- Proliferation and growth factors
- Angiogenesis
- Apoptosis and cell cycle
- Immunology
- Cell adhesion molecules
- Steroids and cytokines
- Protein production and gene expression

Structure changes of eutopic endometrium in women with endometriosis

- Increased heterogeneity in surface epithelium , reduced glandular and stromal mitoses, basal vacuolated cells
- Reduced endometrial thickness
- Altered neuroendocrine cells
- Nerve fibres identified

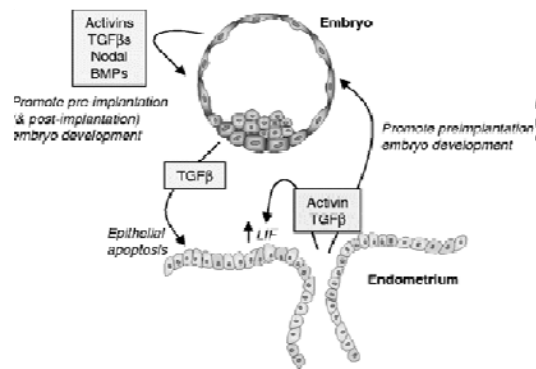


A: preovulatory stage healthy women
B: secretory stage healthy women
C: preovulatory stage endometriotic women
D: secretory stage endometriotic women

Changes in eutopic endometrium of women with endometriosis

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GROWTH FACTORS ENDOMETRIUM IN HEALTHY WOMEN



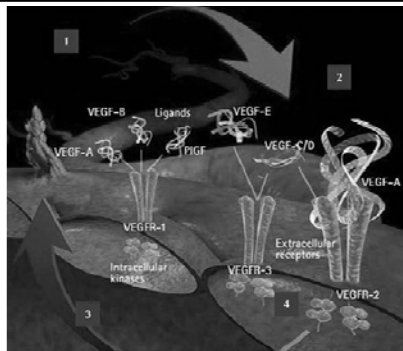
Proliferation – growth factors changes of eutopic endometrium in women with endometriosis

- Increased proliferation of endometrial epithelial, stromal and endometrial cells
- Lower TGFβ1
- Higher activin, IGF, IGF-BP3, HGF, annexin-1
- No differences in endometrial cell proliferative activity
- Reduced crypto

Controversial theories

Changes in eutopic endometrium of women with endometriosis

- Structure and histology
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- 1 Upstream activators stimulate the production of VEGF
- 2 VEGF binds to receptors on endothelial cells
- 3 Angiogenesis is mediated primarily through the interaction of VEGF-A with VEGFR-2
- 4 Other variants of VEGF and the VEGFR play a secondary role in this process

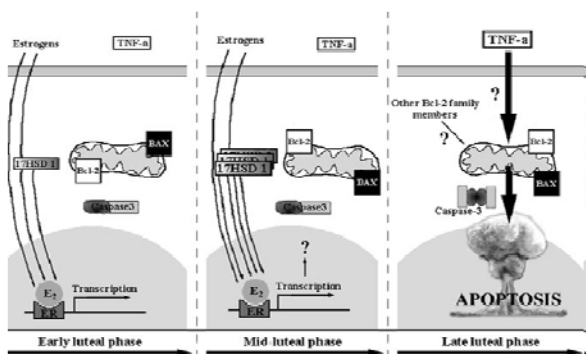
Angiogenesis changes of eutopic endometrium in women with endometriosis

- VEGF elevated in secretory phase, some studies during the proliferative phase
- VEGF-A elevated
- Reduced VEGF receptor-1
- Controversy concerning VEGFR-2
- VEGF – C reduced
- Increased angiopoietin-1 and -2 in the secretory phase
- Higher endoglin positive vessels
- Decreased platelet growth factor – A
- Controversy for thrombospondin – 1 and prokineticin- 1 (angiogenetic factors)

