PRE-CONGRESS COURSE 11

SIG Early Pregnancy

"Pregnancy after ART"

CONTENTS

PRE-CONGRESS COURSE 11 - PROGRAMME

SIG Early Pregnancy

Pregnancy after ART

Course co-ordinator: Roy Farquharson (UK)

Course description: Postgraduate course for clinicians interested in early pregnancy after ART

Target audience: Clinicians, scientists and allied medical professionals

Programme

09.00 - 09.30: Prenatal diagnosis and fetal anomaly after ART – ***M. Bonduelle (B)***
09.30 - 09.45: Discussion

09.45 - 10.15: Recurring pregnancy loss after ART -***H. Carp (IL)***
10.15 - 10.30: Discussion

10.30 - 11.00: Coffee break

11.00 - 11.20: Multiple gestation pregnancies after ART - ***E. Jauniaux (UK)***
11.20 - 11.30: Discussion

11.30 – 11.50: Embryo reduction after ART; state of the art - ***E. Gratacos (E)***
11.50 - 12.00: Discussion

12.00 - 12.20: Maternal age and health risks with ART - ***M. Blott (UK)***
12.20 – 12.30: Discussion

12.30 - 13.30: Lunch

13.30 - 14.00: Obesity and ART outcome – ***J. Bellver (E)***
14.00 – 14.15: Discussion

14.15 – 14.45: Ovarian reserve, ART and early pregnancy loss - ***F. Broekmans (NL)***
14.45 – 15.00: Discussion

15.00 – 15.30: Coffee break

15.30 - 16.00: Endometrial gene expression during ART implantation window - ***J. Horcajadas (E)***
16.00 - 16.15: Discussion

Universitair Ziekenhuis Brussel

Prenatal Diagnosis and Fetal anomaly after ART

Prof Maryse Bonduelle MD, PhD

Centrum voor Medische Genetica

Centrum voor Reproductieve Geneeskunde

Learning objectives

- Evaluate risk of
  - chromosomal anomalies,
  - adverse neonatal outcome
  - malformations
- For the children born after ART

Conflict of interest

- The children's follow-up team of the Vrije Universiteit Brussel got support from different funding resources
  - University Hospital,
  - University Research Council
  - Willy Gepts Foundation
  - unrestricted educational grant from Organon International

Outline lecture

- **Introduction of IVF and ICSI**
- Perinatal outcome
- Prenatal diagnosis
- Major malformations
- Conclusion

Introduction of IVF

- 1978 birth of Louise Brown
  - IVF was introduced into practice with little formal evaluation of the effects on the health of the children
- Register data: reassuring on congenital malformations
  - no increase in malformation rate compared to the general population in different countries
    - Australia, USA, UK, France …

Introduction of IVF

- Neonatal outcome problems seemed primarily related to higher incidence of multiple pregnancies
- But also more frequent in IVF singletons
  - Prematurity higher
    - IVF Australian Collaborative Group, 1985; Doyle et al. 1992; Tan et al. 1992; Olivennes et al. 1993; Verlaenen et al. 1995…
  - Low birthweight rate (<2500g) higher
    - Doyle et al. 1992; Tan et al. 1992; Olivennes et al. 1993; Verlaenen et al. 1995…
  - Higher rate of children small for gestational age
    - Doyle et al. 1992; Olivennes et al. 1993…

Introduction of ICSI

1991 introduction of ICSI

at the Vrije Universiteit Brussel

- concerns re-emerged about the health and well-being of the children
- concerns were related
  - to the invasiveness of the procedure
  - to the type of sperm used
- new studies on IVF and ICSI were undertaken

Outline lecture

- Introduction of IVF and ICSI
- **Perinatal outcome**
- Prenatal diagnosis
- Major malformations
- Conclusion

Adverse neonatal outcome in *IVF singletons*

- Number of controlled / prospective studies on neonatal outcome were performed, controlled for extensive maternal variables

  age, parity, diabetes, hypertension, social class, year of birth, smoking, area of residence…
  - Prematurity risk in singletons < 37 weeks
  - Low birthweight rate <2500g, <1500g
  - SGA risk
- Recently summarized in two meta analysis

Meta analysis on neonatal outcome

Jackson et al. 2004	Helmerhorst et al. 2004
Inclusion criteria 1. IVF singletons 2. >50 % IVF 3. Control for maternal age and parity	Inclusion criteria 1. ART singletons and twins 2. ART vs natural conception 3. Studies with matched (mat age, parity, sociodemographic variables, smoking pre existing disease) and non-matched controls

Meta analysis on neonatal outcome
SINGLETONS

	Jackson **N=12,283 IVF**	Helmerhorst **N=5,361 ART/sing**
Perinatal mortality	OR 2.2 95% CI 1.6-3.3	RR 1.7 95% CI 1.1-2.5
Prematurity	OR 2.0 95% CI 1.7-2.2	RR 2.0 95% CI 1.8-2.3
LBW <2500g	OR 1.8 95% CI 1.4-2.2	RR 1.7 95% CI 1.5-1.9
VLBW <1500g	OR 2.7 95% CI 2.3-3.1	RR 3.0 95% CI 2.1-4.4
SGA	OR 1.6 95% CI 1.3-2.0	RR 1.4 95% CI 1.1-1.7

Meta analysis on neonatal outcome
ART MULTIPLES

	Helmerhorst et al. 2004
Prematurity	RR 1.07 (CI 1.02-1.13)
Very prematurity	RR 0.95 (CI 0.78-1.15)
LBW <2500g	RR 1.03 (CI 0.99-1.08)
SGA	RR 1.27 (CI 0.97-1.65)

ART twins are more comparable to general population

1 meta analysis on 9 studies ART (IVF>>ICSI)

Neonatal outcome after selective single embryo transfer (SET)

- Neonatal outcome in SET embryo's compared to the general population
  - n= 251 singletons after SET
  - compared to 59,535 NC singletons (register)
  - De Neubourg et al. accepted 2006
- Birthweight similar
- Prematurity slightly higher (p = 0.03)
- Stillbirths similar

Good prognosis patients do not have an unfavorable outcome of their singleton baby compared to SC children

Conclusion: ICSI and IVF are a risk factor of adverse perinatal outcome

In ART singletons

- Higher risk of
  - x 2 LBW, VLBW, prematurity,
  - x 1.5 SGA and
  - x 2 perinatal mortality
- No obvious difference between IVF and ICSI
- SET outcome might be better

Conclusion: ICSI and IVF are a risk factor of adverse perinatal outcome

In ART twins

- Perinatal outcome compared to the general population : less obvious difference
- Differences in outcome between singletons and twins compared to SC might be explained by an implantation advantage of multiple pregnancies

Increased risk of ART is mainly related to high rates of multiples undoubtedly leading to worse neonatal outcome

Cause of adverse neonatal outcome ?

- Saunders et al. 1988: infertility as a risk factor
  - Prematurity risk higher in IVF and in patients on waiting list for ART treatment, compared to spontaneously pregnant patients
- Doyle et al. 1992: unexplained / male infertility as a risk factor
  - LBW risk higher in IVF
    - in male factor and unexplained > tubal infertility
    - if more embryos transferred
- Pandian et al. 2001: unexplained infertility as a risk factor
  - Higher pregnancy complications in infertile woman (adjusted for age, parity and fertility treatment) than in general population
  - Complication rate same for ART treated and not-treated infertile women
- Shieve et al. 2002: infertility as a risk factor
  - Surrogate mothers treated with IVF, no increased risk for LBW or VLBW

Prenatal diagnostic testing

Outline lecture

- Introduction of IVF and ICSI
- Perinatal outcome
- **Prenatal diagnosis**
- Major malformations
- Conclusion

Prenatal diagnosis in 1586 ICSI foetuses

Bonduelle et al. 2002

Abnormal results	n	%	Confidence Interval	% General population[1, 2, 3]
■ *De novo*	25	**1.6%***	1.02 - 2.32 %	0.45 - 0.87%
Sex chrom	10	**0.6%***	0.30 - 1.16 %	0.19 - 0.27%
Autosomal	15	0.9%	0.53 - 1.56 %	0.26 - 0.60%
Numerical	8	0.5%	0.22 - 0.99 %	0.14 - 0.33%
Structural	7	0.4%	0.18 - 0.91 %	0.11 - 0.22%
■ Inherited	22	**1.4%***	0.87 - 2.09 %	0.47 - 0.37%
Total	47	3.0%	2.19 - 3.92 %	0.92%

[1] Jacobs, 1992 on 34 910 newborns [2] Ferguson-Smith, 1984 on 52 965 prenatal samples
[3] Hook, 1981, 1984, 1987 on prenatal samples * significant

Prenatal diagnosis in 1586 ICSI foetuses[1]

- Inherited abnormalities 1.4%
- Known risk related to the chromosomal anomalies in the parents (6.3%)
- 17/22 cases paternally inherited
- Preimplantation > prenatal diagnosis

⇨ Informed choice of the parents prior to the procedure

[1]Bonduelle et al. 2002

Prenatal diagnosis in 1586 ICSI foetuses

- Non-inherited (*de novo*) anomalies 1.6%
- Significantly higher than general population (with same age) but absolute risk low
- Related to sperm characteristics
- Severity is variable (termination not always chosen)
- Detectable from 11th week of pregnancy

⇨ Informed choice of the parents
<50% agree to do a prenatal test

Prenatal diagnosis in 1586 ICSI foetuses[1]
de novo anomalies, sperm parameters / origin

- Sperm count (72%)
  < 20.10^6 / ml ⇒ **2.1 %** chromosomal abnormalities
  Fisher Exact 2 tailed test $p < 0.05$
- Sperm motility (83%)
  < 50 % N motility ⇒ **1.9%** chromosomal abnormalities
  Fisher Exact 2 tailed test $p < 0.05$
- Sperm morphology ⇒ **no influence**
  abn < 14 % N or abn ≥ 14 % N morphology
- Sperm origin ⇒ **no influence**

