Pre-congress course 6:

The impact of the reproductive tract environment on implantation success

Late submission

Stockholm, Sweden
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Organised by
Special Interest Group Endometriosis/Endometrium

ESHRE – Pre Congress Course Stockholm 2011

The impact of thrombophilia on endometrial function

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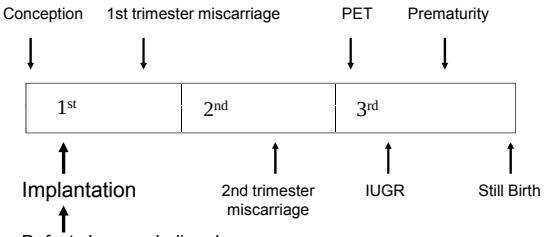
Imperial College London

Implantation spectrum of adverse pregnancy outcome



Recurrent miscarriage

Aetiology

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

New concept of reproductive haemostasis

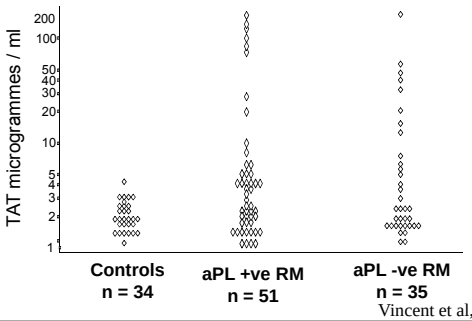
Reproductive actions of thrombin

Thrombin & trophoblast

- ⇧ Angiogenesis
- ⇧ Trophoblast apoptosis
- ⇩ Trophoblast invasion

Defective implantation

Thrombin - antithrombin complexes and miscarriage



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

Clinical features

- Recurrent miscarriage
- Thrombosis
- Thrombocytopenia

Laboratory features*

- Lupus anticoagulant
- Anticardiolipin antibodies (IgG / IgM)

* 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

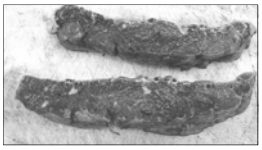
- Screen for both LA and aCL (IgG & IgM) only
- Sample collection and processing- practical points
- Do the best test - international laboratory criteria
- Confirm positive tests - transient positives

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

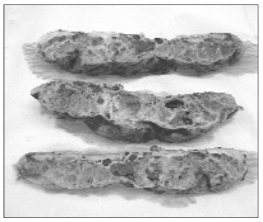
aPL and recurrent miscarriage

- 15% of recurrent miscarriers have aPL – 2% normal obstetric
  Rai et al (1995) Hum Reprod 10(8):2001-2005
- High prospective fetal loss rate of 90%
  First trimester loss after FH activity established
  Rai et al (1995) Hum Reprod 10(12):3301-3304
- Pathogenesis of fetal loss: thrombotic
  De Woolf et al (1982) Am J Obstet Gynecol 142:829-834

aPL and Term placentae

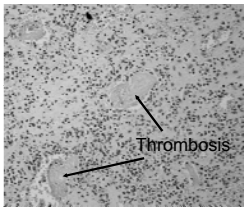


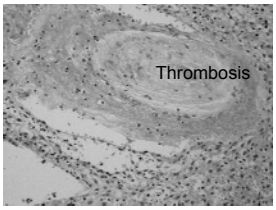
Uncomplicated pregnancy



aPL pregnancy - thrombosis / infarction

Thrombosis in First trimester decidual vessels





Treatment of aPL-related pregnancy loss

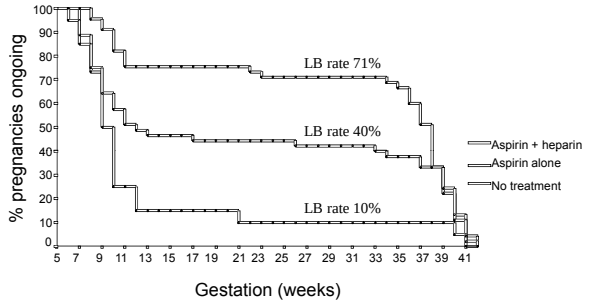
- Aspirin + Heparin therapy significantly improves live birth rate from 10% (untreated pregnancies) to > 70%

