Recurrent Implantation Failure

Professor Antonis Makrigiannakis
Dept of Ob/Gyn
University of Crete, GREECE

WHAT IS RECURRENT IMPLANTATION FAILURE?

What is RIF

- How many attempts?
- How many embryos?
- Age?
- Embryo quality?
- Failure in the presence of potential obstacles?
RECURRENT IMPLANTATION FAILURE

- Failure to achieve a pregnancy after 3 completed fresh IVF-ET cycles (Tan et al 2005)
- Failure of \( \geq 10 \) embryos to implant
- In the era of SET/DET should the definition of RIF be revised

WHY SHOULD IMPLANTATION FAIL TO TAKE PLACE?

Implantation - key event in the establishment of pregnancy

Apposition  Adhesion  Invasion

Embryo

Endometrial stroma

Uterine epithelium

Invading trophoblast

Continuous process from conception to 22 weeks gestation
Difficulties in studying the process of implantation

Successful implantation requires “two-way interaction” blastocyst - endometrium
Non-receptive Endometrium

Receptive Endometrium

Factors implicated in RIF

Hydrosalpinx/Uterine alterations  Ovarian stimulation

Embryos (morphology and genetics)  Uterus (morphology)

Transfer procedure
RECURRENT IMPLANTATION FAILURE

- Embryo
- Endometrium

TREATMENT STRATEGIES FOR RECURRENT IMPLANTATION FAILURE

- Embryo
- Endometrium

EMBRYO

- Preimplantation genetic diagnosis
- Blastocyst transfer
- Assisted hatching
- Co-culture of embryos with endometrium
- Other methods of embryo selection
- Donor oocyte/embryo
TREATMENTS OF PROVEN BENEFIT

embryo: assisted hatching

ASSISTED HATCHING (AH)

• Meta-analysis by Seif et al (2005, Cochrane database) showed that AH produced higher pregnancy rate but not live birth rate

• Meta-analysis by Sallam et al (2003) on women with repeated IVF failure showed that AH produced higher implantation, pregnancy and ongoing pregnancy rates

TREATMENT STRATEGIES FOR RECURRENT IMPLANTATION FAILURE

• Embryo

• Endometrium
**HYSTEROSCOPY**

- RCT by Demirol & Gurgan (2004)
- 421 women with 2 or more IVF failures
- 56 out of 210 (26%) women with normal HSG had intrauterine lesions detected by office hysteroscopy, and treated
- The subsequent pregnancy rate in the treated group (30.4%) and the group with normal hysteroscopy (32.5%) was significantly higher than the group who did not undergo hysteroscopy (21.6%)
HYDROSALPINGS & IVF

- The live birth rate of patients with hydrosalpinges undergoing IVF is only one-half that of women who do not have hydrosalpinges.

- Salpingectomy prior to IVF in women with hydrosalpinges improves pregnancy, implantation and live birth rates.

ENDOMETRIUM

- Hysteroscopy
- Hydrosalpinges
- Reproductive immunology
  - Novel approaches

Histopathology-Immunology of implantation site

T=interstitial EVT, E=intravascular EVT, K=γδNK cells, M=macrophage, L=T cells
The Th1/Th2 theory
Schematic representation of maternal immune response during normal pregnancy

Th1/Th2 balance in Normal Invasion

Th1/Th2 balance in failed invasion
**IVIG ?**

- The only properly conducted prospective RCT by Stephenson & Fluker (2000) involving 51 women with 2 or more IVF failures showed IVIG has no benefit.

**STEROIDS ?**

- A meta-analysis of 13 RCTs by Boomsma et al (Cochrane database 2007) showed no evidence of benefit of routine use in women undergoing IVF +/- ICSI.

**ACA – anticardiolipin antibodies**

- Two studies showed a higher prevalence of ACA in women with RIF (Kaider et al 1996, Qublan et al 2006).

- However, the only RCT on the use of heparin and aspirin in women with RIF tested + for ACA showed no benefit (Stern et al 2003).
Aspirin

• A systematic review and meta-analysis on the use of low-dose aspirin showed no benefit of its use in IVF programme (Gelbaya et al, Human Repro Update 2007)

TREATMENTS OF PROVEN BENEFIT

Endometrium: hysteroscopy
salpingectomy

ENDOMETRIUM

• Hysteroscopy
• Hydrosalpinges
• Reproductive immunology
• Novel approaches
Endometrial Receptivity

Repeated endometrial biopsies in the cycle immediately preceding IVF treatment significantly increased (~doubled) the implantation, pregnancy and live birth rates in women who had one or more IVF failure.

