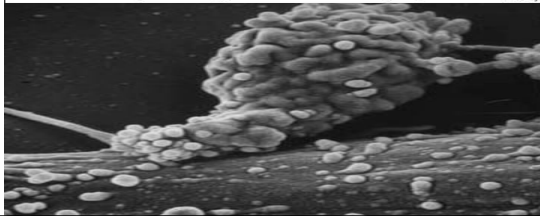
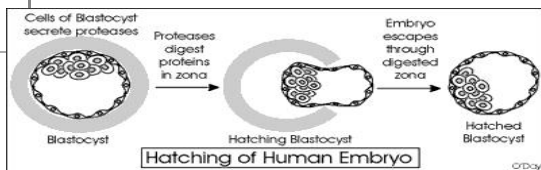


***Cavum uteri –
best place for the embryo***

Atanas Shterev, MD
Ob/Gyn Hospital "Dr. Shterev"
Sofia, Bulgaria

Hatching of human embryo

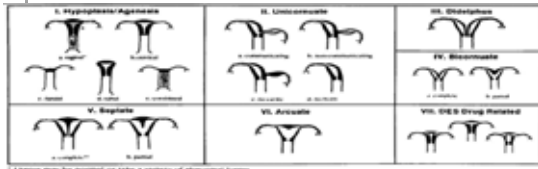


***Factors influencing implantation
in cavum uteri***

- ***Mechanical – congenital or acquired malformations of cavum uteri***
- ***Inflammation***
- ***Immunological factors***
- ***Endometrium structure and communication mother – embryo***
- ***Endometriosis***
- ***PCOS***
- ***Others***

**Mechanical factors
the uterus and fertility**

■ **Congenital**



- Endometrial polyps – over 1,5 cm
- Intrauterine adhesions
- Myoma nodis

**Endometrium structure and
communication mother – embryo**
Endometrial thickness

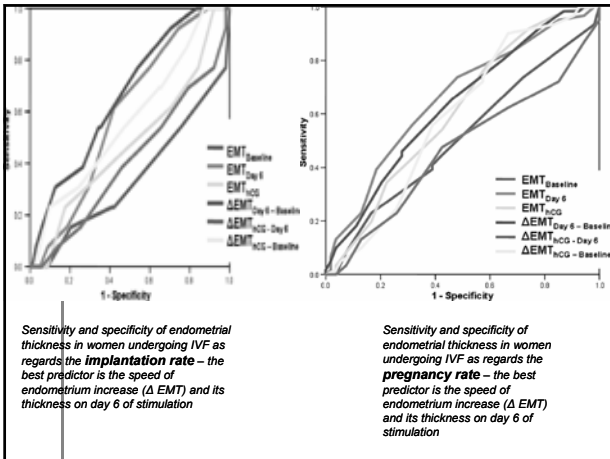
- Under the influence of hormones, the endometrium undergoes some typical changes during the menstrual cycle
- Plenty of clinical trials suggest that the thickness and structure of endometrium are of great significance for the outcome of ART
- However data about the impact of these factors for the outcome of the **intrauterine inseminations** are limited
- **Study** : 249 couples underwent 562 IUI -H. The impact of various factors has been investigated: age, duration of infertility, succession of attempts, sperm analysis deviations, **endometrial thickness**
- **Results**: 15 % pregnancy rate achieved. **As regards the endometrial thickness – it is significantly higher in the pregnant women group compared to the group of non-pregnant women (10,1±3,0 vs. 7,7±3,5).**