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



after Rai et al 1997, BMJ

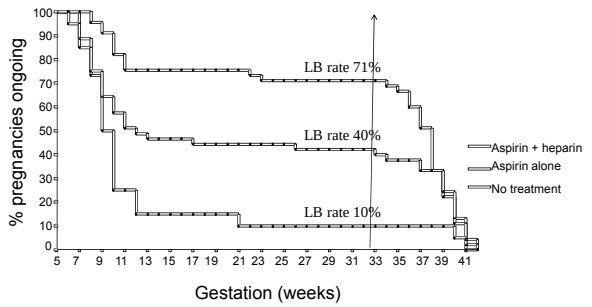
Arthritis & Rheumatism Council

RCT of heparin & aspirin for aPL positive recurrent miscarriage

70% Livebirth rate

Rai, Cohen, Regan BMJ Jan 1997

Antiphospholipid syndrome - effect of treatment



after Rai et al 1997, BMJ

aPL - late pregnancy morbidity despite Rx

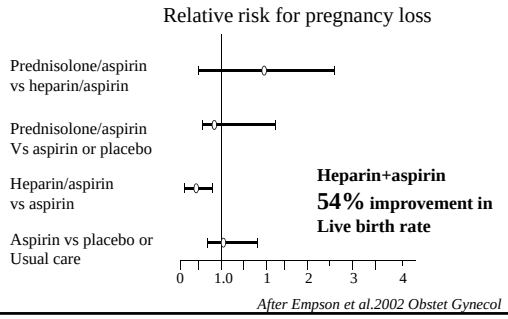
Live birth rate of 74 % after Rx with aspirin & heparin (110/150)
BUT - significant late pregnancy complications

- PIH disorders 17%
- SGA < 2500g 15%
- Placental abruption 5%
- Preterm delivery <37 weeks 24%
- Caesarean section 46%

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

Further clinical studies needed to reduce neonatal morbidity

Treatment of aPL-pregnancies - Meta-analysis



After Empson et al.2002 Obstet Gynecol

Effect of treatment on aPL-pregnancies

Aspirin + Heparin

live birth rate

10% → **70%**

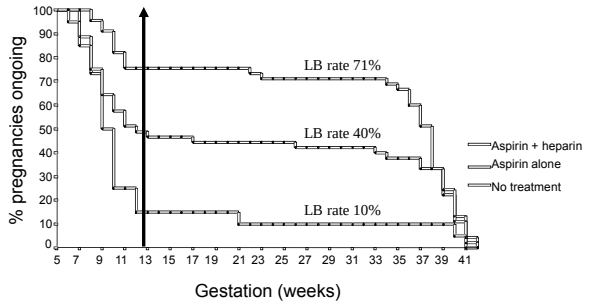
Early miscarriage → late pregnancy complications

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



after Rai et al 1997, BMJ

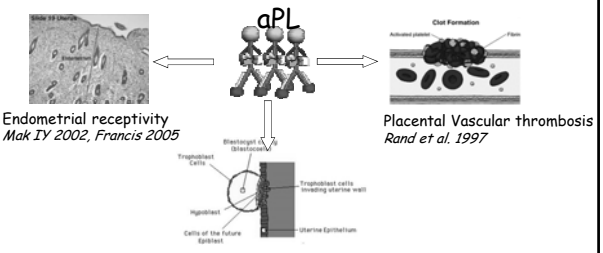
Antiphospholipid syndrome – beyond thrombosis

- 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?

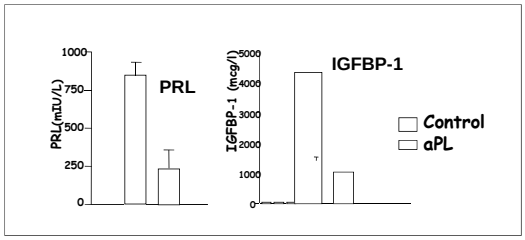


Endometrial receptivity
Mak IY 2002, Francis 2005

Placental Vascular thrombosis
Rand et al. 1997

Trophoblast functions & invasiveness
Katsuragawa et al1997, Di Simone N 2002, Bose 2004