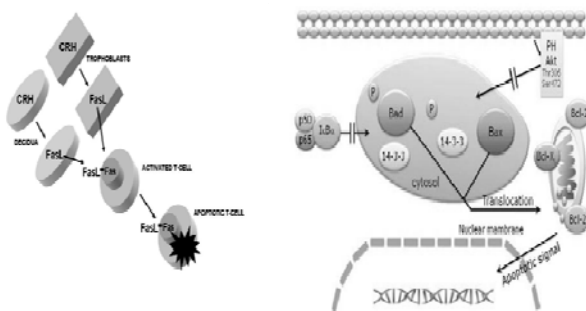
Changes in eutopic endometrium of women with endometriosis

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Eutopic endometrium apoptosis in healthy women



Eutopic endometrium apoptosis in healthy women



Apoptosis and cell cycle changes of eutopic endometrium in women with endometriosis

- Reduced number of apoptotic cells or increased apoptosis in secretory phase **CONTROVERSY**
- Increased or unchanged Bcl-2 **CONTROVERSY**
- Reduced caspase-1 levels
- Increased or reduced p53 levels **CONTROVERSY**
- TNF-α suppresses proliferation of endometrium in healthy women BUT enhances proliferation of endometrium of women with endometriosis
- Increased MCL-1
- Reduction of Bak, some others increased Bax and Bak ratios → anti-apoptotic environment
- Increased proteins for cell survival (p21 activated kinase-1, pERK1/2, cyclin D1)
- Increased levels of c-myc promoting cell growth and proliferation

Changes in eutopic endometrium of women with endometriosis

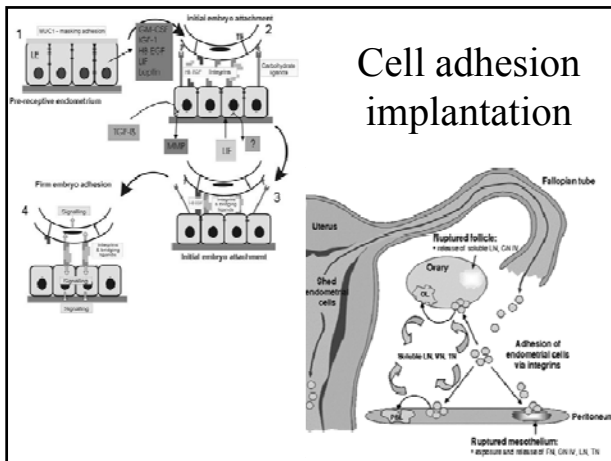
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- Protein production and gene expression

Immunology changes of eutopic endometrium in women with endometriosis

- Increased secretion of complement component C3
- Decreased mitogenicity for autologous lymphocytes
- Fewer T-suppressor /cytotoxic cells and endometrial granulated lymphocytes
- More T helper/inducer cells
- Increased resistance to cytotoxic effect of heterologous lymphocytes
- Increased endometrial IgG and HLA-DR, HLA class I

Changes in eutopic endometrium of women with endometriosis

- Structure and histology
- Proliferation
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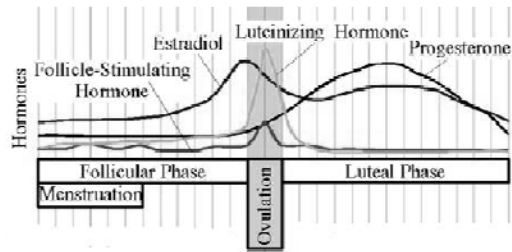
Cell adhesion
changes of eutopic endometrium
in women with endometriosis

■ Controversial data concerning the alterations of integrins, E-Cadherin, ECM molecules, Matrix Metalloproteinase family proteins

Changes in eutopic endometrium of women with endometriosis

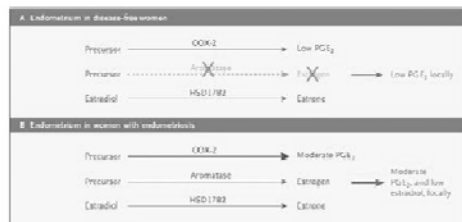
- Structure and histology
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- Cell adhesion molecules
- Steroids and cytokines
- Protein production and gene expression

