

PRE-CONGRESS COURSE 3

**Risk factors for recurrent  
pregnancy loss – more pieces  
of the puzzle.**

Special Interest Group Early Pregnancy  
London - UK, 7 July 2013







# **Risk factors for recurrent pregnancy loss – more pieces of the puzzle**

**London, United Kingdom  
7 July 2013**

**Organised by  
The ESHRE Special Interest Group Early Pregnancy**



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# Course coordinators

Mariëtte Goddijn (The Netherlands)

# Course description

This pre-congress course will address the risk factors involved in recurrent pregnancy loss and focuses on potential therapeutic consequences

# Target audience

Reproductive gynaecologists and physicians



# Scientific programme

## Pregnancies of unknown location (PUL)

*Chairman: Siobhan Quenby - United Kingdom*

- 09:00 - 09:20      Diagnosis solutions for pregnancy of unknown location (PUL) - The role of ultrasound  
*Emma Kirk - United Kingdom*
- 09:20 - 09:40      Diagnosis solutions for pregnancy of unknown location (PUL) - The role of hCG measurements  
*Kurt Barnhart - U.S.A.*
- 09:40 - 10:00      How PULs affect future pregnancy outcomes: new ESHRE guidelines  
*Astrid Marie Kolte - Denmark*
- 10:00 - 10:30      Interactive discussion with the speakers and audience: which diagnostic tests to use; how to inform patients about their prognosis
- 10:30 - 11:00      Coffee break

## Thyroid abnormalities and early pregnancy

*Chairman: Mariette Goddijn - The Netherlands*

- 11:00 - 11:30      When to screen for thyroid function abnormalities?  
*Rosa Vissenberg - The Netherlands*
- 11:30 - 12:00      Thyroid antibodies and miscarriage: clinical trial  
*Arri Coomarasamy - United Kingdom*
- 12:00 - 12:30      Discussion with the speakers and audience: diagnostic tests in setting of scientific studies; RCT design
- 12:30 - 13:30      Lunch

## Societal and life style factors

*Chairman: to be announced*

- 13:30 - 13:50      Life style factors increase the risk of recurrent miscarriage  
*William H. Kutteh - U.S.A.*
- 13:50 - 14:10      The impact of genetic testing for couples with recurrent miscarriage  
*Fleur Vansenne - The Netherlands*
- 14:10 - 14:30      The influence of advanced maternal age: major cause of recurrent pregnancy loss  
*Mary Stephenson - U.S.A.*
- 14:30 - 15:00      Discussion with speakers and audience: how to fight bad habits
- 15:00 - 15:30      Coffee break

## New thoughts

*Chairman: Mariette Goddijn - The Netherlands*

- 15:30 - 16:00      NK cells  
*Siobhan Quenby - United Kingdom*
- 16:00 - 16:30      NICE guidelines 2012 – dissemination and implementation  
*Caroline Overton - United Kingdom*
- 16:30 - 17:00      Discussion with speakers and audience: early pregnancy research networks – bridging the 'pond'

16:30 - 17:00 *Mariette Goddijn - The Netherlands*  
Discussion with speakers and audience: early pregnancy research networks –  
bridging the 'pond'  
*Mary Stephenson - U.S.A.*



## Diagnostic Solutions for Pregnancy of Unknown Location – *The Role of Ultrasound*

Emma Kirk  
MRCOG MD  
Whittington Hospital, London

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### Objectives

1. Define Pregnancy of Unknown Location and subsequent pregnancy outcomes
2. Appreciate the use of ultrasound in diagnosis and management of PULs

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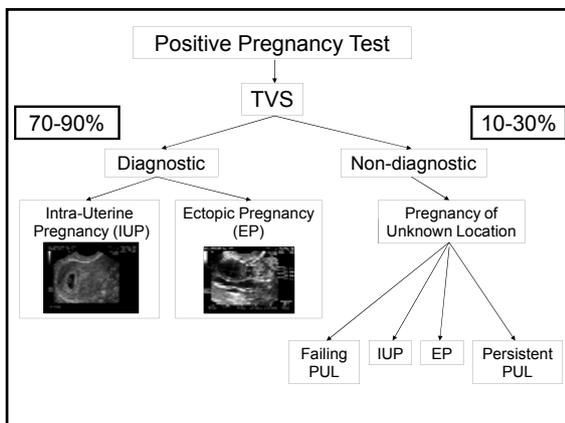
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## Pregnancy of Unknown Location (PUL)

- Positive pregnancy test
- No evidence of an intra-uterine or extra-uterine pregnancy on TVS



Royal College of  
Obstetricians and  
Gynaecologists  
NHS.uk  
THE MANAGEMENT OF EARLY PREGNANCY LOSS  
Updated and revised nomenclature for description  
of early pregnancy events  
Prof C. Campbell<sup>1</sup>, Prof. Scammell<sup>2</sup> and Prof. Akhola<sup>3</sup> on behalf of the 110000 Special  
Interest Group for Early Pregnancy (SIGEP)

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## Pregnancy of Unknown Location

- 5-42% of women attending for USS in Early Pregnancy
- 8-10% in specialized Early Pregnancy Units
- Rates should be < 15%

*International Society of Ultrasound in Obstetrics and Gynecology 2006*

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## Diagnosis

- PUL not a diagnostic term
- Classification term only
- All women need to be followed up in order to determine final clinical outcome

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## Diagnosis



*A woman had an ultrasound examination in very early pregnancy where a diagnosis of 'pregnancy of unknown location' was made, after which serial hCG measurements were arranged. A few weeks later she was admitted to another hospital because of diarrhoea, dizziness, abdominal pain and vaginal bleeding. Repeat ultrasound examination a few hours later queried the presence of a small (9mm) intrauterine sac and a haemoperitoneum. It was decided to perform a uterine evacuation and consider laparoscopy if products of conception were not obtained. An evacuation procedure alone was performed by a junior doctor unfamiliar with the woman, who was then returned to the postoperative ward where she collapsed and died several hours later. Autopsy revealed massive intraperitoneal haemorrhage and a ruptured tubal pregnancy.*

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## The Role of Ultrasound

1. Initial classification

1. Follow-up

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### 1. Classification as a PUL

- Absence of an intra-uterine pregnancy or an ectopic pregnancy
- Clear criteria for diagnosing intra-uterine pregnancies and ectopic pregnancies.

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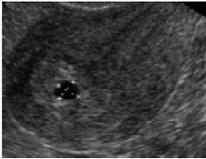
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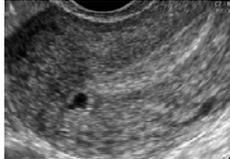
### 1. Classification as a PUL

- PUL or early IUP?

5/40 PV spotting



7/40 PV spotting



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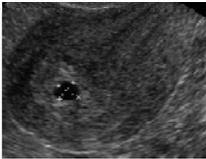
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### 1. Classification as a PUL

- PUL or early IUP?

5/40 PV spotting



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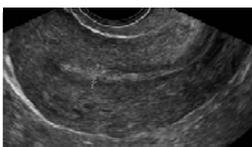
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### 1. Classification as a PUL

- PUL or miscarriage?

9/40 Heavy bleeding with clots



? 6/40 PV spotting



6% incidence of ectopic pregnancy  
*Condous et al., 2005*

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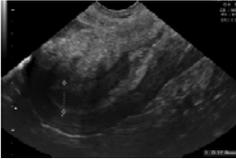
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### 1. Classification as a PUL

- PUL or miscarriage?

? LMP Pain, light bleeding




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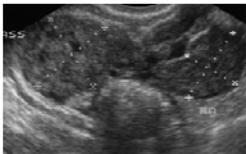
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### 1. Classification as a PUL

- PUL or ectopic pregnancy?

7/40 PV spotting




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### What is a PUL?

		UK	USA
Empty uterus, no signs of an IUP or EP		Yes	Yes
Early intra-uterine gestational sac		No	Yes
Extra-uterine inhomogeneous mass		No	Yes
? Small amount or retained products of conception		? Yes	? Yes

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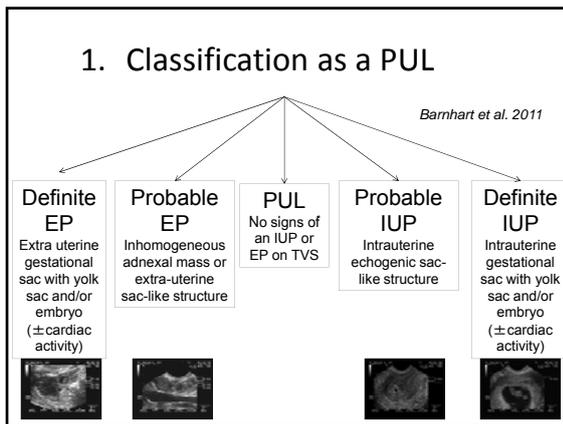
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## 1. Classification as a PUL




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## 2. Follow-up

- Final clinical outcomes
- Management

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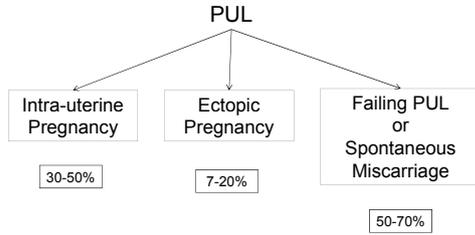
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## PUL Outcome




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## Intra-uterine Pregnancy

		UK	USA
Gestational sac only		Yes	No
Sac with yolk sac		Yes	Yes
Sac with CRL		Yes	Yes
Empty sac (anembryonic)		Yes	No
Delayed miscarriage		Yes	No
Incomplete miscarriage		Yes	No

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## Intra-uterine Pregnancy

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Gestational sac only		Yes	No
Sac with yolk sac		Yes	Yes
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Empty sac (anembryonic)		Yes	No
Delayed miscarriage		Yes	No
Incomplete miscarriage		Yes	No

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## Ectopic Pregnancy

		UK	USA
Sac with a yolk sac /CRL		Yes	Yes
Empty gestational sac		Yes	No
Inhomogeneous mass		Yes	No
No chorionic villi on uterine curettage and rising hCG level		Persisting PUL	Yes

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## Ectopic Pregnancy

		UK	USA
Sac with a yolk sac /CRL		Yes	Yes
Empty gestational sac		Yes	No
Inhomogeneous mass		Yes	No
No chorionic villi on uterine curettage and rising hCG level		Persisting PUL	Yes

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## Failing PUL

## Spontaneous miscarriage

UK	USA
Spontaneous decrease in hCG	Spontaneous decrease in hCG
	Non-viable pregnancy on TVS
	Histological diagnosis of chorionic villi
	No chorionic villi and spontaneous decrease in hCG

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## 2. Follow-up

1. Clinical assessment
2. Expectant management
3. Prediction of outcome
4. Confirmation of outcome
5. Surgical intervention

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## 1. Clinical Assessment

- 5/40 Light PV spotting
- 7/40 Severe lower abdominal pain



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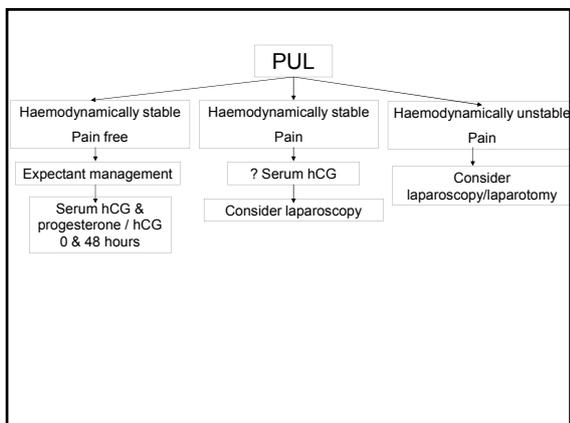
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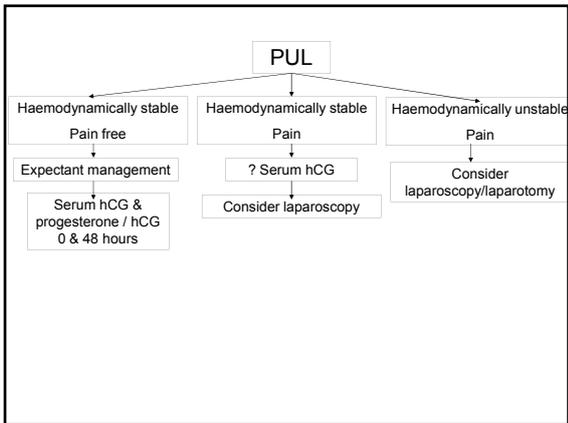
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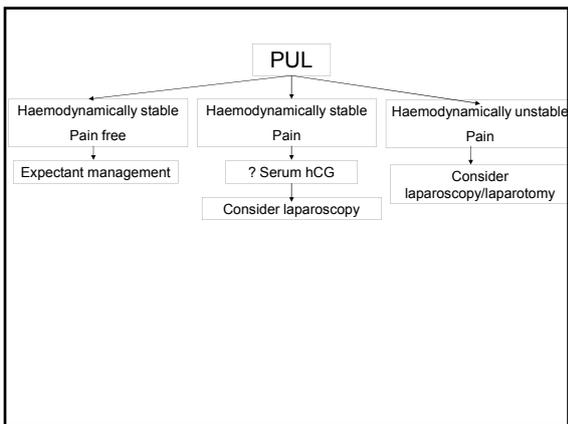
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**2. Expectant Management**

- Majority of women will be relatively asymptomatic and haemodynamically stable
- Expectant management has been shown to be safe
- All women should be counseled about the possible outcomes and ideally given written information

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## 2. Expectant Management

- Majority resolve without intervention
- No consensus on intervention rates
- Reported surgical intervention rates 0.5-11%

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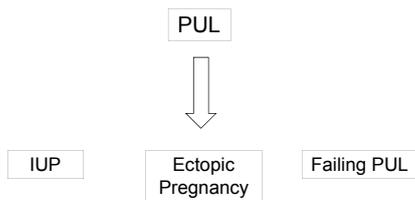
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## 3. Prediction of outcome



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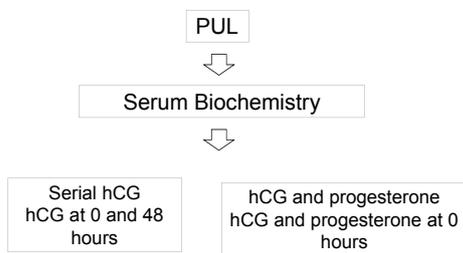
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## 3. Prediction of outcome



Other serum markers evaluated: Cancer Antigen 125, Creatine Kinase, Activin A, Activin B, Inhibin pro- $\alpha$ C-related immunoreactivity, insulin-like growth factor-binding protein. Mathematical models.

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#### 4. Confirmation of outcome

- TVS
- Serum hCG levels
- Urinary pregnancy test
- Histology

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#### 4. Confirmation of outcome

- TVS
- Serum hCG levels
- Urinary pregnancy test
- Histology

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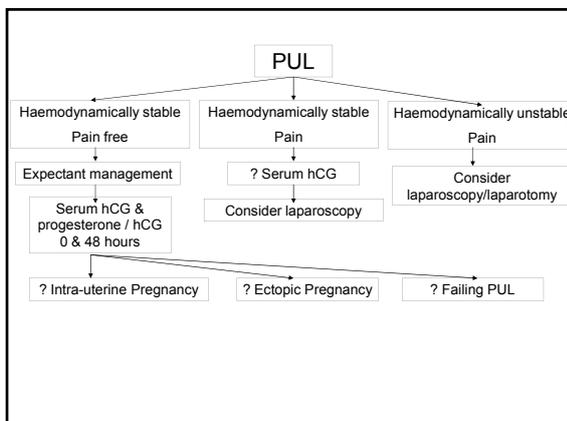
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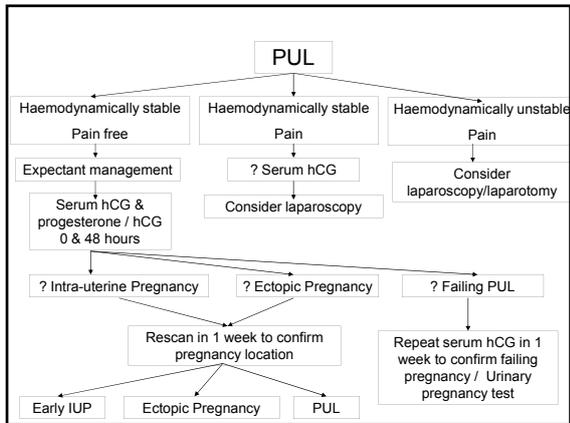
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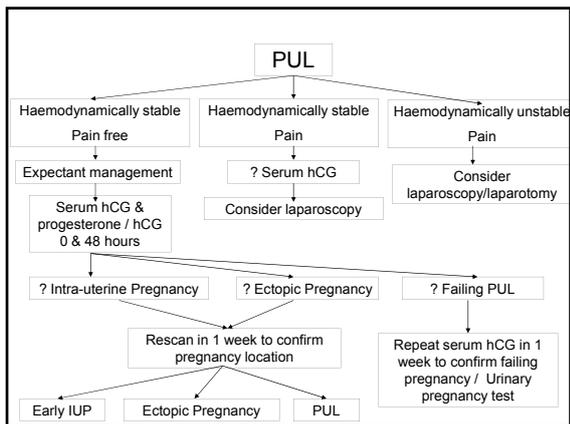
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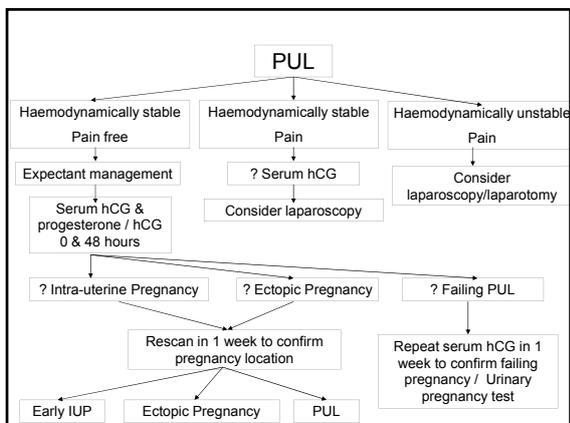
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Summary

**PULs – Role of Ultrasound**

1. Classification as a PUL based on initial USS findings
  
2. USS used to confirm final clinical outcome:
  - Intrauterine pregnancies – viable and non-viable
  - Ectopic pregnancies

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**Diagnosis solutions for pregnancy of unknown location (PUL) - The role of hCG measurements**

- Kurt Barnhart, M.D., M.S.C.E.
  - William Shippen, Jr., Professor of Obstetrics and Gynecology
  - Penn Fertility Care
  - Perlman School of Medicine at the University of Pennsylvania

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**LEARNING OBJECTIVES**

**At the conclusion of this presentation, participants should be able to:**

- Discuss potential pitfalls in the diagnosis of women with a pregnancy of unknown location.
- Integrate new nomenclature for the definitive ultimate diagnosis of women with a pregnancy of unknown location.
- Understand the role of hCG in the evaluation of a woman with a PUL

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**DISCLOSURE**

- Nothing to disclose

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### Utility of Ultrasound Above and Below the Discriminatory Zone

Patients with  $\beta$ hCG level **ABOVE** 1500 mIU/mL at presentation

Ultrasound Diagnosis	Sensitivity	Specificity	+PV	-PV
Intrauterine pregnancy	98%*	90%	96%	96%
Miscarriage	73%*	93%	65%	65%
Ectopic pregnancy	80%*	99%	86%	99%

$\beta$ hCG =  $\beta$  human chorionic gonadotropin; PV = predictive value

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### Utility of Ultrasound Above and Below the Discriminatory Zone (DZ)

Patients with  $\beta$ hCG level **BELOW** 1500 mIU/mL at presentation

Ultrasound Diagnosis	Sensitivity	Specificity	+PV	-PV
Intrauterine pregnancy	33%*	98%	80%	86%
Miscarriage	28%*	100%	100%	47%
Ectopic pregnancy	25%*	96%	60%	85%

Barnhart KT, Simhan H, Kamelle S. Diagnostic accuracy of ultrasound, above and below, the  $\beta$ hCG discriminatory zone. *Obstet Gynecol* 1999; 94(4):583-587.