Seddighi Esmailzadeh et al., Ferti Steril 2007;88:432-7

**Endometrium structure and
communication mother – embryo**
Endometrial thickness

- A number of factors contributes to pregnancy rates
- As regards the endometrium, the probable criteria are the endometrial blood flow, its type and thickness
- Different trials suggest different significance degree of endometrial thickness for the final outcome of ART
- **A trial on 132 cycles** examines the endometrial thickness before stimulation, on day 6 and on the hCG day
- **Results**:
 - in pregnant women the endometrial thickness is higher on day 6 and on the hCG day compared to the non-pregnancy group
 - In the pregnant women group, the degree of increase of endometrial thickness is higher from the baseline to day 6 of stimulation
- **Conclusion**: endometrial response and its thickness at an early stage are a better prognostic criteria for the IVF outcome compared to its thickness at the beginning and the end of stimulation

Grant D. et al., Fertil Steril, 2007;88:74-81



Endometrium structure and communication mother – embryo
Vascularization of endometrium

- *The good vascularization of endometrium is of great importance for its receptiveness as regards the implantation and the good communication between mother and embryo*
- *Disorders in vascularization of endometrium has an impact on the frequency of pregnancy complications such as early abortions, preeclampsia or intrauterine retardation of the foetus*
- *The endometrial preparation for implantation begins during menstrual period and continues after conception, under the influence of estrogens, progesterone, hCG, cytokenes and growth factors, produced by the corpus luteum, the endometrium and the implanting embryo*
- *The implantation receptiveness is a result of the steady interaction of growth factors such as vascular growth factor, vascular-endothelial growth factor, placenta growth factor and its receptors*



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| | <p>Endometrium structure and communication mother – embryo Vascularization of endometrium</p> |
| | <ul style="list-style-type: none"> ■ Study: 32 women with interuptio during the first trimester. A comparison between vascularization and angiogenic factors at decidua basalis, decidua parietalis and decidua at the implantation place level has been made ■ Results: the pregnancy hormones and extravilose trophoblast influence the vascularization through increase of the lumen and the surface of the vessels at mm² and bigger intensity at the implantation place compared to decidua basalis, decidua parietalis. These changes correlate with a difference in gene expression and those of specific receptors in the different types of decidua ■ Conclusion: under the influence of hormones produced during pregnancy and of the extravilose trophoblast, an expression of different genes and receptors is provoked at the different types of decidua and also in different terms of pregnancy. This determines the extent of vascularization of the tissues. The successful implantation is a result of the stable and well-balanced hormonal support of pregnancy <p style="text-align: right; font-size: small;"><i>Margreet Plaisier et al., 2007; 88: 176-187</i></p> |

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| | <p>Endometrium structure and communication mother – embryo endometrium produced substances</p> |
| | <ul style="list-style-type: none"> ■ <i>Leptin – the first known adipocide hormone</i> ■ <i>Influences not only the functions of hypothalamic – pituitary – ovarian axis but also reproductive tissues such as endometrium and placenta</i> ■ <i>The lack or shortage of leptin (genetically or receptor-determined) as well as the increased levels (obesity) are related with reproductive failures</i> ■ <i>The import of exogenous leptin has a reversibile effect and restores the reproductive abilities</i> ■ <i>The normal reproductive function requires optimal leptin levels</i> ■ <i>The prolactin, produced by the endometrial cells (in bigger amounts at the middle and late secretion phases) plays an important role for a successful implantation</i> |

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| | <p>Endometrium structure and communication mother – embryo endometrium produced substances</p> |
| | <ul style="list-style-type: none"> ■ Study: the influence of exogenous leptin on secretion of prolactin from endometrial stromal cells from rat ■ Results: <ul style="list-style-type: none"> - leptin and its receptors are being expressed on the embryo and are important for the embryo – mother dialogue during implantation and is necessary for the pre-implantation development of embryo - In mice trophoblast invasion is stimulated with application of leptin in vitro (experimental model) <p style="text-align: right; font-size: small;"><i>Kamani et al., Feril Steril 2007; 88: 193-9</i></p> |