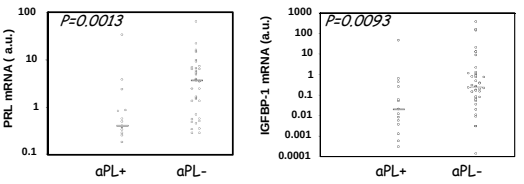
aPL affects Decidual Differentiation – in vitro



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*

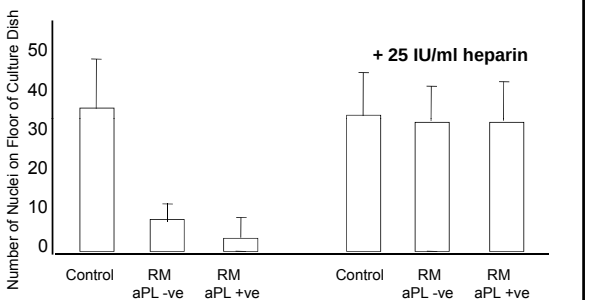


Analysis of 57 timed late-secretory endometrial biopsies using real-time quantitative PCR

Funded JSRC & Save the Baby: Francis et al 2005

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

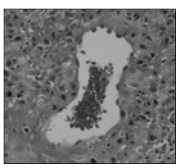
Funded by WHO – Rockefeller – Royal Society

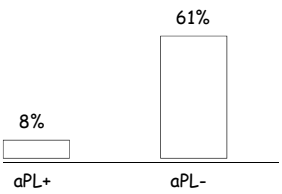


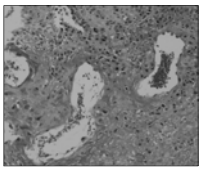
Bose et al 2004, Am J O&G

Defective trophoblast invasion with aPL+

- Extravillous trophoblast OK
- Poor intravascular trophoblast

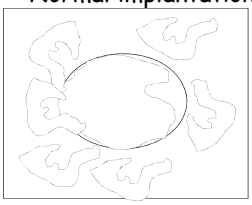






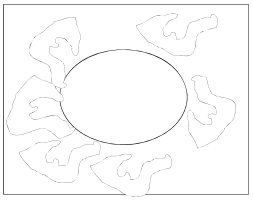
Sebire et al 2002

Normal implantation



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

aPL



Lack of plugging → pressure-related oxidative damage

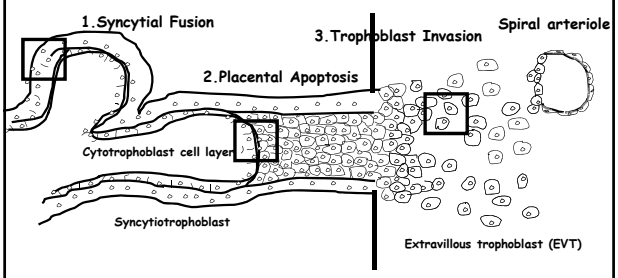
Mechanism?

severe → early pregnancy failure
less severe → 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



Anti Phospholipid Syndrome – evolving story

Systemic autoimmune disease

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Thrombotic disorder

↙ ↘

Acquired thrombophilic Defect

Disordered reproductive immunology

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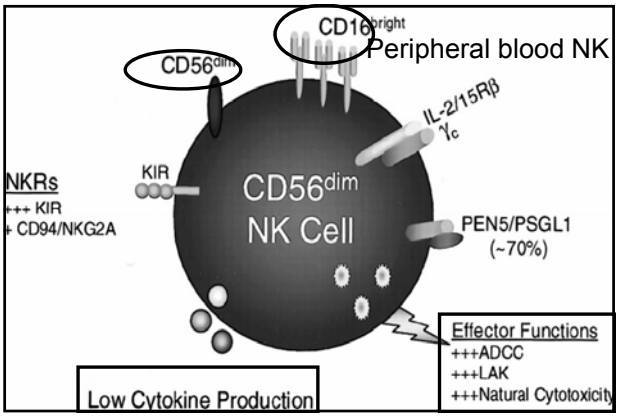
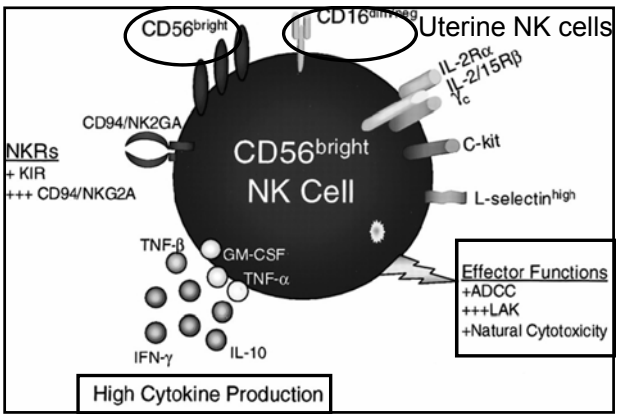
NK cells **Complement**