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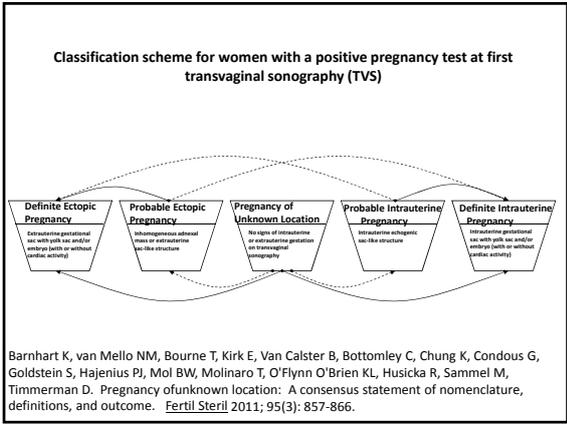
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## Discriminatory Zone

- **What has changed?**
  - IRP has changed so now 1500 first IU is about 1900 4th IU
  - Most women get US in first trimester (even without symptoms)
  - Ruptured EP uncommon, clinician very aware of risk
    - Effort has shifted to avoid interruption of a desired IUP
    - Methotrexate is common and easy to administer
    - More scans = more false positives (false negatives)

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## What is the Discriminatory Zone?

- **Surrogate for gestation age**
  - Level at which normal milestones should be identified (gestational sac): the level does not discriminate location
  - The best DZ is gestational age
    - 5 5/7 weeks ( 40 days) regardless of number of gestations
  - Very wide variation in hCG in first trimester
- **Not all women know their LMP**
  - Maybe off by days, or at times off by 4 weeks
- **DZ may need to be 3000 or higher\***

\*Duobliet P, Benson C. J ultrasound med 2011;30:1637-1642  
\*Metha et al, Radiology 1997;205:569-573

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## Case Presentation

- Your beeper goes off Friday afternoon, before your planned trip to ASRM.
- Your nurse calls you: Ms. Smith called your nurse.
  - Ms. Smith has a home pregnancy test is positive, and she *THINKS* she is about 2 weeks late for her period.
  - She has moderate pain in her left side and has been spotting for 4 days.
  - She is a G4 P0, with three miscarriages in the first trimester.

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### Case Presentation

- Ms. Smith's hCG level is 1000 mIU/mL.
- She is clinically stable.
- This is a desired pregnancy.

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### Normal Rise in hCG

- Fit the curve of women who presented to ED at risk for EP who were definitively diagnosed with a viable IUP
- 293 subjects, 873 observations
  - Average age 24 years
  - Average G 2.4; P 0.8
  - Average hCG value 1000 mIU/mL
- Fit a number of models:
  - Linear, spline, exponential

G = gravida; P = para

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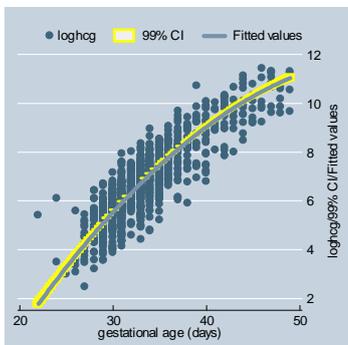
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### Normal Rise in hCG



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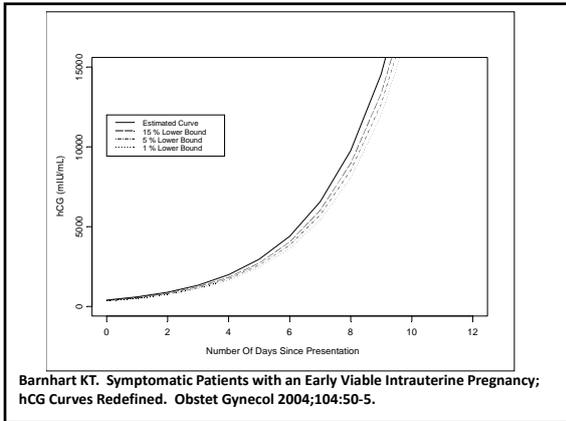
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Increase in hCG value at different days (as a percent of initial value)

quartile	slope	1 day	2 day	3 days	4 days
• 99	1.23	1.23	1.53	1.84	2.26
• 95	1.30	1.30	1.69	2.19	2.84
• 85	1.37	1.36	1.87	2.55	3.48
• 50	1.50	1.50	2.22	3.31	4.94
• 10	1.66	1.66	2.76	4.58	7.60
• 1	1.81	1.81	3.29	5.96	10.80

**Barnhart KT. Symptomatic Patients with an Early Viable Intrauterine Pregnancy; hCG Curves Redefined. Obstet Gynecol 2004;104:50-5.**

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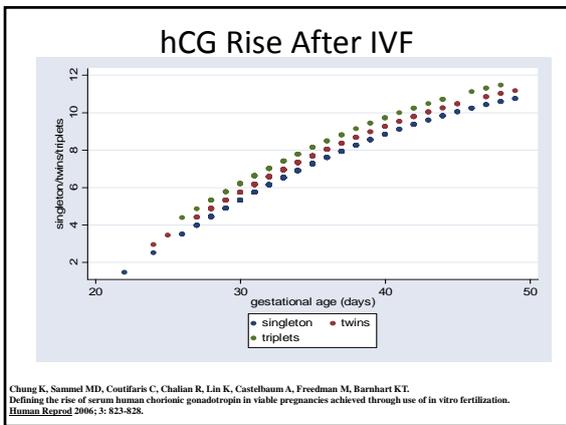
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## Normal Fall in hCG

- Fit the curve of women who presented to ED at risk for EP who were definitively diagnosed with a complete SAB
- 719 subjects, 2914 observations
  - Serum hCG confirmed to be > 5
- Fit a number of models:
  - Linear, quadratic, cuboidal, change point with random intercept and random effect
- Final model was random linear effect dependent on initial hCG value

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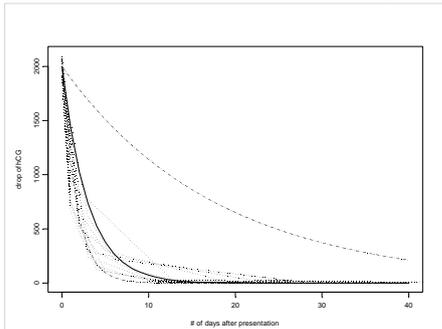
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## Curve of Complete Miscarriage



Barnhart, K. Decline of serum human chorionic gonadotropin and spontaneous complete abortion: Defining the normal curve. *Ob Gyn* 2004;104(5):975-981.

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## Normal Fall of hCG for Complete SAB

Initial hCG value (mIU/mL)	hCG value at 2 days (mIU/mL)	hCG value at 7 days (mIU/mL)	hCG value at 21 days (mIU/mL)	Days to negative hCG
500	256	48	0	19
	447 (21%)	337 (60%)	76	
1000	513	96	0	21
	894	675	308	
2000	1027	193	0	23
	1788	1351	616	
5000	2567	484	5	26
	4470 (35%)	3378 (84%)	1541	

Barnhart, K. Decline of serum human chorionic gonadotropin and spontaneous complete abortion: Defining the normal curve. *Ob Gyn* 2004;104(5):975-981.

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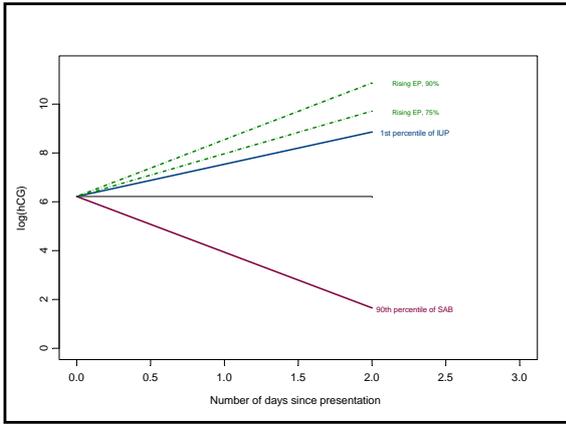
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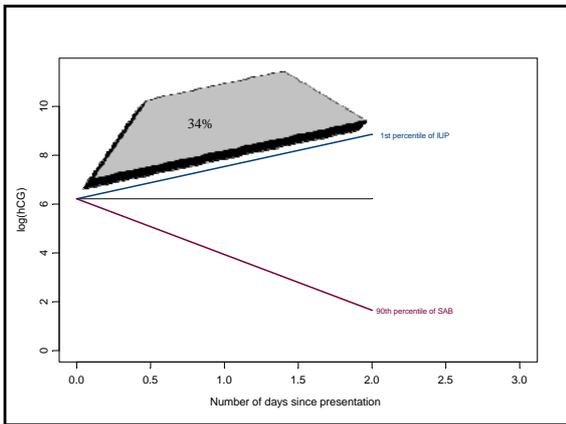
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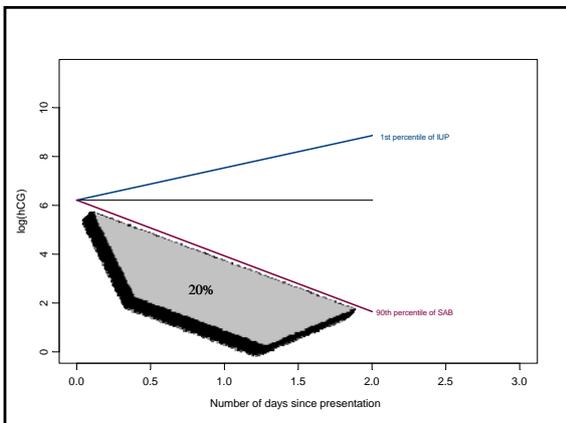
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Performance in Validation Cohort versus Original Cohort						
Expected Two-Day Rise for an IUP	Sensitivity for EP (%)				Mean number of days saved (range) <sup>f</sup>	
	Validation	Original*	Validation	Original	Validation	Original
					2.87	2.64
35% Rise in hCG	83	83	92	95	(0-35)	(0-34)
					3.27	2.85
53% Rise in hCG	91	88	83	90	(0-35)	(0-34)
					3.44	2.94
71% Rise in hCG	92	91	73	78	(0-37)	(0-34)

Morse CB, Barnhart KT et al. Performance of human chorionic gonadotropin curves in women at risk for ectopic pregnancy: Exceptions to the rules. *Fertil Steril* 2012; 97: 101-106.

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Performance in Validation Cohort versus Original Cohort						
Expected Two-Day Rise for an IUP	Number of misclassified EPs (%)		Number of misclassified IUPs (%)		Number of misclassified miscarriages (%)	
	Validation	Original	Validation	Original	Validation	Original
35% Rise in hCG	30 (16.8)	34 (17.3)	20 (7.7)	12 (4.6)	221 (39.0)	222 (28.0)
53% Rise in hCG	16 (8.9)	24 (12.2)	45 (17.4)	26 (10.0)	231 (40.7)	224 (28.2)
71% Rise in hCG	14 (7.8)	18 (9.2)	71 (27.4)	58 (22.2)	236 (41.6)	225 (28.4)

Morse CB, Barnhart KT et al. Performance of human chorionic gonadotropin curves in women at risk for ectopic pregnancy: Exceptions to the rules. *Fertil Steril* 2012; 97: 101-106.

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**How does Misclassification Occur?**

- Of 30 (17%) patients with “missed” EP (classified as IUP or SAB): 24 has “NL rise” and 6 had “NL fall”
  - 6 were diagnosed due to pain (3 ruptured)
  - Rupture was 0.03% of cohort or 1.7% of EP
- Of 22 (8%) patients with “missed” IUP (classified as EP or SAB):
  - 18 had rise less than 35%
  - 2 had change in direction

Morse CB, Barnhart KT et al. Performance of human chorionic gonadotropin curves in women at risk for ectopic pregnancy: Exceptions to the rules. *Fertil Steril* 2012; 97: 101-106.

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## How does Misclassification Occur?

How did hCG mislead us into an error?

Such that we “missed” the IUP?

- 13/20 has findings on US suggesting an IUP
- Many of “abnormal” hCG values were the first 2 values and where below 500
- If one considered a third hCG; 6 were reclassified (correctly) as an IUP
  - BUT 9 EP and 2 SAB were reclassified (incorrectly) as an IUP

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## Practice Patterns Across the Pond

- **USA:** more aggressive strategies in the diagnosis of women at risk for ectopic pregnancy
  - Determine viability by serial hCG and then distinguish spontaneous abortion from that of EP
  - Use of uterine evacuation
  - Little presumptive diagnosis
- **UK:** more conservative approach
  - More liberal use of ultrasound in diagnosis
  - Identify Pregnancy of Unknown location
  - Use first 2 hCG values to predict outcome
  - Little use of surgical intervention, more expectant management

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## Prediction of ectopic pregnancy in women with a pregnancy of unknown location –M4 Model

Condous G, Van Calster B, Kirk E, Haider Z, Timmerman D, Van Huffel S, Bourne T. Prediction of ectopic pregnancy in women with a pregnancy of unknown location. *Ultrasound Obstet Gynecol* 2007;29:680-7.

Table 3 Areas under the receiver-operating characteristics curves (AUC) for Models M4 and M1 to distinguish outcomes in women with a pregnancy of unknown location (PUL)

Predicted outcome/model	AUC (95% CI)		P*
	Training set	Test set	
Failing PUL			0.2531
Model M4	0.991 (0.981–1.000)	0.978 (0.964–1.000)	
Model M1		0.965 (0.948–0.994)	
Intrauterine pregnancy			0.3750
Model M4	0.976 (0.956–0.994)	0.974 (0.954–0.994)	
Model M1		0.949 (0.941–0.953)	
Ectopic pregnancy			0.0301
Model M4	0.941 (0.886–0.993)	0.900 (0.811–0.988)	
Model M1		0.817 (0.719–0.943)	

\*Comparison of test-set AUCs.

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## Validation of M4 with USA data

		Sensitivity	Specificity	AUC (95% CI)
EP	UK (M4)	80.0	88.6	0.900 (0.812, 0.988)
	US	49.0	87.4	0.821 (0.778, 0.865)
	Adjusted US	54.8	87.7	0.830 (0.787, 0.872)
IUP	UK (M4)	85.9	96.3	0.974 (0.954, 0.994)
	US	84.1	92.8	0.961 (0.941, 0.980)
	Adjusted US	81.9	93.1	0.953 (0.930, 0.977)
Failing PUL (UK) / SAB (US)	UK (M4)	87.2	97.5	0.978 (0.954, 1.000)
	US	81.4	83.0	0.933 (0.913, 0.953)
	Adjusted US	83.1	83.1	0.929 (0.907, 0.952)

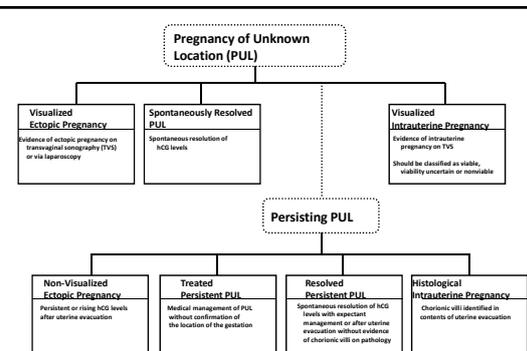
Barnhart KT, Sammel MD, Appleby D, Rausch M, Molinaro T, Van Calster B, Kirk E, Condous G, Van Huffel S, Timmerman D, Bourne T. Does a prediction model for Pregnancy of Unknown Location developed in the UK validate on a US Population? *Hum Reprod* 2010; 25(1): 2434-2440.

## Two hCG values may not be enough

	9.5*	4.4	4.9*
Day 2 vs Day 4	(1.8, 16.7)	(-1.7, 10.4)	(0.5, 9.2)
Day 2 vs Day 7	(0.6, 12.7)	(-1.1, 8.3)	(-0.8, 6.9)
Day 4 vs Day 7	(-4.3, 6.9)	(-5.8, 2.7)	(-0.8, 6.5)

Net Reclassification Index (NRI) is the total net reclassification improvement in EP prediction, calculated as the sum of NRI<sup>EP</sup>, the net reclassification improvement in EP prediction among those with an ultimate EP diagnosis, and NRI<sup>IUP/SAB</sup>, the net reclassification improvement in EP prediction among those with an ultimate IUP or SAB diagnosis.