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| | <p>Endometrium structure and communication mother – embryo <i>Increase of endometrium receptiveness</i></p> |
| | <ul style="list-style-type: none"> ■ \leftarrow-hCG stimulates corpus luteum to produce progesterone during early pregnancy thus supporting implantation and providing systematic communication between mother and embryo ■ During the embryo's forward movement in the fallopian tube and prior to implantation, it directly communicates with the endometrium via different factors. Thus it signals its existence and prepares the receptiveness of the endometrium. No communication of this kind occurs during an embryo transfer ■ Such factors are detected in the supernatants of the embryo's culture medium |

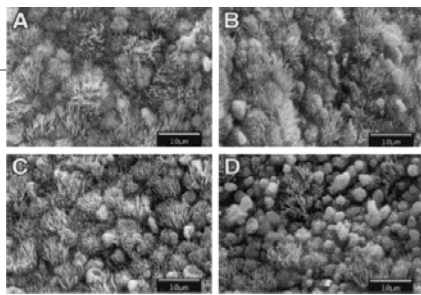
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| | <p>Endometrium structure and communication mother – embryo <i>Increase of endometrium receptiveness</i></p> |
| | <ul style="list-style-type: none"> ■ Study: evaluation of the impact of injecting supernatant in cavum uteri taken from media where embryos were cultivated prior to blastocyst transfer for the outcome of ART. ■ Method: 23 women procedure group versus 25 control group. Women with one or more than one failures. ■ Results: the level of implantation and pregnancy and the \leftarrow-hCG values are statistically significantly higher in the group of patients with endometrial stimulation with supernatant compared to the control group <p style="text-align: right; font-size: small;"><i>Sakae Goto et al., Fertil Steril 2007; 88: 1339-43</i></p> |

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| | <p>Endometrium structure and communication mother – embryo <i>pinopods and implantation window</i></p> |
| | <ul style="list-style-type: none"> ■ For the successful implantation-the embryo and the endometrium should reach a simultaneous maturity ■ Endometrial maturity is regulated by the ovarian hormones E2 and P4 and make the endometrial receptiveness ready for the implanting embryo ■ Endometrial morphology is often a subject of histological researchers for its maturity and implantation readiness whereas great expectations are assigned to the evaluation of presence of pinopods on apical surface of endometrial cells detected via electronic microscope |

Endometrium structure and communication mother – embryo
pinopods and implantation window

- **Study:** evaluation of the availability of pinopods on endometrial tissue on 25 women with regular cycles, 15 with regular period, 9 – interrupted, 1 on GnRH and hormonal treatment
- **Results:** pinopods are available during the whole luteal phase of menstrual period, up to 11 g. w. and during hormonal treatment
- **Conclusion:** pinopods can be discovered in the endometrium, put under the progesterone influence for a long period of time and thus cannot be a marker for the implantation window of the human endometrium

Clair Quinn et al.: Fertil Steril; 2007; 87: 1015-21



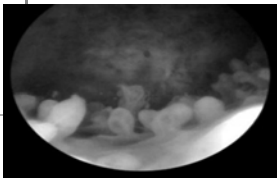
Pinopod expression was scored as follows: (A) absence of pinopods; (B) covering <25%; (C) covering 25%–50%; or (D) covering 50% of surface epithelium.

Inflammatory changes
Chronic endometritis

- The chronic endometritis (CE) is often without symptoms or occurs with weak and unspecific complaints – pelvic pain, dysfunctional uterine bleedings, dyspareunia
- Although it is clinically asymptomatic, CE may **compromise female reproductive abilities for spontaneous or in vitro conception**, to provoke spontaneous abortion or premature delivery
- Histologically it is diagnosed on the great number of neutrophils and plasmatic cells infiltrating the endometrium
Ettore Ciccinelli et al., F&S, 2007; 89: 677
- Tuberculous infection – specific endometrial view – whitish precipitation on endometrium and scarce ovulation. After a 6 month antibiotic treatment – different situation. After a year – spontaneous conception and a delivery to term
Atul Kumar et al.: F&S; 89: 701

