



Azienda Ospedaliera  
Universitaria Senese



**Università degli Studi di Siena**  
1240-2010: 770 years of history



**Siena, Piazza del Campo**



**Siena, Policlinico Universitario "Santa Maria alle Scotte"**



# Endometriosis: symptoms



Infertility

Pain

# Endometriosis and infertility: possible mechanisms

❖ Follicular environment and ovulation

❖ Changes in peritoneal fluid

❖ Adhesions between peritoneum and pelvic organs

❖ Eutopic endometrium abnormalities

❖ Implantation disorders



# Hormones and inflammation: endometriosis

Estrogens

Progesterone

+

-

Growth factors

Cytokines  
Chemokines

Free radicals  
(Oxidative stress)

Prostaglandins

Endometrial cell

Cell proliferation and differentiation

Immune function and inflammation

Apoptosis

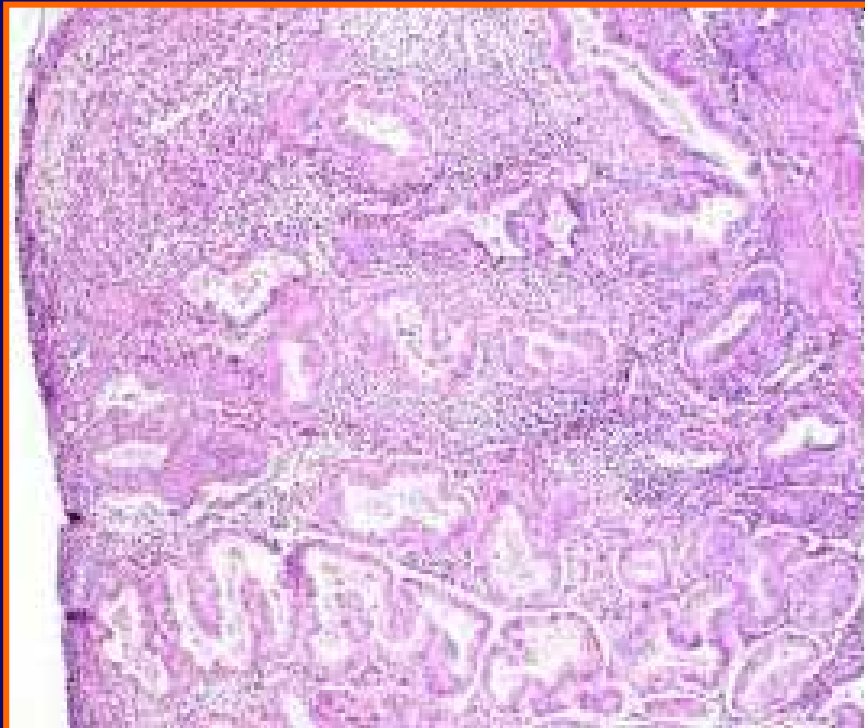
Invasion / neoangiogenesis

Endometriotic cell

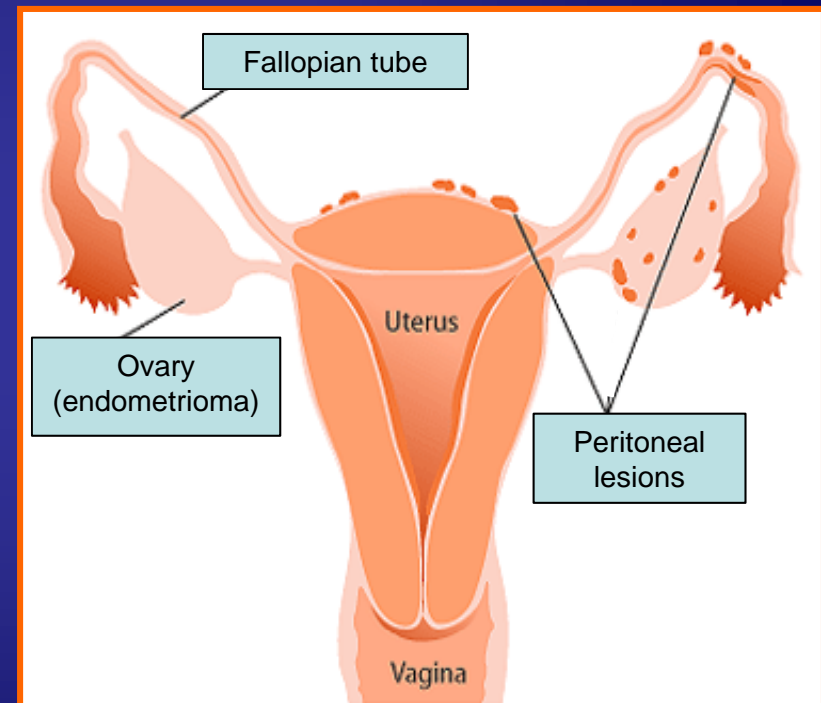
Chronic  
Inflammation

Genetic/epigenetic influences

# Endometriosis: tissues studied



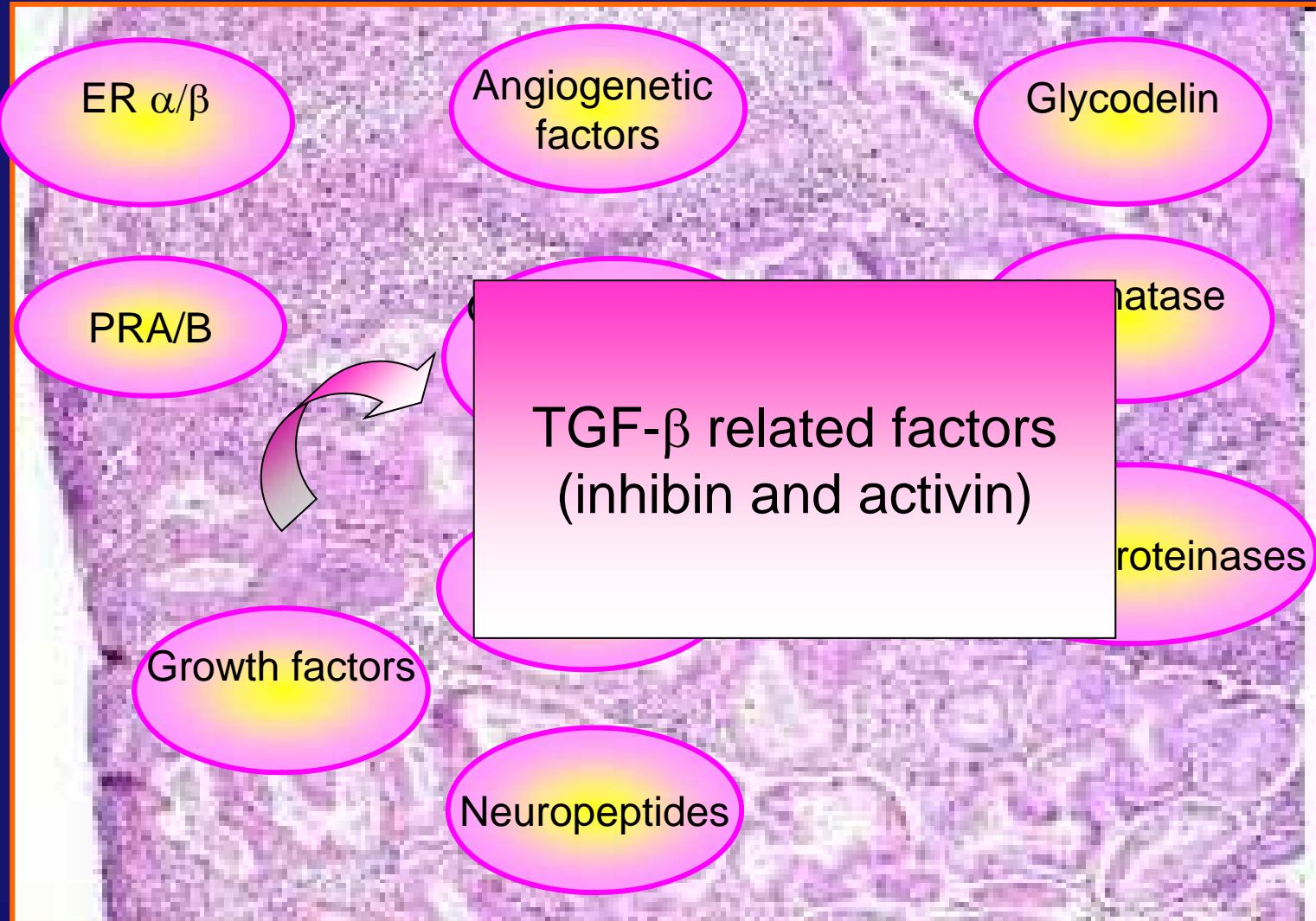
Eutopic endometrium



Ectopic endometrium

But also: - Gastrointestinal lesions  
- Urinary tract

# Eutopic and ectopic endometrium: hormone receptors, factors and enzymes

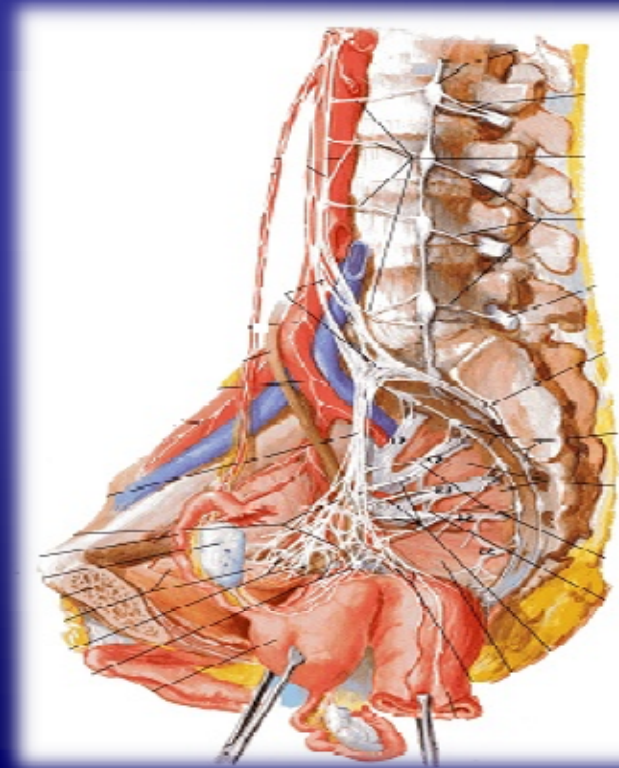
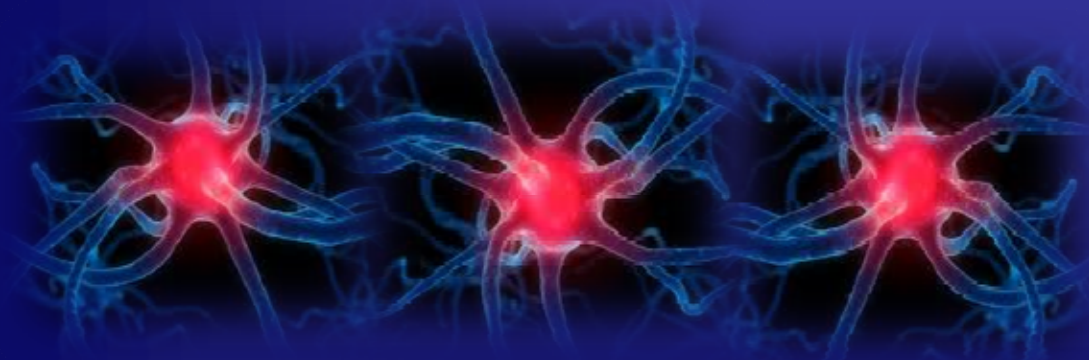