[1]Bonduelle et al. 2002

Prenatal diagnosis ICSI fetuses / anomalies in relation to sperm origin[1]

	de novo	inherited
• Ejaculated sperm[1]	1.7%*	1.4%
• n =1469 (prenatal)	(25)	(22)
• Epididymal sperm[2]	0%*	0.0%
• n = 61 (pre- and postnatal)	(0)	(0)
• Testicular sperm[2]	2.0%*	0.5%
• n = 198 (pre- and postnatal)	(4)	(1)

[1]Bonduelle et al., 2002
[2]Bonduelle et al., 2008

* not significant

Prenatal diagnosis in ICSI in Literature Belgium and France

	Testicular de novo + inherited	ICSI total de novo + inherited	Testicular vs General Pop
Belgium	n = 198	n = 1496	
de novo	4 (2.02%)	25 (1.7%)	0.45% (OR 4.6; 95%CI 1.7-12.4)
inherited	1 (0.51%)	22 (1.4%)	0.47% (OR 1.1; 95%CI 0.2-7.7)
	2.5%	3.1%	
France	n = 201	n= 2332	*Test vs ICSI
	(5)*	(16)	p = 0.02
	2.5%	0.7%	

Chromosomal anomalies in relation to sperm origin / quality

- Increased aneuploidy rate in sperm when severe testicular failure
  - Levron et al., 2001; Burello et al., 2002; Palermo et al., 2002
  - Gianarolli et al. 2005. Higher aneuploidy compared to the general population in testicular sperm compared to ejaculated sperm
- Higher aneuploidy rate in MESA / TESE embryos compared to ICSI embryos from normospermic patients
  - Gianarolli et al. 2000
- Higher incidence of mosaicism in TESE embryos
  - Silber et al. 2003 Immature centrosome leading to errors in mitosis?

Chromosomal anomalies in relation to sperm origin / quality

- No difference in non-obstructive azospermia / normal spermatogenesis azospermia patients
  - Mateizel et al. 2002. n =17 NOA; 26 OA
    NO difference in chromosomal abnormality in patients with severe testicular failure vs normal spermatogenesis except for more aneuploidy for chromosome 18
- Higher aneuploidy rate in preimplantation NOA embryos
  - Silber et al. 2003
- Lower implantation rate of NOA embryos compared to OA
  - Vernaeve at al., 2002

Outline lecture

- Introduction of IVF and ICSI
- Perinatal outcome
- Prenatal diagnosis
- **Major malformations**
- Conclusion

Meta analysis on malformation in ART[1]

Major malformation	N° studies	Pooled OR	CI 95%
• All • Reviewer selection	25 7	**1.32** **2.01**	1.20-1.45 1.49-2.69
Singletons only	15	1.31	1.17-1.46
Adjusted[2]	19	**1.29**	1.19-1.39
IVF only	12	1.94	1.50-2.50
ICSI only	5	1.28	1.14-1.43

[1] Hansen et al. 2005 ART technologies and the risk of birth defects a systematic review
[2] Adjusted for maternal age, parity, infant sex (some), not for plurality

Meta analysis on malformation in ART

- Hansen et al. 2005 (7 studies)
  OR Adjusted **1.29** (95% CI 1.19-1.39)
- Rimm et al. 2004 (19 studies)
  OR all ART children **1.29** (95% CI 1.01-1.67)
  OR singletons ICSI vs SC 1.33 (95% CI 0.90-1.95)
  OR singletons IVF vs SC 1.51 (95% CI 0.85-2.7)

ICSI vs IVF as a risk factor of MM

- Major malformation rate in ICSI is comparable to IVF
  - Bonduelle 2002 **OR 0.93**
  - Ericson 2001 **OR 1.35**
  - Hansen 2004 **OR 0.96**
  - Odereid 2003 **OR 1.02**
- Meta analysis of 4 selected studies ICSI vs IVF:
  - Lie 2005 **OR 1.12** (CI 0.97-1.28)
    No increase of cardiovascular malformations, musculo-skeletal, hypospadias, NTD, oral cleft

Major malformations definitions

- ICD-10 codes for malformations
- Major malformation defined as malformation causing functional impairment and/or requiring surgical correction

  Remaining malformations were classified as minor
- Internal guidelines to code for major/minor

1

Major malformations in Brussels study[1]

	ICSI	IVF
Maj. malform.	96 **(3.4%)**[1]	112 **(3.8%)**[1]
Number	n = 2840	n = 2955

[1]Bonduelle et al. 2002
[2] Cochram Mantel Haenzel test p = 0.402 n.s

The same in both groups

Malformations in ICSI: sperm parameters / origin

• Sperm conc.[1]	≥ 5.10^6 /ml	2.8 %[3]
Sperm conc.[1]	< 5.10^6 /ml	3.8 %[3]
• Ejaculated sperm[2]	n = 2530	3.3 %[3]
• Testicular[2]	n = 518	5.0 %[3]
• Epididymal[2]	n = 182	4.4 %[3]

[1]Bonduelle et al. 2002
[2]Bonduelle et al. update 05/2008 of children born after TESE
[3]Fisher's Exact Test n.s

Malformations in ICSI: sperm origin

	Liveborns	Major malf
ICSI[2]	n = 2530	3.3 %[1]
Testicular[2]	n = 518	4.0 %[1]
• NOA	n = 168	4.2 %[1]
• OA	n = 360	5.3 %[1]

[1]Fisher's Exact Test non significant
[2]Bonduelle et al. update 5/2008 of children born after TESE

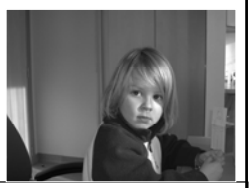
Malformations in ICSI literature / epididymal & testicular sperm

	Epididymal	Testicular	ICSI	Statistics
Belgium	n = 182 liveb 4.4%	n = 518 liveb 5.0%	n = 2530 liveb 3.3%	**n.s.**
France A France B	n = 546 preg 2.2% OR=1.30 [0,95-1,84]	n = 201 preg 4.0%[2]	n = 2332 preg 2.5%[2]	**[2]n.s.** **significant**
Germany	n = 26 liveb 3.8%	n = 229 liveb 9.1%[3]	n = 3199 liveb 8.4%[3]	**[3]n.s.**

Belgium Bonduelle et al., update 2008
France A De Mouzon et al oral comm., 2005, Fivnat et al. 2007
Germany Ludwig et al., Hum Reprod, 2003

Long term FU studies on ICSI

Bonduelle et al. 2005
- Prospective controlled on singletons
- Multicentre EU study at 5y
  - 1515 ICSI, IVF and SC
  - Medical
  - Cognitive
  - Behavioral



Major malformations at 5 years

	ICSI n 540	IVF n 437	Control n 538	p-value
Neonatal	3.3%	2.1%	1.9%	ns
Childhood	**3.0%**[1]	2.3%	0.4%[1]	[1]0.001
Total major malformation	**6.3%**[2]	4.3%	2.2%[2]	[2]0.001

Major malformations at 5 years

Increase in ICSI > IVF > control children

- Not detected at birth
- Partially due to increased defects in uro-genital system
- Higher malformation rate in ICSI boys 8.2% > girls 3.6%

Growth at 5 years

- Subgroups
  - Epididymal and testicular
  - Ejaculated <1 million/ml
  - Ejaculated 1- 4.99 million/ml
  - Ejaculated 5- 19.9 million/ml
  - Ejaculated > 5 million/ml
- No difference in growth and cognitive development

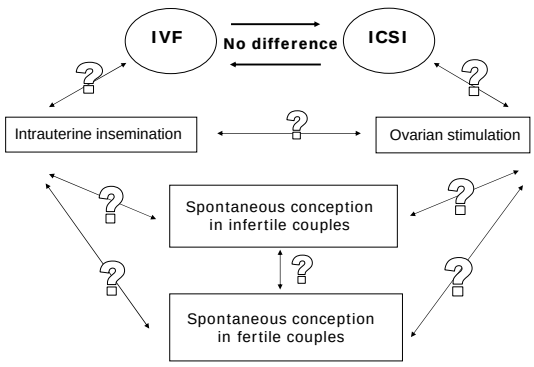
[1] Wennerholm et al. H Reprod 2005

Outline lecture

- Introduction of IVF and ICSI
- Perinatal outcome
- Prenatal diagnosis
- Major malformations
- **Discussion**
- Conclusion

How to further answer questions?

- **Do infertility treatments have a direct effect on adverse outcomes?**
- *Only **ICSI and IVF** risk is well documented*
- Insufficient data on **ovarian stimulation**, intra-uterine insemination and spontaneous conception in infertile couples, data needed
- Sufficient data indicating that **sub-/ infertility *per se*** are a risk factor (malformations increased by "the time to pregnancy")
- Data on **embryo manipulation** (biopsy, assisted hatching, polar body biopsy) still insufficient, further studies needed
- **Culture** conditions might play a role in imprinting disturbances, careful monitoring is needed



Comparisons ART subgroups

"Insufficient data on **ovarian stimulation**, intra uterine insemination and spontaneous conception in infertile couples, data needed"

- Klemetti et al. 2005 Comparison on Major malformations between 3 groups
  - IVF, ICSI and Frozen embryo transfer
  - Other ART : Ovulation induction with and without insemination
  - General population

Comparisons ART subgroups[1]

	IVF / ICSI N = 4 459	Adjusted*	Other ART N = 4 467	Adjusted
TOTAL	**OR 1.52** (CI 1.3-1.8)	**OR 1.3** (CI 1.1-1.6)	**OR 1.24** (CI 1.0-1.5)	NS (OR 1.2)
Singleton boys	OR 1.79 (CI 1.4-2.3)	**OR 1.63** (CI 1.2-2.2)	NS	NS
Uro genital		OR 2.46 (CI 1.5-4.1)		
Musculo skeletal		OR 1.75 (CI 1.1-2.8)		

*Adjusted for mat age, socio econ, region [1] Klemetti et al. 2005

Conclusion: what to say to the parents of ART children?