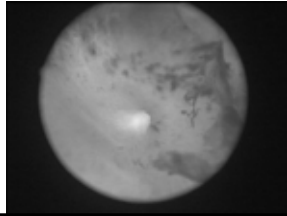
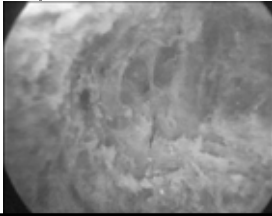
**Inflammatory changes
Chronic endometritis**

- A prospective **study** on 2190 women who underwent hysteroscopy following different indications investigated the causes of chronic endometritis
- The hysteroscopy signs hiperemia, mucose oedema and micropolypose have 93,4% diagnostic precision. In such cases a lavage from cavum uteri has to be examined
- **Results:**
 - In 73% at least one microorganism has been detected
 - The most frequent contagious agents are common bacteria: *E. coli*, *Strept.*, *Staphyl.*, *Enter. Faec.*, and *Mycoplasma*
 - *Ureaplasma urealyticum* – 10%, *Chlamydia* – only 2,7%;
 - Only in 32,6% equal contagious agents have been isolated from the cavum uteri and the vagina



Hysteroscopy view of chronic endometritis – endometrium is thick, oedematose, hiperemic, covered with micropolyps less than 1 mm

Chronic tuberculosis endometritis – prior to an following anti-tuberculosis therapy



**Inflammatory changes
Treatment of hydrosalpings with Essure**

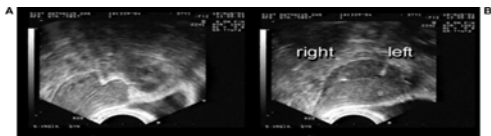
- The negative impact of hydrosalpings on the IVF outcome has been many times demonstrated by a series of studies – direct embryotoxic effect, mechanical wash-out of the cavum during implantation, changes in endometrial receptiveness
- Salpingectomy or electrocoagulation of fallopian tubes via laparoscopy or laparotomy – pros and cons
- The contemporary approach – the Essure system for proximal occlusion of the ostiums of the affected fallopian tubes with plastic implants



Inflammatory changes
Treatment of hydrosalpings with Essure

- **Study:** to evaluate the effect of the Essure system in 5 women with hydrosalpings prior to IVF
- **Results:** the Essure system has been successfully placed bilaterally in 2 women and in other 2 – unilaterally. Without complications. No pregnancies achieved
- **Conclusions:** the placement of the Essure system via hysteroscopy is minimally invading occlusion of the proximal ostium of fallopian tubes. It is specially recommended to patients who are contraindicated for laparotomy or laparoscopy

Jason Hittkari et al., Fertil Steril: 2007;88: 1663-6



Inflammatory changes
Treatment of hydrosalpings with Essure

- **Case report I:** the placement of a system in 3 women has resulted in normal and on term pregnancies; delivery occurred in 37 g. w. and 39 g. w. During the first trimester the implants have been visualized well separated from amniotic membranes on at least 10 mm of horizon-decidual tissue under US control
- **Case report II:** After an occlusion of the ostiums of inflamed fallopian tubes secondary to hydrosalpings with the system Essure
- The two pregnancies were studied, ran without complications with normal term delivery.

J. Korin et al.: Fertil Steril; 2007: 87: 1212

A. Whitney Moses, Fertil Steril: 2007: 89: 724

Immunologic factors
Cell mechanisms

- CD4+CD25+ subpopulation of T-cells is described as responsible for the suppression of autoimmune response and tolerance towards allogenic organ transplants
- The embryo as a carrier of paternal genes is alien for the maternal organism and therefore accepted as an allograft. Pregnancy is a real challenge to tolerate the alien paternal genes
- Nature has created mechanism for the embryo to avoid immunologic attacks from the maternal organism, but pregnancy can be threatened if the maternal immune system is activated during the smallest signal of imbalance in its own immunosuppressive factors