# Neuroendocrine factors in endometriosis



Nerve fibres

In women with endometriosis, there is abnormal sprouting of nociceptors in the endometrium and in peritoneal endometriotic lesions



Jabbour et al. SGI 2010

# Neuroendocrine factors in endometriosis



Neuroendocrine cells

Substance P

VIP

Synaptophysin  
(SYN)

Neuropeptide Y

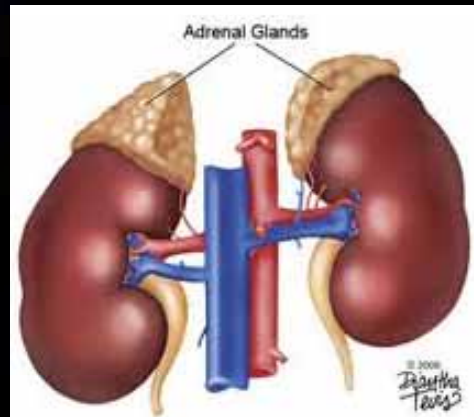
Calcitonine gene  
related protein

neuron-specific  
enolase (NSE)

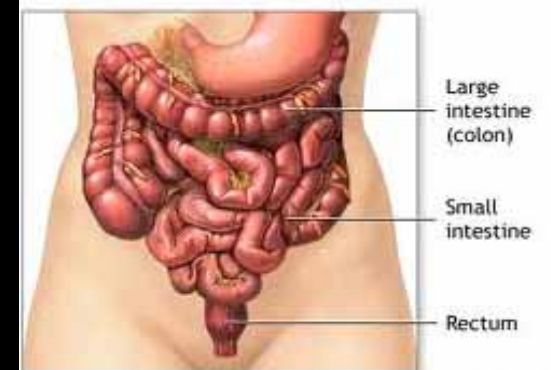


# Neuroendocrine system is localized in various organs and tissues

Neurohormones  
Neuropeptides  
Neurotransmitters



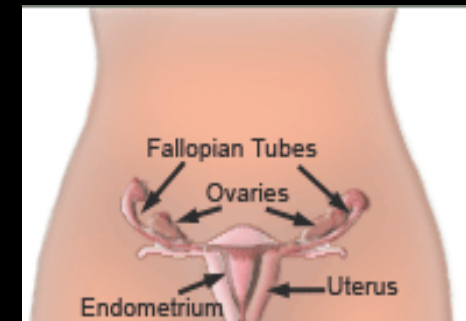
Adrenal glands



Gastrointestinal tract



Neuroendocrine organs



Reproductive tract

# Endometrium and neuroendocrine factors

Prolactin

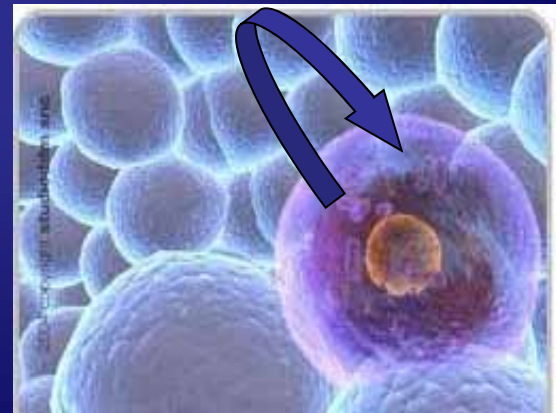
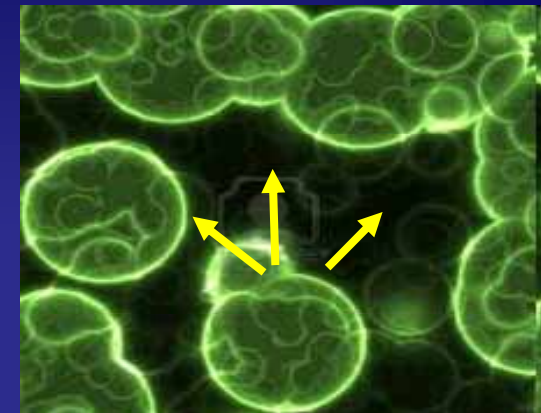
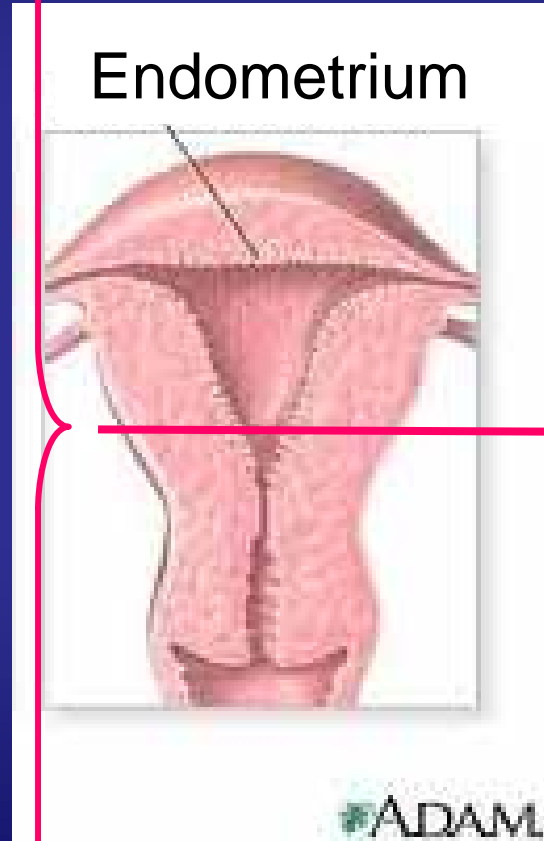
CRH

Urocortins

Relaxin

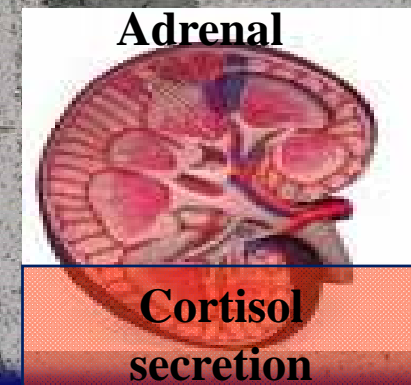
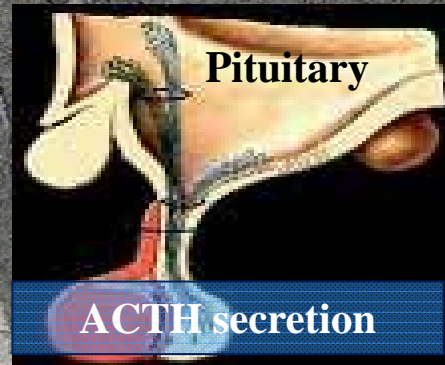
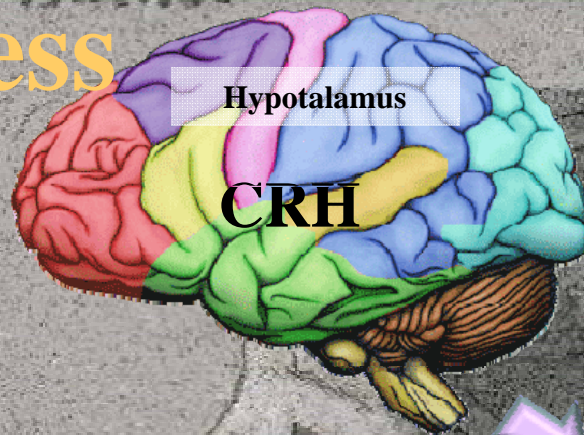
Substance P