- Information to be discussed before pregnancy
  - Multiple pregnancies remain most important risk factor
  - Slightly higher risk of premature (8% instead of 4%) and lighter neonates (x2) also in singletons
  - For ICSI children slightly higher risk of inherited chromosomal anomalies in relation to parental chromosomes and 3 times more *de novo* (1-2%) anomalies related to sperm quality
  - Slightly higher risk (OR 1.3) of major malformations at birth (or 3.5% instead of 2.5%) compared to general population
    - mostly in relation to maternal age, infertility and underlying parental disease
    - but an effect of ART and other factors cannot be excluded

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- Children and parents
- Multidisciplinary team of the Centres for Medical Genetics and for Reproductive Medicine
  - two full-time research nurses
  - paediatricians-geneticists
  - gynaecologists
  - psychologists
  - data managers
  - cytologists, molecular biologists, embryologist
- Support from University Hospital, University Research Council, Willy Gepts Foundation, unrestricted educational grant from Organon

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Recurrent Pregnancy Loss After ART

Prof. H.J.A. Carp, M.B.,B.S. FRCOG.
Sheba Medical Center, Tel Hashomer
& Tel Aviv University, Israel

Conflict of Interest

The author has no financial interest, nor received any perks for any part of this presentation

Plan of Lecture

- Association between RPL & infertility
- Fetal abnormalities leading to RPL & infertility
- APS, pregnancy loss & infertility
- Alloimmune factors & cytokines in RPL & infertility
- Investigation of RPL
- Treatment
  - Progesterone
  - hCG supplementation
  - Anticoagulants
  - Intravenous immunoglobulin
  - PGS/PGD
  - Gamete donation
  - Surrogacy

Association between RPL & ART

- Concurrent infertility in 32% of RPL (Clifford et al, 1994).
- 74 RPL patients referred to ART for subsequent infertility after assessment
- 182 patients seen for RPL after ART (of 2316)
- Incidence of MA is 15% after ART (Schieve et al, 2003), 40% after age 43 (Turner et al, 2003)
- Do the causes of infertility cause RPL?
- Do the causes of RPL cause infertility?

Causes of Infertility & Pregnancy Loss

Recurrent Pregnancy Loss

- Abnormal embryo
  - Structural anomalies incompatible with life
  - Chromosomal anomalies
- Hostile maternal environment
  - Uterine
  - APS
  - Thrombophilia
  - Alloimmune
  - Infection
  - Endocrine

Implantation Failure

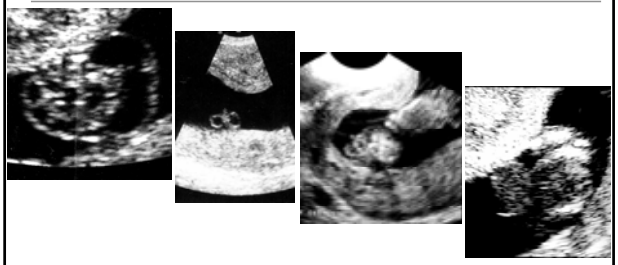
- Abnormal embryo
  - Chromosomal anomalies
- Hostile maternal environment
  - Endocrine
  - Defects in endometrial receptivity

Fetal Abnormalities

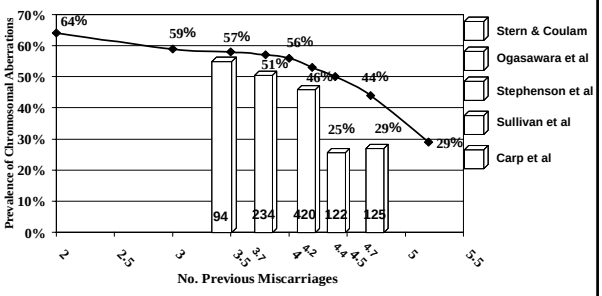
Structural Anomalies

- 70% of miscarriages are blighted ova
- Ultrasound only visualizes empty sac
- Embryoscopy has shown developmental defects in 200/233 missed abortions (85%) (Philipp et al, 2003), including:- anencephaly, encephalocele, spina bifida, syndactyly, pseudosyn-dactyly, polydactyly, cleft hand and cleft lip.
- 56/221 (25%) karyotyped embryos eukaryotypic.

Structural Anomalies: Ultrasound



Karyotyping of Abortus in RPL



Karyotyping of Abortus in ART

- Using PGD techniques
- 29% of morphologically normal embryos are chromosomally abnormal (Munne et al, 1995)
- Morphologically abnormal embryos may have normal chromosomes (Harper et al, 1995)
- 29% of blastocysts may be mosaic (Bielanska et al, 2000)
- Using CGH, only 3 of 12 cells were entirely normal (Voullaire et al, 2000)

Antiphospholipid syndrome

Modified Sapporo Criteria

Clinical	Laboratory
▪ Venous or Arterial Thrombosis	▪ IgG ACA >40gPLu on 2 occasions 12 weeks apart
▪ Recurrent Pregnancy Loss – ≥1 unexplained fetal death, >10 weeks, morphologically normal – Premature delivery, due to placental insufficiency, severe PET or eclampsia – 3 unexplained consecutive abortions <10 weeks	▪ Positive LA Test on 2 occasions 12 weeks apart – APTT, KCT, RVVT ▪ Anti-B_2GP-1 antibody, IgG and/or IgM on 2 occasions 2 weeks apart, measured by ELISA

Additional aPL

- aPE antibodies may be risk factor for early fetal losses (affect trophoblast formation) & mid-to-late pregnancy loss (due to binding to PE-kinionogen complexes resulting in thrombin-induced platelet aggregation (Sugi et al, 2004).
- aPS. Due to apoptosis PS exteriorised raising antibodies. Apoptotic microparticles may act as nidus for thrombosis.

aPL: Evidence of Causation of Pregnancy Loss / Infertility

- Binding of aPL to β2GP1, may lead to breakdown of phospholipid adhesion molecules in trophoblast & subsequent prothrombotic effects (Lyden et al, 1992).
- aPL reported to affect implantation, placentation, and early embryonic development (Shurtz-Swirsky et al, 1993; di Simone et al, 2000).
- aPL significantly reduce hCG release and trophoblast invasiveness. (Shurtz-Swirsky et al, 1993; di Simone et al, 2000).
- aPL inhibit trophoblast differentiation "in vitro" (Quenby et al, 2005)

Evidence Opposing Association Between APS & Infertility (1)

- Hornstein et al. (2000), - meta-analysis of studies of and IVF
  - No significant association between aPL & pregnancy rate or live birth rate. OR = 0.99 & 1.07, respectively.
- ASRM, practice committee bulletin (2006) based on this metaanalysis
  - aPL testing not warranted in IVF
  - treatment not indicated in seropositive patients.
  - Clinical pregnancy & live birth rates were 57% and 46% in aPL positive patients, compared with 49.2% & 42.9%, in aPL negative patients.

Evidence Opposing Association Between APS & Infertility (2)

- Mardesic et al, (2000). No relationship found between aPLs and establishment of IVF pregnancy. Concluded that aPLs do not influence fertilization rates.
- Eldar-Geva et al, (1999) 173 sera of IVF patients analyzed for ACl, aPS, PA & aPG. 56 patients with $\geq$ 2 failed IVF cycles evaluated for LA,
  - Neither presence of antibodies nor the number of positive antibodies affected IVF success.
  - Multiple failed IVF cycles not associated with positive aPLs.
  - None of 18 patients with multiple failed IVF cycles tested positive for LA.

Supportive Evidence (1)

- 27.9% of infertile patients positive for at least one aPL, (unexplained infertility, ovarian dysfunction, endometriosis or IVF failure),

  14.8% had at least 2 positive aPLs. (Kaider et al, 1999)

  (PC most frequently occurring antibody followed by PG & PA)
- Egbase et al. (1999) retrospectively evaluated 1027 IVF cycles. 6.6% of women with 1^{o} infertility positive for aPL. Concluded that aPL testing justified after 2 IVF/ET attempts.

Supportive Evidence (2)

- Bakimer et al, (1992) immunized BALB/c mice with human monoclonal antibody. Lower fecundity rate observed in immunized females (21% vs. 48%) ($P < 0.005$).
- Sher et al. (2000) reported a direct relationship between aPE, aPS, & increased NK cell activity in non-male-factor IVF patients. 88% of patients positive for aPE & aPS had increased NK cell activity, compared to 12-25% in controls. Hence, aPL's might be markers of abnormal activation of cellular immunity.