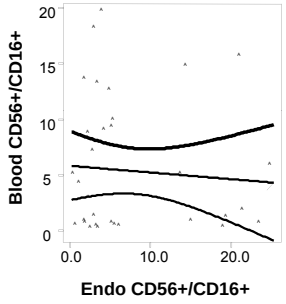




Natural killer cells – part of the innate immune system

- 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 leukocte 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



R-Square = 0.01

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

Quenby et al Hum Reprod 1999; Clifford et al Hum Reprod 1999

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

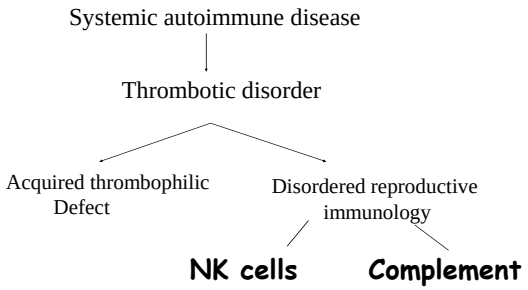
Recurrent Miscarriage

- 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



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 (Pierangeli, 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)

aPL and complement mediated damage

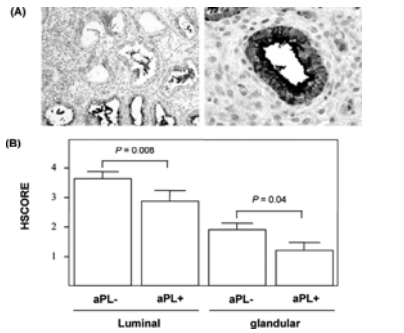
Murine model of aPL – pregnancy loss
– passive transfer of human aPL

- Heparin prevents aPL induced fetal loss by inhibiting complement activation rather than its antocagulant activity (Girardi, Redecha & Salmon)
- Targeted complement inhibitory therapies needed

CHANGING PARADIGM: AN EVOLUTIONARY PERSPECTIVE

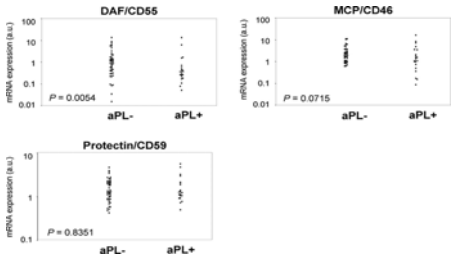

Reproductive performance:	excellent	poor
Estrous behaviour:	yes	no
Intercourse induced ovulation:	yes	no
Embryonic aneuploidy:	no	yes
Embryonic diapause:	yes	no
Multiple implantation:	yes	no
Embryonic control of maternal response:	yes	no
Invasiveness:	low	high
Menstruation:	no	yes