J. Zee, KT Barnhart Et al, 2012 ARSM



Barnhart K, van Mello NM, Bourne T, Kirk E, Van Calster B, Bottomley C, Chung K, Condous G, Goldstein S, Hajenius PJ, Mol BW, Molinaro T, O'Flynn O'Brien KL, Husicka R, Sammel M, Timmerman D. Pregnancy of unknown location: A consensus statement of nomenclature, definitions, and outcome. *Fertil Steril* 2011; 95(3): 857-866.

### Summary

- Most women with an abnormal early gestation are diagnosed with ultrasound
- A single hCG can not help with viability or location
- Serial hCG values can assist in identification of viability
- Case with slow increase or clearance are at risk for EP (but may need more than 2 values)
- hCG values are NOT diagnostic

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### Take Home Message

**A single value of hCG cannot determine location or viability of gestation**

**A single hCG, regardless of its level, does not justify presumptive treatment for ectopic pregnancy using methotrexate or other medical/surgical means**

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# How pregnancies of unknown location (PULs) affect future pregnancy outcome

## - new ESHRE guidelines

Astrid Marie Kolte, MD, PhD fellow  
Recurrent Miscarriage Unit, Fertility Clinic 4071  
University Hospital Copenhagen, Rigshospitalet  
Denmark

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### Conflict of interest

I declare that I have no commercial or financial interests pertaining to the subject of this presentation or its content.

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### Learning objectives

- To give an overview of definitions of early pregnancy events
- To present current knowledge of PULs and their impact on prognosis for recurrent miscarriage
- To discuss guideline recommendations concerning PULs.

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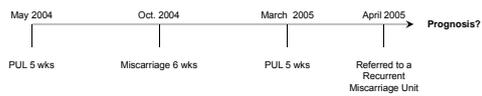
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## Clinical problem

### • Patient 1, 33 years



### • Patient 2, 32 years



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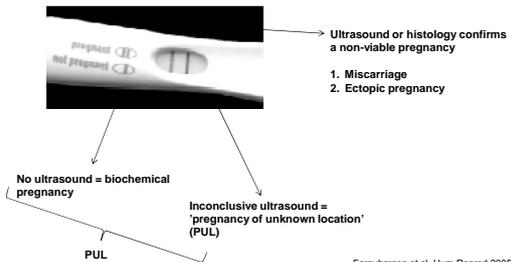
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## Early pregnancy loss – definitions (I)



Farquharson et al. Hum Reprod 2005  
Barrhart et al. Fertil Steril 2011

Astrid Marie Kolte, University Hospital Copenhagen, Denmark

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## What is recurrent miscarriage? Definitions (II)

"Recurrent pregnancy loss is (...) defined by two or more failed pregnancies" **ASRM Practice committee 2013**

"Recurrent miscarriage [is] defined as the loss of three or more consecutive pregnancies" **RCOG 2011**

"Recurrent miscarriage is defined as three or more consecutive miscarriages before 22+0 weeks gestation" **DSOG 2009**

"We refer to recurrent miscarriage (...) if a woman has had two or more objectified miscarriages" **NVOG 2007**

"Recurrent miscarriage (RM) is traditionally defined as three or more consecutive miscarriages occurring before 20 weeks post-menstruation" **ESHRE 2006**

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### PULs and recurrent miscarriage – definitions (III)

"Pregnancy is defined as a clinical pregnancy documented by ultrasonography or histopathologic examination" **ASRM** Practice committee 2013

"Miscarriage is defined as the spontaneous loss of a pregnancy before the fetus reaches viability" **RCOG** 2011

"The miscarriages should be confirmed by a positive hCG and at least one by ultrasound and/or histology" **DSOG** 2009

"These miscarriages (...) do not include (...) biochemical pregnancies" **NVOG** 2007

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### PULs: aetiology

- Intrauterine miscarriage
- Spontaneously resorbed ectopic pregnancy

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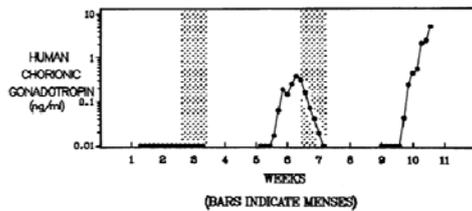
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### Subclinical pregnancy



Wilcox et al Environ health perspec. 1987

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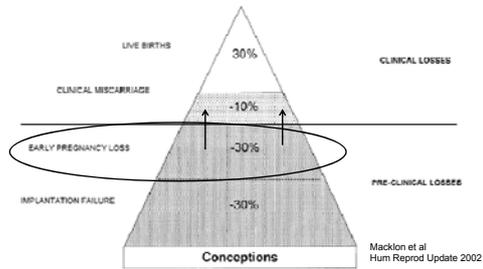
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## Pregnancy loss iceberg



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## Hypothesis

If PULs have a negative impact on the chance of subsequent live birth, then PULs should be part of the definition of recurrent miscarriage.

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### Pregnancies of unknown location have an important prognostic impact in women with unexplained recurrent miscarriage

Kolte AM<sup>1</sup>, van Oppenraaij RH<sup>2</sup>, Quenby SJ<sup>3</sup>, Fairquharson RG<sup>4</sup>, Stephenson MP<sup>5</sup>, Goddijn M<sup>6</sup>, Christiansen OB<sup>1,7</sup> on behalf of the ESHRE Special Interest Group Early Pregnancy

<sup>1</sup>Recurrent Miscarriage Unit, Fertility Clinic 4071, University Hospital Copenhagen, Rigshospitalet, Denmark

<sup>2</sup>Department of Obstetrics and Gynaecology - sub division Obstetrics & Prenatal Care, Erasmus MC, Rotterdam, The Netherlands

<sup>3</sup>Clinical Sciences Research Institute, University Hospital Coventry, Warwick Medical School, Warwick, UK

<sup>4</sup>Department of Obstetrics and Gynaecology, Liverpool Women's Hospital, Liverpool, UK

<sup>5</sup>Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, USA

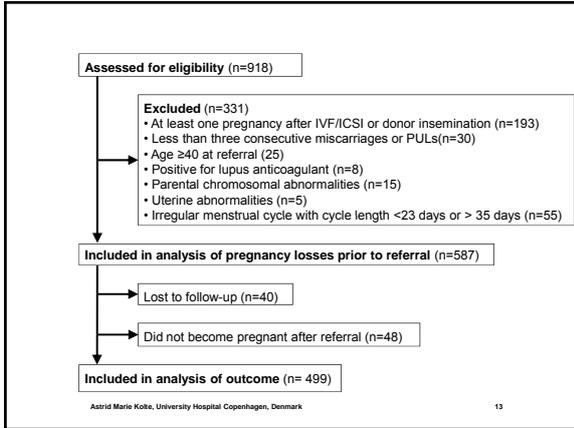
<sup>6</sup>Center for Reproductive Medicine, Department of Obstetrics and Gynaecology, Academic Medical Center, Amsterdam, the Netherlands

<sup>7</sup>Department of Obstetrics and Gynaecology, Aalborg Hospital, Aalborg, Denmark

Submitted to Human Reproduction February 2013

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## Pregnancy history

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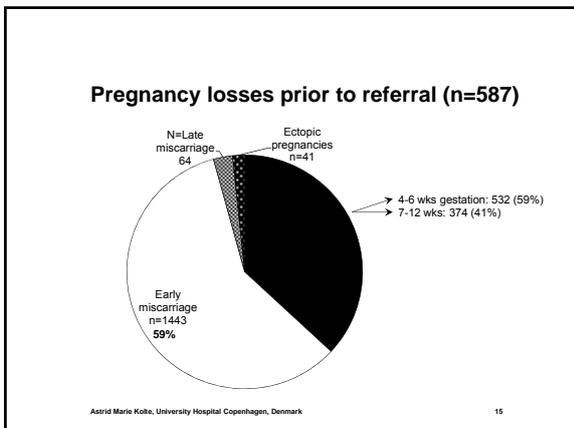
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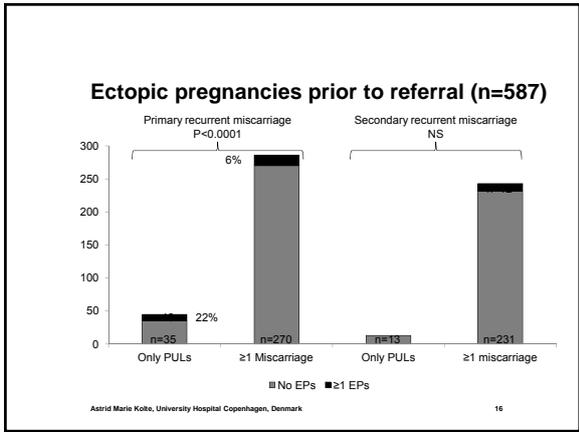
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### Characteristics for PULs

	4-6 weeks gestation (n=77)			7-12 weeks gestation (n=46) 37%		
	u-hCG <sup>a</sup> , home	u-hCG, GP <sup>b</sup>	s-hCG <sup>c</sup>	u-hCG, home	u-hCG, GP	s-hCG
No TVS <sup>d</sup>	34	9	10	13	8	3
TVS	3	2	19	4	4	14

<sup>a</sup>u-hCG: Urinary hCG measurement, <sup>b</sup>GP: General practitioner, <sup>c</sup>s-hCG: Serum hCG measurement, <sup>d</sup>TVS: Transvaginal sonography

31% (Total TVS: 24) | 48% (Total TVS: 22)

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### Chance of live birth

Astrid Marie Kolte, University Hospital Copenhagen, Denmark

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### Chance of live birth

	RR (95% CI)
<b>All (n=499)</b>	
Age at index pregnancy <sup>a</sup>	0.98 (0.96;0.99)
Miscarriage <sup>b</sup>	0.88 (0.81;0.95)
PUL <sup>c</sup>	0.91 (0.84;0.98)
<b>BMI (n=312)</b>	<b>37%</b>
BMI <20	1.12 (0.89;1.40)
BMI 20-24	1 (reference)
BMI >24	1.02 (0.77;1.34)
BMI ≥30	1.03 (0.80;1.33)

<sup>a</sup>Index pregnancy: The first pregnancy after referral; <sup>b</sup>Miscarriage: Histologically or ultrasonically confirmed intrauterine pregnancy loss before 12 weeks gestation; <sup>c</sup>PUL: Positive hCG without definitive diagnosis on location

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### Conclusions & guideline recommendations

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PULs constitute 37% of all pregnancies reported by RM patients at first consultation

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•Women with no confirmed  
intrauterine miscarriages have a  
higher frequency of EPs.

•IVF as treatment? RCT?

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•PULs and miscarriages have the  
same prognostic impact on live  
birth (RR 0.90)

•Increasing age is a negative  
prognostic factor for live birth (2%  
p.a.)

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**PULs have the same impact on  
the chance of live birth as  
miscarriages and should  
therefore be included in the  
definition of recurrent  
miscarriage.**

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The Fertility Clinic  
Copenhagen University Hospital  
Rigshospitalet  
Denmark

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## When to screen for thyroid function abnormalities?



GHRI Center for reproductive medicine

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## When to screen for thyroid function abnormalities?



Rosa Vissenberg  
PhD student

*Conflict of interest*  
No commercial or financial interests pertaining to the subject of this presentation or its content.

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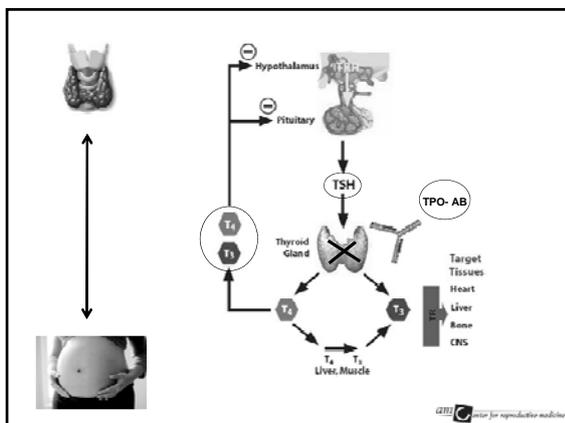
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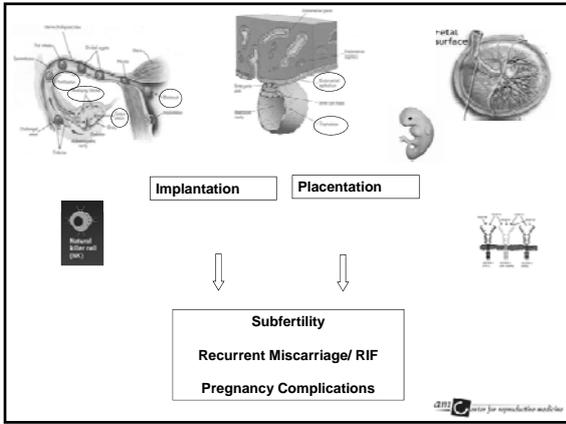
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**Guidelines**

**Hyperthyroidism**  
 Propylthiouracil (PTU) or  
 Methimazole (MMI)

**Hypothyroidism**  
 Levothyroxine (T4)

The complex block contains text about thyroid guidelines, logos for the Dutch Thyroid Association and nvog, and a small image of a medicine bottle. At the bottom right, there is a logo for 'GRIJ' (Gynaecological and Reproductive Institute).

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**What if symptoms are missing?**

The complex block features the text 'What if symptoms are missing?' and three small images: a person's face, a person holding a pill, and a person holding their head in pain. At the bottom right, there is a logo for 'GRIJ' (Gynaecological and Reproductive Institute).

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**Debate screening**



YES    NO



GUM Centre for reproductive medicine

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**Debate screening**



Subfertility  
Recurrent Miscarriage  
Pregnancy

YES    NO



GUM Centre for reproductive medicine

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**Guidelines – Subfertility**

	No routine measurement
	No routine measurement
	No routine measurement

GUM Centre for reproductive medicine

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### Guidelines – Recurrent Miscarriage



Not mentioned



Withdrawn



Not mentioned



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### Guidelines - Pregnancy

#### First trimester screening of risk patients



1. Previous thyroid dysfunction
2. Irradiation of the neck or goiter
3. Family history of thyroid disease
4. TPO-Ab
5. Dysthyroid symptoms
6. DM type 1 or other immune diseases
7. Unexplained subfertility
8. Miscarriage or preterm birth



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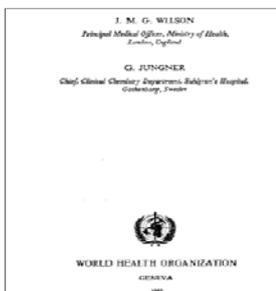
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### WHO Screening Criteria



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1. The condition should be an important health problem

The condition	Incidence
Hyperthyroidism	0.1-0.4%
Hypothyroidism	0.6%
Subclinical hypothyroidism (SCH)	2.0-3.0%
Thyroid autoimmunity → TPO	8.0-14.0%

van den Boogaard et al. Hum Reprod Update 2011

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1. The condition should be an important health problem

**Subclinical hypothyroidism**

**Pregnancy complications**

Preeclampsie  
OR 1.68 (95% CI 1.09-2.6)

Perinatal mortality  
OR 2.73 (95% CI 1.59-4.7)

↓ intelligence scores

van den Boogaard et al. Hum Reprod Update 2011

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1. The condition should be an important health problem

**Subclinical hypothyroidism**

*Possible association with:*

Subfertility ( OR 4.0, 95% CI 1.7-9.8)

van den Boogaard et al. Hum Reprod Update 2011  
Abalovich et al. Gynecol Endocrinol 2007

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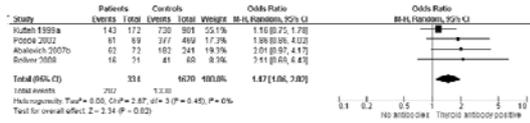
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1. The condition should be an important health problem

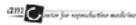
### Thyroid autoimmunity

#### Unexplained subfertility

Figure 2. Forest plot of Odds Ratio's and 95% Confidence Interval of pooled studies comparing euthyroid thyroid antibody positive patients with euthyroid antibody negative controls according to the risk of unexplained subfertility.



van den Boogaard et al. Hum Reprod Update 2011




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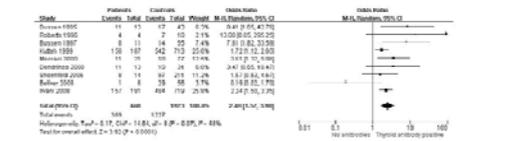
1. The condition should be an important health problem

### Thyroid autoimmunity

#### Recurrent miscarriage



Incidence 8-36%



van den Boogaard et al. Hum Reprod Update 2011




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1. The condition should be an important health problem

### Thyroid autoimmunity

#### Pregnancy complications

##### Miscarriage

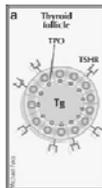
OR 3.7 (95% CI 1.8-7.6)

##### Preterm birth

OR 1.9 (95% CI 1.1-3.5)

##### Postpartum thyroid disease

OR 12 (95% CI 5.6-24)



van den Boogaard et al. Hum Reprod Update 2011




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## WHO Criteria

1. The condition should be an important health problem



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## 2. There should be an accepted treatment for patients with recognized disease

### Subclinical hypothyroidism

Recurrent miscarriage  
Pregnant population



No evidence effective treatment

Vissenberg et al. *Human Reprod Update* 2012  
Lazarus et al. *NEJM* 2012



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## 2. There should be an accepted treatment for patients with recognized disease

### Subclinical Hypothyroidism

Subfertile population

- ↑ Delivery rate
- ↑ Fertilized oocytes
- ↑ Implantation rate
- ↓ Miscarriage rate
- Clinical pregnancy rate – ns

Limitation: no live birth rate as outcome

Velkeniers et al. *Human Reprod Update* 2013



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2. There should be an accepted treatment for patients with recognized disease

**Thyroid autoimmunity**

Subfertility  
 Recurrent miscarriage  
 Pregnancy



No evidence effective treatment

Visserberg et al. Human Reprod Update 2012




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**WHO Criteria**

- 1. The condition should be an important health problem
- 2. There should be an accepted treatment for patients with recognized disease.