Barash et al, Fertil Steril 2003

Intrauterine PBMCs administration

Intrauterine administration of autologous PBMCs promote clinical pregnancy, implantation and live birth rates in patients with repeated failure of
IVF-embryo transfer

41.2% vs 11.1% and 23.4% vs 4.1% and 35.3% vs 5.5%

Yoshioka et al, 2006

Does Intrauterine Administration of PBMCs Pretreated with CRH Promote Implantation Rates in Patients with RIF?
Background

- Implantation sites in rat uterus contain increased CRH concentrations.
  Makrigiannakis et al, JCEM 1995
- EVT cell line expressing FasL induces apoptosis of activated T-lymphocytes
  Kauna et al, JCEM 1999

IVF PROCEDURE

SUBJECTS

97 cycles in 106 patients
All patients had experienced 3 or > failures of IVF-embryo transfer therapy without poor ovarian reserve (FSH< 12 mIU/ml)

EMBRYO CULTURE

After fertilization was confirmed the day after fertilization (day 1), the zygotes were cultured for another 2 days. For blastocyst transfer, embryos were further cultured in Blastocyst Medium with 10% of SPS. 2 or 3 blastocysts were transferred to the uterine cavity on day 5.
PREPARATION AND INTRAUTERINE ADMINISTRATION OF PBMCs

PBMCs isolation by Ficoll-Hypaque centrifugation
PBMCs collection from the interphase layer and wash with RPMI 1640
PBMCs incubation (RPMI 1640 supplemented with 10% SPS) in the presence of hCG and CRH for 48h
2 days after oocyte retrieval, freshly isolated PBMCs were immediately combined with 2-day cultured PBMCs and suspended in PBS
Administration of this cell suspension to the uterine cavity using an embryo transfer catheter

<table>
<thead>
<tr>
<th>Characteristics and clinical outcome of the patients under 38</th>
<th>CRH-PBMC treated</th>
<th>PBMC treated</th>
<th>Non-treated</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>33.1 ± 2.07</td>
<td>32.2 ± 2.17</td>
<td>32.1 ± 2.08</td>
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<tr>
<td>Number of IVF embryos number previous attempts</td>
<td>5.20 ± 1.04</td>
<td>5.0 ± 1.30</td>
<td>5.30 ± 2.06</td>
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<tr>
<td>Endometrial thickness on day of oocyte retrieval</td>
<td>10.7 ± 2.40</td>
<td>11.5 ± 1.30</td>
<td>9.5 ± 1.2</td>
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<tr>
<td>Number of embryos transferred</td>
<td>2.00 ± 1.00</td>
<td>3.0 ± 0.34</td>
<td>2.91 ± 0.41</td>
</tr>
<tr>
<td>Number of good quality embryos</td>
<td>1.91 ± 0.40</td>
<td>1.9 ± 0.30</td>
<td>2.01 ± 0.30</td>
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<td>Clinical pregnancy rate</td>
<td>70.9%</td>
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DOES INTRAUTERINE ADMINISTRATION OF PBMCs PRETREATED WITH CRH & hCG PROMOTE IMPLANTATION RATES IN PATIENTS WITH RIF?

YES!

POSSIBLE EXPLANATIONS?

Intrauterine PBMCs administration & IVF outcome in RIF patients

CRH & cytokine production

CRH added to primary cultures of PBMCs significantly increased IL-6 (Th2-type immunity) release and decreased IFN-γ (Th1-type immunity) levels in a dose dependent manner

Angioni et al, Life Sci 1993; Makrigiannakis et al in press

CRH & development of embryos

CRH is expressed in human embryos
CRHR1 is expressed in human embryos
(unpublished data)
CRH & endometrium

- CRH induces stromal decidualization and potentiates the decidualizing effect of progesterone. (Makrigiannakis et al., 1999)

- CRH regulates local modulators of the decidualization process; it inhibits the enhancer PGE2, induces the inhibitor interleukin (II) 1 and stimulates the inducer IL-6. (Zoumakis et al., 2000; Makrigiannakis et al., 1999)

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**Natural Selection of Human Embryos: Deciduating Endometrial Stromal Cells Serve as Sensors of Embryo Quality upon Implantation**

(DeBakker et al., 2000)

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**The extended role of the Decidualised Endometrium**

- Embryonal receptivity
- Embryo quality control
- Immunomodulation
- Trophoblast invasion
- Tissue haemostasis
- Oxidative stress resistance
- Menstruation
From Implantation window to Selection window

The Embryo Selection Hypothesis

The endometrium has an important role in embryo selection
- Decidual cells selectively recognize impaired embryos and inhibit implantation
- Undecidualised cells do not mount such a response.
- Ability of stromal cells to express the decidual phenotype is impaired in women with recurrent miscarriage

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Thank you for your attention