Pre-congress course 6:

The impact of the reproductive tract environment on implantation success

Late submission

Stockholm, Sweden
3 July 2011

Organised by
Special Interest Group Endometriosis/Endometrium
The impact of thrombophilia on endometrial function

Lesley Regan MD FRCOG
Department of Obstetrics & Gynaecology,
Imperial College Healthcare (I)
St Mary’s Hospital, London W2 1NY, UK

Implantation spectrum of adverse pregnancy outcome

Conception 1st trimester miscarriage PET Prematurity

↑ ↓ ↓ ↓
1st 2nd 3rd

↑ Implantation 2nd trimester miscarriage IUGR Still Birth

Defects here underlie adverse pregnancy outcomes at all gestations

Recurrent miscarriage

Aetiology
- genetic
- anatomical
- infection
- endocrine
- environmental
- immune
- thrombophilia
- unexplained

New concept of reproductive haemostasis
Reproductive actions of thrombin

Thrombin & trophoblast

↑ Angiogenesis

↑ Trophoblast apoptosis

↓ Trophoblast invasion

Defective implantation

---

Thrombin - antithrombin complexes and miscarriage

![Graph showing TAT micrograms/ml across different groups: Controls (n = 34), aPL +ve RM (n = 51), aPL -ve RM (n = 35).]

Vincent et al, Lancet 1998

---

Anti Phospholipid Syndrome - evolving story

Systemic autoimmune disease

↓ Thrombotic disorder

Acquired thrombophilic Defect

Disordered reproductive immunology

NK cells

Complement
Antiphospholipid syndrome

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Laboratory features*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent miscarriage</td>
<td>Lupus anticoagulant</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>Anticardiolipin</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>antibodies (IgG / IgM)</td>
</tr>
</tbody>
</table>

* 2 positive tests greater than 6 weeks apart

Prevalence aPL = 15% (n=6500) 2004 audit data

Caveats in Screening for aPL

- aPL - family of >20 antibodies directed against phospholipid binding proteins
  - Screen for both LA and aCL (IgG & IgM) only
  - Sample collection and processing - practical points
  - Do the best test - international laboratory criteria
  - Confirm positive tests - transient positives

Screen for 20 aPL with 95% cutoff; 64% chance of spurious +ve test

aPL and recurrent miscarriage

- 15% of recurrent miscarriers have aPL – 2% normal obstetric

- High prospective fetal loss rate of 90%
  First trimester loss after FH activity established

- Pathogenesis of fetal loss: thrombotic
aPL and Term placentae

Uncomplicated pregnancy

aPL pregnancy - thrombosis / infarction

Thrombosis in First trimester decidual vessels

Thrombosis

Treatment of aPL-related pregnancy loss

◆ Aspirin + Heparin therapy significantly improves live birth rate from 10% (untreated pregnancies) to > 70%
  
  Rai et al (1997), ARC funded RCT
  Kutteh et al (1996), controlled
  Backos et al (1999), observational
RCT : Rai, Cohen & Regan; BMJ 1997

- 90 women > 3 consecutive miscarriages
- Vast majority positive for lupus anticoagulant
- Aspirin 75 mg from positive pregnancy
- Unfractionated heparin when FH seen
- All pregnancies analysed, no patient crossovers
- Live birth 71% (32/45) aspirin/heparin
  42% (19/26) aspirin OR =3.4 CI=1.4-8.1

Antiphospholipid syndrome - effect of treatment

Gestation (weeks)

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%

% pregnancies ongoing

Gestation (weeks)

Aspirin + heparin
Aspirin alone
No treatment

LB rate 71%
LB rate 40%
LB rate 10%

after Rai et al 1997, BMJ

Arthritis & Rheumatism Council

1999-1998
Preventing recurrent miscarriage
RCT of heparin & aspirin for aPL positive recurrent miscarriage
70% Livebirth rate
Rai, Cohen, Regan
BMJ Jan 1997
Antiphospholipid syndrome - effect of treatment

Gestation (weeks)

after Rai et al 1997, BMJ

aPL - late pregnancy morbidity despite Rx

Live birth rate of 74% after Rx with aspirin & heparin (110/150)

BUT - significant late pregnancy complications

- PIH disorders
- SGA < 2500g
- Placental abruption
- Preterm delivery < 37 weeks
- Caesarean section