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- 3. Facilities for diagnosis and treatment should be available.
- 4. There should be a detectable early stage
- 5. There should be a suitable test
- 6. The test should be acceptable to the population
- 7. The natural history of the condition should be adequately understood
- 8. There should be an agreed policy on whom to treat as patients
- 9. The costs should be balanced against the benefits
- 10. The risks, both physical and psychological, should be less than the benefits

Center for Reproductive Medicine

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**WHO Criteria**

- 1. The condition should be an important health problem
- 2. There should be an accepted treatment for patients with recognized disease.
- 3. Facilities for diagnosis and treatment should be available.

Center for Reproductive Medicine

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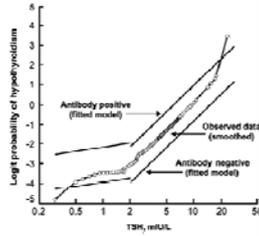
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#### 4. There should be a detectable early stage

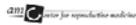
↑ Level of TSH

TPO-Ab – annual risk 2.1%

Whickham Survey



Van derpump et al. Clinical endocrinology 1995



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#### WHO Criteria

- 1. The condition should be an important health problem
- 2. There should be an accepted treatment for patients with recognized disease.
- 3. Facilities for diagnosis and treatment should be available.
- 4. There should be a detectable early stage



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#### WHO Criteria

- 1. The condition should be an important health problem
- 2. There should be an accepted treatment for patients with recognized disease.
- 3. Facilities for diagnosis and treatment should be available.
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- 5. There should be a suitable test



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### WHO Criteria

- ✓ 1. The condition should be an important health problem
- ✗ 2. There should be an accepted treatment for patients with recognized disease.
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### WHO Criteria

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- ✓ 6. The test should be acceptable to the population
- ✓ 7. The natural history of the condition should be adequately understood




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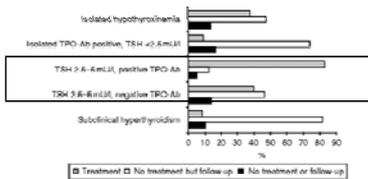
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### 8. There should be an agreed policy on whom to treat as patients



TSH > 2.5mU/L: treatment T4  
(evidence poor)



Vaidya et al. EJE 2012  
de Groot et al. JCEM 2012




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8. There should be an agreed policy on whom to treat as patients



TSH > 2.5mU/L: treatment T4  
(evidence poor)



TSH > 2.5 mU/L and TPO-Ab: T4  
TSH > 4.0 mU/L : treatment T4



TSH > 4.0 mU/L : treatment T4




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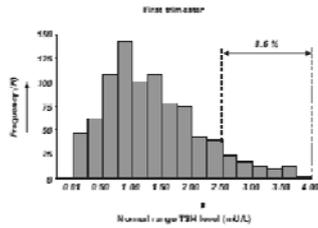
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8. There should be an agreed policy on whom to treat as patients

Population specific reference intervals



Medici et al. JECM 2012




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WHO Criteria

- 1. The condition should be an important health problem
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### WHO Criteria

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- ✗ 8. There should be an agreed policy on whom to treat as patients
- ✗ 9. The costs should be balanced against the benefits



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### WHO Criteria

- ✓ 1. The condition should be an important health problem
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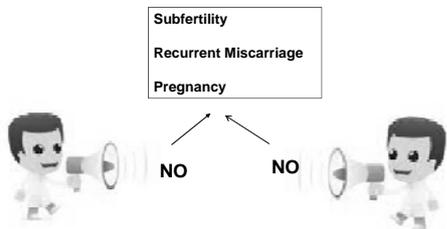
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### Conclusion



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## WHO Criteria

The condition should be an important health problem

- 2. There should be an accepted treatment for patients with recognized disease.
- 3. Facilities for diagnosis and treatment should be available.
- 4. There should be a detectable early stage
- 5. There should be a suitable test
- 6. The test should be acceptable to the population
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## Evidence & Gaps

Treatment effects subclinical hypothyroidism pregnancy

### CATS trial

IQ level 3 yr

Obstetric outcomes



RCT for treatment of subclinical hypothyroidism in a subfertile population- effect on Live Birth Rate



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## Evidence & Gaps

Treatment effects thyroid autoimmunity

### T4-LIFE trial



<http://www.studies-obsgyn.nl/T4-LIFE>

### Tablet-trial:

live birth rate



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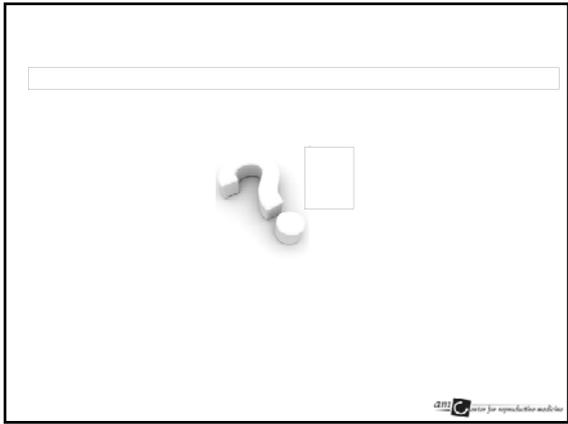
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**Thyroid antibodies and miscarriage: Clinical trial**  
Arri Coomarasamy  
University of Birmingham

UNIVERSITY OF BIRMINGHAM  
Birmingham Women's NHS Foundation Trust

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**Conflict of Interest**

- I declare that I have no commercial or financial interests pertaining to the subject of this presentation or its content

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**Agenda**

- **What** is it?
- **Why** are we doing it?
- **How** are we doing it?
- **Where** are we with it?

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**TABLET**  
Efficacy of Levothyroxine Treatment on Pregnancy and Neonatal Outcomes in Women with Thyroid Antibodies

MRC Health Research Council

£1.4m

UNIVERSITY OF BIRMINGHAM

Birmingham Women's NHS Foundation Trust

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### TABLET Trial: What is it?

Primary objective: To test the hypothesis that

- ...in **euthyroid women with thyroid peroxidase antibodies (TPO)**,
- ...**levothyroxine** (50mcg, oral, once daily), started pre-conceptually and continued to the end of pregnancy,
- ...compared with placebo,
- ...**increases the proportion of women who attain a live birth beyond 34 completed weeks of gestation by at least 10%.**

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### TABLET – Why?

nature CLINICAL PRACTICE  
ENDOCRINOLOGY  
& METABOLISM

**Table 2** Percentage of euthyroid women with and without thyroid autoantibodies who miscarry

Study and country	Number of patients (% TAI)	Proportion of patients who miscarried (%)		P-value*	Characteristics of study group
		TAI	No TAI		
Stagnam-Green et al. (1963), <sup>38</sup> US	552 (19.6%)	17.0	11.4	0.01	Unselected population
Gilboa (1991), <sup>40</sup> Belgium	729 (8.2%)	13.8	8.3	<0.001	Unselected population
Lejeune et al. (1993), <sup>41</sup> Belgium	363 (6.3%)	22.0	8.0	<0.001	<14 weeks' gestation
Pratt et al. (1993), <sup>42</sup> US	42 (31.0%)	67.0	32.0	NA	Recurrent miscarriages
Singh et al. (1996), <sup>43</sup> US	487 (22.0%)	32.0	16.0	0.002	Pregnancy achieved with ART
Bussen and Steck (1995), <sup>44</sup> Germany	66 (17.0%)	36.0	7.0	<0.01	Recurrent miscarriages
Ijima et al. (1997), <sup>45</sup> Japan	1,179 (10.0%)	10.4	6.5	<0.01	Unselected population
Engle et al. (1998), <sup>46</sup> US	145 (33.0%)	19.0	17.0	>0.05	Recurrent miscarriages
Kim et al. (1998), <sup>47</sup> Korea	79 (29.1%)	20.0	11.4	<0.01	Pregnancy achieved with ART
Kulbicki et al. (1999), <sup>48</sup> US	800 (29.8%)	22.0	11.5	0.01	Two or more consecutive miscarriages
Muller et al. (1999), <sup>49</sup> Netherlands	173 (14.0%)	33.0	19.0	0.29	Pregnancy achieved with ART
Bussen et al. (2000), <sup>49</sup> Germany	49 (30.6%)	34.2	8.3	0.001	Failure to conceive after three cycles of IVF
Dendrinos et al. (2000), <sup>50</sup> Greece	45 (32.9%)	37.0	13.0	<0.01	Recurrent miscarriages
Rushworth et al. (2000), <sup>51</sup> UK	870 (19.0%)	22.0	12.0	NA	History of miscarriage
Bagis et al. (2001), <sup>52</sup> Turkey	876 (12.3%)	20.0	14.1	<0.001	Unselected population
Poppe et al. (2001), <sup>53</sup> Belgium	234 (14.0%)	33.0	23.0	<0.01	Pregnancy achieved with ART
Sieiro Netto et al. (2004), <sup>54</sup> Brazil	534 (5.4%)	10.3	2.0	<0.001	Unselected pregnant young women
Ningro et al. (2005), <sup>55</sup> Italy	404 (15.0%)	32.0	20.0	<0.001	Pregnancy achieved with ART
Ningro et al. (2007), <sup>56</sup> Italy	564 (11.7%)	13.8	2.4	<0.01	Pregnant women

Abbreviations: ART, assisted reproductive technologies; IVF, in vitro fertilization; NA, not applicable; TAI, thyroid autoantibodies.

Poppe K et al. (2008)

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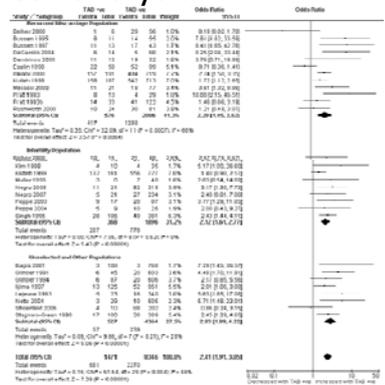
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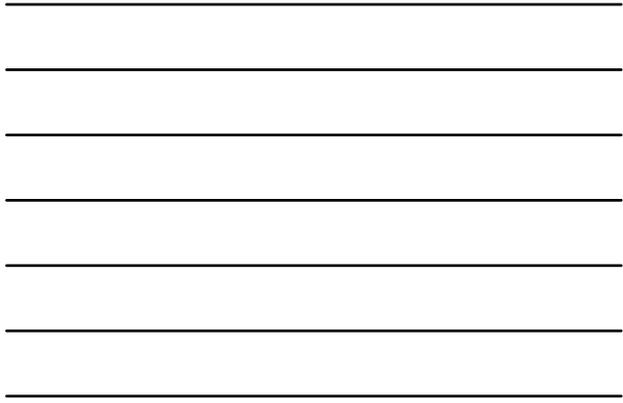
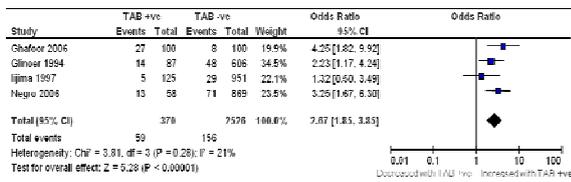
# Our own meta-analysis

BMJ 2011

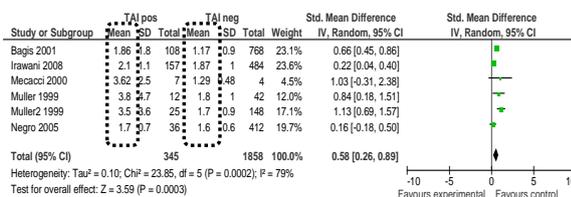


# Pre-term birth

BMJ 2011



# TSH?



## Thyroid replacement: Study 1 Negro, HR, 2005

- **Population:** 86 women TPO +ve undergoing ART (TPO +ve rate was 15%)
- **Intervention:** levothyroxine
- **Comparison:** placebo
- **Outcome:** pregnancy, miscarriage rate
- **Design:** RCT
- **Findings**
  - Pregnancy rate: 56% vs 49% NS
  - Miscarriage rate: 33% vs 52% NS – Type II error?

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## Thyroid replacement: Study 2 Negro 2006 Journal of clinical endocrinology & Metabolism

- **Population:** 115 Euthyroid women TPO +ve (screened from unselected population) (TPO +ve rate was 11.7%)
- **Intervention:** levothyroxine
- **Comparison:** untreated
- **Outcome:** miscarriage rate , obstetric outcome
- **Design:** RCT
- **Findings**
  - Miscarriage rate: 3.5% vs 13.8% (24% in TPO -ve group [n=86g]!!!!)
  - Preterm birth: 7% vs 22.4%




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## Do we need a trial?

- **Clinician Survey** (to see if there is collective uncertainty – equipoise)
  - Now over 183 responses (1/3 gynaecologists; 1/3 obstetricians; 1/3 endocrinologists)
  - > 85% will randomise
- **Patient survey** – support
- **EP-CSG** – Support
- **MA** – support
- **BTF** – support

**TABLET** study




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## TABLET: Where are we?

- 129 randomised.
- T4Life Trial



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**LIFE STYLE FACTORS INCREASE THE RISK OF RECURRENT MISCARRIAGE**

*William H. Kutteh, M.D., Ph.D., H.C.L.D.  
Clinical Professor, Vanderbilt University*



William H. Kutteh, MD, Ph.D. Raymond W. Kr, MD



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**LEARNING OBJECTIVES**

At the conclusion of this presentation, participants should be able to:

1. Discuss the lifestyle issues that influence the outcome of pregnancy.
2. Screen all patients for obesity, alcohol use, tobacco use, and caffeine use.
3. Counsel patients about the harmful effects of certain lifestyle factors on successful pregnancy outcome.
4. Understand the effect of maternal age and number of prior losses on predicting future live births

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**DISCLOSURES**

- Research Support - Finox
- Research Support - Merck
- Owner/Director - Reproductive Lab

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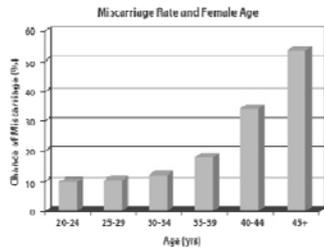
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### Spontaneous Pregnancy Loss: Role of Maternal Age



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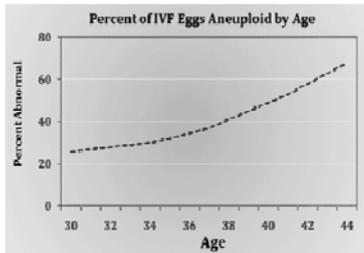
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### Spontaneous Pregnancy Loss: Role of Maternal Oocyte Aneuploidy




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### Spectrum of Pregnancy Loss



- Pregnancy of Unknown Location (PUL)
- Early embryonic (< 6 wks)
- Embryonic (> 6 to 9 wks)
- Fetal loss (> 9 to 20 wks)
- Miscarriage (< 20 wks)
- Stillbirth (> 20 wks)

Silver et al. Obstet Gynecol 118: 1402-1408, 2011.

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## What about Lifestyle Factors?

Effects on the Risk of miscarriage

- Obesity
- Tobacco
- Caffeine
- Ethanol



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## CONTROVERSIES

- How much alcohol is safe before pregnancy?  
*"I will stop drinking when I get pregnant"*
- Why pressure me about my weight?  
*"My overweight friends had babies"*
- How many cigarettes are safe while pregnant?  
*"I'll stop smoking when I get pregnant"*

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## Obesity Trends Among U.S. Adults Between 1985 and 2010

- **Obesity:** Body Mass Index (BMI) of 30 or higher.
- **Body Mass Index (BMI):** A measure of an adult's weight in relation to his or her height, specifically the adult's weight in kilograms divided by the square of his or her height in meters.



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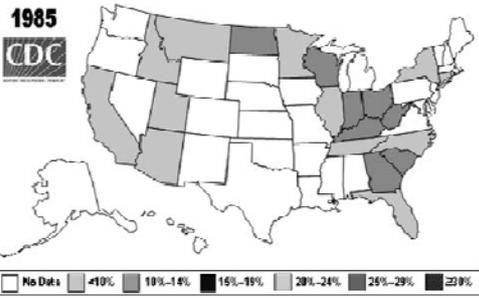
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### Obesity in US Adults - 1985



Percent of Obese (BMI ≥ 30) in US Adults

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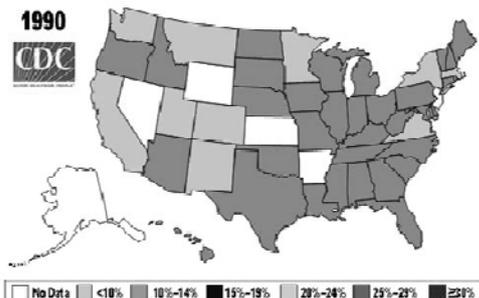
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### Obesity in US Adults - 1990



Percent of Obese (BMI > 30) in US Adults

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### Obesity in US Adults - 1995



Percent of Obese (BMI > 30) in US Adults

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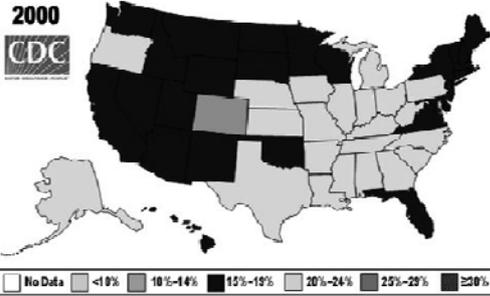
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### Obesity in US Adults - 2000



Percent of Obese (BMI > 30) in US Adults

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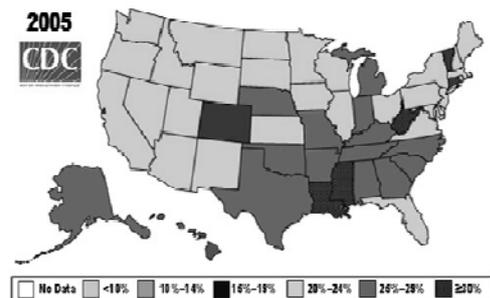
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### Obesity in US Adults - 2005



Percent of Obese (BMI > 30) in US Adults

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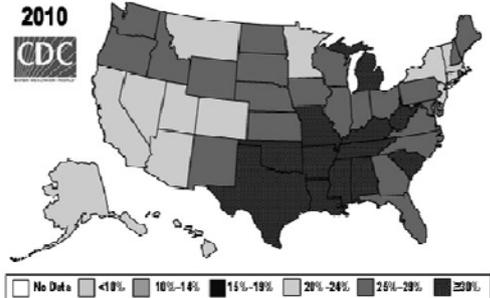
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### Obesity in US Adults - 2010



Percent of Obese (BMI > 30) in US Adults

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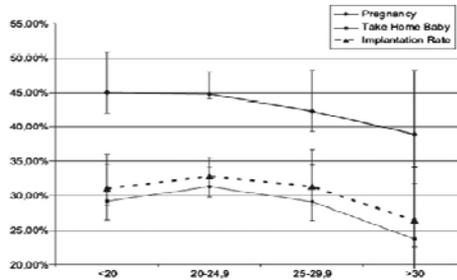
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### Pregnancy, Implantation, and Take Home Baby Rates based on BMI



Penzias AS. Recurrent IVF Failure: other factors. Fertil Steril 97:1033-1038, 2012

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### Overweight (BMI >25) and miscarriage

- Retrospective study of 393 women undergoing IVF with single blastocyst transfer
- Cases: 169 women with BMI  $\geq 25 \text{ kg/m}^2$
- Controls: 224 women with BMI 18.5-24.9
- More than double the risk of miscarriage in women with BMI  $>25 \text{ kg/m}^2$  vs controls (OR=2.4 CI=95%, 1.6-3.8 p=.001)

Rittenberg V, Sobaleva S, Ahmad A, et al. Influence of BMI on risk of miscarriage after single blastocyst transfer. Hum Reprod. 26:2642-50, 2011.