Antibody Screen in Infertility

(Shoenfeld, Carp et al, 2006)

	Patients	Controls	OR. (95% CI)
Prothrombin	22/69 (31.9%)	10/120 (8.3%)	5.15 (2.12-12.74)
aPL	8/69 (11.6%)	3/120 (2.5%)	5.11 (1.18-25.35)

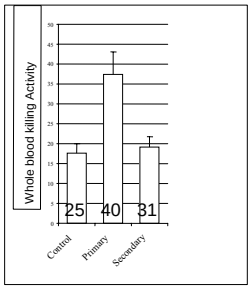
Multi centre study looking at prevalence of various autoantibodies in 269 patients with reproductive failure

Alloimmune Factors

Cellular Mechanisms: NK cells

- NK cells physiologically accumulate in decidua in 1st trimester & regress towards the myometrium at 13 weeks. Hence, NK probably not pathological
- Role may be immunosurveillance, mediation of angiogenesis (Hanna, et al, 2006) or remodeling of spiral arteries to uteroplacental arteries (Guimond et al, 1998).
- Trophoblast induces apoptosis in NK cells by HLA-G & FasL
- Hence trophoblast resistant to NK cells.
- If cytokine activated, (LAK), attacks trophoblast

Natural Killer Cells: Relevance in RPL



p=0.001

- Increased NK cells in peripheral blood in RPL (Emmer et al , 2000)
- Increased NK cells in luteal phase endometrial biopsies in RPL (Clifford et al, 2000)

NK Cells: Clinical Relevance

	Abortion Rate	Abnormal Karyotype
■ NK >1SD	17/24 (71%)	2/12 (17%)
■ NK <1SD	9/44 (20%)	3/5 (60%)

(Aoki et al, 1993)

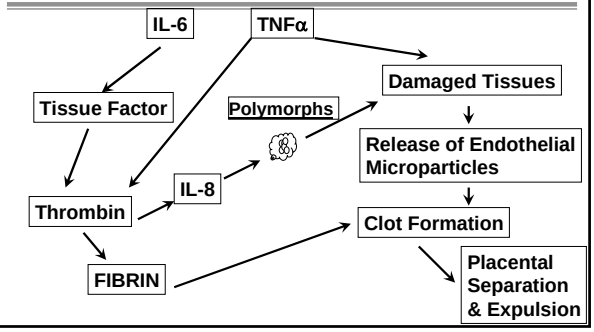
	Predictive value for pregnancy loss.	Abnormal Karyotype
■NK > 12%	87%	22%
■NK < 12%,		68%

(Coulam et al, 1995) 114 patients studied prospectively

Cytokines In Infertility & Pregnancy

Cytokine	Infertility	Pregnancy
GM-CSF	Trophoblast proliferation	Trophoblast proliferation
EGF		hPL & hCG
IFNγ	Remodelling of spiral arteries, Induce MHC expression	Remodelling of spiral arteries
IL-4 IL-10		Inhibits IFN γ, Inhibits Prothrombinase
IL-1	Stimulates IL-6, IL-8, LIF, TNFα,PGE2, PGF	
IL-6	Releases hCG	Releases Tissue Factor, Initiates clotting, Releases hCG
TNFα	ActivatesNK cells, Mediate Apoptosis	ActivatesNK cells, Mediate Apoptosis, Initiate clotting
TGFβ2	Inhibits NK activation, Inhibits placental differentiation	Inhibits NK activation, Inhibits placental differentiation
IL-15	Increases trophoblast invasion, Modulates MMP-1 Maintains uNK cells	Increases trophoblast invasion, Modulates MMP-1 Maintains uNK cells
LIF	Essential for implantation, Cytotrophoblast differentiation	Cytotrophoblast differentiation
IL-18	Prevents implantation	
IL-3	Cytotrophoblast differentiation	Cytotrophoblast differentiation

Cytokines Affecting Coagulation



APS and Cytokines

- IL-3 decreased in APS (Shoenfeld et al, 1998)
- Administration of IL-3 reduces fetal loss in experimental APS (Fishman et al, 1993)
- Alteration of Th-1/Th-2 balance may be involved in effect of antiidiotypic antibodies on APS (Krause et al, 1999)
- TNF-α levels were significantly higher in patients with APS than healthy controls (Bertolaccini et al, 2001; Borodin et al, 2002)
- Elevated levels of IL-6 and TNF-α & a trend to lower IFN-γ were found in patients with definite APS. (Forastiero et al, 2005)

Investigation of RPL

Current Practice

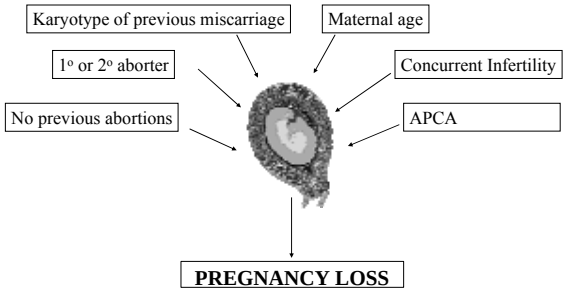
- Investigate parents for a list of causes
- Any abnormal result diagnosed as the cause and treated
- Outcome of subsequent pregnancy compared to outcome without treatment

Comparison of Protocols for Investigation of RPL

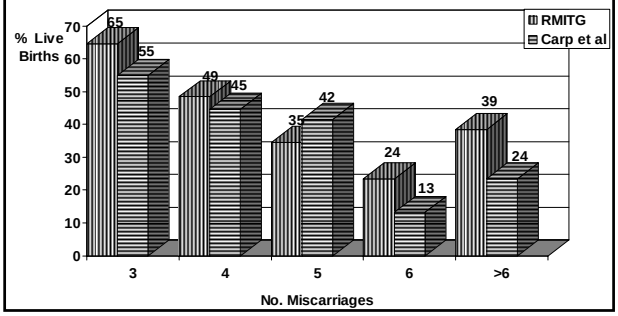
Investigation or treatment	RCOG protocol	ACOG protocol	ESHRE protocol
Parental karyotyping	Recommended	Recommended	Recommended
APS assessment (aCL and LA)	Recommended	Recommended	Recommended
Fetal karyotyping	Recommended	Insufficient evidence	Trial required
Uterine cavity assessment	Recommended by ultrasound or hydrosonography	Insufficient evidence	Recommended by ultrasound or hysterosalpingography
Luteal phase investigation	—	Insufficient evidence	Insufficient evidence: trials required
Bacterial vaginosis	Insufficient evidence	Not recommended	—
Hereditary thrombophilias	Insufficient evidence	Insufficient evidence	Recommended as advanced investigation
Thyroid function	Not recommended	Not recommended	Recommended
Glucose challenge test	Not recommended	Not recommended	Recommended
TORCH testing	Not recommended	Not recommended	Not recommended
Alloimmune testing	Not recommended	Not recommended	Insufficient evidence

Carp. Recurrent Pregnancy Loss, Causes Controversies & Treatment, 2007

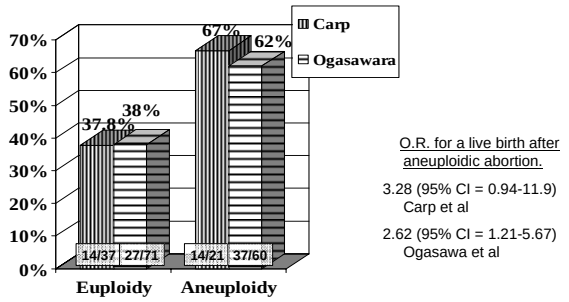
Factors Predicting Subsequent Live Birth



Live Birth Rate in Untreated Patients



Subsequent Pregnancy According to Karyotype of Abortus

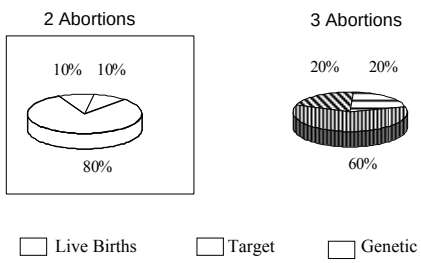


O.R. for a live birth after aneuploidic abortion.

3.28 (95% CI = 0.94-11.9) Carp et al

2.62 (95% CI = 1.21-5.67) Ogasawa et al

Good Prognosis Patients



Investigation Of Recurrent Pregnancy Loss (1)

History

- Accurate details, past missed abortions, blighted ova, abortions of live embryos, mixed pattern of losses, 1º, 2º, or 3º
- Medical history, diabetes, thyroid disease, infertility.
- Karyotype of previous abortions (if available)
- Cycle length
- Treatment in past pregnancies.

Examination

- Vagina and cervix, vaginal septum, one or two cervices.
- Is cervix wide or torn?
- Notch at fundus of uterus indicating bicornuate.
- Presence of fibroids.

Investigation Of Recurrent Pregnancy Loss (2)

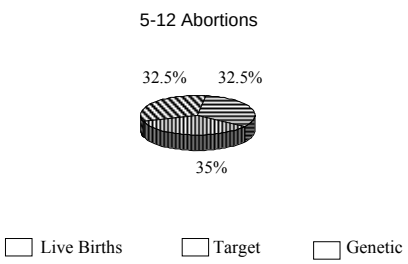
Investigations

- Hysteroscopy, hydrosonography or 3-D ultrasound.
- Autoantibody screen for ANA, ACA, LA.
- Thrombophilia screen, APCR, MTHFR, Factor II.
- Thyroid function and glucose challenge test if indicated.

Treatment

- Treatment usually empiric
- If uterine septum – resect
- Treat APS with anticoagulants
- Thrombophilia treated with anticoagulants
- Hormone supplements used empirically

Poor Prognosis Patients



Investigation Of Poor Prognosis Patients

Investigations

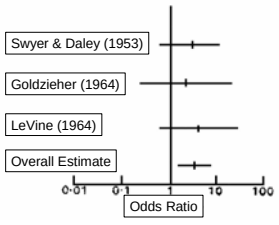
- Hysterosalpingogram or hysteroscopy.
- Autoantibody screen for ANA, ACA, LA.
- Thrombophilia screen, APCR, MTHFR, Factor II.
- Thyroid function and glucose challenge test if indicated.
- **APCA or NK Cells**
- **Karyotype of Parents**

Treatment

- If uterine septum – resect
- Resistant APS treated with IVIg, or surrogacy
- If karyotypically normal, Paternal leucocyte immunization
- If karyotypically normal, PLI and IVIg fail - surrogacy
- If two karyotypically abnormal embryos - PGD

Treatment

Metaanalysis on Progesterone Support (Daya, 1989)



	Progesterone	Controls
Live Births	39/50 (78%)	24/45 (53%)

- Only 3 placebo controlled trials from 1953-1964
- When 3 trials analysed together, absolute benefit 25%
- No trial matched for predictive factors, or chromosomal aberrations
- No matching for fetal heart, or start of treatment

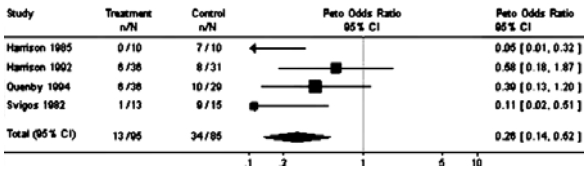
p=0.01, OR = 3.09 (1.28-7.52)

Actions of hCG

- Pregnant women's lymphocytes express hCG receptors (Lin et al, 1995)
- hCG may prevent T-cell activation at maternal-fetal interface (Lei et al, 2006).
- hCG influences TNFα and IL-6 secretion (Uzumcu et al,1998), increases IL-1β secretion, and inhibit IL-2 expression (Shaffer et al, 1992).
- U-hCG contains LIF which regulates trophoblast differentiation
- Promotion of angiogenesis via VEGF. (Zygmunt et al, 2002)
- Stimulates corpus luteum to produce progesterone
- PGE2 production and stimulates 17β -estradiol secretion (Han et al, 1996)
- hCG involved in differentiation of endometrial stromal cells to decidua, (Han et al, 1996).
- hCG regulates smooth muscle cell gap junctions in the pregnant human myometrium inhibiting myometrial contractions (Ambrus & Rao, 1994).