Vasoactive  
Intestinal peptide



CRH is a neuropeptide originally localized in central nervous system

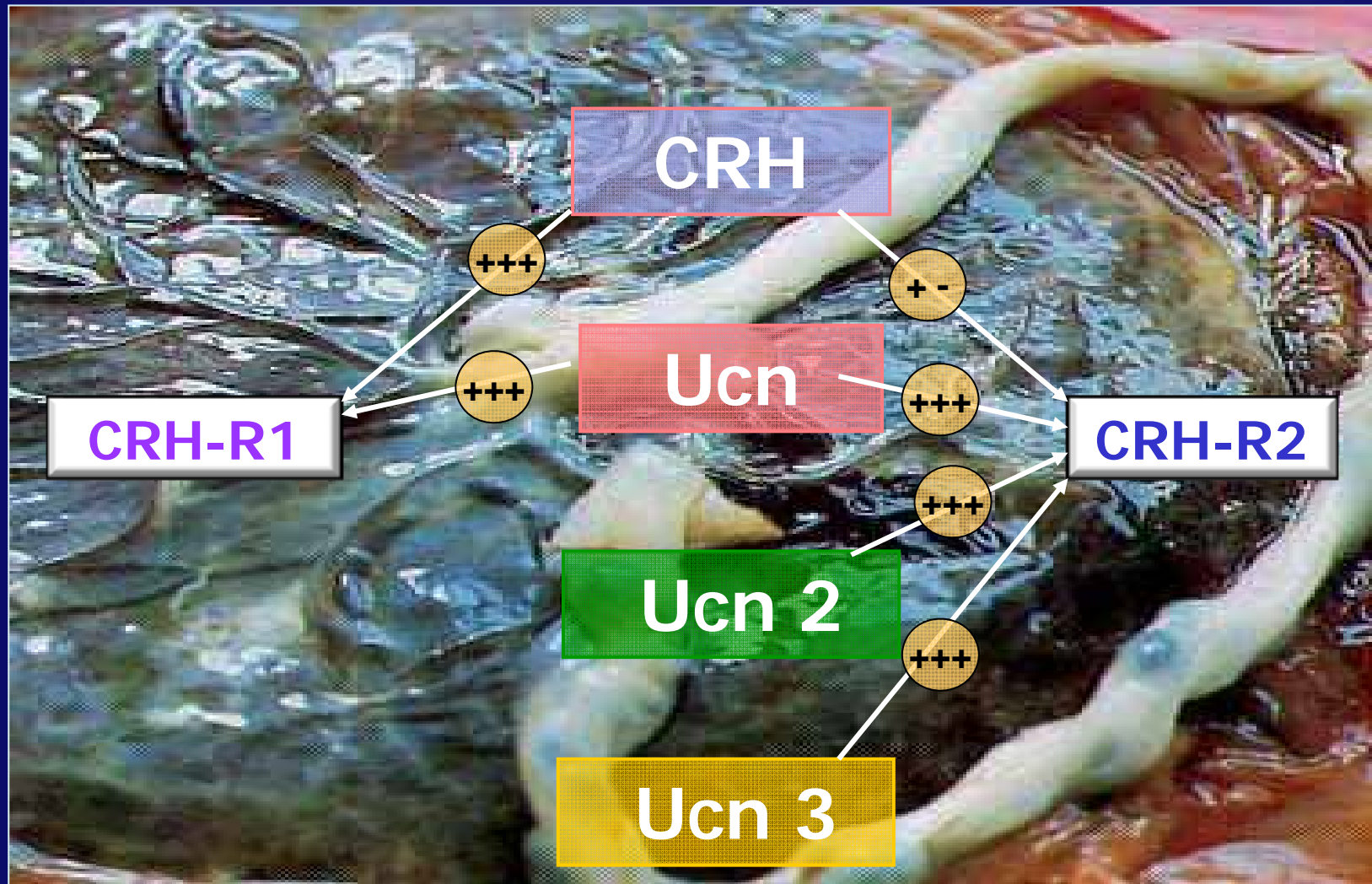
Stress



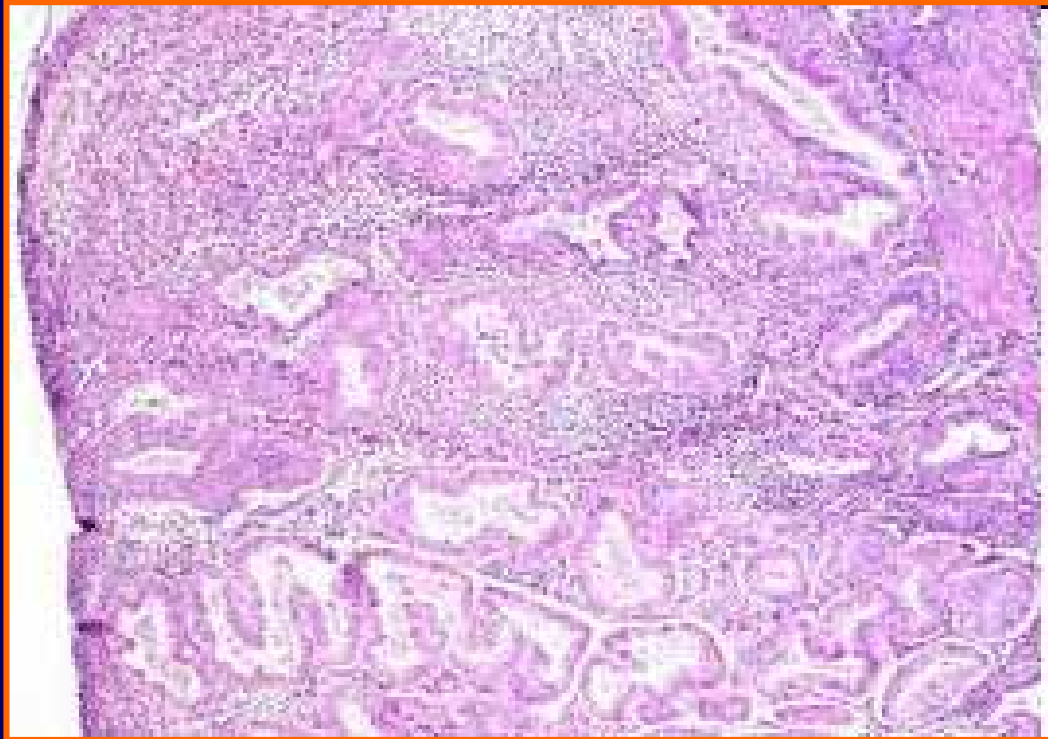
# CRH-family

	amino acids	% CRH sequence homology
<b>CRH</b>	<b>41</b>	
<b>urocortin</b> <i>Vaughan et al., Nature 1995</i>	<b>40</b>	<b>42</b>
<b>urocortin 2 (or stresscopin related peptide)</b> <i>Reyes et al., PNAS U S A. 2001</i>	<b>38</b>	<b>31</b>
<b>urocortin 3 (or stresscopin)</b> <i>Lewis et al., PNAS U S A. 2001</i>	<b>38</b>	<b>26</b>

# CRH- Receptors affinity



# Human endometrium express and localize



CRH

Urocortin

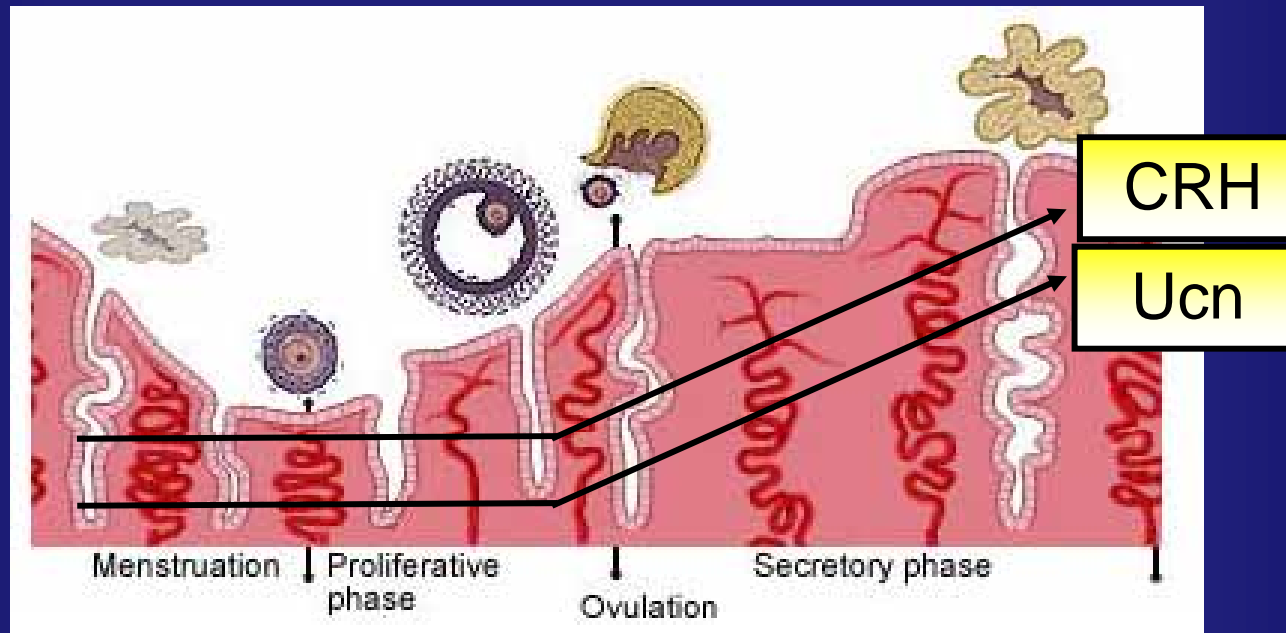
Urocortin 2

Urocortin 3

CRH-R1

CRH-R2

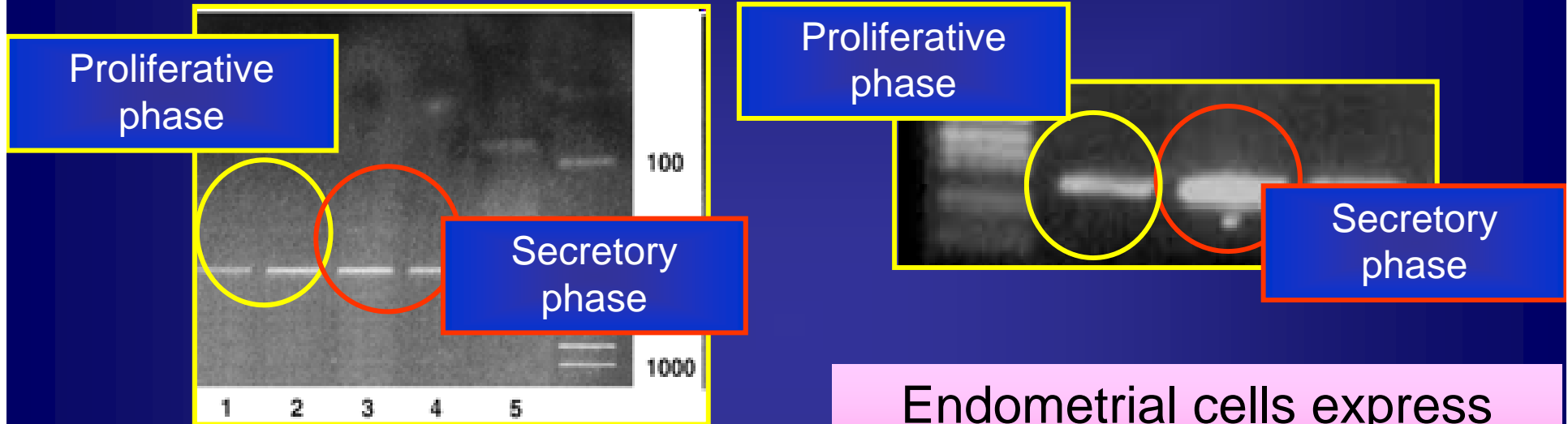
## Endometrial CRH/Ucn and menstrual cycle



CRH mRNA expression is higher during secretory phase  
(Zoumakis et al. 2001)

Ucn mRNA expression is higher during secretory phase  
(Torricelli et al, Hum Reprod 2007)

# Endometrial cells and CRH receptors



Endometrial cells express CRH-R1 mRNA in both phases of menstrual cycle

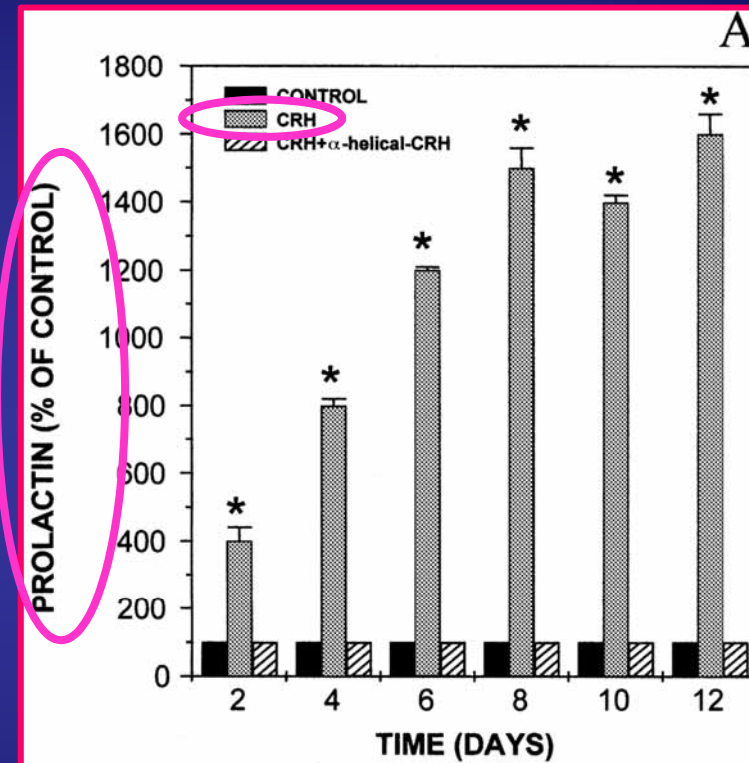
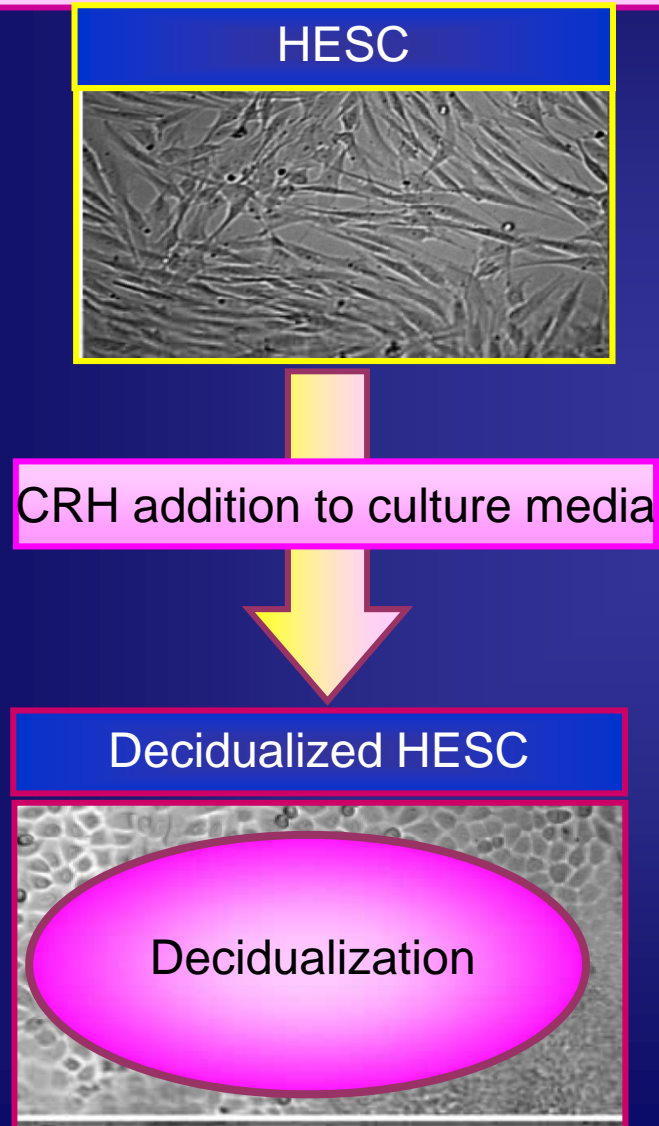
Endometrial cells express CRH-R2 mRNA in both phases of menstrual cycle