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### Euploid miscarriage and Body Mass Index

Retrospective study of 204 miscarriages sent for chromosome testing based on BMI

		Euploid	Noneuploid	P-value
Age	<35 yrs	51%	40%	0.009
	$\geq 35$ yrs	32%	68%	
BMI (kg/m <sup>2</sup> )	<25	37%	63%	0.040
	$\geq 25$	53%	47%	

Conclusion: Obesity associated with an increased rate of euploid miscarriage

Landres IV, Milki AA, Lathi RB. Hum Reprod 25:1123-1126, 2010.

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## Spontaneous pregnancy and Obesity Increased risk of Miscarriage

- Systematic review of published studies
- Six studies with a cohort of 28,538 women
- In women with a BMI of >30kg/m, the probability of spontaneous miscarriage was 27% higher
- In women with recurrent miscarriage who had a BMI of >30kg/m, probability of spontaneous miscarriage was 7% higher

Boots C, Stephenson MD. Does obesity increase the risk of miscarriage in spontaneous conception. *Semin Reprod Med* 29:507-513, 2011.

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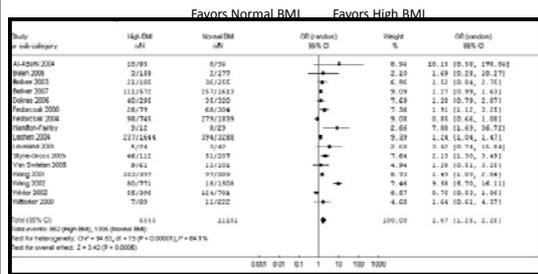
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## OR of Miscarriage regardless of the Method of Conception



Metwally M et al. Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? *Fertil Steril* 90: 714-726, 2008.

Copyright © 2008 American Society for Reproductive Medicine

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## Obesity (BMI >30) and miscarriage

- Obesity has been shown to be a risk factor for miscarriage
- Obesity has been shown to be an independent risk factor for first trimester miscarriage
- Miscarriage association is strongest in women with morbid obesity (BMI > 40 kg/m<sup>2</sup>)
- Increased risk may be linked to a generalized increase in systemic inflammatory responses

Smith ML, Schust DJ. Endocrinology of Recurrent Pregnancy Loss. *Semin Reprod Med*. 29:482-490, 2012.

Johnson AR, Milner JJ, Makowski. The inflammation highway: metabolism accelerates inflammatory traffic in obesity. *Immunol Rev* 249:218-238, 2012.

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## Obesity and Effect on Sperm

Possible role for increased miscarriage

- Cross sectional study of 305 males presenting to a urology clinic
- Cases: 187 overweight males ( $\geq 25 \text{ kg/m}^2$   $\leq 30$ )  
36 obese males ( $\geq 30 \text{ kg/m}^2$ )
- Controls: 82 normal weight males ( $< 25 \text{ kg/m}^2$ ).
- Performed semen analysis and sperm DNA fragmentation assay
- Percentage of DNA damage higher in obese groups ( $p = 0.004$ )  
7.7 (4.9-10.5) in BMI  $\geq 30 \text{ kg/m}^2$   
4.7 (4.0-5.3) in BMI  $\geq 25 \text{ kg/m}^2$   $\leq 30$   
4.4 (3.3-5.5) in BMI  $< 25 \text{ kg/m}^2$

Fariello RM, et al. Association between obesity and alteration of sperm DNA integrity and mitochondrial activity. BJU Int. 110:863-867,2012

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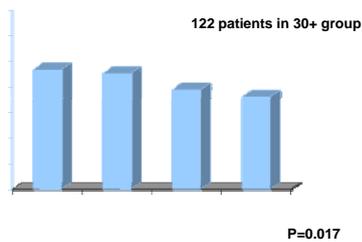
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## BMI and Uterine Receptivity in Oocyte Recipients

Potential role in Miscarriage



Bellver et al. Fertil Steril. Obesity and poor reproductive outcome: the potential role of the endometrium 87:1098-1101, 2007

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## Obesity and Oocyte and Embryo quality

- Prospective , multi-center study of women undergoing IVF
- 487 patients and 1417 cycles
- Correlations with increased BMI were:
  - Increased cancelled cycles
  - fewer oocytes retrieved
  - fewer embryos available
  - lower odds of clinical pregnancy
  - decreased live birth rate

Pinborg A et al. Influence of female bodyweight on IVF outcome. Reprod BioMedicine Online 23:490-499, 2011.

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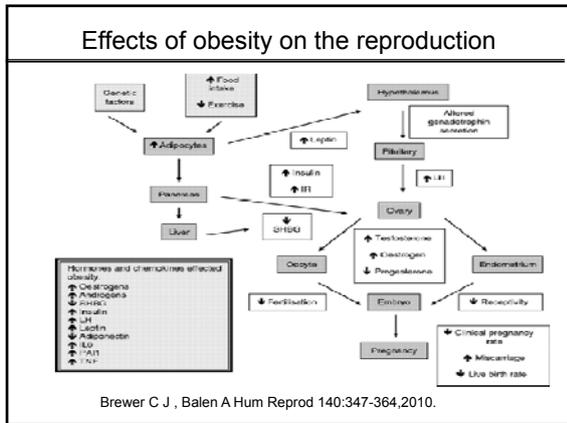
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### Metformin and Miscarriage

- 197 obese PCOS women in Pakistan
- Cases conceived on metformin and continued throughout pregnancy
- Controls conceived without metformin or stopped metformin in early pregnancy
- Miscarriage rate 8.8% on metformin vs 29.9% in controls (p<0.001)

Nawaz FH. Gynecol Obstet Invest 69: 184-9, 2010

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### Metformin and Miscarriage

- Prospective, single center study
- Cases: 98 pregnant, hyperinsulinemic PCOS treated with metformin  $\geq 1700$  mg/day to 37 weeks
- Controls: 110 normal pregnant women
- Comparable Apgars, birth weight and birth length
- Miscarriage rate 9.1% on metformin vs 20 % in controls
- Less gestational HTN and DM compared to controls

DeLeo V et al. Eur J Obstet Gyn Reprod Biol 63-6, 2011.

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## Smoking in the US

- 30% reproductive aged women smoke
- 35% of reproductive aged men smoke
- Only 22% of female reproductive health care providers were aware of the deleterious effect on fertility

ASRM Committee Opinion Fertil Steril. 98:1400-1406, 2012

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## Smoking and Fertility

- 60% more likely to be infertile (CI=1.34-1.91)
- Require nearly twice the number of IVF attempts to conceive
- Menopause occurs 1 to 4 years earlier
- Basal FSH significantly higher
- Increased miscarriage (natural & IVF)



ASRM Committee Opinion Fertil Steril. 98:1400-1406, 2012  
ESHRE Task Force Hum Reprod 25:578-583,2010.

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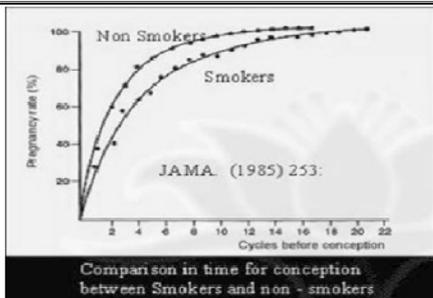
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## Time to conception Based on Smoking



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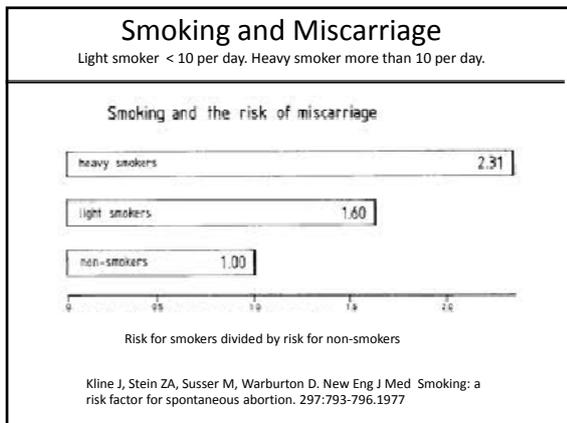
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### Smoking and Miscarriage

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- Smoking increased miscarriage in natural and IVF conceptions
- Accounted for 16% of miscarriages of inner-city women age 14 to 39
- Vasoconstrictive and antimetabolic effects may lead to placental insufficiency, embryonic and fetal growth restriction and demise
- Proportion of diploid oocytes in the ovary increases with the number of cigarettes smoked per day

Augood C, Duckitt K, Templeton AA. Hum Reprod 13:1532-1539,1998  
Zenzes MT,Wang P, Casper RF. Hum Reprod 10:3213-3217,1995.  
Ness RB, et al. NEJM 340:333-339,1999.

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### Smoking Increases IVF Miscarriages

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- Retrospective study 8323 women undergoing IVF  
Smokers=3617, Non Smokers= 4706
- Smoking was associated with a significantly lower delivery rate  
OR= .72, (95% CI 0.61- 0.84)
- Smoking was associated with a higher miscarriage rate compared  
21.4% vs 16.4% (p=.02)
- Adjusted effect of smoking on live birth was stronger than an increase in female age with >10years from 20-30  
OR= .78 (95%CI .63-.96)

Lintsen AM, Pasker-de jong PC, De boer EJ, et al. Effects of subfertility cause, smoking and body weight on the success rate of IVF. Hum Reprod. 20:1867-75, 2005.

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**Smoking and Miscarriage**  
Odds ratio of miscarriage per pregnancy.

- Meta-analysis of 7 studies of women undergoing IVF
- 211 smokers and 1688 non-smokers
- Miscarriage defined as any loss before 20 weeks
- Smokers defined as any amount of active smoking
- Smoking patients demonstrated significantly lower odds of live birth per cycle  
OR 0.54, 95% CI 0.30 to 0.99
- Smokers had a significantly higher odds of spontaneous miscarriage  
OR 2.65, 95% CI 1.33 to 5.30

Waylen A et al. Hum. Effects of cigarette smoking upon clinical outcomes of assisted reproduction: a meta-analysis Reprod. Update 2009;15:31-44

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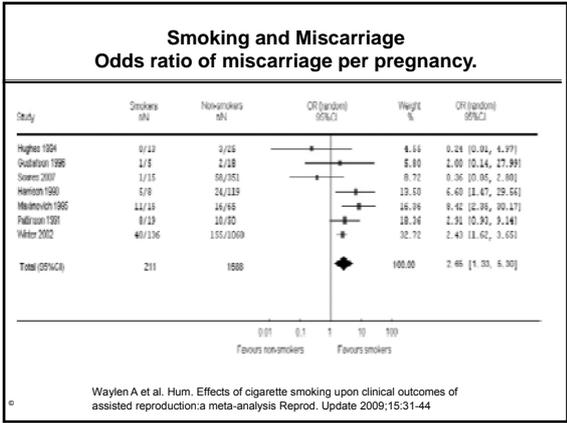
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**Cigarette smoking and uterine receptivity**  
Possibly due to implantation failure

- Retrospective study of reproductive aged women
- Cases: 44 heavy smokers (>10/day)
- Controls: 741 non heavy smokers (0-10/day)
- Pregnancy rates significantly lower in heavy smokers vs non heavy smokers (34.1% vs 52.2 %)

SR Soares, C Simon, J Remohi, and A Pellicer. Cigarette smoking affects uterine receptiveness Hum Reprod. 22: 543-547, 2007.

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## Proposed FDA Warning

Smoking during pregnancy can increase the risk of miscarriage, stillborn or premature infants, infants with low birth weight and an increased risk for sudden infant death syndrome (SIDS).



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## Caffeine May Double Miscarriage Risk: Study

Jan 21, 2008 9:28 AM CST

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## Amounts of Caffeine in Beverages

**Comparing caffeine**  
Milligrams of caffeine in a serving of various beverages:

Coffee (8 oz.)*	150 mg
Starbucks mocha ("tall" size, 12 oz.)	95
Red Bull (8 oz.)	80
Iced tea (12 oz.)*	70
Coke (12 oz.)	54
Hot cocoa (8 oz.)*	15

\* Amount of caffeine will vary depending on how it is made.  
*The Chronicle*

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Sources of Caffeine		
Product	Serving Size	Milligram (mg) of Caffeine per serving
Brewed Coffee	8oz / 237 mL (1 cup)	135.0
Roasted & Ground, Percolated	8oz / 237 mL (1 cup)	118.0
Roasted & Ground, Filter Drip	8oz / 237 mL (1 cup)	179.0
De-caffeinated Coffee	8oz / 237 mL (1 cup)	3.0 - 5.0
Black Tea	8oz / 237 mL (1 cup)	48.0
Green Tea	8oz / 237 mL (1 cup)	30.0
Cola Beverage, Regular	12 oz / 355 mL (1 can)	36.0 - 46.0
Cola Beverage, Diet	12 oz / 355 mL (1 can)	39.0 - 50.0
Chocolate Milk	8 oz / 237 mL	8.0
Candy, Milk Chocolate	1 oz / 28g	7.0
Red Bull® Energy Drink	250 mL (1 can)	80.0
General Coffee Cup Sizes		
Small (Short)	8 oz	
Medium (Tall)	12 oz	

Adapted from Health Canada's Caffeine in Food (<http://www.hc-sc.gc.ca/fn-an/securett/active/caf/food-ca-faliments-eng.php>)

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### Maternal caffeine consumption during pregnancy and risk of miscarriage

- Prospective Cohort Study
- Cases: 635 pregnant women with caffeine consumption of  $\leq 200$  mg/d and 164 pregnant women with consumption of  $>200$ mg/d
- Controls: 264 pregnant women, no caffeine
- Risk of miscarriage compared with no caffeine  
OR = 1.42 (95% CI 0.93-2.15) if  $\leq 200$  mg/d  
OR = 2.23 (95% CI 1.34-3.69) if  $> 200$  mg/d

Weng X, Odouli R, Li DK. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gynecol. 2008;198(3):279.e1-8.

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### Caffeine intake and Miscarriage

- Nested case control study
- Case: Women drink  $\geq 75$  mg/d of caffeine
- Controls: 206 Women drink  $<75$  mg/d
- Increased spontaneous abortion with adjusted OR of 1.26, 1.45, 1.44, 1.72 for prepregnancy intake of 75-300, 301-500, 501-900 and  $>900$  mg of caffeine compared to controls.

Tolstrup JS et al. Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion? Hum Reprod. 18:2704-10,2003.

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## Caffeine intake and Miscarriage

- >200-300 mg/day (2-3 cups/day) may increase the risk of miscarriage
- >500 mg/day (>5cups) decreases fertility
- “Overall, moderate caffeine consumption (2 cups of coffee/day or its equivalent) before or during pregnancy has no apparent adverse effects on fertility or pregnancy outcomes”

ASRM Committee Opinion. Fertil Steril epub 2013

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## Alcohol Use and Pregnancy

Among women in the US seeking pregnancy:

- 54.9% reported alcohol use
- 12.4% reported binge drinking
- 12.5% continue to drink during pregnancy



ACOG Committee Opinion #422. December 2008

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## Alcohol Use in US Women Ages 18 to 44, 1991 to 2005



Binge drinking = >5 drinks in one day in the past 30 days

Behavioral Risk Factor Surveillance System, US. 2009

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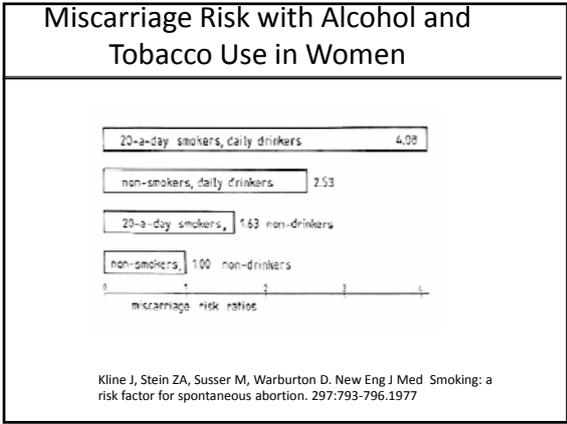
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### Alcohol use and Miscarriage

- Associated with an increased risk of miscarriage
- A few as five (5) alcoholic drinks per week significantly increase the risk for first trimester miscarriage
- When combined with cigarette smoking, alcohol use may increase the risk of miscarriage 4-fold

Harlap S, Shiono PH. Alcohol, smoking, and incidence of spontaneous abortions in the first and second trimester. Lancet. 2:173-178, 1980.  
Kline J, Shroat P, Stein ZA, Susser M, Warburton D. Drinking during pregnancy and spontaneous abortion. Lancet 2: 176-180, 1980.