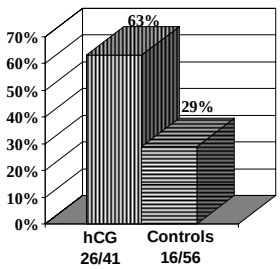
hCG Supplementation

(Pattison & Scott, Cochrane Systematic Review, 1995)



- Benefit 26.3%
- OR for miscarriage = 0.26 (0.14-0.52)
- ≥ 2 miscarriages, No. miscarriages not quoted
- All trials used u-hCG, not r-hCG

hCG ≥ 5 Abortions

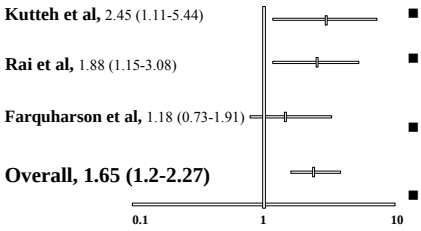


- 5 or more consecutive miscarriages
- Absolute benefit 34%

OR = 4.33 (95% CI 1.7-11.3)

- hCG cannot be used in OHSS

Metaanalysis on Heparin & LMWH



- No measurement of β2GP-1 antibodies
- No correction for chromosomal aberrations
- No correction for structural malformations
- Overall benefit 22%

Role of Aspirin

(Empson et al, 2002)

	Aspirin	Placebo
Cowchock & Reece, 1997	10/11 (91%)	7/8 (87.5%)
Pattison et al, 2000	16/20 (80%)	17/20 (85%)
Tulppala et al, 1997	26/33 (78.8%)	28/33 (84.9%)
Total	52/64 (81.3%)	52/61 (85.2%)

- 135 patients
- Aspirin alone did not significantly reduce pregnancy loss, RR = 1.05, (95% CI, 0.66 - 1.68)
- Aspirin has not been assessed on obstetric complications or thromboses

Non Anticoagulant Actions of Heparin / LMWH

- Heparin increases serum TNF-BP-I. Hence protecting against systemic harmful manifestations (Lantz et al, 1991)
- LMWH inhibits TNFα production (Baram et al, 1997)
- Thrombosis results in vein wall inflammatory response. Both heparin & LMWH limit anti-inflammatory response. (Downing et al, 1998)
- In vitro, heparin restres ability of trophoblast to secrete hCG, which is inhibited by aPL (Di Simone et al, 1997)
- Heparin inhibits apoptosis of villous trophoblast induced by IFN-γ & TNF-α. (Hills et al, 2006)

Heparins in Infertility with aPL

(Sher et al, 1998)

- 687 aPL+ women underwent 1050 IVF cycles. 477 births with Heparin/Aspirin (46%), compared to 22 (17%) births in 127 IVF cycles without H/A.

IVIg autoantibodies & Cytokines

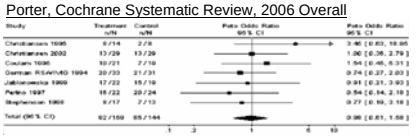
- IVIG inhibits production of IL-2, IL-10, TNF-α & IFN-γ, (Th-1) from peripheral blood mononuclear cells in culture. (Andersson et al, 1996)
- Circulating levels of TNF-α & IL-1β decreased after IVIg in Guillain-Barre syndrome (Sharieff et al, 1999).
- Proportion of IFN-γ producing (Th1) and IL-4-producing (Th2) cells and Th1/Th2 ratio compared before and after IVIg. IVIg enhanced proportion of Th-2 producing cells (Graphou et al 2003)
- Modulation of cytokine levels following IVIg is due to interference with cytokine secretion or cytokine-specific blocking antibodies, rather than direct infusion of cytokines (Sherer et al, 2001)
- IVIg lowers levels of autoantibodies, e.g. ACA, LA

IVIg & NK Cells

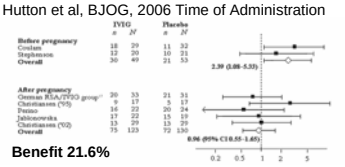
- NK cells down-regulated by IVIG (Ruiz et al, 1996; Kwak et al, 1996; Szereday et al, 1999; Perricone et al, 2006; Roussev et al, 2007)
- Down regulation associated with improved outcome (Aoki et al, 1993; Emmer et al, 2000; Kotlan et al, 2006)

IVIG in RPL: Randomized Trials

Porter, Cochrane Systematic Review, 2006 Overall



Hutton et al, BJOG, 2006 Time of Administration



Benefit 21.6%

IVIg in IVF Failure

(Clark et al, 2006)

- Meta-analysis of 3 RCTs of IVIG in IVF failure shows a significant increase in the live birth rate per woman
  (OR = 2.55, 95% CI 1.19-5.49)
- Benefit = 16.7% (p=0.012)

IVIg in Infertility with aPL

(Sher et al, 1998)

- 121 aPL+ women with aPE or aPS no live births after 2 IVF attempts with H/A, received IVIg in addition to H/A in 3rd IVF cycle. Birth rate 41% when aPS or aPE present compared to 17% with H/A alone.
- IVIg did not improve IVF outcome with other aPL.

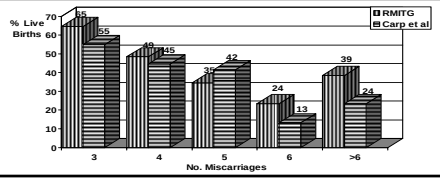
Pregnancy Rates With PGS

ART used for Infertility. RPL is probably only indication for which ART is used in fertile patients.

	No. Pregnancies
Rubio et al, 2003	23/67 (34%)
Wilding et al, 2004	58/276 (21.1%)
Platteau et al, 2005	15/63 (24%)
Total	96/406 (23.6%)

PGS For RPL: Live Births

	No. Women	Cycles	No. Miscarriages	Pregnancies	Live Births
Rubio et al, 2003	71	67	2.9	23	19
Platteau et al, 2005	49	31	4.7	15	10
Total	120	98	3.6	39	29 (74%)



Problems of PGS Techniques

- Only FISH can be used for PGS due to speed
- FISH allows 5, 7 or 9 chromosomes to be analysed, not 23.
- Embryo cannot be diagnosed as normal
- Classical karyotype takes too long to be practical
- CGH is expensive, time taken does not allow transfer in same cycle.
- If CGH, analysis and freezing.
- Pregnancy rate reduced after PGS (Ankum et al, 2007)

Which Patients with RPL need PGS?

Human Reproduction Page 1 of 4 May 6, 2004

OPINION

ART in recurrent miscarriage: preimplantation genetic diagnosis/screening or surrogacy?

H.J.A.Carp[1,3], M.Dirnfeld[2], J.Dor[1] and J.G.Grudzinskas[2]

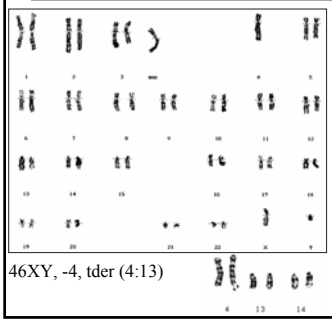
- Repeat fetal aneuploidy
- Parental chromosomal aberrations with associated aberration in fetus
- Older Patients

Repeat Embryonic Karyotyping

	Repeat Aneuploidy
Carp et al, 2004	8/43 (19%)
Sullivan et al, 2005	3/30 (10%)
Total	11/73 (15.1%)

62 of 73 (84.9%) subsequent abortions euploid
3 patients with 3 subsequent aneuploid abortions
In repeat aneuploidy PGD seems to be treatment of choice

PGD Patient



46XY, -4, tder (4:13)

- Age: 38
- **Obstetric History:**
  2 Live Births, 6 Miscarriages
  10-12 weeks
- **APCA:** Positive.
- **Other features:**
  No APS, thrombophilia, or other cause.
- **Parental Karyotype:**
  46XX 46XY t(14:13)(p11:q12)
- **Subsequent Pregnancy:**
  Missed abortion
  10 weeks, FHL

Role of Ovum Donation

- In 92 cycles of ED 64 implantations. 30 (32.6%) viable pregnancies, 34 (37.0%) were miscarriages. (Simon et al, 1999)
- ED in 8 RM couples, woman low responder to gonadotrophins 12 ED cycles performed. Pregnancy rate (75%), delivery rate (66.6%) Miscarriage rate per cycle, 11.1%. (Remohi et al, 1996)
- Tel Hashomer registry shows 4 cases of egg donation.
- No series in literature
- Has role if all embryos abnormal at PGD

Ovum Donation Patient

- **Obstetric History:** Age: 37. 4 previous miscarriages
- **Other features:** No APS, ANF, Thrombophilia, or other cause. Hysteroscopy NAD.
- **Parental Karyotype:** 46XX 46XY
- **Treatment** IVIG 30g.
- **Subsequent Pregnancy:** **I.** Biochemical pregnancy
  II. Missed Abortion - Culture failure
- **PGS** **8 embryos – all abnormal when tested with 9 probes**
- **Treatment** **Ovum Donation**

Role of Sperm Donation

- Tel Hashomer registry shows 62 cases of change in male partner of 1925 patients, 22 had 3 partners. 1 had 5 partners.
- Change of male partner did not prevent subsequent miscarriages.
- No series in literature

Results of Surrogacy

- Few reports of surrogacy in RM
- Raziel (2000) reported a normal live birth in a patient with 24 prior pregnancy losses.
- Author has advised surrogacy in 2^{o} aborter with 12 miscarriages, and 1^{o} aborter with 6 miscarriages & triplets of 25 weeks, died from prematurity. In both cases the surrogate delivered normal twins.
- Logic of surrogacy in patients with large numbers of miscarriages is due to the poor prognosis and low incidence of chromosomal aberrations.