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| | <h2 style="margin: 0;">Immunologic factors</h2> <h3 style="margin: 0;">Cell mechanisms</h3> |
| | <ul style="list-style-type: none"> ▪ A study comprises 25 women with recurrent implantation failures of unclear etiology origin and a control group of 34 women with normal ongoing pregnancies. It investigates the role of CD4+CD25+ subpopulation of T-cells in peripheral blood and in the decidua level in regards to the immunologic tolerance of the maternal organism towards the embryo ▪ Results: <ul style="list-style-type: none"> - In the group of women with recurrent abortions, significantly lower levels of CD4+CD25+ T-cell were discovered both in peripheral blood and the decidua. The percentage of CD25+ cells in CD4+ T-cell population at decidua level is lower compared to the control group - In the control group the percentage of CD25+ cells in CD4+ T-cell population at decidua level is much higher than the peripheral blood while in the group of women with recurrent abortions there is no major difference ▪ Conclusion: The human CD4+CD25+ T-cell population plays a major role in tolerating embryo allogenes and probably participates in pregnancy support. Data shows that CD4+CD25+ subpopulation of T-cell population can be a new approach for the treatment of habitual abortions of unclear etiology |

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| | <h2 style="margin: 0;">Immunologic factors</h2> <h3 style="margin: 0;">Antiphospholipid antibodies</h3> |
| | <ul style="list-style-type: none"> ▪ Habitual spontaneous abortions, described as three or more recurrent abortions prior to 20 g. w., are a multifactorial disorder, often of unclear etiology ▪ Immunologic disorders such as stirring of antiphospholipid antibodies are often correlated with recurrent spontaneous abortions as they provoke a trophoblast damage and thrombosis |

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| | <h2 style="margin: 0;">Immunologic factors</h2> <h3 style="margin: 0;">Antiphospholipid antibodies</h3> |
| | <ul style="list-style-type: none"> ▪ Study: The levels of lupus-anticoagulant antibodies (LAC) and antibodies against β2-glycoprotein I, annexin V and cardiolipin (ACA) were studied in 200 women with habitual abortions during different terms of their pregnancy ▪ Results: <ul style="list-style-type: none"> - Antiphospholipid antibodies can have different levels and different relationship in respect to each pregnancy trimester - ACA are related with early, while annexin V and LAC antibodies - with late abortions, the combination between them leads respectively to early and late pregnancy complications - The levels of LAC vary during pregnancy and during their continuous increase, or in combination with the increased annexin V levels, enhances the risk of habitual spontaneous abortions ▪ Conclusion: the screening of AC and LAC as well as those of annexin V during pregnancy and with appropriate treatment assigned, may decrease the frequency of recurrent early and late pregnancy complications <p style="font-size: small; margin-top: 10px;">Kathleen K. et al., Fertil Steril 2007; 88: 1462-4</p> |

PCOS
The Role of Homocysteine

- Major part of women with PCOS is insulin-resistance with a compensatory hyperinsulinemia
- This reflects in increased serum levels of homocysteine
- Homocysteine is related with reproductive failures (implantation failures and early abortions) because of: oxidative stress of the vascular endothelium, thrombocytes activation, reduction of blood flow at the endometrium level
- The administration of metformin in such patients favourably influences insulin resistance but also decreases the vitamin B12 and folic acid levels. This respectively leads to increased levels of homocysteine
- Necessity for a combined administration of vitamins and metformin for reducing the homocysteine levels in insulin resistant PCOS

PCOS
The Role of Homocysteine

- **Study:** 102 women with PCOS and insulin resistance treated with metformin, vitamin B12 and a combination therapy
- **Results:** the combination of metformin and vitamin B12 leads to better results as regards the ongoing pregnancies due to the increase of the homocysteine level
- **Conclusion:** women with PCOS and insulin resistance have increased homocysteine levels which can be closely related to the decreased implantation levels and frequent abortions even in the cases of optimal stimulation or IVF
- Supplementing vitamin B12 and metformin in combination contributes to the sensitive decrease of plasma homocysteine levels and improvement of reproductive results