Karteris *et al.*, *JCE&M* 2004

Di Blasio *et al.*, *JCE&M* 1997

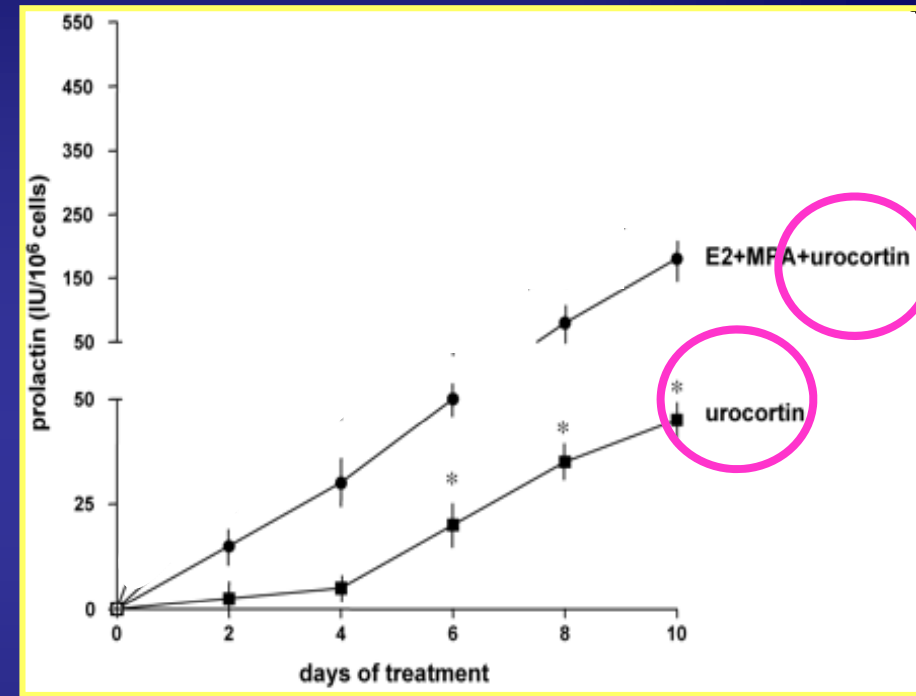
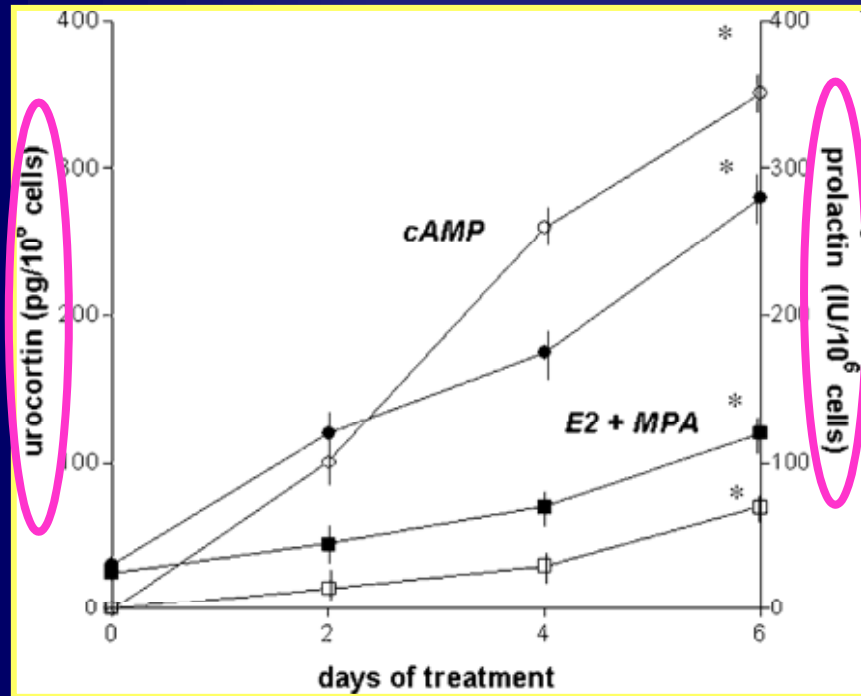


# CRH stimulates decidualization in endometrial stromal cells



Zoumakis *et al.*, *Mol Hum Rep* 2000

# Ucn stimulates decidualization in endometrial stromal cells

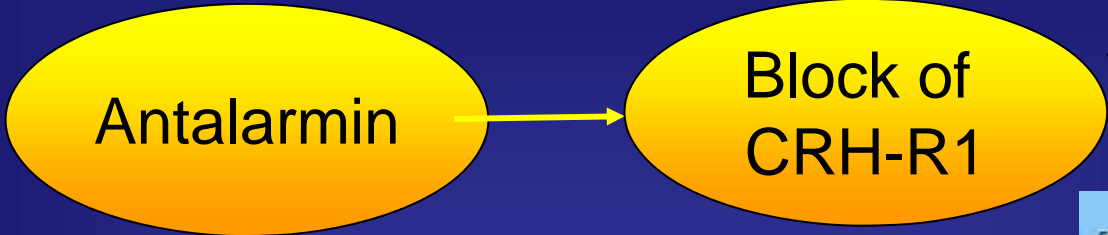


Ucn and prolactin concentration  
Increase during in vitro  
decidualization

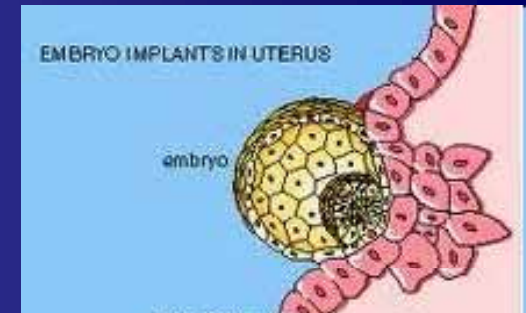
Ucn stimulates  
decidualization process

# CRH/CRH-R1 are involved in embryo implantation

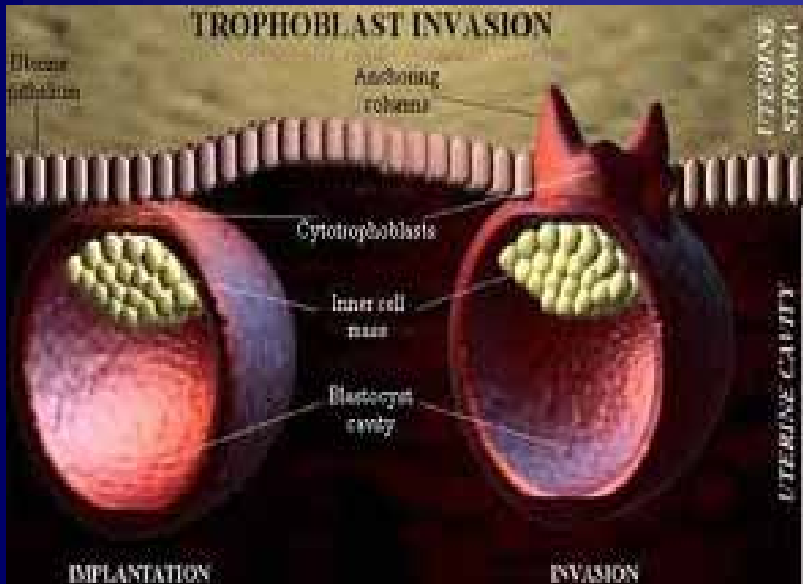
Mouse model



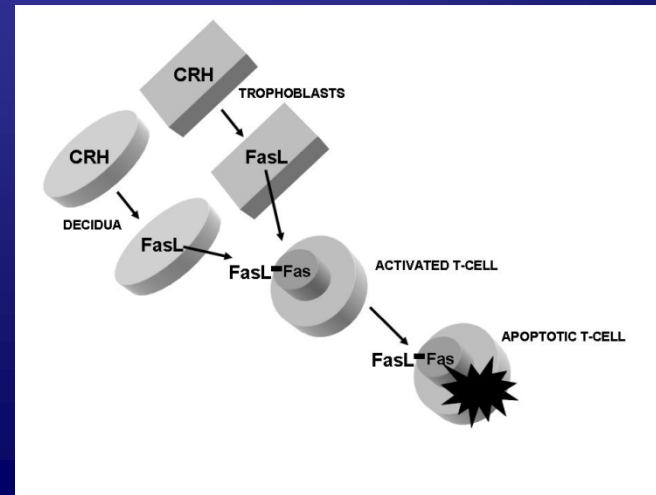
Makrigiannakis *et al.*, Am J Reprod Immun 2004