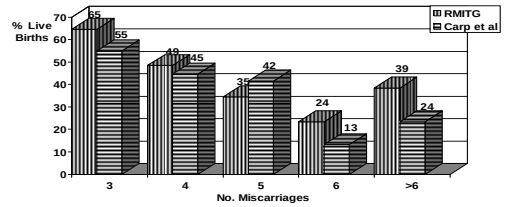
Surrogacy Patient

- **Obstetric History:** Age: 40. PROM 20 weeks, 2 x IUFD 20weeks, hypertension & Gest Diabetes. Missed 14 weeks. From 4 cycles ZIFT
- **Other features:** No APS, ANF, Thrombophilia, or other cause. Hysteroscopy NAD. CRP 44.7
- **Parental Karyotype:** 46XX 46XY
- **Treatment** IVF for 22 months infertility
- **Subsequent Pregnancy:** **I.** 8th cycle IVF, Aspirin 100mg. TOP 22 weeks for PET with HELLP **Advised Surrogacy**
  II. Spontaneous, Enoxaparin 40mg. Aspirin 100mg. NT Normal, Shirodkar Suture, PET & Gest Diabetes @ 18 weeks. Fetal death & PROM 20 weeks

Parental Chromosomal Aberrations: Subsequent Pregnancies

Subsequent Pregnancies	Live Births	Mean No.Abortions
164	78 (47.5%)	3.76

Carp et al, Goddijn et al, Ogasawara et al



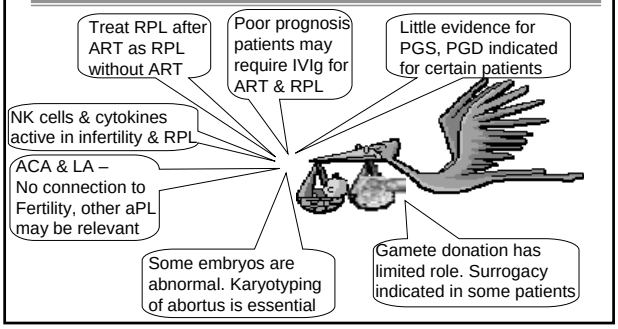
Parental Chromosomal Aberrations: Subsequent Fetal Karyotype

Karyotype of Abortus (Carp et al, 2005)

Embryos	Euploid	Aneuploidy
39	17 (43%)	10 (26%) Balanced, 5 (13%) Unbalanced, 7 (18%) Numeric (5 trisomies, 2 Monosomy X)

- In parental chromosomal aberrations, embryo should be karyotyped to reach accurate diagnosis.
- Parental karyotyping is a poor substitute for embvryonic karyotyping

Thank You for Listening



MULTIPLE GESTATION PREGNANCIES AFTER ART

Eric Jauniaux
TWIN CLINIC
UCL EGA Institute for Women's Health

LEARNING OBJECTIVES

- TO CONFIRM THE LINK BETWEEN ART AND MPG.
- TO DESCRIBE THE PERINATAL RISKS ASSOCIATED WITH MPG.
- TO EVALUATE THE COSTS ON HEALTH CARE OF MPG RESULTING FROM ART.
- TO DISCUSS THE IMPACT OF SINGLE ET.

TWINS in Medicine

(May 2008)

<u>Pub-Med:</u> Twin pregnancy = 21195 hits
(17180 in Jan 05)

<u>Google:</u> Twin pregnancy = 1.910000 hits
(973000 in Jan 05)

SPONTANEOUS TWINS

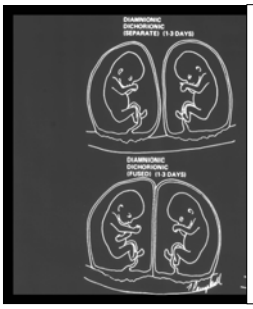
SPONTANEOUS TWINNING RATE

=> 1.6%

->1.2% DZ

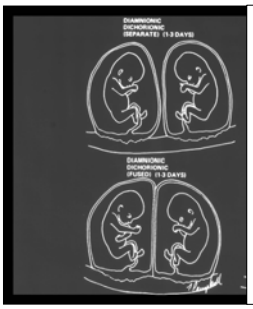
-> 0.4% MZ

DZ TWINNING



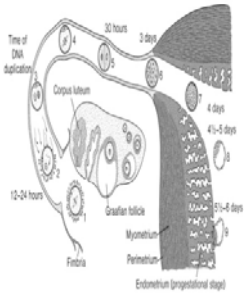
- **Fertilization of 2 ovulated oocytes (DiDi).**
- **Associated with multiple ovulation.**
- **Frequency varies between races (Asians< whites< blacks). Yarubas have 4.5 % twins (90% are DZ)**

DZ TWINNING RATES (TR)



- **Linked to racial or individual secretion in FSH/LH production.**
- **FSH level is higher in Nigeria (4.5%TR) vs (1.2% TR in Scotland) => Sun light ?**
- **DZ TR in ART are FSH dose-dependent.**

Monochorionic DZ Twinning

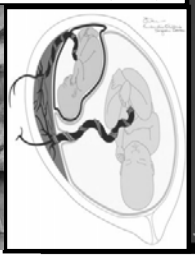


- **Very rare in natural DZ twinning (< 1%).**
- **May result from trophoectoderm cell fusion of 2 different blastocysts at or just before implantation.**
- **Incidence may increase with IVF blastocyst culture techniques.**

MZ TWINNING

Spontaneous division of 1 egg. Constant throughout the World. Maternal genetic component ? Teratogens? ART.

T2T TRANSFUSION SYNDROME (25%)



ART TWINS

IATROGENIC TWINNING (ART)

=> 15%

(Bardis et al; Fertil Steril 2005)

Ovulation induction=> 1/5

- IVF (3-7% per ET) => 4/5 (10% MZ)

⇒ART= 60% INCREASE IN TWIN BIRTHS

(Between 1980 and 2001)

=> TWINNING RATE = 3-4% OF ALL BIRTHS

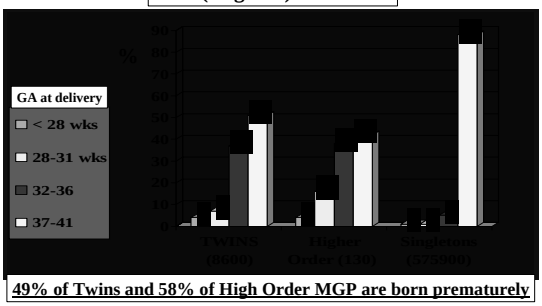
TWINS: ANTENATAL & PERINATAL COMPLICATIONS

ANTENATAL COMPLICATIONS:
- PRETERM LABOUR & DELIVERY (PTD)
- MATERNAL DISORDERS
  - ~ PRECLAMPSIA
  - ~ METABOLIC (Diabetes, Anaemia…)

OB PERINATAL COMPLICATIONS:
- Em C-SECTION
- PPH

MGP & PTD

DoH (England) 2003-2004



49% of Twins and 58% of High Order MGP are born prematurely

TWINS: PTD

- 42-55 % OF TWINS ARE BORN < 37 WKS & 7-8% ARE BORN < 32 WKS (EHSRE) & AVERAGE BIRTH WEIGHT = 2.5 Kg (US & EUROPE).

- TWINS= 12-15% OF ALL PTD & 15% OF ALL NEONATAL DEATH (UK). 25% PTD (US)

TWINS & NICU

@ The main cause of neonatal death in twins is pulmonary immaturity (63%).
Glinianaia et al., Twin Research, 1998 & BJOG, 2000

@ Twin pregnancies delivered at 36 weeks are 13 times more likely to require NICU.
Udom-Rice et al., J Perinatol, 2000

TWINS & PERINATAL OUTCOME: ADDITIONAL RISK FACTORS (2004)

@ Natural vs ART:
Maternal risks are comparable
(Pinborg et al., Acta OG Scand 2004)
Neonatal outcome are comparable
(Pinborg et al., Hum Reprod 2004)
Perinatal mortality is 40% lower in ART
(Hemmerhorst et al., BMJ 2004)

@ Maternal age: Higher risk < 18 & > 40 years

TWINS & PERINATAL OUTCOME: ADDITIONAL RISK FACTORS (2004)

@ Social background: Poor attendance to ANC & poor diet (Anaemia) = higher risk of PTD.

@ Nulliparity: Deliver 0.9 wk earlier than parous.
(Higher rate of Preeclampsia and PROM)
@ Discordant growth: Higher risk of IUD (DZ >> MZ)

@ Maternal smoking: increases the RR of PTD.

@ Male fetuses: have a higher incidence of PTD.

MGP: RISK OF PREECLAMPSIA

Sibai et al., 2000 AJOG

Multicentric prospective study of 684 twins vs 2946 singletons (NICH).