Morey S. et al., Fertil Steril, 2007; 88: 227-30

PCOS
**the influence of insulin sensibilizator
Pioglitazone**

- Hypothalamus – pituitary – adrenal axis (HPA) is hyperfunctional in central and peripheral levels in women with PCOS. Hyperinsulinemia is connected with hyperactivity of suprarenal ACTH.
- **Study:** To investigate the role of insulin in the distress of hypothalamus – pituitary component of HPA axis in women with PCOS through hormonal and lipid tests. TTT Tests and the influence of insulin sensitizer Pioglitazone after a 4 month treatment
- **Results:** Pioglitazone does not influence ACTH and the cortisole response of corticotropin-releasing factor. However, following treatment, a reduction of corticotropin-releasing hormone dependent production of androstenidion and 17OH-progesterone released from the adrenal gland has been reported
- **Conclusions:** In PCOS insulin may increase the production of androgens through a direct influence on peripheral glands, without sensitive effect on pituitary response of corticotropin-releasing hormone. **The insulin sensitizor Pioglitazone favourably influences the process.**

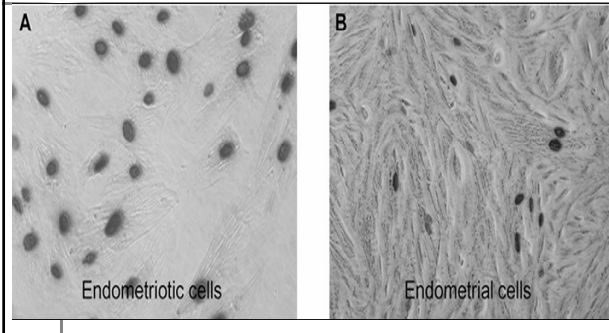
Daniela Romualdi et al., Fertil Steril, 2007; 88: 131-8

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| | <h2>PCOS</h2> |
| | <ul style="list-style-type: none"> ▪ Although PCOS is associated with insulin resistance and compensatory hyperinsulinemia in 50-70% of the cases, affecting the syndrome with insulin-sensitizing agents (e.g. metformin) with the objective to improve ovulation is controversial lately ▪ Adiposities, treated with cinnamon in vitro, improve the absorption of glucose and glycogen synthesis ▪ In humans, the cinnamon extract taken p.o. reduces fast sugars, triglycerides, LDL and total cholesterol levels in patients with type 2 diabetes ▪ Study: 15 women with PCOS and insulin resistance who were administered a treatment with cinnamon extract in capsules 1000 mg/per day for 8 weeks ▪ Results: cinnamon extract improves the insulin sensitiveness in PCOS patients without diabetes and improves the glucose absorption <p style="text-align: right; font-size: small;">Jeff G. Wang et al.; Fertil Steril, 2007; 88: 240-3</p> |

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| | <h2>ENDOMETRIOSIS</h2> <h3>Gene therapy</h3> |
| | <ul style="list-style-type: none"> ▪ Endometriosis is the presence of endometrium outside cavum uteri ▪ It is chronically recurrent disease, characterized with pelvic pain and infertility ▪ It affects between 5% and 10% of all women in reproductive age. In infertile women – 20 – 40% ▪ Endometriosis is an estrogen-dependant disease and contemporary treatment with gonadoliberrine analogues aims at decreasing the estrogen levels close to the levels of the menopausal women. However it is related with some adverse reactions such as low bone density, etc. ▪ Changes in immune system also are of major importance for the pathogenesis of endometriosis – high levels of cytokins, angiogene substances and growth factors (VEGF, IL-6, MCP-1), in a peritoneal wash-out. They provoke cell proliferation of endometroid cells in the peritoneum, enhanced angiogenesis of peritoneum, typical for endometriosis, adhesions of endometroid foci toward peritoneal membrane |