Hormones in endometrium of healthy women



Steroids and Cytokines changes of eutopic endometrium in women with endometriosis

- No difference in estrogen nor progesterone receptor expression
- Elevated production of IL-1 β , IL-13, IL-15, macrophage-stimulating protein
- Controversial data for :IL-6, IL-8, MCP-1



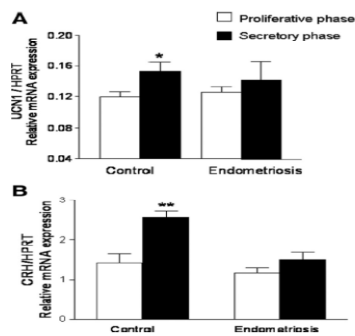
Changes in eutopic endometrium of women with endometriosis

- Structure
- Proliferation
- Apoptosis
- Immunity
- Cell adhesion molecules
- Proteases
- Steroids and cytokines
- Protein production and gene expression

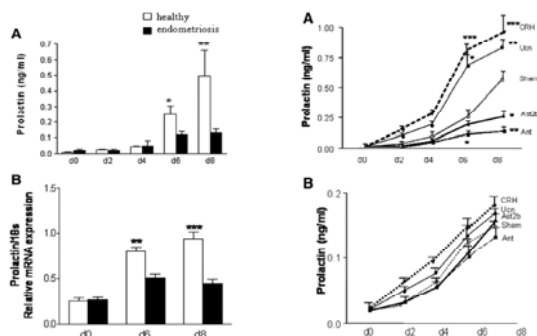
Proteins and genes changes of eutopic endometrium in women with endometriosis

- Elevated production of CA-125
- Absence of HOX gene expression
- Increased production of CRH, UCN

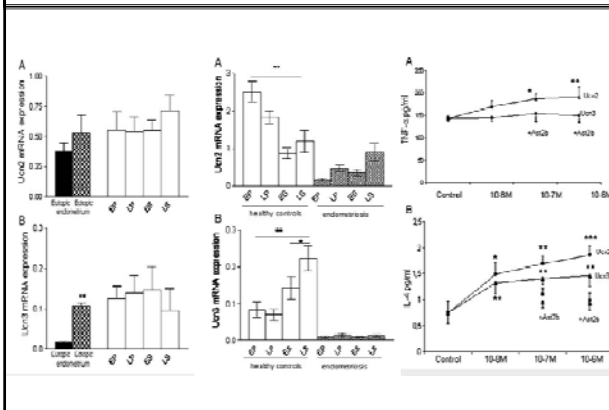
Impaired CRH and Urocortin expression and function in eutopic endometrium of women with endometriosis (Novembri R. et al, 2011)



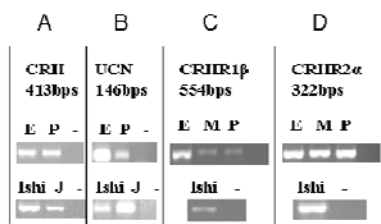
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UCN2 and UCN3 in endometriosis(Novembri R. et al , 2011)

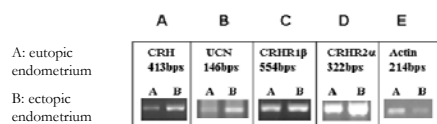


OVEREXPRESSION OF CRH, UCN, CRHR1 AND CRHR2 IN ECTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS (Vergetaki A. et al , in process)

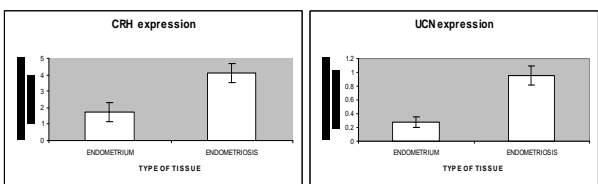


E: endometriotic tissue, P: placental tissue, M: myometrial tissue
 Ishi: Ishikawa cell line, JEG3: human choriocarcinoma cell line, - : negative control