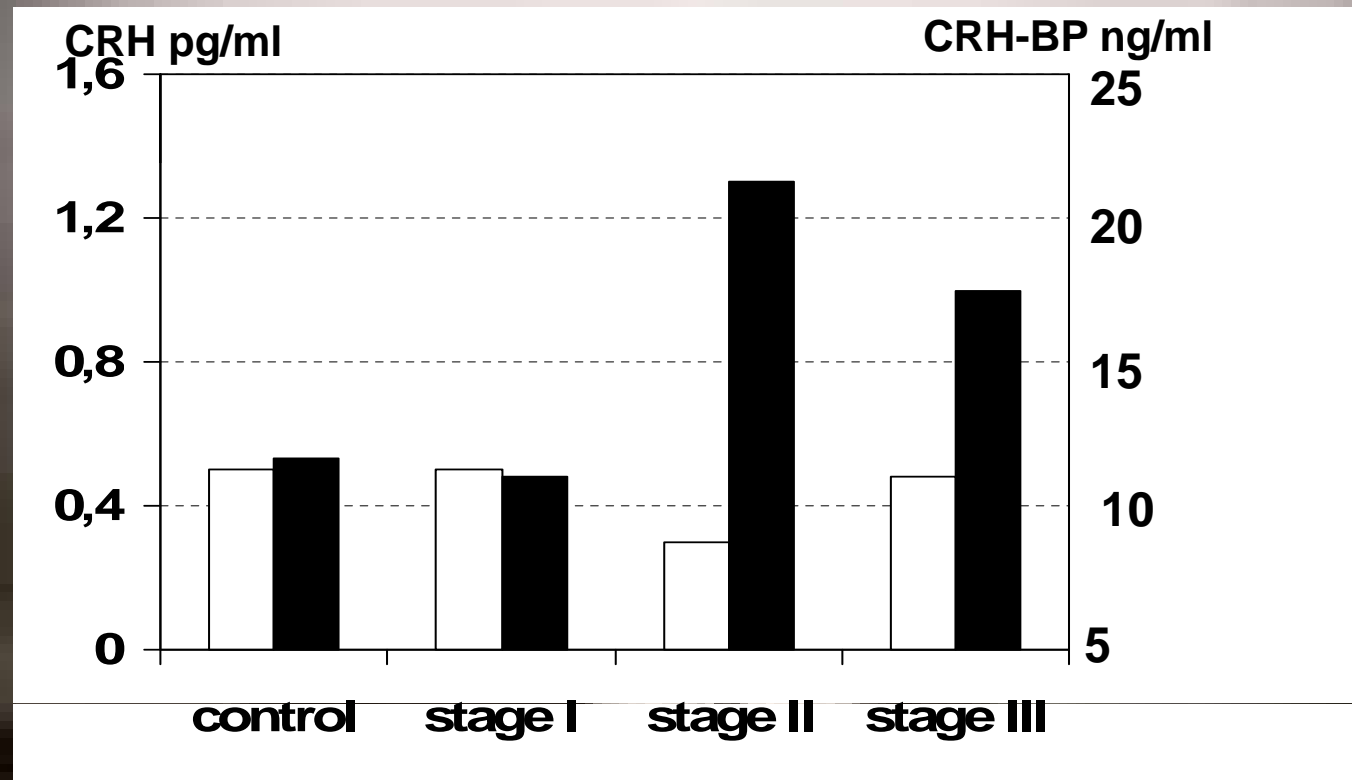
Implantation failure 70%



Kalantaridou *et al.*, Crit Rev in Clinic Lab Sci 2007

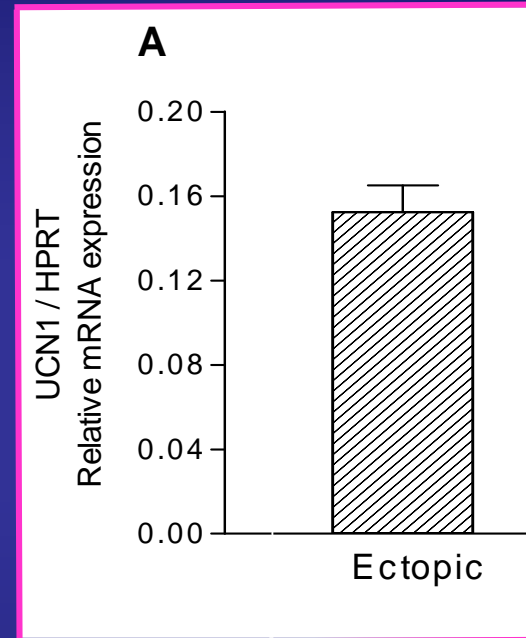
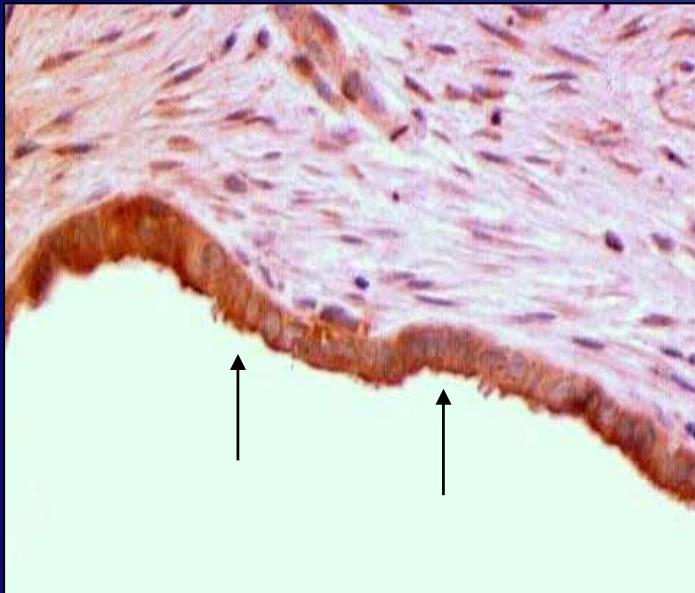


# Endometriosis and CRH



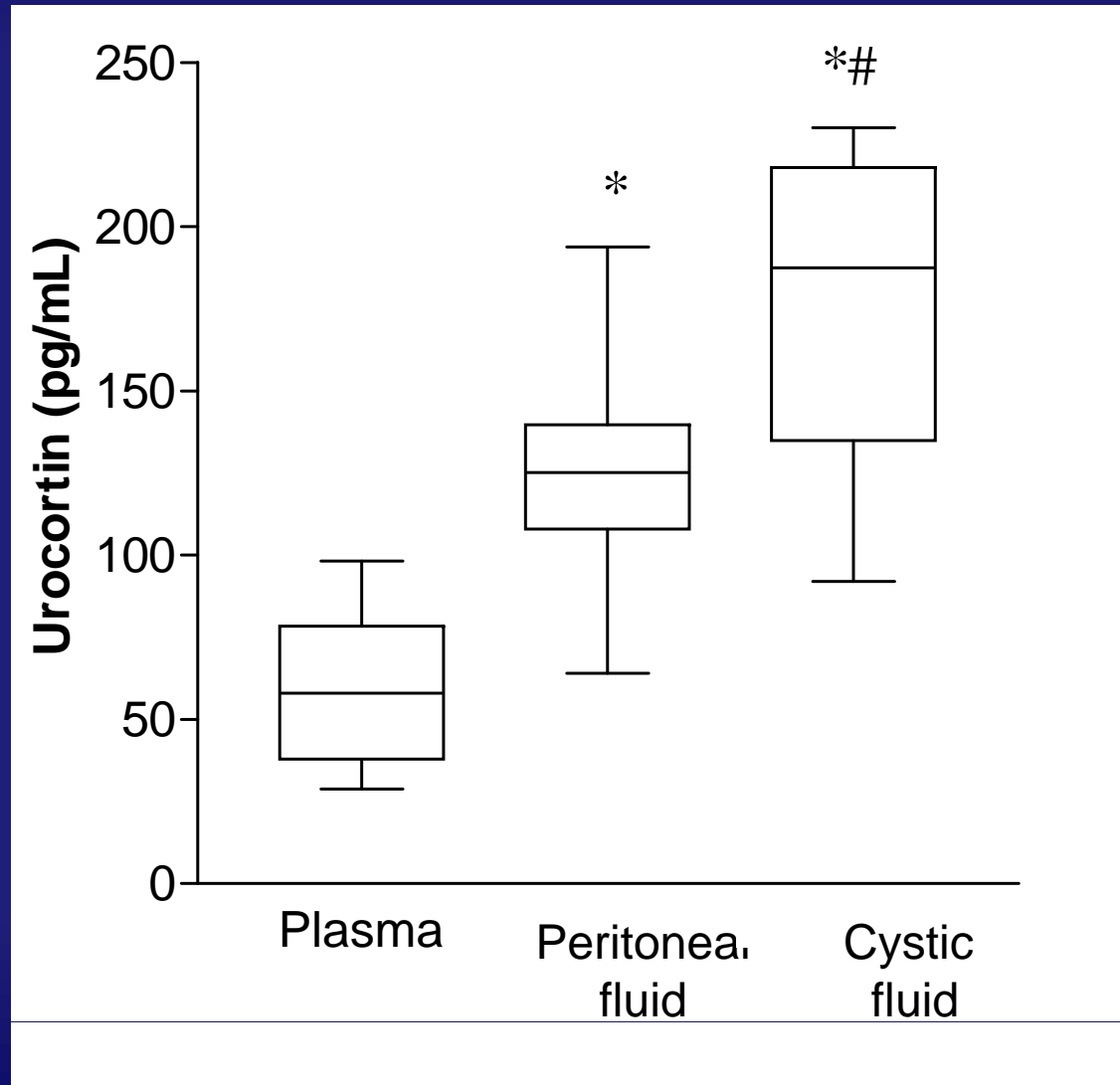
# Endometriosis and Ucn

Ectopic tissue

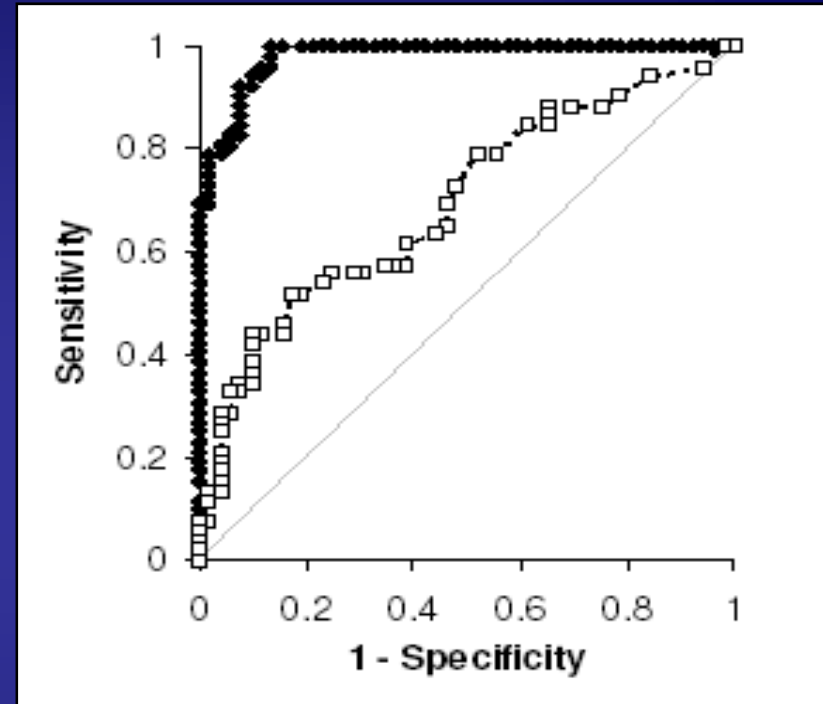
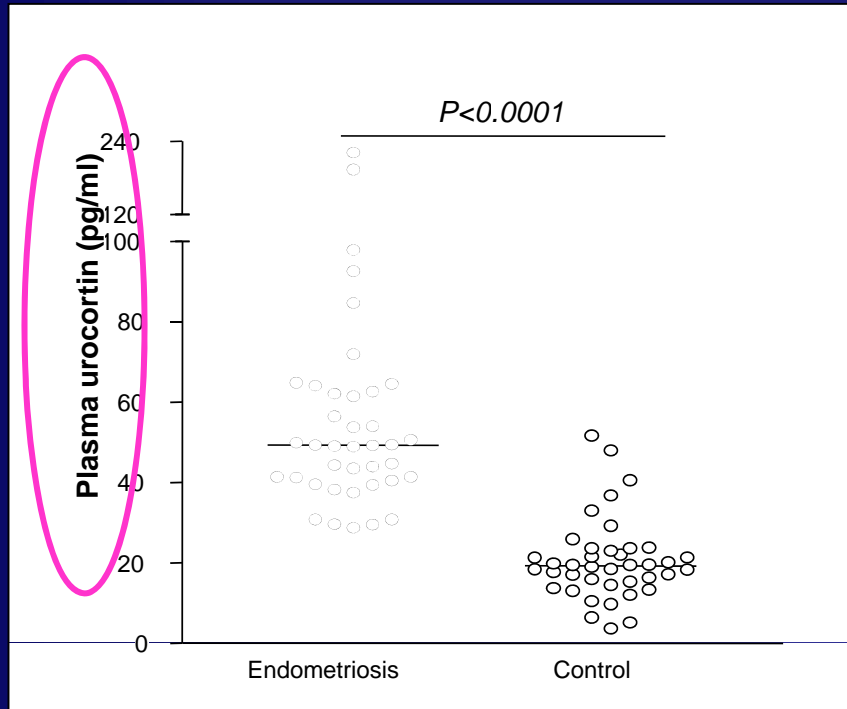