Backos et al. 1999, BJOG; 106:102 -107

Further clinical studies needed to reduce neonatal morbidity

Treatment of aPL-pregnancies - Meta-analysis

Relative risk for pregnancy loss

After Empson et al 2002 Obstet Gynecol
Effect of treatment on aPL-pregnancies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Live Birth Rate</th>
<th>Early Miscarriage</th>
<th>Late Pregnancy Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin + Heparin</td>
<td>10% ↓ 70%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PAPS - Updated Clinical Criteria

- ≥ 3 consecutive miscarriages < 10 weeks
- ≥ 1 unexplained death of a morphologically normal fetus ≥ 10 weeks
- ≥ 1 PTD before 34 weeks because of severe PET or placental insufficiency

Consensus statement aPL workshop, Wilson 1999

Antiphospholipid syndrome - effect of treatment

LB rate 71%  LB rate 40%  LB rate 10%

GBJ after Rai et al 1997, BMJ
**Antiphospholipid syndrome – beyond thrombosis**

- Most important treatable cause of RM

- Mechanism(s) of pregnancy loss
  - abnormal decidualisation
  - abnormal trophoblast function - poor invasion & hormone production

  ➔ adverse effects on embryonic implantation

  Di Simone et al, Ripo, Mak Francis, Brosens, Bose

**Where are these Pathological Effects of aPL Exerted?**

- Placental Vascular thrombosis: Rand et al 1997
- Endometrial receptivity: Mak IY 2002, Francis 2005

**aPL affects Decidual Differentiation – in vitro**

- aPL inhibits PRL and IGFBP-1 production by Endometrial stromal cells

  Mak I et al. 2002 J Clin Endocrinol Metab
Antiphospholipid Antibodies inhibit Endometrial Differentiation - *in vivo*

Analysis of 57 timed late-secretory endometrial biopsies using real-time quantitative PCR

Funded JSRC & Save the Baby: Francis et al 2005

Matrigel invasion assay - aPL sera Reduces Trophoblast Cell Survival
Unfractionated Heparin Promotes Trophoblast Cell Survival

Funded by WHO – Rockefeller – Royal Society

Defective trophoblast invasion with aPL+  
• Extravillous trophoblast OK  
• Poor intravascular trophoblast

8%  
61%  

aPL-  aPL+  

Sebire et al 2002
Normal implantation

Mechanism?
plugging of maternal circulation
limits blood flow
pressure-related & oxidative damage

Lack of plugging
pressure-related oxidative damage
early pregnancy failure
PET, IUGR, PTD

Actions of heparin

- Anticoagulant
  - Potentiates action of Antithrombin

- Non-anticoagulant actions
  - Restores trophoblast invasive properties Bose et al. 2004
  - Prevents trophoblast apoptosis Sullivan & Hills, Bose 2006
  - Restores placental hCG production Di Simone et al. 1997, 1999
  - Immunomodulation of cellular immunity, antagonises IFN gamma production Francis et al. 2004, 06

Where are these Pathological Effects of aPL Exerted

1. Syncytial Fusion
2. Placental Apoptosis
3. Trophoblast Invasion
Anti Phospholipid Syndrome – evolving story

- Systemic autoimmune disease
- Thrombotic disorder
  - Acquired thrombophilic defect
  - Disordered reproductive immunology
- NK cells
- Complement
Natural killer cells – part of the innate immune system

- Peripheral blood NK cells
  - 10 - 15% of peripheral blood lymphocytes
  - Unique phenotypic characterisation and function
  - Two main expression markers CD 56 and CD 16
  - 90% CD 56 dim CD16+ → CYTOTOXIC

- Uterine NK cells
  - 70% of the decidual leukocyte population in implantation window
  - CD 56 bright CD 16 -ve → immunoregulatory cytokines
  - Limit trophoblast invasion of decidua and spiral arteries

No Correlation between peripheral blood and endometrial NK cell number in RM women

Rai, Regan in press

Peripheral & uterine NK cells – correlation ?