- **Preeclampsia => RR 2.6 (95% CI= 2.0-3.4)**
- **FGR => 15% (vs 7%)**
- **ABRUPTIO => 5% (vs 0.7%)**

Conde et al., O&G 2000: 15484 MGP (870,000 S) WHO
RR for eclampsia = 3.0 (95% CI, 2.9, 3.3)
RR for preclampsia = 2.2 (95% CI, 1. 9, 2.5)

TWINS: MATERNAL METABOLIC DISORDERS

DIABETES: Higher in Triplets (>25%) than in Twins (5-6%) but the risk of gestational diabetes is similar in twins and singletons. (US)

HYPOTHYROIDISM: No evidence of increased incidence in twins.

ANAEMIA: *x2 & Hb levels decrease more rapidly in Twins than in singleton after 24-28 weeks.*

TWINS & PPH

WHO Latin American Centre for Perinatology and Human Development (O&G 2000)

Outcome of 885,338 pregnancies including 15,484 twins =>
RR 2.0 (95% CI, 1.9, 2.0) for PPH

Blood should be available for all twin deliveries (ACOG guidelines)
Active management of third stage with oxytocin (SOGC)
Misoprostol (Routine UCLH)

MGP: FETAL PERINATAL MORTALITY & MORBIDITY

¶ **NEONATAL MORTALITY (FGR & PTD)**
- TWINS= X 5-7
- TRIPLETS= X > 9

¶ **MORTALITY AT 1 YEAR: X > 6**

¶ **CEREBRAL PALSY**
- TWINS= X 3-7 (OR < 32WKS 20X THAN > 36 WKS)
- TRIPLETS= > X 10

ESHRE 1999, Acta Ob Gyn Scand 2004

Compared to singletons

MGP: MATERNAL PERINATAL MORTALITY

TWINS: 3 X (OR: 2.9; 95% CI 1.4-6.1)
TRIPLETS: 6 X

ESHRE 1999

Compared to singletons

MGP: COSTS

¶ **TWINS: 60,000 $** ESHRE 1999 £ x 3-4 (2008)

¶ **TRIPLETS: 170,000-300,000 $**

¶ **QUADRUPLETS: >300,000 $**

MGP: COSTS

Cost analysis of singleton vs twin pregnancies after IVF: => From 6 wks to Post-Partum => <u>Medical cost per twin is > 5 times higher than per singleton.</u>

(Lukassen et al Fertil Steril, May 2004)

MGP: COSTS

Cost analysis of singleton vs twin & Triplet pregnancies after IVF (NHS):
- Singleton: 3313£
-Twin: 9112£
- Triplet: 32,354£
=> <u>56% direct cost of IVF pregnancies</u>.

(Ledger et al BJOG, 2006)

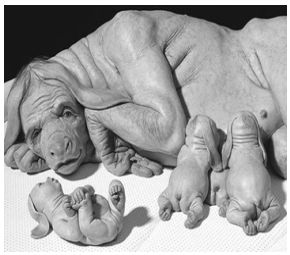
REDUCING ART TWINS

<u>Pandian et al., COCHRANE REVIEW 2007</u>

- **MGP rate is significantly lower after single ET (OR 9.97 vs 38.19; p<0.001).**
- **Clinical pregnancy and LB rates are lower with single than with double ET (2.08 vs 3.50 & 1.90 vs 3.22).**
- **LB rate after quadruple ET is not different than after double ET.**
- **Single ET in women < 36 years has decreased MGP from 29% to 6% in Belgium** (Van Landuyt et al., RBM on line 2006).

=> FINANCIAL & HUMAN COSTS (DEVELOPING COUNTRIES?)

THANK YOU



OBESITY AND ART OUTCOME

José Bellver, MD

***Instituto Valenciano de Infertilidad. Valencia. Spain*.**

jbellver@ivi.es

* No commercial and/or financial relationships with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices

Learning objectives

1- Deleterious effects of obesity on reproduction

2- Impact of obesity on spontaneous pregnancies and those achieved by ovulation induction and IVF

3- Effect of obesity during the different trimesters of pregnancy

4- The role of the oocyte and embryo

5- Male obesity and reproduction

6- Endometrial disturbance in obesity

7- Weight management for improving reproductive performance

Epidemiology

"Western lifestyle"

Reduced exercise

Changes in dietary composition

Increased caloric intake

USA & Europe (women) : 60 % overweight (≥ 25 kg/m^2)
30 % obese (≥ 30 kg/m^2)
6% morbidly obese (≥ 35 kg/m^2)

Australia (women): 52% overweight or obese

Year 2000: 300 million obese adults

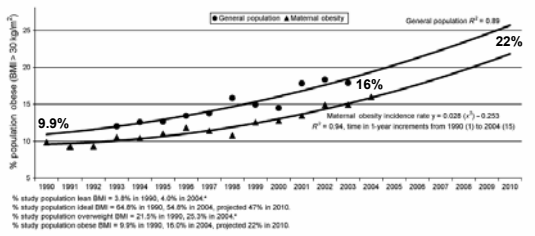
International Obesity Task Force and European Association for the Study of Obesity, 2002; Norman et al, 2004; Hedley et al, 2004; Hall and Neubert, 2005; Catalano, 2007

Epidemiology

Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36 821 women over a 15-year period

BJOG 2007;114:187–194.

N Heslehurst,[a] LJ Ells,[a] H Simpson,[b] A Batterham,[a] J Wilkinson,[c] CD Summerbell[a]



Health consequences

- **General: ↑ morbidity and mortality**
  - **→ Cardiovascular and cerebrovascular disease**
  - **→ Type II diabetes**
  - **→ Sleep apnoea**
  - **→ Osteoarthritis**
  - **→ Gastrointestinal diseases**
  - **→ Cancer**
- **Women (childbearing age):**
  - **→ Menstrual disorders (oligo-amenorrhoea)**
  - **→ Infertility (anovulation, hyperandrogenism)**
  - **→ Increased risk of miscarriage**
  - **→ Increased maternal morbi-mortality**
  - **→ Increased foetal/ neonatal morbi-mordidity**
  - **→ Lower livebirth rate**

Norman & Clark, 1998; Pasquali et al, 2003; Hall and Neubert, 2005; Dokras et al, 2006

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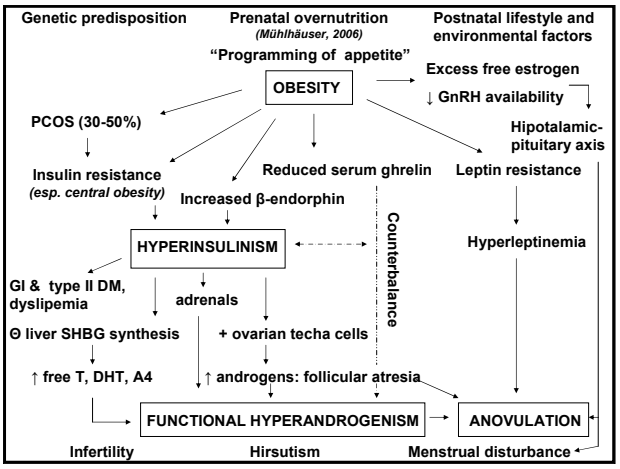

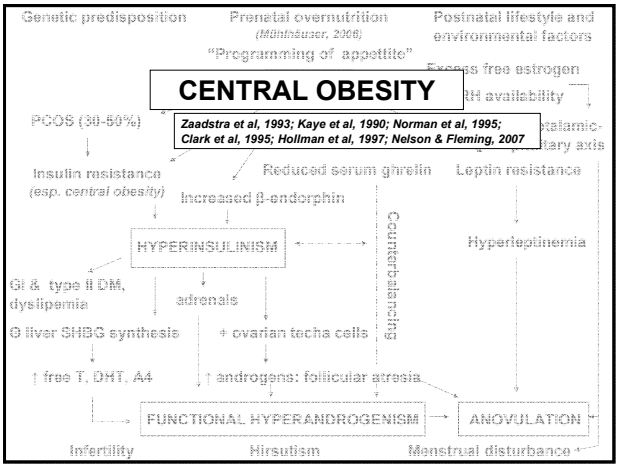

ACHIEVEMENT OF PREGNANCY

- Spontaneous pregnancy
- Ovulation induction
- IVF / ICSI

Obesity and spontaneous conception

- **Risk for ovulatory infertilty: OR: 2.1- 3.1**

  Green et al, 1988; Rich-Edwards et al, 1994; Grodstein et al, 1994; Clark et al, 1998

- **Reduced fecundability (ovulatory): OR: 0.69- 0.82 (0.66*)**

  Lake et al, 1997; Jensen et al, 1999; Gesink Law, 2007

- **Increased time to pregnancy: OR: 2.2- 11.5**

  Bolumar et al, 2000; Hassan and Killick, 2004

The effect of obesity on fecundity persists for women with regular menstrual cycles

Jensen et al, 1999; Bolumar et al, 2000; Hassan and Killick, 2004; Gesink Law, 2007

Anovulation despite regular menses? Ova with reduced fertilization potential? Endometrial abnormalities?

