Implantation: The Immune System Partakes in the Maternal-Embryo Interaction

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Fertilization and Early Embryonic Development

- Oviduct
- Uterus
- Ovary

Fertilization

Images of developing embryos at different stages.
Window of Implantation (WOI)

Adopted from Salmonsen et al, 2009
Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures

Simon et al., 1998
Ledee-Bataille et al., 2002
Fatemi and Popovic-Todorovic, 2013
Implantation is the rate-limiting step for the success of ART.
Study of the expression profile of some specific genes in the uterus throughout the menstrual cycle

12 patients treated for infertility who volunteered to undergo uterine biopsy

11 of these patients conceived in the following cycle of IVF/ET
A total of 134 patients who responded positively to hormonal stimulation and produced fertilizable eggs

An experimental group of 45 volunteers who underwent uterine biopsy prior to their IVF/ET cycle

A control group of 89 patients subjected to the IVF/ET protocol without pretreatment

All patients exhibited similar baseline characteristics
All patients underwent at least 4 previous failing IVF cycles
Endometrial Biopsy Substantially Increases Pregnancy Rate in Patients Undergoing IVF

Barash et al. 2003, Fertil. Steril. 79: 1317-1322

Control patients (n=89)

Biopsy-treated patients (n=45)

Implantation rate: 14.2% vs. 27.7%, P=0.00011
Rate of clinical pregnancies: 30.3% vs. 66.7%, P=0.00009
Rate of live births: 23.6% vs. 48.9%, P=0.016
Favorable influence of local injury to the endometrium in intracytoplasmic sperm injection patients with high order implantation failure

Local injury of the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates

Endometrial local injury improves the pregnancy rate among recurrent implantation failure patients undergoing IVF/ICSI: a randomized clinical trial

Promoting implantation by local injury to the endometrium

Does local endometrial injury in the nontransfer cycle improve the IVF-ET outcome in the subsequent cycle in patients with previous unsuccessful IVF/ICSI: a randomized controlled pilot study

Impact of a single endometrial injury on assisted reproductive technology outcome: a preliminary observational study

Endometrial scratching performed in the non-transfer cycle and outcome of assisted reproduction: a randomized controlled trial

Endometrial injury may increase the clinical pregnancy rate in normoresponders underwent long agonist protocol intracytoplasmic sperm injection cycles with single embryo transfer
Other forms of local injury increase endometrial receptivity in human

**Hysteroscopy**

Mooney and Milki, Fertil Steril 2003, 79:637

Demirol and Gurgan, Fertil Steril 2004, 8:590

Rama Raju et al., Arch Gynecol Obstet 2006, 274:160

**Curettage**

Friedler et al., Lancet 1993, 341:1213
Hypothesis

Local injury of the endometrium increases its receptivity by provoking inflammation.
Inflammation

Injury

Cytokines/chemokines

Monocytes

Macrophages  Dendritic cells (DCs)

Proliferation, differentiation and remodeling of the tissue
Uterine Dendritic Cells are Crucial for Successful Embryo Implantation


Experimental Model
### Analysis of the effect of uDC depletion on embryo ISs

<table>
<thead>
<tr>
<th>E5.5</th>
<th>CD11c:DTR + DTx</th>
<th>Control\textsuperscript{A} (CD11c:DTR + PBS/WT + DTx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. mice with resorptions/no IS</td>
<td>13 (68%)</td>
<td>1 (5%) [1/0]</td>
</tr>
<tr>
<td>No. mice with impaired IS</td>
<td>6 (32%)</td>
<td>0 [0/0]</td>
</tr>
<tr>
<td>No. mice with normal IS</td>
<td>0</td>
<td>18 (95%) [12/6]</td>
</tr>
<tr>
<td>No. total mice</td>
<td>19</td>
<td>19 [13/6]</td>
</tr>
</tbody>
</table>

\textsuperscript{A}Note that controls were composed of PBS-treated CD11c:DTR transgenic mice and DTx-treated WT mice, with number indicated in the square brackets.