“Examination of peripheral NK cells will not tell us what is happening in the uterus.
This is akin to estimating the number and activity of black cabs in Trafalgar Square by analysing red mini-cabs circulating on the M 25”

Moffett, Regan, Braude; BMJ 2004; 329; 1283-5
### Recurrent miscarriage - uNK cells

- Increased uterine CD56+ NK cells in recurrent miscarriers
  

- Extensive coverage in all media and www.internet – Infertility and miscarriage associated with "raised" NK cells
  - numerous anecdotal reports
  - Advocates push suppression with steroids, IVIG, TNFa drugs

- Higher uterine NK cells in RM women compared to controls, but no future pregnancy prognostic value
  
  Tuckerman, Laird, Li et al 2007

### Potential immunomodulatory agents

- White cell transfusions
- Steroids
- IVIG
- Anti TNF
- Progesterone
- Heparin

Reproductive failure always emotive issue
History keeps on repeating itself BUT
Are we any wiser?

### NK Cell KIR - KAR genotyping

- Maternal uNK inhibitory receptors, Fetal HLA-C

Genotype resulting in maximum effect on uNK cell inhibitory receptors significantly higher in women with pre-eclampsia

Hiby et al 2004

Suggests that overly inhibited uNK cells cause trophoblast to prematurely cease remodelling spiral arteries leading to pre-eclampsia AND unexplained recurrent miscarriage

Hiby, Moffett, Regan et al, 2008

Future considerations - Fetal genotype, paternal testing
Recurrent Miscarriage

• Maternal NK Cell KIR – KAR genotyping
• Paternal HLA-C on trophoblast

maternal KIR AA genotype with Paternal HLA-C2 combination significantly increased

Consider sperm donation to avoid recurrent miscarriages of spontaneous and IVF pregnancies ???

Anti Phospholipid Syndrome - evolving story

Systemic autoimmune disease

Thrombotic disorder

Acquired thrombophilic Defect

Disordered reproductive immunology

NK cells Complement

aPL and complement mediated damage

Murine model of aPL – pregnancy loss – passive transfer of human aPL

► aPL preferentially targeted at decidua and trophoblast
► Activate complement – generate split products that induce thrombosis (Paranjek, Girardi)
► Generates C3a and C5a
► Recruitment of inflammatory cells
► Pro-inflammatory response and tissue damage in placenta → fetal death or growth restriction (Girardi, Salmon, Redecha)
Heparin prevents aPL induced fetal loss by inhibiting complement activation rather than its anticoagulant activity (Girardi, Redecha & Salmon)

Targeted complement inhibitory therapies needed

---

**CHANGING PARADIGM: AN EVOLUTIONARY PERSPECTIVE**

- Reproductive performance: excellent/poor
- Estrous behaviour: yes/no
- Intercourse induced ovulation: yes/no
- Embryonic aneuploidy: no/yes
- Embryonic diapause: yes/no
- Multiple implantation: yes/no
- Embryonic control of maternal response: yes/no
- Invasiveness: low/high
- Menstruation: no/yes

---

Immunohistochemical detection of endometrial decay-accelerating factor (DAF/CD56) expression in women with RPL

Real-time quantitative (RTQ)-PCR analysis of transcripts that encode for the complement regulatory proteins decay-accelerating factor (DAF/CD55), membrane cofactor protein (MCP/CD46) and protectin/CD59

Complement regulatory factors and miscarriage

- Collaboration with Professor Tim Goodship (Newcastle)

- Hypothesis: complement regulatory factor H
  membrane cofactor protein MCP
  Decay Accelerating factor CD59

  Act as susceptibility factors for RM

- Analysis Using haplotype tagging SNPs

- Translational potential: Offers novel therapeutic intervention – complement inhibitors / statins

STRIKING SUPERFICIAL SIMILARITIES…..BUT ALSO FUNDAMENTAL DIFFERENCES

PHASES OF THE FEMALE CYCLE

Proliferative Secretory Menstrual

Phase:

Cycle days:

Implantation window

Decidualization

Mother must signal its competence to the implanting embryo

Mother must discriminate between competent and incompetent embryos
EMBRYO SELECTION HYPOTHESIS: EPIDEMIOLOGICAL EVIDENCE

Late implantation = exponential increase in miscarriage rate

EMBRYO SELECTION HYPOTHESIS: MOLECULAR EVIDENCE

PROK1 - A PRO-IMPLANTATION CYTOKINE

PRL - A CLASSICAL DECIDUAL MARKER

Conception

Prematurity

1st trimester miscarriage

PET

2nd trimester miscarriage

Still Birth

Defects here underlie adverse pregnancy outcomes at all gestations
Pre-conceptual endometrial gene expression