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### Two drinks/week Increase the Risk of Miscarriage

- 92,719 women in Danish National Birth Cohort
- Determined risk of first trimester miscarriage (<13 weeks) and fetal death (13 to 16 weeks)

DRINKS /WEEK	% IN THIS GROUP	LOSS < 13 WEEKS	LOSS 13-16 WEEKS
None	55%	1.0	1.0
2 to 3.5	43%	1.66 (1.43-1.92)	1.57 (1.3-1.9)
More than 4	2%	2.82 (2.27-3.49)	1.73 (1.24-2.41)

Andersen AM et al. Moderate alcohol intake during pregnancy and risk of fetal death. Int J Epidemiol. 41:405-413, 2012.

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## Summary of Lifestyle Factors

Risks of miscarriage increase 1.5 -2 fold

- Tobacco (>10/day)
- Ethanol (> 2/week)
- Obesity (BMI > 30)
- Caffeine (> 2-3 cups/day)



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**The impact of genetic testing for couples with recurrent miscarriage**

Fleur Vansenne, MD, PhD  
 Department of Clinical Genetics  
 Academic Medical Center, Amsterdam

ESHRE-meeting  
 London 2013




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**Conflict of interest**

- I declare that I have no commercial or financial interests pertaining to the subject of this presentation or its content




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**Learning objectives**

- Overview of karyotyping in recurrent miscarriage
- Efficacy from doctors' perspective
- Efficacy from patients' perspective
- Impact of genetic testing for patients in terms of anxiety, depression and distress




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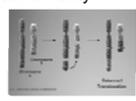
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## Genetic testing in recurrent miscarriage

- Chromosome abnormality riskfactor for recurrent miscarriage (RM)
- 2-5% of couples with RM carry a chromosomal abnormality
  - Balanced reciprocal translocation
  - Robertsonian translocation
- Risk for unbalanced offspring
  - Miscarriage
  - Stillborn
  - Live born with congenital malformations
- Offered invasive prenatal diagnosis in susequent pregnancies



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## Genetic testing in recurrent miscarriage

- Karyotyping advised after two or more, not necessarily consecutive miscarriages
- Adopted by guidelines (until 2010):



Royal College of  
Obstetricians and Gynaecologists  
Improving the way the world's women's health care



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## Efficacy of parental karyotyping?

- Doctor/health-care centered
- Frequency of identification of carrier couples
  - Risk factors for carrier status
  - Subsequent pregnancies
  - Obstetric outcome
  - Risk of unbalanced offspring



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## Identification of carrier couples\*

- Karyotyping in the Netherlands
- 1992-2001: 11971 couples karyotyped
- 382 carriers identified → 3.1%
- Identification of risk factors for carrier status?
  - Higher number of miscarriages
  - Younger age at second miscarriage
  - Recurrent miscarriage in parents
  - Recurrent miscarriage in siblings

\*Franssen et al. *BMJ* 2005




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## Subsequent pregnancies\*

- 1992-2001: 278 carrier couples, 427 non-carrier couples
- Follow-up at least 24 months after karyotyping

**Table 2** Reproductive outcome after parental chromosome analysis in couples with recurrent miscarriage.\* Values are numbers (percentages) of couples unless otherwise indicated

Reproductive outcome	Carrier couples (n=247)	Non-carrier couples (n=409)	Difference in % (95% CI)§	P value
Failure to conceive	8 (3.2)	19 (4.6)	-1.4 (-4.4 to 2.0)	0.38
One or more miscarriages	120 (48.6)	122 (29.8)	18.8 (11.1 to 26.3)	<0.01
One or more terminated pregnancies	9 (3.6)	9 (2.2)	1.5 (-1.2 to 3.4)	0.29
One or more ectopic pregnancies	3 (1.2)	13 (3.2)	-2.0 (-4.3 to 0.7)	0.11
One or more stillbirths	3 (1.2)	6 (1.5)	-0.3 (-2.1 to 2.2)	0.79
One or more children who died postpartum	1 (0.4)	4 (1.0)†	-0.6 (-2.1 to 1.4)	0.41
One or more ill or handicapped children	2 (0.8)	11 (2.7)‡	-1.9 (-4.0 to 0.5)	0.09
One or more healthy children	205 (83.0)	344 (84.1)	-1.1 (-7.2 to 4.6)	0.71

\*Franssen et al. *BMJ* 2006




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## Risk unbalanced offspring\*

- 278 carrier couples → 550 pregnancies
- 4 unbalanced fetal karyotypes (0.7%)
- 3 detected at invasive prenatal diagnosis (PND) → 2 aborted
- 1 couple refrained from invasive PND
- 2 children with unbalanced karyotype born
- → uptake of invasive PND?

\*Franssen et al. *BMJ* 2006; Vansenne et al. *Fertil Steril* 2010




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## Patients' perspective

CONGENO-study

- CONsequences of GENOtyping in reproductive medicine

- Multicenter
- 7 Academic medical centers in the Netherlands
- 01/2006 - 07-2009
- Prospective, longitudinal, index-control study
- 3 Questionnaires
- Both RM and subfertile couples (poor semen quality)



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## Inclusion criteria

- Recurrent miscarriage
  - $\geq 2$  miscarriages, not necessarily consecutive
- Poor semen quality
  - $< 1.10^6$  spermcells per ejaculate
- Both groups
  - Sufficient knowledge of Dutch language
  - Unaware of genetic test result at inclusion



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## Selection of participants

- Identification of carrier couple in the lab  $\rightarrow$  index couple
- Selection of first two couples karyotyped after index  $\rightarrow$  control couples
- Referring gynaecologist or urologist contacted
- Couples contacted for participation



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## Methods

- Questionnaire study
  - T0: before disclosure (baseline)
  - T1: 3 months after disclosure
  - T2: 12 months after disclosure
- Both partners invited



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## Questionnaire T0

- Anxiety (STAI)
  - 20 questions on Likert scale 1-4 →sumscore 20-80
- Depression (BDI-II-NL)
  - 21 questions on Likert scale 0-3 →sumscore 0-63
- Knowledge and awareness genetic test
- Perceived risks potential outcomes (VAS-scale)
- Comparison to Dutch reference population\*



\* De Weerd 2001, Van der Does 2002



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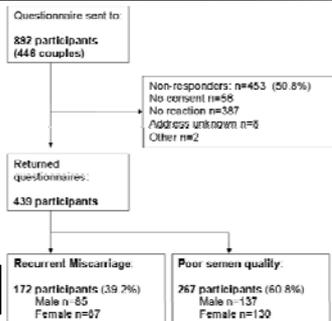
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## Inclusion



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## Baseline characteristics\*

**TABLE 1**  
Baseline characteristics and obstetric history of participants with recurrent miscarriage and male subfertility (n=439 participants)

Baseline characteristics (participants)	Recurrent miscarriage n=172	Male subfertility n=267
Sex		
Men	85 (49%)	137 (51%)
Women	87 (50%)	130 (49%)
Mean Age (SD)	35.4 (5.1)	34.1 (6.4)
Education		
Primary	4 (2%)	14 (5%)
Secondary	89 (51%)	154 (58%)
High	72 (42%)	84 (31%)
Other	4 (2%)	2 (1%)
Religious affiliation		
None	89 (52%)	129 (48%)
Christian	55 (32%)	71 (27%)
Other	18 (11%)	33 (12%)
Obstetric history (couple)	Recurrent miscarriage n=172	Male subfertility n=267
Start attempting to conceive		
< 6 months	1 (1%)	2 (1%)
6 months - 1 year	17 (10%)	8 (3%)
1 - 2 years	39 (23%)	55 (21%)
> 2 years	38 (22%)	66 (25%)
Median no. previous pregnancies (IQR)	5 (2-4)	0 (0-0)
Median no. previous miscarriages (IQR)	2 (1-3)	0 (0-0)
Median no. live born children (IQR)	0 (0-1)	0 (0-0)

\*Vansenne 2011, *Reprod Biomed online*



## Anxiety and depression

	Recurrent miscarriage n=172	Poor semen quality n=267	Dutch reference population	p-value
Total score BDI				
Median (IQR) (range)	5 (0-11) (0-44)	3 (0-7) (0-41)	5 (0-8)	
Total score STAI state				
Mean (SD)	36.6 (12.2)	34.6 (10.1)	35.5 (10.2)	
Male	32.4 (9.0)	32.0 (9.2)		
Female	40.0 (13.8)	36.3 (10.8)		<0.01
Total score STAI trait				
Mean (SD)	36.4 (11.5)	33.2 (10.3)	35.6 (10.4)	
Male	32.8 (9.1)	32.0 (9.7)		
Female	40.0 (12.6)	34.4 (10.9)		<0.01

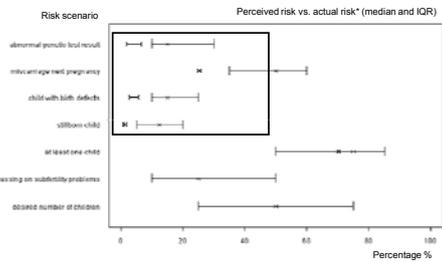


## Knowledge about the genetic test

	Recurrent miscarriage n=172 (%)	Poor semen quality n=267 (%)
Aware of standard genetic testing		
No	101 (59%)	155 (58%)
Yes	70 (41%)	110 (42%)
Information received about genetic testing		
No	61 (36%)	87 (32%)
Yes	110 (64%)	177 (67%)
What is tested		
Antibodies	0 (0%)	0 (0%)
Clotting factors	11 (6%)	1 (0%)
Changes in DNA or chromosomes	68 (40%)	117 (45%)
Something else	17 (10%)	26 (10%)
Don't know	60 (35%)	111 (42%)
Possible to ask questions		
No	117 (69%)	160 (61%)
Yes	32 (19%)	47 (18%)
Don't remember the genetic test discussed	21 (12%)	57 (21%)



## Perceived Risks RM group



\* Brigham 1999, Franssen 2006




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## Questionnaires T1 and T2

- Anxiety (STAI)
  - 40 questions on Likert scale 1-4 →sumscore 40-160
- Depression (BDI-II-NL)
  - 21 questions on Likert scale 0-3 →sumscore 0-63
- Distress (IES-R)
  - 22 items on Likert scale 0,1,3,5 →sumscore 0-110
- Comparison to Dutch reference population\*



\* De Weerd 2001, Van der Does 2002




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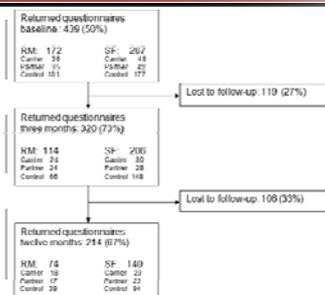
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## Inclusion




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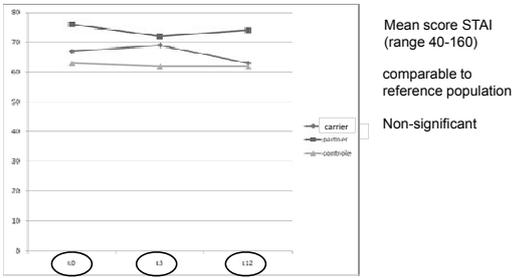
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## Anxiety men




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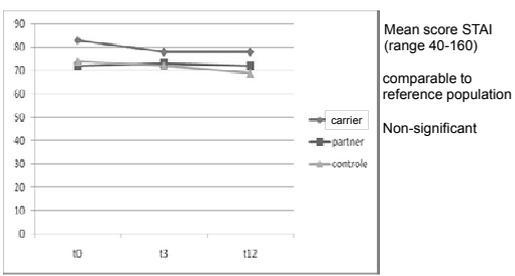
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## Anxiety women




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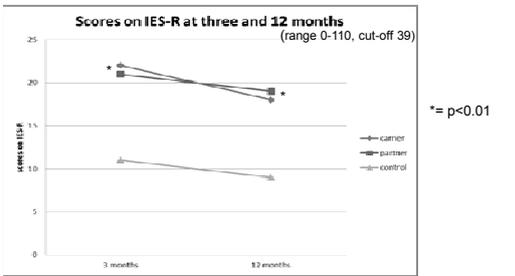
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## Distress




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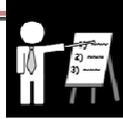
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## Conclusions

1. Unaware of genetic test performed
2. Overestimation of potential risks
3. Disclosure genetic test result does not lead to more anxiety or depressive feelings
4. Increase in distress, persists for longer time
5. Balanced against potential benefits, before offering genetic testing



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## Karyotyping in RM 2013

1. Guideline 2011 → karyotyping no longer advised after RM
2. Guideline withdrawn



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## Acknowledgements

- Study-group:  
- Center for Reproductive Medicine M. Goddijn, F. van der Veen, J. Langerijs
- dept. of Clinical Genetics M.C. van Maarse, B. Redeker, S. Srijder
- dept. Clinical Epidemiology C. de Borge, P.M. Bossuyt
- Participating centers:
  - University Medical Center Utrecht
  - University Medical Center Leiden
  - Vrije Universiteit Medical Center
  - Erasmus Medical Center Rotterdam
  - University Medical Center Groningen
  - University Medical Center Nijmegen
- And of course all participants!



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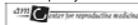
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**The Influence of Advanced Maternal Age:  
Major Cause of Recurrent Pregnancy Loss**

Mary D. Stephenson, MD, MSc, ELAM\*  
Professor and Head

**UIC** Department of Obstetrics  
UNIVERSITY OF ILLINOIS AT CHICAGO and Gynecology  
COLLEGE OF MEDICINE

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**Disclosure**

- I have no conflict of interest

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**Objectives**

- For RPL and advanced maternal age (AMA):
- Compare the frequency and type of chromosome errors with AMA
  - Present an AMA-dependent cost-saving algorithm to determine when a RPL evaluation is warranted
  - Discuss the impact of AMA on the frequency of RPL/Translocation carriers

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### What is Recurrent Pregnancy Loss?

• ASRM Practice Committee Opinion (2012):

RPL:  $\geq 2$  more failed clinical pregnancies, documented by ultrasound or histopathology

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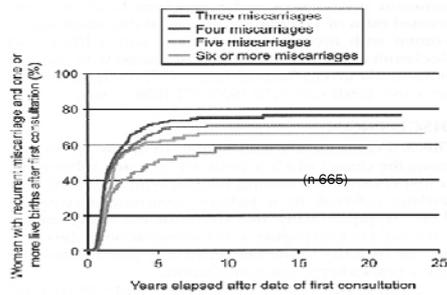
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### Chance of Live Birth Based on Number of Prior Miscarriages



Lund et al, Obstet Gynecol 2012

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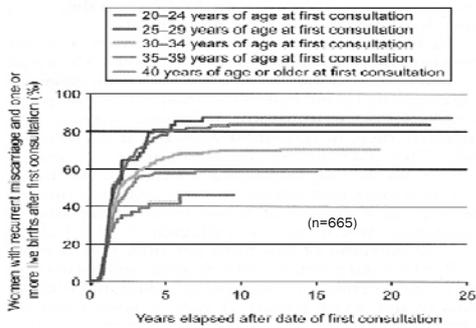
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### Chance of Live Birth based on Maternal Age



Lund et al, Obstet Gynecol 2012

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General Reproductive Population: All ages

Gestational Age	Risk of Pregnancy Loss	Chromosome errors
Preclinical (< 6 wks)	30-50% <sup>1,2</sup>	70% <sup>5</sup>
Clinical (6 to <10 wks)	15% <sup>3</sup>	50% <sup>3</sup>
Fetal (≥ 10 wks)	2-3% <sup>4</sup>	5% <sup>4</sup>

<sup>1</sup>Edmonds et al, 1982; <sup>2</sup>Wilcox et al, 1988; <sup>3</sup>Jacobs et al, 1987; <sup>4</sup>Simpson, 1990; <sup>5</sup>Ohno et al. 1991

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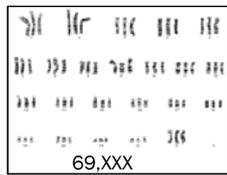
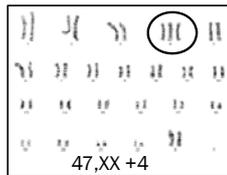
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Types of Miscarriage Chromosome Errors

- ✓50% trisomy ↑AMA
- ✓20% polyploidy
- ✓18% monosomy X
- ✓4% structural rearrangement: balanced or unbalanced
- ✓2% other



Jacobs et al, Human Genetics 1987

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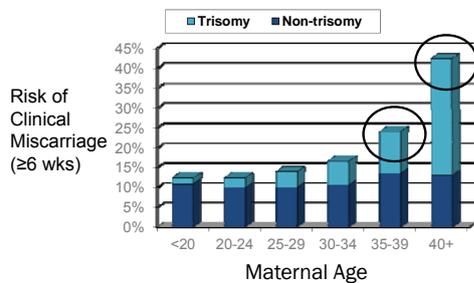
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Clinical Miscarriage and Advancing Maternal Age

Hassold and Chiu, Hum Genet 1985



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& Gynecology

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### Recurrent Miscarriage: Chromosome Testing

	Number of miscarriages	Chromosome errors	46,XX/46,XY miscarriages
Stern et al. 1996	94	57%	?
Ogasawara et al. 2000	114	49%	?
Carp et al. 2001	125	29%	?
Stephenson et al. 2002	420	46%	1.1

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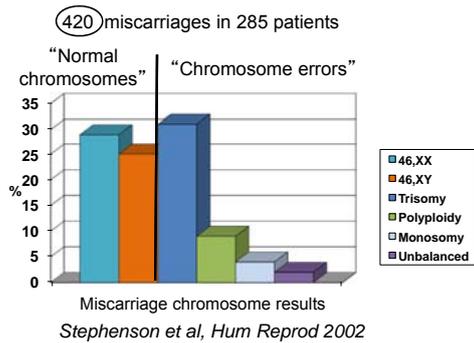
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### Cytogenetic Analysis of Miscarriages From Couples With Recurrent Miscarriage