Ectopic tissue express Ucn mRNA

# Urocortin levels in ovarian endometriosis

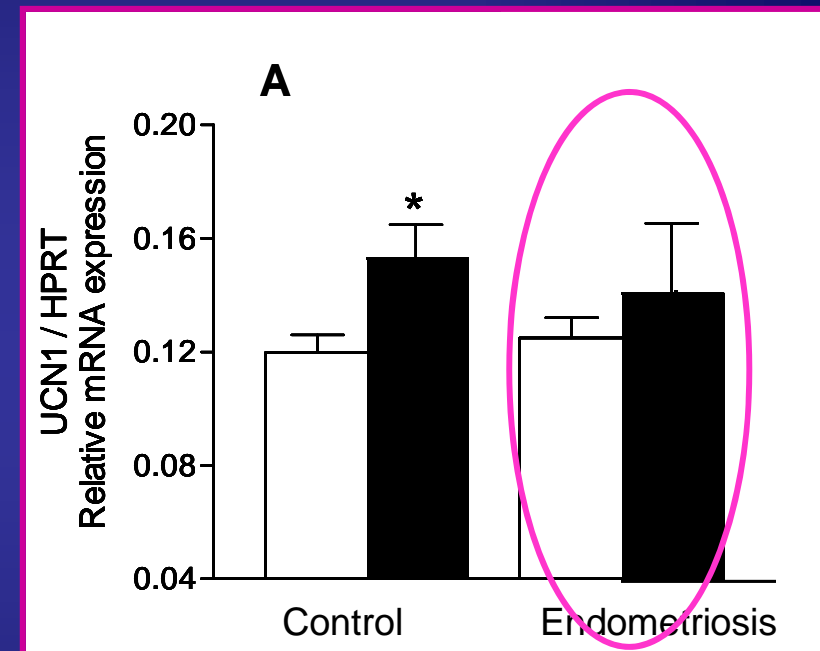
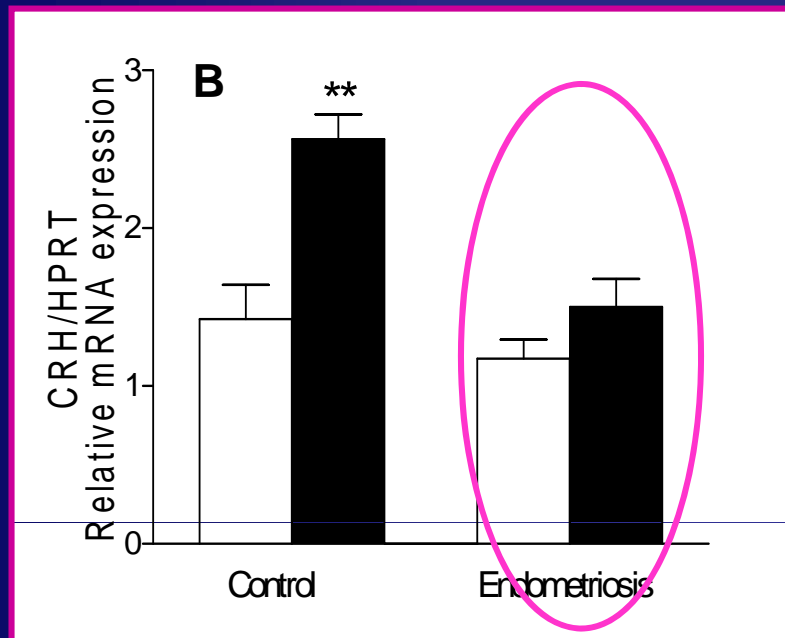


# Endometrium and Ucn



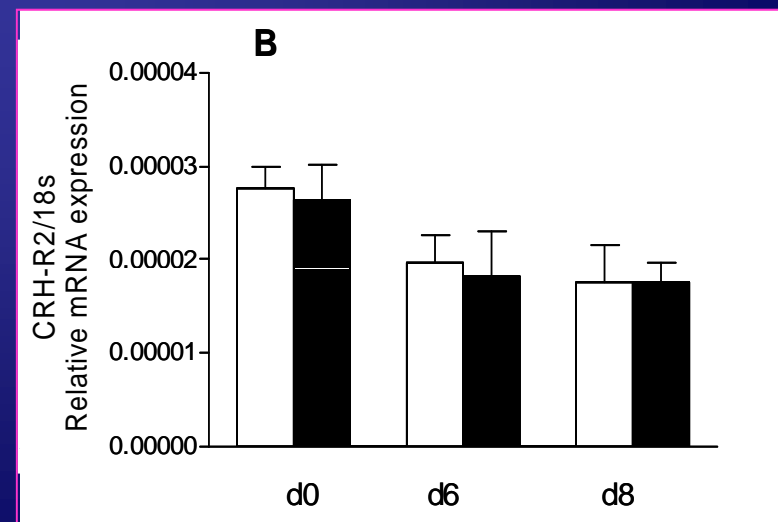
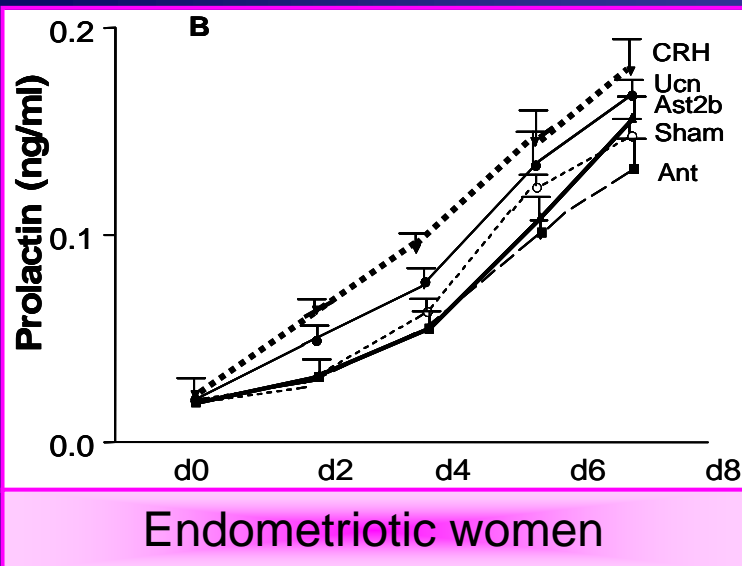
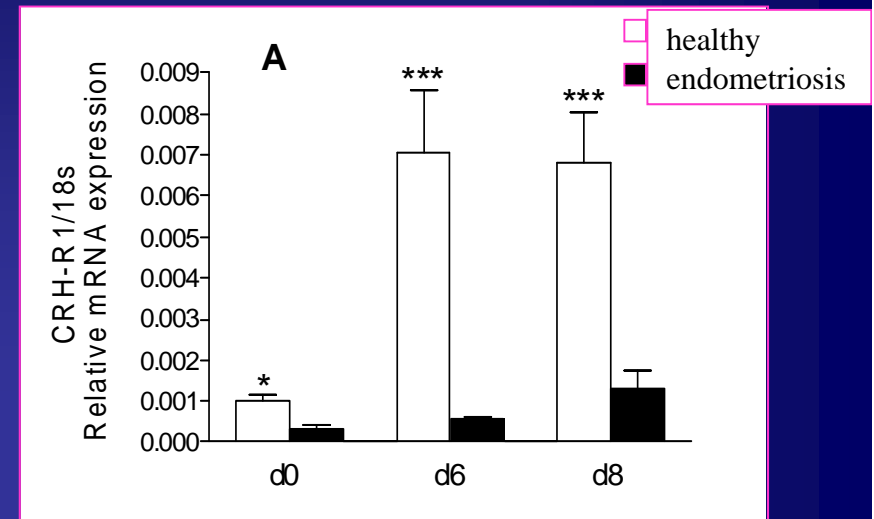
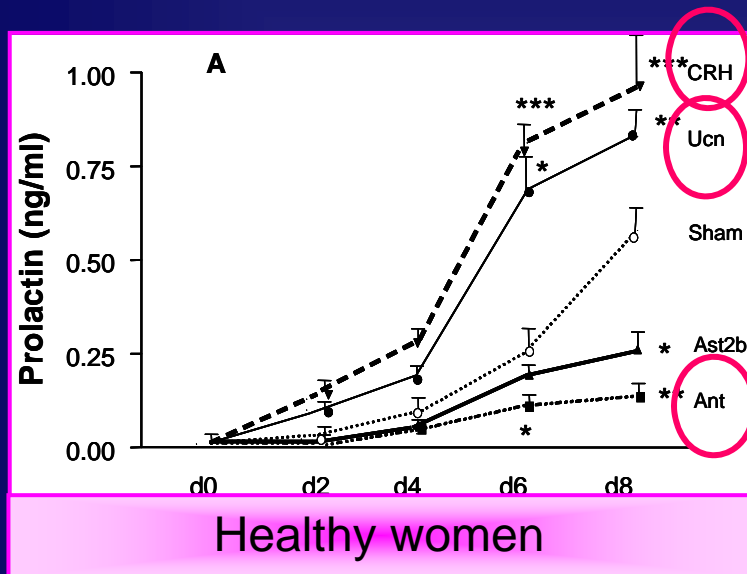
Ucn level detects 88% of cases of endometrioma with a specificity of 90%

# Eutopic endometrium in endometriosis: impaired CRH and Ucn mRNA expression





# Eutopic endometrium in endometriosis: role of CRH and Ucn in *in vitro* decidualization



## Role of CRH and Ucn in endometriosis

CRH and Ucn showed aberrant cycle-related changes in endometriosis

The lack of increase of CRH and Ucn during secretory phase may be involved in a deranged decidualization

Endometriotic cells show a deranged expression of CRH-R1



Infertility

# CRH-family

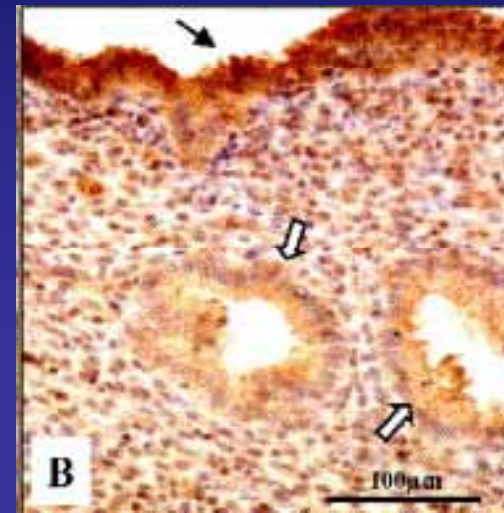
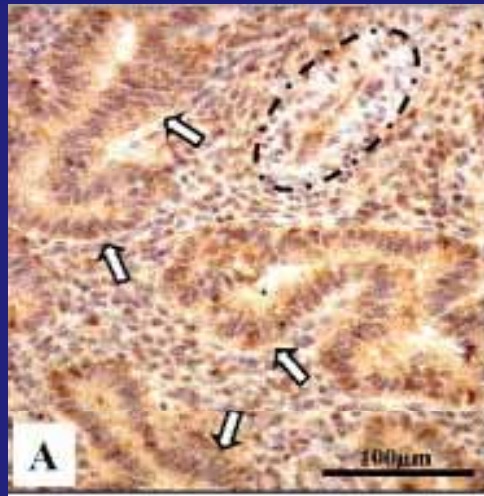
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<b>urocortin 3 (or stresscopin)</b> <i>Lewis et al., PNAS U S A. 2001</i>	<b>38</b>	<b>26</b>