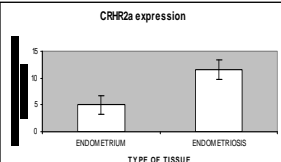
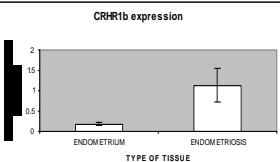
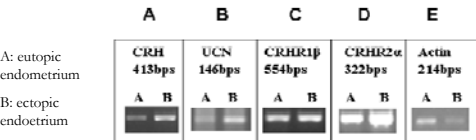
OVEREXPRESSION OF CRH, UCN, CRHR1 AND CRHR2 IN ECTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS (Vergetaki A. et al , in process)



A: eutopic endometrium
 B: ectopic endometrium

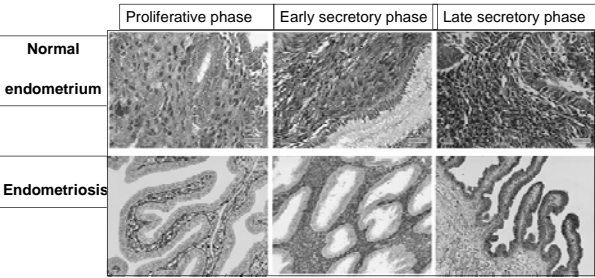


OVEREXPRESSION OF CRH, UCN, CRHR1 AND CRHR2 IN ECTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS (*Vergetaki A. et al, in process*)

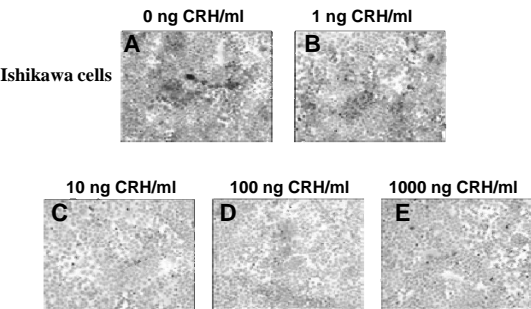


THE EFFECT OF CRH ON GALECTIN-1 PATTERN IN ENDOMETRIOSIS AND GALECTIN-1 EXPRESSION IN ECTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS (*Jeschke U. et al, in process*)

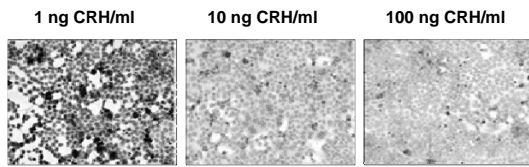
Downregulation of galectin-1 in endometriotic tissue



THE EFFECT OF CRH ON GALECTIN-1 PATTERN IN ENDOMETRIOSIS AND GALECTIN-1 EXPRESSION IN ECTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS (*Jeschke U. et al, in process*)



**THE EFFECT OF CRH ON GALECTIN-1 PATTERN IN
ENDOMETRIOSIS AND GALECTIN-1 EXPRESSION IN
ECTOPIC ENDOMETRIUM OF WOMEN WITH
ENDOMETRIOSIS (*Jeschke U. et al, in process*)**



Hec1B cell line

Conclusions

- The association of endometriosis and infertility can be attributed to both ovarian and endometrial etiologies
- Ovarian dysfunction can lead to bad quality oocytes and thus embryos
- Both ectopic and eutopic endometria from patients with endometriosis differ from normal endometrium

Conclusions

- Endometriosis is inducing a microenvironment against implantation and early fetal development.
- The role of local stress in the endometrium of endometriosis patients may substantially explain the increased rate of IVF failures
- Further experiments using animal models could elucidate the role of CRH in endometriosis.

CONCLUSION

Abnormal eutopic endometrium



Decidualization – Implantation
problems



Infertility

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