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### Comparison of Trisomies

✓General reproductive population  
Trisomy 16, 22, 21, 15, 13  
*Jacobs et al, Human Genetics 1987*

✓Recurrent miscarriage cohort  
Trisomy 15, 16, 22, 21, 14, 13  
*Stephenson et al, Human Reprod 2002*

Adjusted for AMA, frequency of trisomies was identical  
→ No evidence of recurrent trisomy

*Hassold et al, Hum Genet 1985 vs  
Stephenson et al, 2002*

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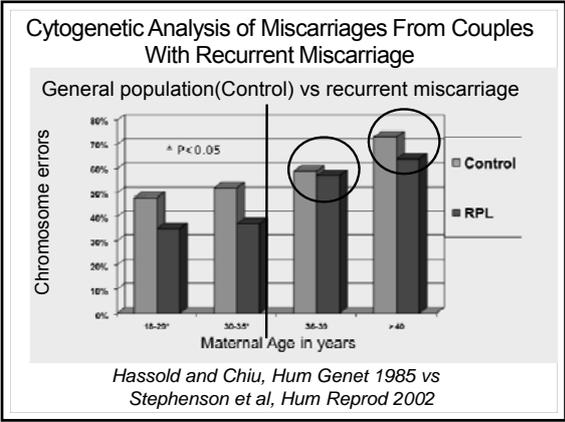
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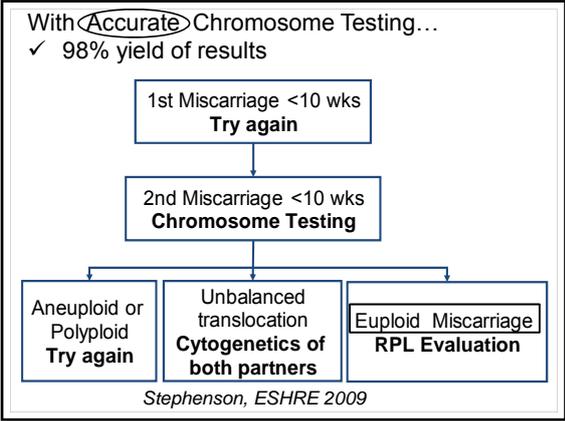
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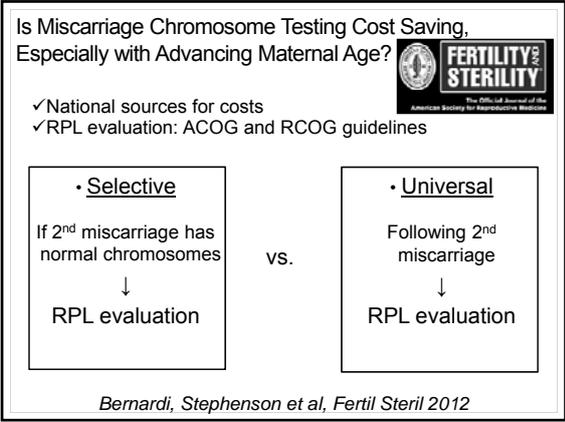
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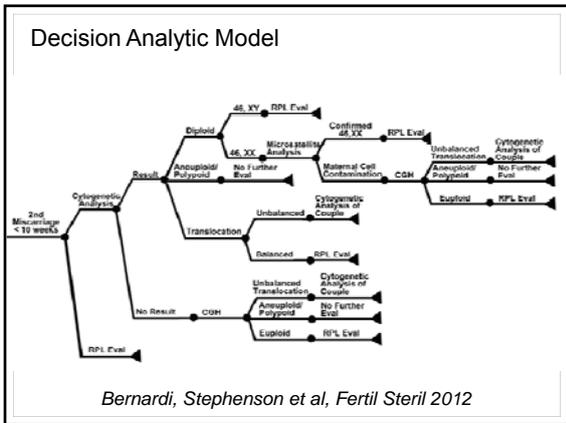
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### Is Miscarriage Chromosome Testing Cost Saving?

*Bernardi, Stephenson et al, Fertil Steril 2012*

Strategy	Estimated cost	Cost savings
<b>Universal RPL evaluation</b>	\$4,507	
<b>Selective RPL evaluation when 2<sup>nd</sup> miscarriage euploid</b>		
All maternal ages	\$3,352	\$1,155
18-35 years	\$3,766	\$794 ↑
36-39 years	\$2,973	\$1,534 ↑↑
>40 years	\$2,598	\$1,909 ↑↑↑
Yes! Especially with advancing maternal age		

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### RPL/Translocation Carriers: 3-5% of RPL Couples

*Stephenson and Sierra, Hum Reprod 2006*

Prospective study of 40 translocation carriers with a history of RPL

- ✓ 40% had concomitant RPL factors

**Management: IVF/PGD or treat concomitant factors?**

Normal chromosomes 14 and 21  
Chromosomes 14q21 and 21q14

Reciprocal translocation

two acrocentric chromosomes  
a Robertsonian translocation  
the short arms (lost)

Robertsonian translocation

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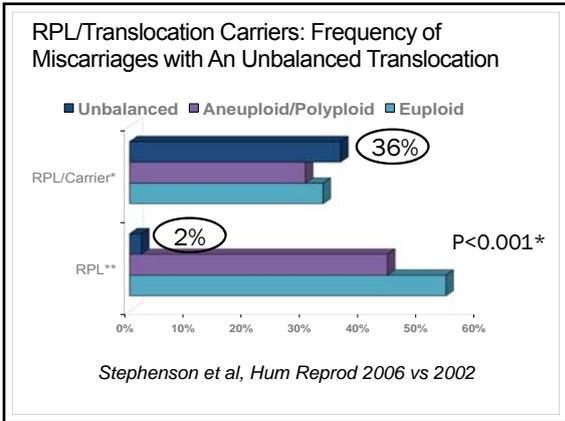
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Fertility and Sterility  
Volume 95, Issue 1, January 2011  
A critical look at the evidence does not support PGD for translocation carriers with a history of recurrent losses  
May D Stephenson, M.D., M.Sc.  
Marette Goddijn, M.D., Ph.D.

	Intervention	Cumulative live birth rate
Fischer et al. 2010	IVF/PGD	31% (60/192)
Franssen et al. 2006	Tx other factors, close monitoring	83% (205/247)
Stephenson et al. 2006	Tx other factors, close monitoring	65% (26/40)
Goddijn et al. 2004	Tx other factors, close monitoring	72% (18/25)

Higher live birth rate with treating concomitant RPL factors

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### Selective Parental Testing For Translocations

*Franssen et al, BMJ 2005*

Age at 2 <sup>nd</sup> misc	Sibling Hx RPL	+ve Parents RPL Hx		-ve Parents RPL Hx	
		≥3 misc	2 misc	≥3 misc	2 misc
<23 yrs	Yes	10%	7.5%	7.5%	5%
	No	5.5%	4%	4%	3%
23-24 yrs	Yes	10%	7%	7%	5%
	No	5.5%	4%	4%	3%
34-37 yrs	Yes	6%	4%	4%	3%
	No	3%	2%	2%	1.5%
37-39 yrs	Yes	4%	3%	3%	2%
	No	2%	1.5%	1.5%	1%
≥39 yrs	Yes	2%	1%	1%	1%
	No	1%	0.5%	0.5%	0.5%

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## Summary

For RPL and advanced maternal age:

- Trisomic miscarriages increase exponentially with AMA, in sporadic and recurrent pregnancy loss
- Miscarriage chromosome testing is cost-saving, especially with AMA
- Marked decrease of RPL/Translocation carriers with AMA, especially with no family history of RPL

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## NK cells

Professor Siobhan Quenby MD FRCOG  
Professor of Obstetrics University of Warwick  
Honorary Consultant University Hospitals Coventry and  
Warwickshire NHS Trust  
Director of the BRU in Reproductive Health

THE UNIVERSITY OF  
WARWICK

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- I have no conflict of interest to declare

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## Learning Objectives

- To gain insights into:
  - Biological significance of high uNK cell density
  - Benefits and limitations of testing for high density of uNK cells
  - Possible treatments for women with high uNK cell density

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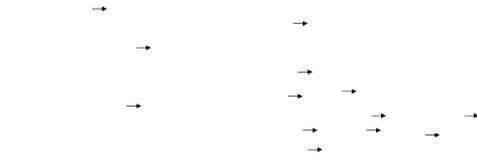
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## uNK cells in endometrium



Control patient with normal pregnancies  
 uNK cells more numerous in luteal phase endometrium  
 Quenby et al, 1999,2005; Clifford et al, 1999, Tuckerman et al., 2007

Patient who had idiopathic RM (miscarriages)  
 ten miscarriages

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## Mechanism of fetal loss when uNK cells density is high

- Direct killing?
- Excessive oxygenation?
- High uNK cell density result of poor decidualisation?

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## Maternal uNK interact with fetal trophoblast

- Have correct receptors
- Certain HLA-C/KIR combinations associated with
  - PET, RM, IUGR
  - Hiby et al., 2008
- Lack killing ability

Trophoblast	uNK cells
Antigen	Receptor
HLA-E	CD94 NKG2
HLA-C	KIRs
HLA-G	ILT-2,+ILT 4KIR2DL4
?	NKp44

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## Nature Medicine 2006

Hanna et al., NATURE MEDICINE 2006

Editorial:

### Killers become builders during pregnancy

Philippe Le Douarin & Julie Tibbax

Considering natural killer cells might be best known for their ability to destroy and roam target cells, but in the pregnant state of women these cells seem to have a positive role, regulating foetal development and angiogenesis (pages 1095–1096)

NOT DIRECT KILLING

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## Mechanism of fetal loss when uNK cells density is high

### — Direct killing?

- Excessive oxygenation?
- High uNK cell density result of poor decidualisation?

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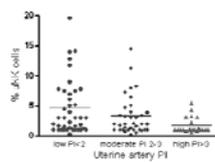
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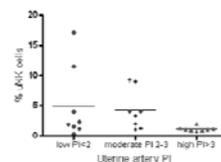
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## More blood uNK cells more blood flow



Recurrent miscarriage



Recurrent IVF failure

Quenby et al., 2009

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Journal of Reproductive Immunology 77 (2008) 37–42

Endometrial vascularity by three-dimensional power Doppler ultrasound and cytokines: a complementary approach to assess uterine receptivity<sup>††</sup>

Nathalie Lédée<sup>a,\*</sup>, Gérard Chaouat<sup>a</sup>, Valérie Serazin<sup>b</sup>, Raouf Lombroso<sup>a</sup>, Sylvie Dubanchet<sup>c</sup>, Pierre Oger<sup>a</sup>, Nabli Louati<sup>b</sup>, Yves Ville<sup>d</sup>

<sup>a</sup> Service de Gynécologie Obstétrique et Médecine de la Reproduction, Paris, France  
<sup>b</sup> INSERM U756, Unité Paris-Saclay, CERM-SOINS, F-92140 Clamart, France  
<sup>c</sup> Service de Médecine, Centre Hospitalier Intercommunal Paris-Saclay, Centre de Recherche en Gynécologie, Gynécologie, Paris, France  
<sup>d</sup> Centre de Recherche en Médecine de la Reproduction, Paris, France

Received 22 December 2006; received in revised form 25 May 2007; accepted 19 July 2007

Increased IL15 = Increased uNK=Increased blood flow

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**Mechanism of fetal loss when uNK cells density is high**

- ~~Direct killing?~~
- Excessive oxygenation?
  - Possible lack of direct evidence
- High uNK cell density result of poor decidualisation?

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OPEN ACCESS Freely available online

PLoS ONE

**Natural Selection of Human Embryos: Impaired Decidualization of Endometrium Disables Embryo-Maternal Interactions and Causes Recurrent Pregnancy Loss**

Madhuri Salker<sup>1</sup>, Gijb Teklenburg<sup>2,4</sup>, Mariam Molokhia<sup>2</sup>, Stuart Lavery<sup>1</sup>, Geoffrey Trew<sup>1</sup>, Tepchongchit Hojarepong<sup>5</sup>, Melon J. Mardani<sup>6</sup>, Amali U. Lokugamage<sup>5</sup>, Raj Rai<sup>1</sup>, Christian Landies<sup>3</sup>, Bernard A. J. Roelen<sup>6</sup>, Siebhan Quenby<sup>7</sup>, Ewart W. Kuijck<sup>8</sup>, Annemieke Kavelaars<sup>9</sup>, Cobi J. Heijnen<sup>9</sup>, Lesley Regan<sup>1</sup>, Nick S. Mecklen<sup>2,5</sup>, Jan J. Brosens<sup>1,4</sup>

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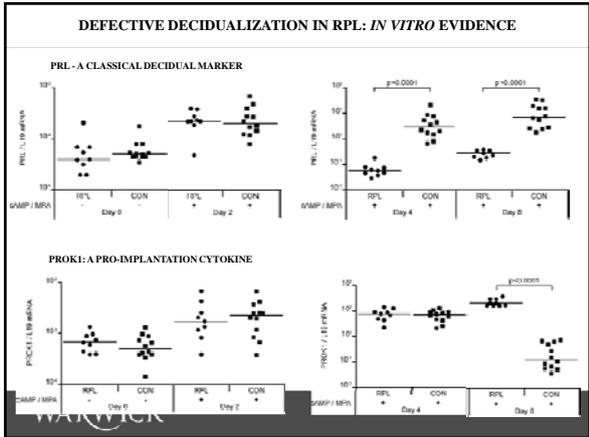
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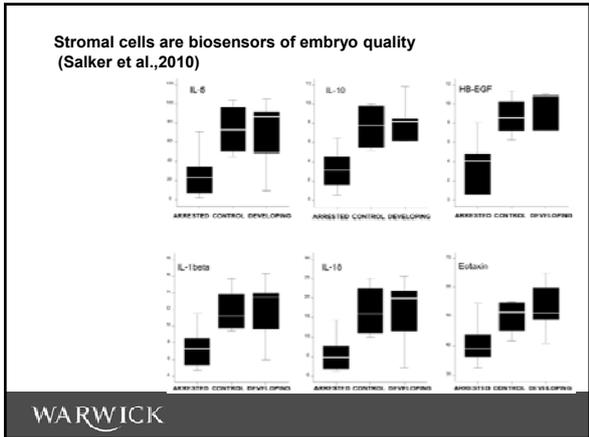
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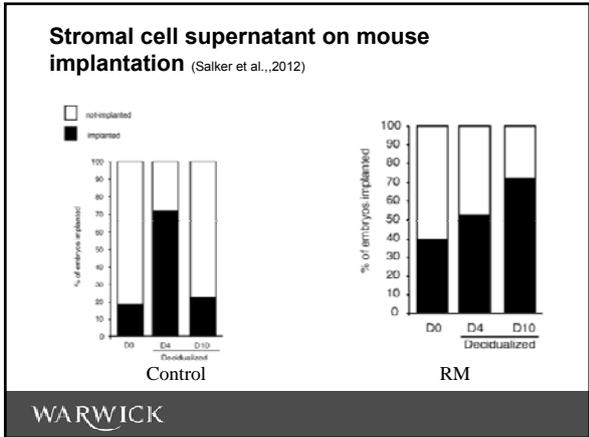
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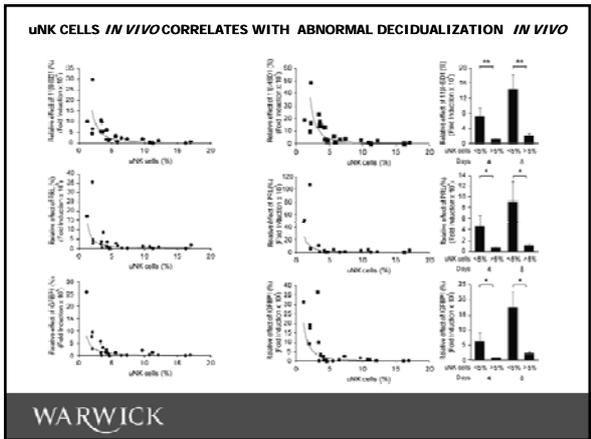
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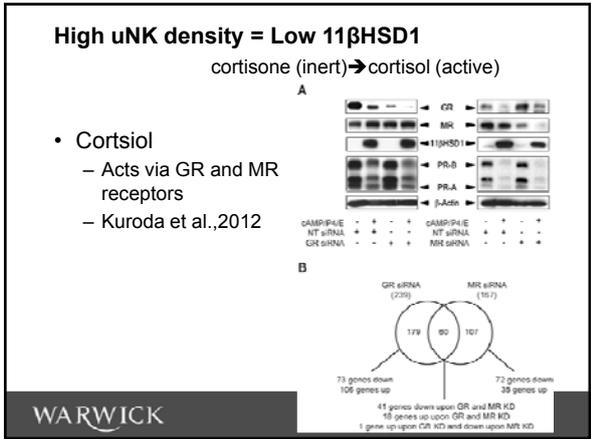
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**What do GR and MR control ?**

- Deacetylation & methyltransferase complexes
- Lipid droplet formation
- Vit A pathway

Kuroda et al., 2012

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## Mechanism of fetal loss when uNK cells density is high

### • Direct killing?

- Excessive oxygenation?
  - Possible lack of direct evidence
- High uNK cell density result of poor decidualisation?
  - Yes

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Human Reproduction, Vol.28, No.8 pp. 1971–1980, 2011  
Advanced Access publication on May 25, 2011 doi:10.1093/humrep/der164

human reproduction

META-ANALYSIS Early pregnancy

## Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review

A.W. Tang<sup>1\*</sup>, Z. Alfirevic<sup>1</sup>, and S. Quenby<sup>2</sup>

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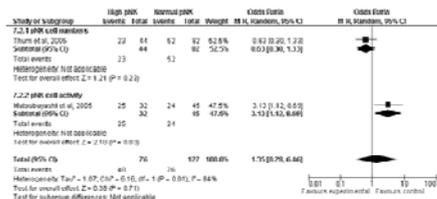
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## Peripheral NK Cells in Infertility

- Odds of implantation failure after ART with high pre-pregnancy peripheral NK cell parameters in women with infertility



N = 203

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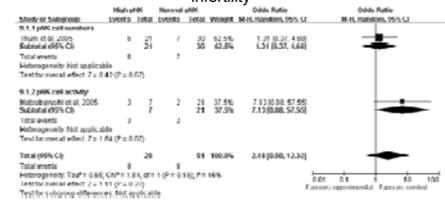
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## Peripheral NK Cells in Infertility

•Odds of miscarriage (after implantation success from ART) with high levels of pre-pregnancy peripheral NK cell parameters in women with infertility



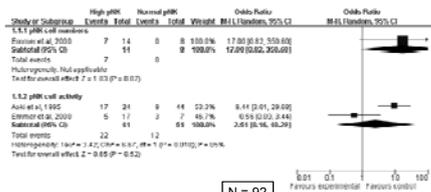
N = 79

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## Peripheral NK Cells in RM

•Odds of miscarriage with high pre-pregnancy peripheral NK cell parameters in women with idiopathic RM



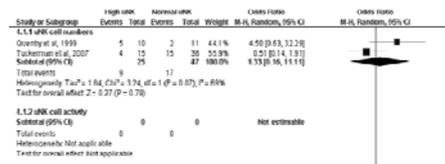
N = 92

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## Uterine NK Cells in RM

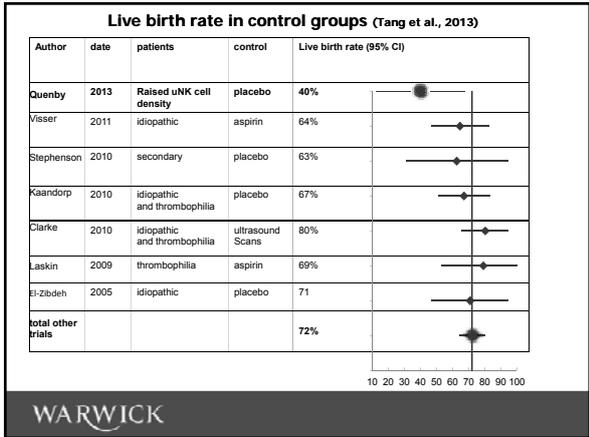
•Odds of miscarriage with high levels of pre-pregnancy uterine NK cells in women with idiopathic RM



N = 72

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## Treatment?