# Endometrium and Ucn2/Ucn3

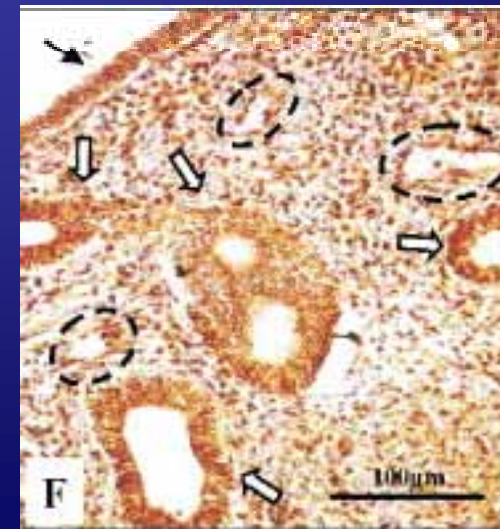
Proliferative phase

Secretory phase

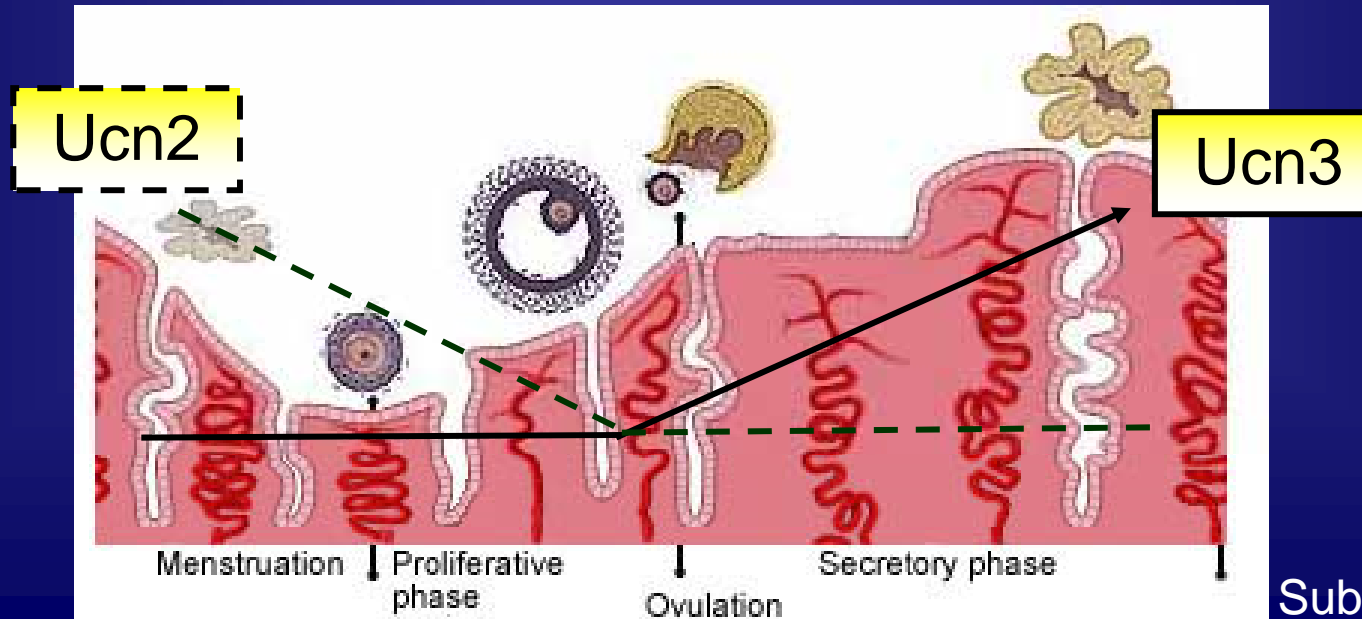
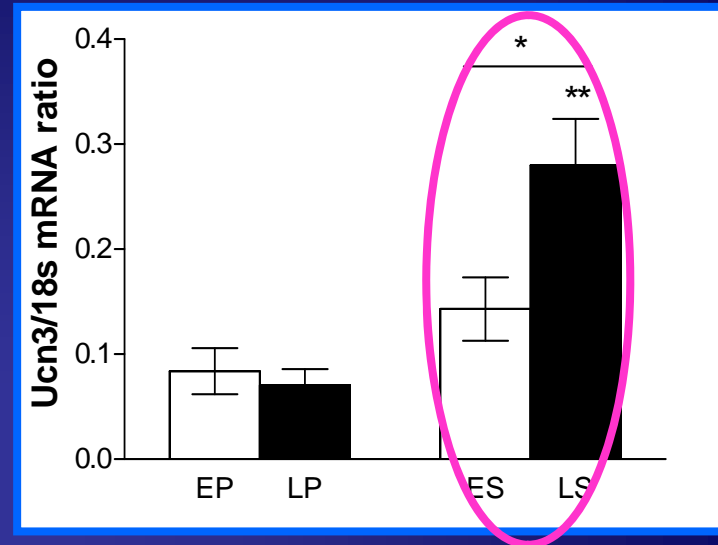
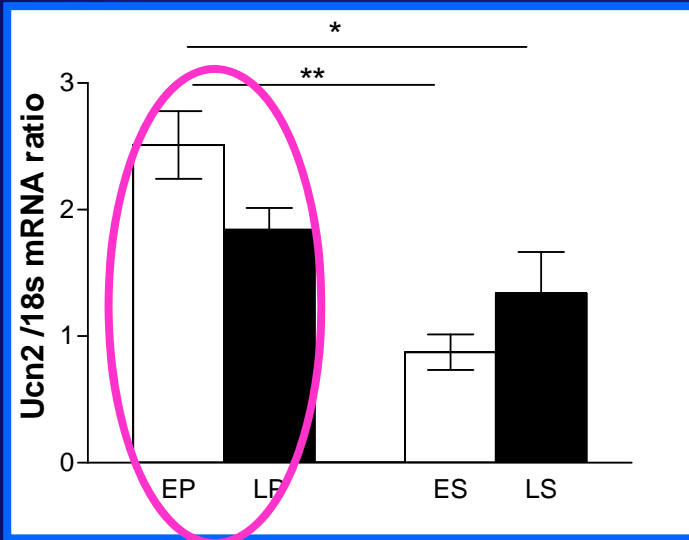
UCN2



UCN3



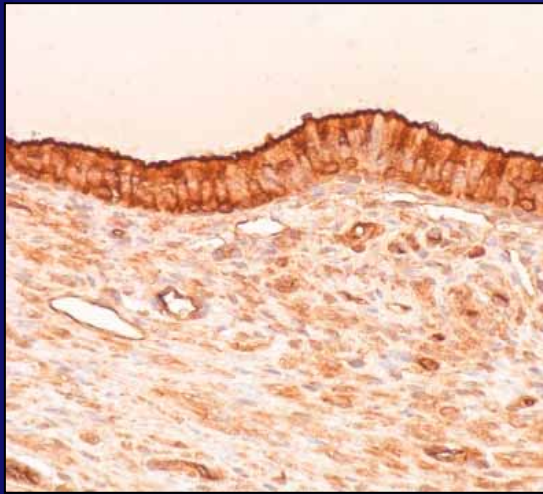
# Endometrium and Ucn2/Ucn3



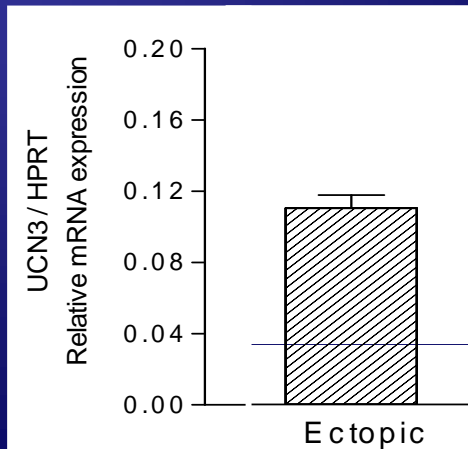
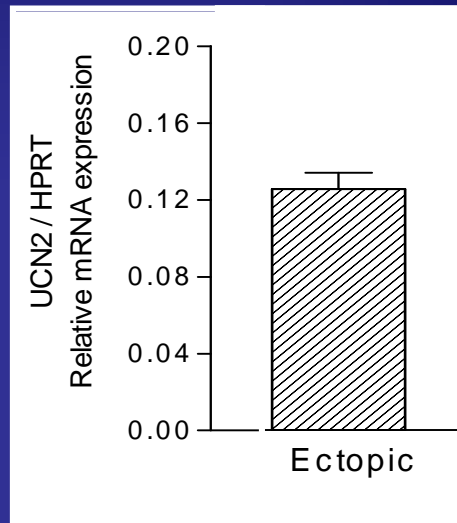
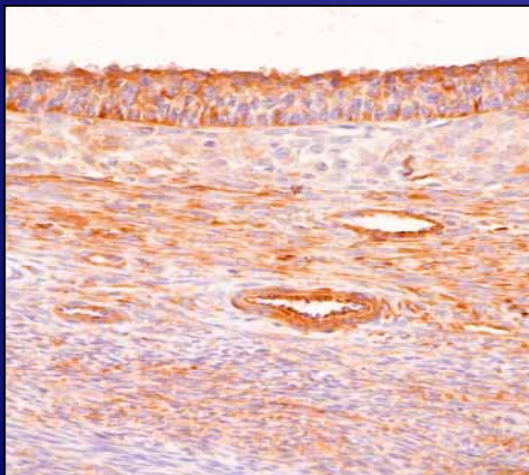
# Endometriosis and Ucn2/Ucn3