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



Francis, J. et al. Mol. Hum. Reprod. 2006 12:435-442; doi:10.1093/molehr/gal048

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

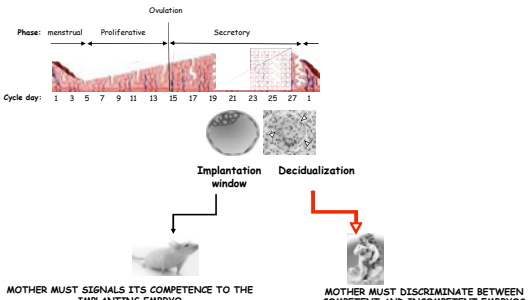


Complement regulatory factors and miscarriage

- 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

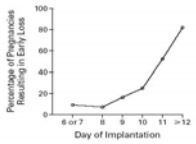
STRIKING SUPERFICIAL SIMILARITIES..... BUT ALSO FUNDAMENTAL DIFFERENCES



MOTHER MUST SIGNALS ITS COMPETENCE TO THE IMPLANTING EMBRYO

MOTHER MUST DISCRIMINATE BETWEEN COMPETENT AND INCOMPETENT EMBRYOS

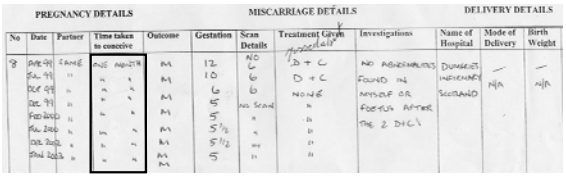
EMBRYO SELECTION HYPOTHESIS: EPIDEMIOLOGICAL EVIDENCE




The New England Journal of Medicine

TIME OF IMPLANTATION OF THE CONCEPTUS AND LOSS OF PREGNANCY

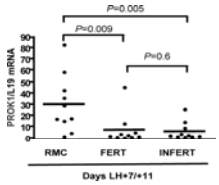
Late implantation = exponential increase in miscarriage rate


PREGNANCY DETAILS | MISCARRIAGE DETAILS | DELIVERY DETAILS

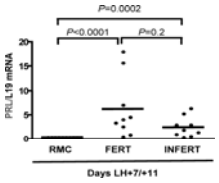
No	Date	Partner	Time taken to conceive	Outcome	Gestation	Scan Details	Treatment Given	Investigations	Name of Hospital	Mode of Delivery	Birth Weight
8	Apr 99	SAME	ONE MONTH	M	12	NO	D + C	NO ABNORMALITIES	DUMFRIES	—	—
	Sep 99	"	"	M	10	6	D + C	FOUND IN	INFIRMARY	N/A	N/A
	Oct 99	"	"	M	6	6	NONE	MYSELF OR	SCOTLAND		
	Dec 99	"	"	M	5	NO SCAN	"	FOETUS AFTER			
	Feb 2000	"	"	M	5	"	"	THE 2 D+C'S			
	Mar 2000	"	"	M	5½	"	"				
	Oct 2002	"	"	M	5½	"	"				
	Sept 2003	"	"	M	5	"	"				

EMBRYO SELECTION HYPOTHESIS: MOLECULAR EVIDENCE

PROK1 - A PRO-IMPLANTATION CYTOKINE

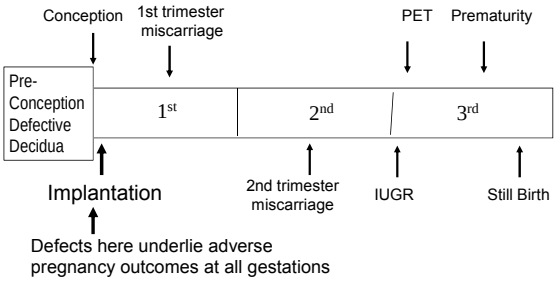


PRL- A CLASSICAL DECIDUAL MARKER



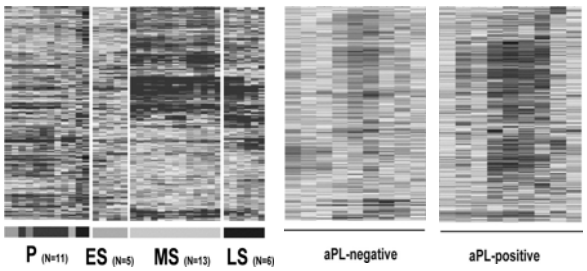
	RPL	INF
No. of patients	9	12
Age (yr)	34.1 ± 3.6	33.0 ± 4.9
Parity	0.4 ± 0.7	0.4 ± 1
Miscarriages	3.9 ± 1.2	0.1 ± 0.3*
Days from LMP	16.2 ± 7.9	17.0 ± 5.6
Time to treatment (days)	8.3 ± 4.1	8.0 ± 5.1