- “A specific assay to diagnose immune-mediated early pregnancy loss and a reliable method to determine which women might benefit from manipulation of the maternal immune system are urgently needed”
  - Porter et al, cochrane 2006

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## Case History

- 17 consecutive miscarriages
- No Cause found
- Most NK cells in study n=40
- Preconceptual prednisolone 5mgs
- Two further miscarriages
- Higher dose (prednisolone 20mg)
- Live Birth aged 42
  - (IUGR 32/40)
- Alive and well age 4 years
  - Quenby et al., 2004

Henderson TA, Saunders PT, Moffatt King A, Groome NP, Critchley HO. Sarcoid receptor expression in uterine natural killer cells. J Clin Endocrinol Metab 2002;95:910-9.

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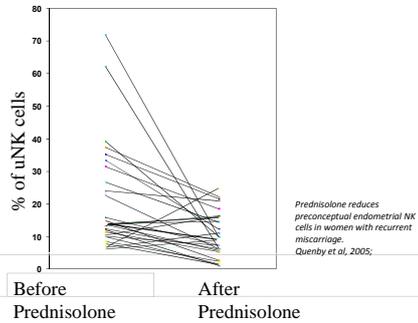
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### Effect of Prednisolone on uNK cells




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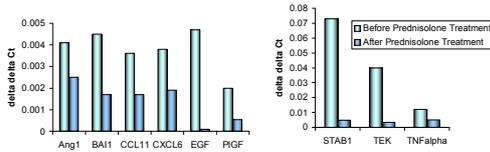
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### Prednisolone treatment reduces endometrial angiogenic growth factor expression at LH+7



Lash et al 2012

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### Prednisolone Trial

Evaluate if prednisolone therapy during the first trimester of pregnancy is able to reduce the risk of miscarriage and improve live birth rates in women with RM and high uterine natural killer (uNK) cells density

Pilot phase: to assess feasibility of recruitment, integrity of trial procedures and preliminary data for accurate power calculations  
Tang et al., 2013 in press

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## Study Population

- Recruitment – August 2008 to August 2010
- Inclusion Criteria
  - $\geq 3$  consecutive miscarriages with no cause found (idiopathic)
  - $\leq 40$  years old
  - $\geq 5\%$  uNK cells at day LH +6 to +9 (mid-luteal)
- Exclusion Criteria
  - Known cause for recurrent miscarriage
  - Contraindications to steroid therapy: hypertension, diabetes, mental health problems or obesity with BMI  $>35$
  - Decline consent to randomisation

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## Study Design – Randomisation

- Randomised to either prednisolone (50%) or placebo (50%)
  - Confirmed 4-8 weeks pregnant
- Treatment regime
  - 4 tablets for 6 weeks, 2 tablets for 1 week, 1 tablet for 1 week
  - Active tablets has 5mg of prednisolone
- Monitoring in pregnancy (*in addition to routine antenatal care*)
  - Reviewed and scanned every 2 weeks until 14 weeks gestation
  - Growth scans at 28 weeks and 34 weeks gestation
  - Post-delivery follow-up at 6 weeks

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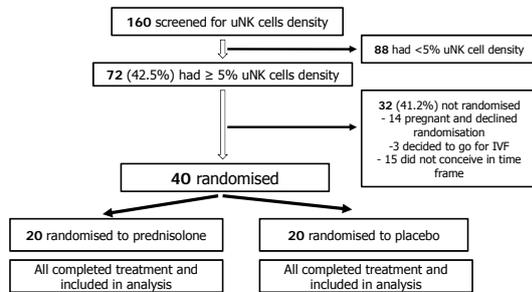
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## Trial Flow Chart



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### Baseline characteristics of women randomised

	Prednisolone (N=20)	Placebo (N=20)
Age in years (Mean)	34	33
% uNK cells (Mean (Range))	8.3 (5-22.8)	7.2 (5-18.3)
BMI (Mean)	26.1	25.6
Women with previous live birth (No.)	4	3
Mean number of previous early miscarriages	4	5
Women with previous 2 <sup>nd</sup> trimester miscarriage (No.)	0	2
Women with previous ectopics (No.)	1	2
<b>Current Pregnancy</b>		
Folic Acid Intake	20	19
Aspirin Intake	4	5
Sec present at randomisation	15	17
FH present at randomisation	3	1

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### Side effects of Steroids

Side Effects (Count (%))	Prednisolone (N=20)	Placebo (N=20)	Relative Risk (95% CI)
Acne	4 (33.3)	2 (25)	1.33 (0.32-5.64)
Bruising	0	0	-
Flushing	4 (33.3)	1 (12.5)	2.67 (0.36-19.71)
GI problems	4 (33.3)	2 (25)	1.33 (0.32-5.64)
Insomnia	5 (41.7)	1 (12.5)	3.33 (0.47-23.47)
Infections	0	0	-
Joint pain	0	1 (12.5)	0.23 (0.01-5.05)
Mood changes	3 (25)	2 (25)	1.00 (0.21-4.71)
Others (headaches, nausea, increased appetite, palpitations)	7 (58.3)	2 (25)	2.33 (0.64-8.49)

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### Clinical Outcomes

Outcomes	Prednisolone (N=20)	Placebo (N=20)	Relative Risk (95% CI)
<b>Livebirths (%)</b>	<b>12 (60)</b>	<b>8 (40)</b>	<b>1.5 (0.79-2.86)</b>
Delivery <37 weeks (%)	1 (8.3)	0	3.00 (0.13-69.52)
Vaginal Delivery	3 (25)	4 (50)	0.75 (0.19-2.93)
Caesarean Section Delivery	9 (75)	4 (50)	2.25 (0.83-6.13)
Birthweight (mean)	3516g	3547g	-
Admission to SCBU	1 (8.3)	1 (8.3)	1.00 (0.07-14.90)

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## Clinical Outcomes

Outcomes	Prednisolone (N=20)	Placebo (N=20)	Relative Risk (95% CI)
Miscarriages (%)	8 (40)	12 (60)	0.67 (0.35-1.27)
Biochemical loss	2	1	
Empty gestation Sac	2	3	
Fetal loss	4	6	
Trisomy 22	(1)	(1)	
Normal karyotype	(2)	(2)	
Ectopic (treated methotrexate)	0	2	

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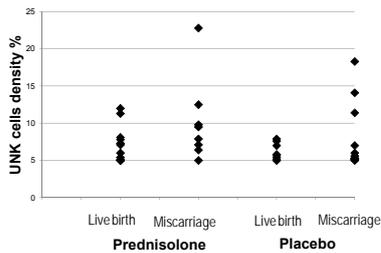
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## uNK cells an pregnancy outcomes



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## Can we test and treat endometrium?

- Close
- Progesterone and prednisolone?
- When?
  - Start of decidualisation 7 days after ovulation?
  - Make endometrium more selective?

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- Jan Brosens
- Keiji Kuroda
- Madhuri Salker
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  - Gendie Lash
- LWFT
  - Aie-Wei Tang
  - Lisa Heathcote
  - Jo Drury



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*Bringing to life the best in women's health care*

**NICE guidelines 2012  
Dissemination and implementation**  
Mrs Caroline Overton

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**Declaration of Interest** 

Mrs Caroline Overton  
St Michael's University Hospital Bristol

Chair Association of Early Pregnancy Units (AEPU)  
Member of the NICE GDG on miscarriage & ectopic pregnancy  
Medical advisor for Endometriosis UK  
Consultant for Swiss Precision Diagnostics

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THE ASSOCIATION OF EARLY PREGNANCY UNITS  
**Early Pregnancy Information Centre**

**[www.earlypregnancy.org.uk](http://www.earlypregnancy.org.uk)**

Via the website  
Email [aepu@rcog.org.uk](mailto:aepu@rcog.org.uk)  
RCOG, 27 Sussex Place, Regent's Park, London NW4 7RG

*AEPU: support through the cycle of pregnancy*




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## NICE 2012 Miscarriage



- Key change in guidance
- Expectant management of miscarriage for 7-14 days having considered safety & acceptability
- 50% miscarry spontaneously within 7-14 days of diagnosis
- Anti D is not required for expectant or medical management of miscarriage less than 13 weeks

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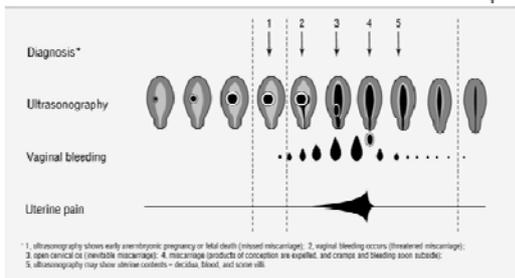
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- Severe haemorrhage or pain
- Signs of infection: purulent discharge, Pyrexia >37.5°C, tender uterus, white cell count >15
- Haemolytic disease or blood dyscrasia
- Twins or more
- Inability to understand written English and/or difficulty in accessing help
- Anaemia
- Women in late first trimester (63 days)
- Women more than 13 weeks by gestation
- No emergency gynaecology provision




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- The RCT papers report the outcomes and follow-up data of seven trials, conducted in the **UK** (2 trials: Chipchase, J. et al. 1997 and Smith, L.F.P. et al. 2009 / Trinder, J. et al. 2006), **Australia** (Shelley, J.M. et al. 2005), **Sweden** (Nielsen, S. et al. 1995/ Nielsen, S. et al. 1996/ Blohm, F. et al. 1997 and Nielsen, S. et al. 1999), **The Netherlands** (Wieringa-de Ward, M. et al. 2002/ Wieringa-de Ward, M. et al 2002) and **Hong Kong** (Ngai, S.W. et al. 2001).
- All studies compared expectant management with medical and/or surgical management of miscarriage (both of which isolated or in combination were defined as "active" by the GDG), and reported at least one priority outcome. The trials were all conducted in developed countries, and their populations include women with missed miscarriages and/or women with ongoing miscarriages.

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### Expectant management

- 238/632 (35%) unplanned intervention
- 2% infection
- 13% gastrointestinal side effects
- 1.6% need for a blood transfusion
- 7-14 bleeding days
- 0-5 days in pain
- 49% unplanned admission

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### Expectant versus active management

No difference in

- Infection rates
- Bleeding time for expectant and medical
- Duration and severity of pain
- Satisfaction
- Anxiety scores
- Fertility or Live birth rate

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• The RCT papers report the outcomes and follow-up data of sixteen trials, conducted in the **UK** (2 trials: Demetroulis, C. et al. 2001; Smith, L.F.P. et al. 2009 / Trinder, J. et al. 2006), **Australia** (Shelley, J.M. et al. 2005), **Austria** (Egarter, C. et al. 1995), **Burkina Faso** (Dao, B. et al. 2007), **China** (Fang, A. et al. 2009), **Egypt** (Dabash, R. et al. 2010), **Finland** (Niinimäki, M. et al. 2006), **Hong Kong** (1 trial: Chung, T.K.H. et al. 1999 / Lee, D.T.S. et al. 2001 / Tam, W.H. et al. 2005), the **Netherlands** (1 trial: Graziosi, G.C.M. et al. 2004 / Graziosi, G.C.M. et al. 2005a / Graziosi, G.C.M. et al. 2005b), **South Africa** (2 trials: de Jonge, E.T.M. et al. 1995; Moodliar, S. et al. 2005), **Tanzania** (Shwekerela, B. et al. 2007), **Turkey** (Sahin, H.G. et al. 2001), and the **USA** (2 trials: Davis, A.R. et al. 2007 / Harwood, B. and Nansel, T. 2008 / Zhang, J. et al. 2005; Muffley, P.E. et al. 2002). The qualitative study is the follow-up to an RCT conducted in the UK, including both participants and non-participants of the trial (Smith, L.F. et al. 2006). The partially randomised trial included both women who had chosen their method of management, and those who had been randomised to medical or surgical management (Hinshaw, H.K.S. 1997).

• All studies compared medical and surgical management of miscarriage, and reported at least one priority outcome. The trials were conducted in both developed and developing countries, and their populations include women with missed miscarriages and/or women with ongoing miscarriages.

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**Medical versus surgical management**  
 No difference in

- Unplanned visits to a medical facility
- Infection
- Need for a blood transfusion
- Satisfaction, social function, mental health, subsequent live birth rate

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**Medical versus surgical management**

- Higher rate of unplanned intervention 36% versus 5%
- Higher rate of gastrointestinal side effects
- Longer duration of bleeding
- Longer duration and more severe pain
- Higher rate of admissions 18% versus 8%

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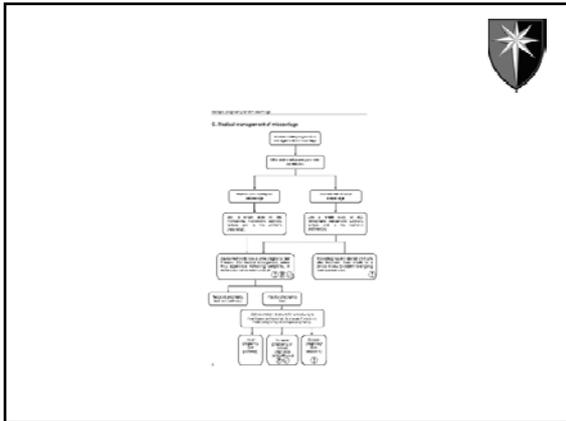
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**Areas with general consensus:**

**Fear:** There was near uniform fear of intervention, especially anaesthetic and a perception of hospitalisation and surgery as traumatic events

**Predictability:** Women wanted a predictable end, so they could get on with their lives, and they wanted their management and symptoms to have a predictable course.

**Need for more information:** Women felt they did not know what to expect in terms of bleeding and pain, and wanted more details on the timing, duration and effects of interventions.

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## Decidual cast



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Check pregnancy test after three weeks



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## Follow-up



- Personalized
- Cancel routine follow-ups especially dating scans and antenatal appointments

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## Confidential Enquiries

- One death due to anaphylaxis to opioid analgesia administered by a paramedic
- 5 deaths due to infection associated with miscarriage

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- It's not a "retained product of conception". For us, as soon as we see those two lines on the pregnancy test, that is **OUR** baby growing inside of me.
- I had an ERPC last month and have never been able to call it that. I've always said 'surgical management of my miscarriage'
- What's an ERPC? Is it the same as a D&C?

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National survey results 2012  
Surgical management of miscarriage (SMM) should replace ERPC

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## Choice



- It is no longer acceptable to offer only surgical management for women diagnosed with miscarriage.
- Expectant management is at least as effective as medical management for women with incomplete miscarriage.
- Many women would prefer the options of expectant or medical management.

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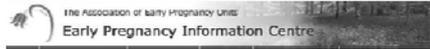
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[www.earlypregnancy.org.uk](http://www.earlypregnancy.org.uk)

Via the website [www.earlypregnancy.org.uk](http://www.earlypregnancy.org.uk)  
Email [aepu@rcog.org.uk](mailto:aepu@rcog.org.uk)  
RCOG, 27 Sussex Place, Regent's Park, London NW4 7RG

*AEPU: support through the cycle of pregnancy*



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**You can now register for these upcoming ESHRE Campus events:**

- Application and challenges of emerging technologies in preimplantation and prenatal diagnosis  
12-13 September 2013 - Prague, Czech Republic
- Female genital tract congenital malformations: new insights in an old problem  
27-28 September 2013 - Thessaloniki, Greece
- Introducing new techniques into the lab  
4-5 October 2013 - Barcelona, Spain
- Polycystic ovary syndrome: A new look at an old subject  
25-26 October 2013 - Rome, Italy
- Infections from conception to birth: role of ART  
7-8 November 2013 - Berlin, Germany
- Endoscopy in reproductive medicine  
20-22 November 2013 - Leuven, Belgium
- From early implantation to later in life  
28-29 November 2013 - Brussels, Belgium

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- Premature ovarian insufficiency  
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# NOTES