Thrombophilias & Fetal Loss

**Acquired** (autoimmune)
- Antiphospholipid syndrome - established cause of recurrent fetal loss & placental pathology

**Inherited**
- APCR, FVLleiden, hyperhomocysteinaemia, Protein C, S & AT 111 deficiency - established major causes of thrombosis
- Recent association with fetal loss, preeclampsia, IUGR

**Genetic thrombophilia & fetal loss - Meta-analysis**

<table>
<thead>
<tr>
<th>Genetic factor</th>
<th>Early RM</th>
<th>Recurrent late loss</th>
<th>Non-recurrent late loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL</td>
<td>0.24(1.1-3.5)</td>
<td>0.03(0.1-0.8)</td>
<td>0.24(1.0-5.8)</td>
</tr>
<tr>
<td>PT</td>
<td>0.24(0.9-6.2)</td>
<td>0.004(1.0-4.8)</td>
<td>0.24(1.0-5.8)</td>
</tr>
<tr>
<td>PS</td>
<td>4.16(0.3-77.6)</td>
<td>7.3(1.2-42)</td>
<td>4.16(0.3-77.6)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>0.24(1.1-3.5)</td>
<td>0.03(0.1-0.8)</td>
<td>0.24(1.0-5.8)</td>
</tr>
<tr>
<td>PC</td>
<td>0.24(0.9-6.2)</td>
<td>0.004(1.0-4.8)</td>
<td>0.24(1.0-5.8)</td>
</tr>
</tbody>
</table>

*After Rey et al. 2003 Lancet*
Thrombophilic defects & pregnancy loss

- Complex interaction between inherited + acquired risk factors
- 15% of the Western population carry > 1 of these defects
- Presence of a thrombophilic defect does not always lead to pregnancy complications

The ability to identify thrombophilic defects has outstripped our understanding of the mechanisms of pregnancy loss

New tests needed to identify the pregnancies at risk

Thrombophilia in Pregnancy

The challenge we face

Genotype or Phenotype?

Maternal or Fetal Inheritance?

How can we best predict adverse pregnancy outcome?

Thromboelastography (TEG)

- Global assessment of whole blood haemostasis in one hour from a single blood sample
- Parameters give kinetics of formation, strength and stability of blood clot
- Accurate, reproducible and inexpensive test
- Overcomes limitations of conventional haemostasis tests
Thrombosis
Fibrinolysis

The Thromboelastograph

Late miscarriage
n = 241
Early miscarriages
n = 820
Normal controls
n = 51

MA is significantly increased amongst women with RM compared with controls

Increases in MA during pregnancy predict pregnancy loss
Aspirin & other NSAID’s

• NO improvement in LB rate for unexplained RM  
  Tulpalla et al 1997; Rai et al 2000
• Preconception usage associated with higher 
  miscarriage rate  
  Nielsen et al 2001, Li et al 2003
• Improves LB rate for RM with prothrombotic 
  tendencies eg.TEG  
  Rai et al, In press
• Risk of fetal gastroschisis - avoid empirical use  
  Werler et al 2003, Kozer et al 2002

Raised TEG & RM: A role for Aspirin 150mg

Prospective pregnancy outcome study  
Rai, Regan,Aziz et al: In press 2010

Thrombophilia and Recurrent Pregnancy Loss

Reproductive haemostasis - Progress report 2011

• Shift in emphasis from single dominant cause to importance 
  of multiple “hits”
• Development of global tests of haemostasis
• Prothrombotic markers detectable in non-pregnant state
• Fetal genotype may help to determine pregnancy outcome
• Health implications during and beyond reproductive years
Implantation spectrum of adverse pregnancy outcome

<table>
<thead>
<tr>
<th>Pre-Conception</th>
<th>1st trimester</th>
<th>PET</th>
<th>Prematurity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defective Decidua</td>
<td>Implantation</td>
<td>2nd trimester</td>
<td>IUGR</td>
</tr>
</tbody>
</table>

Defects here underlie adverse pregnancy outcomes at all gestations.

Thank you