Ectopic lesions express and localize Ucn2 and Ucn3 mRNA

UCN2

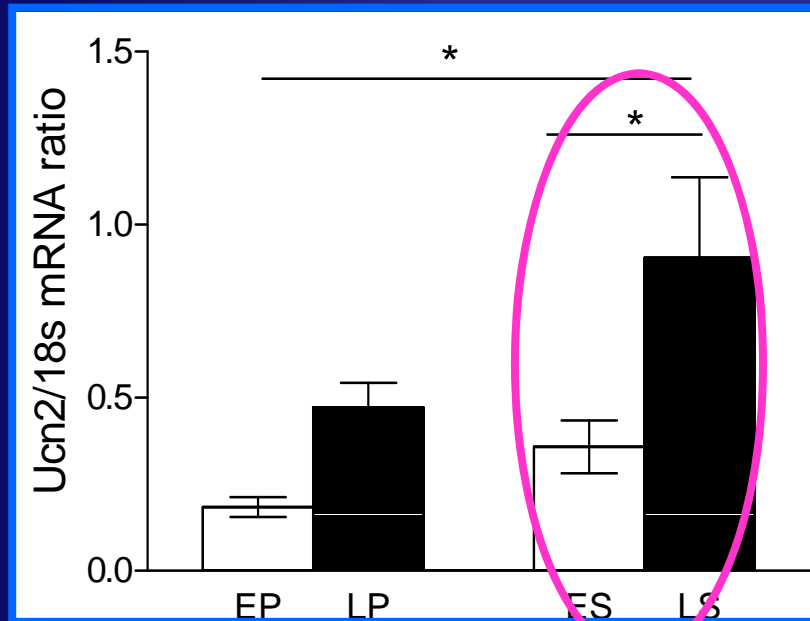


UCN3

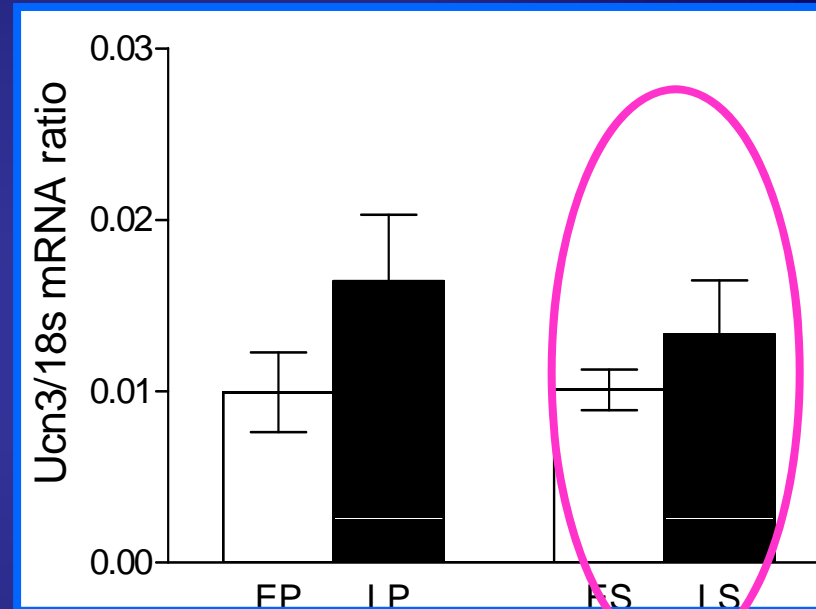


Submitted 2010

## Ucn2 /Ucn3 in endometrium of endometriotic women



Ucn2 mRNA expression showed the highest expression in the late secretory phase



Ucn3 mRNA expression did not change during endometrial cycle

# Urocortin2 and inflammation



Intestinal tract



Proinflammatory  
action

Moss et al. Gut 2007

Urocortin 2

Antiinflammatory  
action



Placenta



Proinflammatory  
action

Submitted 2010



# Urocortin 3 and inflammation



Intestinal tract

Saruta et al., Peptides 2005

Urocortin 3



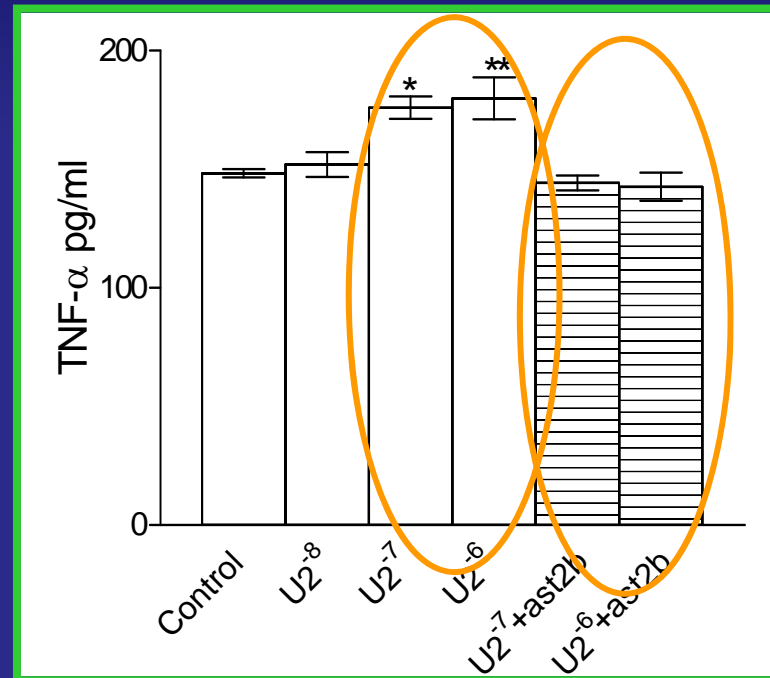
Placenta

Submitted 2010

Antiinflammatory  
Action



## Effect of Ucn2 in cultured endometrial stromal cells on cytokines secretion

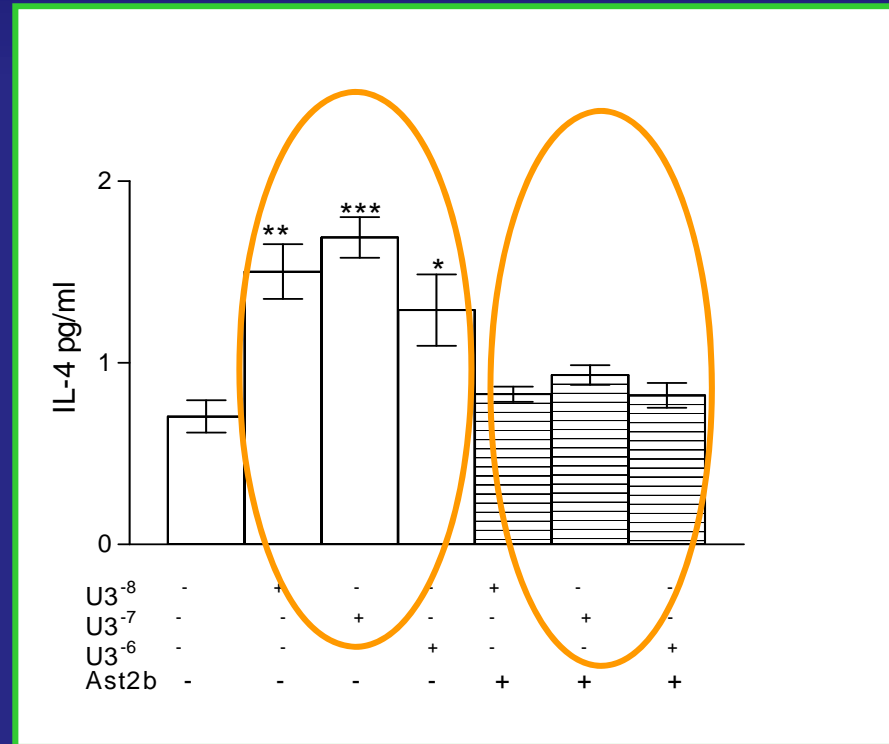


Ucn2 treatment increased TNF- $\alpha$  release in a dose dependent manner

Astressin 2b reversed Ucn2 effects



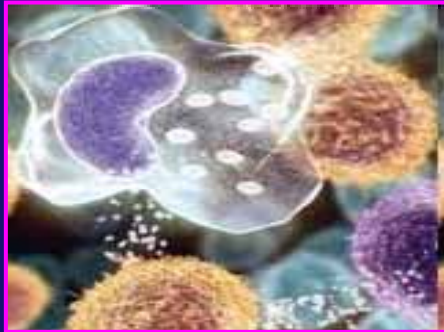
## Effect of Ucn3 in cultured endometrial stromal cells on cytokines secretion



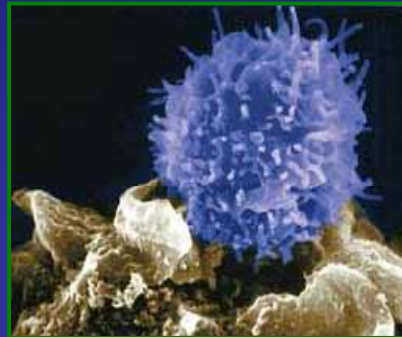
Ucn3 treatment increased IL-4 release

Such effect was reversed by astressin 2b

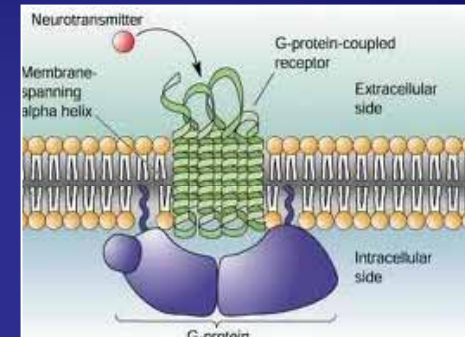
# Neuroendocrine elements in endometriosis



CRH



Urocortin

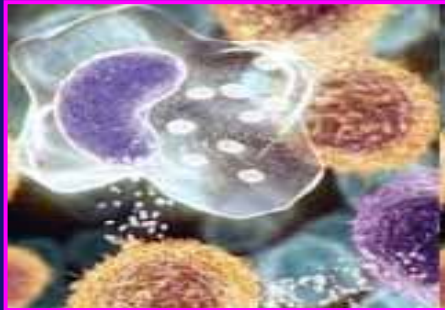


CRH-R1

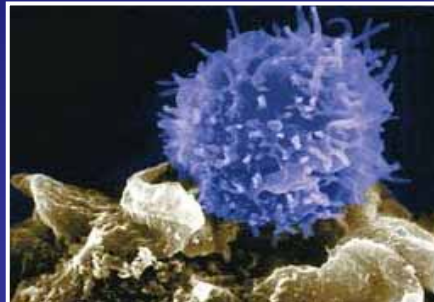
Decidualization

Infertility

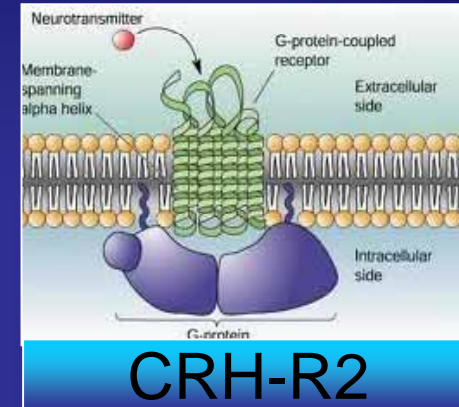
# Neuroendocrine elements in endometriosis



Urocortin 2



Urocortin 3



CRH-R2

Inflammation

Pain

# Acknowledgements

## Laboratory group



## Clinicians



# Key points of established knowledge

1. Endometrial dysfunction is a cause of infertility in endometriosis
2. Increased abortion rate in endometriosis is related to endometrial dysfunction
3. Estrogen/Progesterone act on endometrial functions through locally produced growth factors/peptides and the alteration of this paracrine actions occurs in endometriosis
4. Endometrial cells implant in peritoneum is driven by immunomodulatory factors, facilitating neoangiogenesis and decreasing apoptotic process
5. Ectopic endometrium expresses some of the growth factors/peptides functionally produced by eutopic endometrium, with an overfunction of proinflammatory pathways

## Unresolved areas

**A. Increased estrogen activity or progesterone resistance remain to be demonstrated in a large scale study by gene mutations / polymorphism evaluations**

**B. Has ectopic endometrium identical characteristics of the eutopic tissue?**

**C. The use of endometrial cells or factors for the noninvasive diagnosis of endometriosis is under investigation**

**D. The endometrial action of estroprogestins in the treatment of endometriosis is not yet defined**



# Further research questions (1)

**Epidemiology:** it is not clear how is really endometriosis diffuse in the Developing Countries

**Classification:** is deep infiltrating endometriosis a separate disease or just a developmental stage?

**Pathogenesis:** How critical are the serum estrogen/progesterone level changes in creating the endometrial substrate for endometriosis?

How relevant are the epigenetic influences on estrogen/progesterone receptors?

Is infertility related to specific endometrial pathways dysfunction?

**Diagnosis:** Endometrial cytology may have a future for the diagnosis of endometriosis? Urinary or serum markers have sufficient specificity? Does uterine doppler analysis reveal some specific changes?

## Further research questions (2)

### Treatment:

Do surgical procedures contribute to spread endometriotic cells?

Does it exist an optionally timing for the surgical treatment of endometriosis?

Is induction of ovulation a treatment at risk for women with endometriosis?

How to prevent the recurrency?

Women's Health: How is the gestational outcome?

How is he climateric period?

How is the postmenopausal period in Women with endometriosis?