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| | <h2>ENDOMETRIOSIS</h2> <h3>Gene therapy</h3> |
| | <ul style="list-style-type: none"> ▪ A challenge to the endometriosis therapy is to find out a way for blocking the estrogen activity at an endometriosis level without affecting the system level of estrogens and respectively to avoid the heavy adverse reactions such as osteoporosis and loss of fertility ▪ Study: investigating the possibility to transform endometriosis cells through adenoviral vector ▪ Results: through a change of receptors on the endometriosis cells through the action of adenoviral vector in vitro, a decrease of proliferation and intensity of apoptosis of endometriosis cells and decreased production of cytokines. Adenoviral vectors alter endometriosis cells through provoking the expression on their surface of negative mutations of the estrogen receptors (altered estrogen receptors) which impedes the identification of estrogen from the endometriosis cells, respectively reducing the effect of estradiol on the spot without affecting its plasma levels ▪ Conclusions: Adenoviral –mediated gene therapy may become a potential therapeutic opportunity for endometriosis in future. <p style="text-align: right; font-size: small;">Essam-Eldin, Fertil Steril, 2007; 88: 462-71</p> |

(A): Endometriotic cells. (B): Endometrial cells.



ENDOMETRIOSIS *Pioglitazone therapy*

- *Endometriosis is also related with local inflammation*
- *The levels of some interleukins /IL-8, IL-6 and tumor-necrosing factor - α / are increased in peritoneal liquid in women with endometriosis*
- *The tumor-necrosing factor - α and IL-8 exacerbates the proliferation of endometrial stromal cells*
- *PPARs- γ (peroxisome proliferator-activated receptor- γ) are cell receptors which lately have been related with the regulation of inflammation, respectively the levels of inflammation factors*

ENDOMETRIOSIS *Pioglitazone therapy*

- **Study:** *the influence of Pioglitazone (PPARs- γ ligand) on the concentration of tumour-necrosing factor - α – induced production of IL-8 in endometrial stromal cells*
- **Results:** *the presence of PPARs- γ in endometrial stromal cells and Pioglitazone suppress the tumor-necrosing factor - α – induced production of IL-8 in endometrial stromal cells*
- **Conclusion:** *Pioglitazone can be an attractive treatment opportunity for endometriosis*

Yoko Ohama, Fertil Steril, 2008; 89: 311-7

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| | <i>Endometrial receptiveness factors in egg donation</i> |
| | <ul style="list-style-type: none"> ■ <i>Recipient's age – aggravated blood supply, vessels sclerosis, decreased number of E receptors, accumulation of extra cellular matrix</i> ■ <i>BMI – worsened implantation conditions and more abortions</i> ■ <i>Parameters of endometrial response:</i> <ul style="list-style-type: none"> – <i>Endometrium thickness</i> – <i>Serum E2 levels during endometrial maturation</i> – <i>Continuity of estrogen exposition</i> – <i>Application of estrogens and progesterone</i> – <i>Hypophysis suppression or activation</i> ■ <i>Smoking</i> ■ <i>Hydrosalpingis</i> ■ <i>Endometriosis</i> ■ <i>Adenomyosis</i> <p style="text-align: right; font-size: small;"><i>Sergio Soares et al., Fertil Steril, 2007; 89: 491-501</i></p> |

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| | In conclusion |
| | <p>It is obvious that implantation and normal development of pregnancy depend not only on the good embryo but also on the good conditions at a certain time in cavum uteri.</p> <p>The contributing factors in this process may vary and are numerous.</p> <p>Studying the good implantation conditions for the embryo, undoubtedly contributes to the better treatment of infertility.</p> <p>Today, we, the specialists in reproductive medicine have broad opportunities for the support of such good conditions.</p> |