Implantation spectrum of adverse pregnancy outcome



Defects here underlie adverse pregnancy outcomes at all gestations

Pre-conceptual endometrial gene expression



Thrombophilias & Fetal Loss

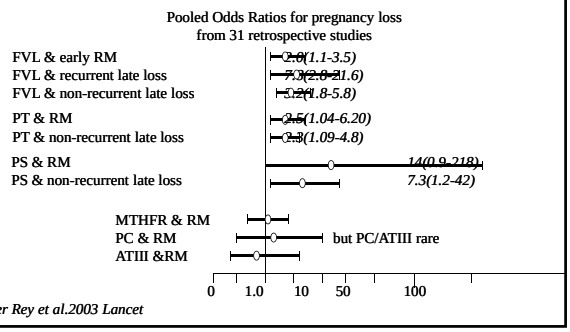
Acquired (autoimmune)

- Antiphospholipid syndrome - established cause of recurrent fetal loss & placental pathology

Inherited

- APCR, FVLeiden, 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



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

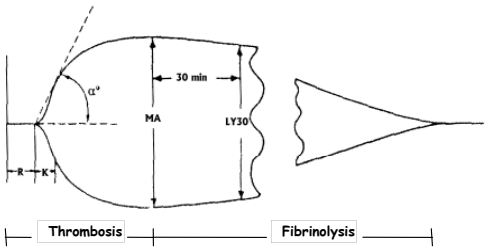
Genotype or Phenotype ?

Maternal or Fetal Inheritance ?

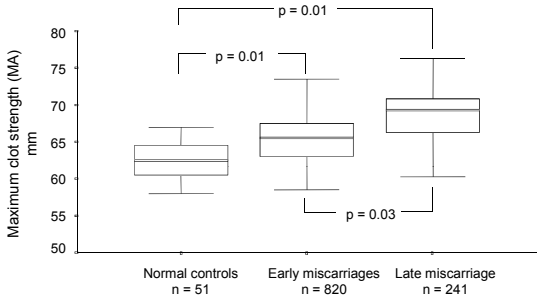
How can we best predict adverse pregnancy outcome ?

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

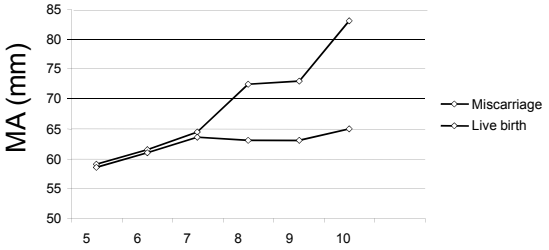


The Thromboelastograph



MA is significantly increased amongst women with RM compared with controls

Increases in MA during pregnancy predict pregnancy loss

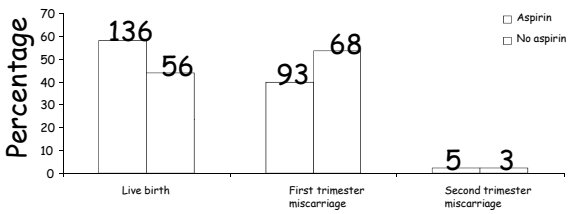


Weeks gestation

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



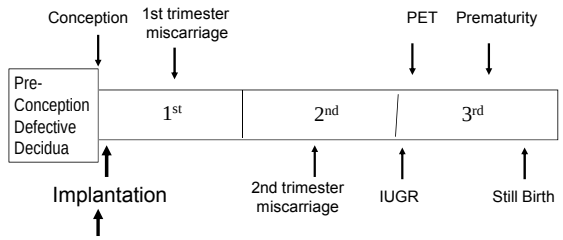
Prospective pregnancy outcome study
Rai, Regan,Aziz et al: In press 2010

Thrombophilia and Recurrent Pregnancy Loss

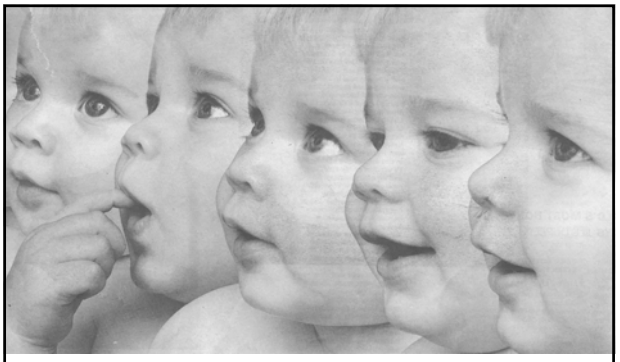
Reproductive haemostasis - Progress report 2011

- 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



Defects here underlie adverse pregnancy outcomes at all gestations



Thank you