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*Plaks et al. J Clin Invest, 2008*
Our overall goal was to define the role of the immune system in improving implantation in biopsy-treated IVF patients.
Objectives

- To examine the effect of biopsy treatment on the production of cytokines as well as on the recruitment of immune cells in human endometrium
- To decipher the mechanism by which these cytokines and immune cells may facilitate endometrial receptivity
Experimental Design

Days of cycle

Experimental

Control

Endometrial samples

- Cytokine expression screen
- Abundance of immune cells

IVF treatment
Endometrial Biopsy Increases the Expression of Pro-inflammatory Cytokines

Gnainsky, Granot et al, Fertil Steril 2010
Endometrial Biopsy Elevates the Abundance of Macrophages and Dendritic Cells

Gnainsky, Granot et al, Fertil Steril 2010
Does this increase correlate with successful pregnancy?
Biopsy-induced Inflammatory Events are Associated with Positive Pregnancy Outcome

Gnainsky, Granot et al, Fertil Steril 2010
Experimental Design

Days of cycle

1  8 - 12  19  21  23

Endometrial samples

Stromal cells/epithelium

Incubation with TNFα

24h

- Cytokine expression profile
- Monocytes attraction assay
**TNFα Increases the Expression of Injury-induced Cytokines by Endometrial Stromal Cells**

**GROα**

<table>
<thead>
<tr>
<th>Control</th>
<th>TNFα</th>
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<tbody>
<tr>
<td>Pg/ml</td>
<td>±</td>
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**MIP-1B**

<table>
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<tr>
<th>Control</th>
<th>TNFα</th>
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<tbody>
<tr>
<td>Pg/ml</td>
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**IL-8**

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<tr>
<th>Control</th>
<th>TNFα</th>
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<tr>
<td>Pg/ml</td>
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**IL-6**

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<th>Control</th>
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<td>Pg/ml</td>
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**MCP-1**

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<th>Control</th>
<th>TNFα</th>
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<tr>
<td>Pg/ml</td>
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**RANTES**

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<th>Control</th>
<th>TNFα</th>
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<td>Pg/ml</td>
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**INFγ**

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<th>Control</th>
<th>TNFα</th>
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<td>Pg/ml</td>
<td>±</td>
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Gnainsky, Granot et al, Reproduction, 2014
The Effect of TNFα-induced Cytokines Secretion on Monocyte Migration

Conditioned medium of stromal cells

Gnainsky, Granot et al, Reproduction, 2014
Maturation of Monocytes

THP-1 (monocytes) → Macrophages → Mature DC → Immature DC

Analysis of cytokine secretion
# The Effect of Macrophages and DCs on the Expression of Implantation-associated Genes

### Apposition and Attachment

**MIP-1B**

Expressed by endometrial epithelium and decidual cells in the mid-secretory phase and early pregnancy  
*Jones et al., 2004*

Attracts macrophages and dendritic cells during inflammation  
*Sallusto and Lanzavecchia, 1999*

**CHST2**

Generates functional endometrial carbohydrate ligands that bind embryonic L-selectin  
*Genbacev et al., 2003*

### Adhesion

**OPN**

Involved in the attachment between the integrins of the human endometrium (avb3) and that on the trophoblast  
*White et al. 2003*

**Integrin αvβ3**

Necessary for implantation in human and mice; expressed by the endometrium as well as the blastocyst  
*Dey et al., 2004*
Macrophages Increase Expression of
Apposition-associated Genes by Endometrial Cells

Gnainsky, Granot et al,
Reproduction, 2014
Dendritic Cells Stimulate the Expression of Adhesion-associated Genes by Epithelial Cells

N=5 experiments. Each experiment was performed on the cells isolated on days 8-12 from different patients.

Gnainsky, Granot et al, Reproduction, 2014
DCs Increase the Attachment of Embryo-like Spheroids on Human Epithelial Cells

Embryo like spheroids = trophoblast cell line (SW.71)
Endometrial biopsy

Enhancement of cytokine expression (GROα, MIP-1B)

Recruitment of monocytes, macrophages & DCs (MIP-1B, GROα, MCP-1, RANTES, IL6)

Differentiation of the endometrial cells (OPN, Intgαβ3, CHST2)

Embryo attachment to a receptive endometrium

Successful implantation
Conclusions

• Endometrial biopsy triggers inflammatory events, essential for acquisition of uterine receptivity.

• These events do not take place in patients with repeated implantation failures, in the absence of this mechanical intervention.
The immediate implications of our study

• Lessens the need to transfer more than a single embryo in IVF patients, avoiding the subsequent severe complications of multiple birth-pregnancies

• Reduces the number of repeated cycles of treatment, lowering the massive exposure to stimulating hormones
**Long term implications**

**Identification of the components associated with improved implantation**

- Will lead to the establishment of biomarkers for the prediction implantation competence

- Will allow to define new potential targets for intervention and develop novel clinical tools to treat infertility when implantation fails
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Macrophages and DCs Upregulate the Expression of Implantation-associated Genes

Apposition and Attachment

- MIP-1B
- CHST2

Adhesion

- OPN
- Integrin αvβ3

Gnainsky, Granot et al, Reproduction, 2014