Ovulation induction in PCOS obese women

*GONADOTROPHINS; CC**

- Ovarian response:

- *** Lower response to gonadotrophins / CC**
- *** Larger doses of gonadotrophins / CC**
- *** More days of stimulation**
- *** Less ovulation**
- *** More abandoned and cancelled cycles**

Sinergistic effect of obesity and IR

Lobo et al, 1982; Chong et al, 1986; Polson et al, 1989*; McClure et al, 1992; Hamilton-Fairley et al, 1992; Homburg et al, 1996; White et al, 1996; Kousta et al, 1997*; Fridstom et al, 1997; Imani et al, 2000*; Mulders et al, 2003; Al-Azemi et al, 2004*; Balen et al, 2006*

- Pregnancy rates:

- *** Unaffected:** *McClure et al, 1992; Dickey et al, 1997*; Mulders et al, 2003; Balen et al, 2006*
- *** Reduced:** *White et al, 1996; Al-Azemi et al, 2004**

Ovulation induction in non-PCOS obese women

GONADOTROPHINS

<u>IUI (donated or partner sperm)</u>

*** With/without regular menses:**

- 0.1 unit ↑ waist-hip ratio: OR (conception per cycle) 0.70
CENTRAL OBESITY *Zaadstra et al, 1993*

*** Regular menses, ovulatory women:**

- **Greater gonadotrophin dose**
- **Lower E2 levels**
- **More days of stimulation**
- **Increased follicular asynchrony** *(Kably-Ambe et al, 1999)*
- **BUT normal cycle fecundity**

Fuh et al, 1997; Loh et al, 2002; Dodson et al, 2006

Strategies to improve fertility (anovulatory obese women)

Weight loss (exercise + diet): ≥ 5% (better abdominal fat)	Metformin	Bariatric surgery (gastric by-pass or banding) *When ≥ 40 kg/m2 or 35 kg/m2 with comorbid conditions*
↓ T, FI & insulin resp to G ↑ SHBG	↓ FI & androgens	Menstruation & pregnancy restoration
Improv. menstr regularity Reduction hirsutism	Ovulation improvement when hyperandrogenism	- Numerous surgical complications - Poorer perinatal outcome & maternal complications - Expensive - Long-term follow-up data on offspring ? - Higher rates of infertility?
Improvement ovulation & pregnancy rates: 81-92%	Modest weight loss with high doses	
Restoration of ovulatory cycles: - Related to caloric restriction - Mediated by reduction in IR (≈ central obesity)	- < efficacious for ovulation in obese PCOS ♀ - No clear effect in morbid obesity (>37 kg/m²)	

Kiddy et al, 1992; Bilenka et al, 1995; Pirwany et al, 1999; Martin et al, 2000; Pasquali et al, 2003; Norman et al, 2004; Lord et al, 2003; Nelson & Fleming, 2006; Sheiner et al, 2006; Catalano, 2007; Mehri, 2007

Ovarian response in IVF/ICSI

- > gonadotrophin requirement, with/without PCOS
- In long and short COH protocols
- Longer ovarian stimulations
- Higher cancelation rate
- Also in ovum donation*

Crosignani et al, 1994; Soderstrom-Anttila et al, 1996; Wittemer et al, 2000; Loveland et al, 2001; Loh et al, 2002; Mulders et al, 2003; Spandorfer et al, 2004; Fedorcsák et al, 2004; Dokras et al, 2006; Ku et al, 2006; van Swieten, 2005; Dechaud et al, 2006*

- ↓ periovulatory intrafollicular hCG concentration *Carrell et al, 2001*
- Lower bioavailability of injected (sc or im) hCG *Chan et al, 2003*
- Lower E2 peak level (hCG day)

Lashen et al, 1999; Nichols et al, 2003; Spandorfer et al, 2004; Dokras et al, 2006

Ovarian response to gonadotrophins is negatively correlated with the BMI

Ovarian response in IVF/ICSI

State of "gonadotrophic resistance": theories

- Reduced absorption of drugs
- Altered pharmacokinetics → Lower FSH effective concentrations → Fewer selected oocytes → Fewer collected oocytes → Higher FSH doses required
- Leptin: a) Related to the amount of adipose tissue
  b) inhibits the stimulatory effect of FSH on steroid synthesis of granulosa *in vitro*
  c) ↑ intrafollicular concentracions: relative resistance to gonadotrophins during COH for IVF in PCOS women

Soderstrom-Anttila et al, 1996; Fedorcsák et al, 2000; Remorgida et al, 2003; Bellver et al, 2006

Outcome in IVF/ICSI

IMPLANTATION RATES

- **Reduced:** *Loveland et al, 2001; Nichols et al, 2003*
- **Not affected:** *Dechaud et al, 2006; Dokras et al, 2006; Fedorcsák et al, 2004*

PREGNANCY RATES

- **Reduced:** *Halme et al, 1986; Loveland et al, 2001; Carrell et al, 2001; Ku et al, 2006*
  - *** OR: 0.53** *Nichols et al, 2003*
  - *** Each ↑1 BMI unit, OR ↓ by 0.84** *Ferlitsch et al, 2004*
  - *** When WHR > 0.80** *Wass et al, 1997*
  - *** From 25 (OR: 0.81) to ≥ 35 kg/m^2 (OR: 0.50)** *Wang et al, 2000*
- **Not affected:** *Lashen et al, 1999; Wittemer et al, 2000; Loh et al, 2002; Spandorfer et al, 2004; Dokras et al, 2006; van Swieten, 2005; Dechaud et al, 2006*

Conflicting results

*** Type of treatment**
*** Incompletely characterized or unstratified patient heterogeneity**
*** Inconsistent definitions of obesity and normal weight**
*** Combination of overweight and obesity in the same study group**
*** Type of obesity (central vs non-central)**
*** Disregard for the influence of the obese spouse on PR**
*** Retrospective nature of the studies**
*** Small sample sizes: low statistical power**

Effect of overweight and obesity on assisted reproductive technology—a systematic review

Human Reproduction Update, pp. 1–12, 2007

A. Maheshwari[1,4], Lawrize Stofberg[2] and S. Bhattacharya[3]

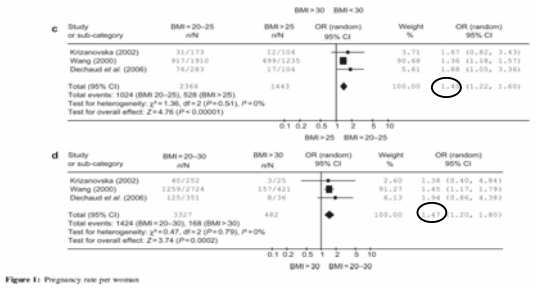

Figure 1: Pregnancy rate per woman

FIRST TRIMESTER OF PREGNANCY

Miscarriage and obesity

- **Spontaneous conception:**
  - **Increased early miscarriage (6-12 w) (OR: 1.2)**
    Clark et al, 1998; Lashen et al, 2004
  - **Increased recurrent early miscarriage (OR: 3.5)**
    Bussen et al, 1999; Lashen et al, 2004
- **Ovulation induction (with/without PCOS):**
  - **Increased miscarriage rates (OR: 2.0-3.0)**
    Bohrer and Kemmann, 1987; Hamilton-Fairley et al, 1992; Franks and Hamilton-Fairley, 1994; Mulders et al, 2003; Ramsay et al, 2006
- **IVF / ICSI:**
  - **Increased miscarriage rates (OR: 1.7-2.2): ↑ with BMI**
    Fedorcsák et al, 2000; Loveland et al, 2001; Wang et al, 2002; Fedorcsák et al, 2004
  - **Increased risk in PCOS only when obesity**
    Wang et al, 2001
  - **Not affected***
    Lashen et al, 1999; Wittemer et al, 2000; Nichols et al, 2003; Roth et al, 2003

*↓ *sample size*

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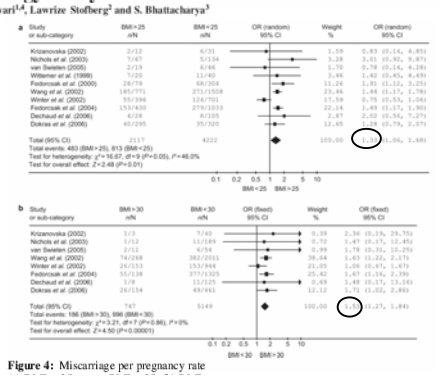


Figure 4: Miscarriage per pregnancy rate

Miscarriage: Theories

Biochemical and clinical miscarriages (<12 w)

* *Association with PCOS*
  Balen et al, 1993
* *Impaired insulin resistance at the time of conception*
  Lashen et al, 2004
* *Abnormal corpus luteum function*
  Sherman & Korenman, 1974; Fedorcsák et al, 2000
* *Related to female infertility (less frequent in ICSI)*
  Orvieto et al, 2000; Wang et al, 2001
* *Poor oocyte or embryo quality / development*
  Kawamura et al, 2002; Winter et al, 2002; Fedorsák & Storeng, 2003; Lashen et al, 2004
* *Abnormal endometrial receptivity*
  Alfer et al, 2000; González et al, 2000
* *Uterus exposure to higher E2 concentrations in IVF*
  Valbuena et al, 1999; Wang et al, 2001; Wang et al, 2002

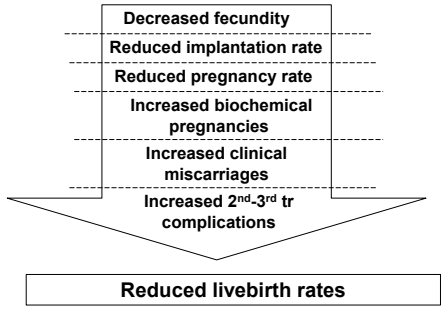
SECOND AND THIRD TRIMESTERS OF PREGNANCY

Pregnancy complications

Maternal complications	Fetal complications
- Hipertension & preeclampsia	- Congenital malformations (x 2-3)
- Gestational diabetes	- Preterm delivery (& postterm)
- Urinary infections	- Sudden intrauterine death
- Induction of labor	- Perinatal death
- Assisted vaginal delivery	- Macrosomy
- Cesarean section	- Shoulder dystocia
- Wound infection	- NICU admission
- Postpartum bleeding	- Obesity / cardiovascular / DM II in adolescence & adulthood
- Thromboembolism	
- Anaesthetic problems	
- Longer hospitalization	Cost of hospital antenatal care: x 5
- Death	

Galtier-Dereure et al, 1995; Kabiru et al, 2004; Andreasen et al, 2004; Linné, 2004; Hall- Neubert, 2005; Nelson & Fleming, 2006; Yu et al, 2006; Catalano & Ehrenberg, 2006; Ramsay et al, 2006

Obesity and reproductive outcome



Livebirth rates after IVF/ICSI

75% when BMI < 25 kg/m^2
63% when BMI ≥ 25 kg/m^2 **p =0.04**

Fedorcsák et al, 2000 (n = 383)

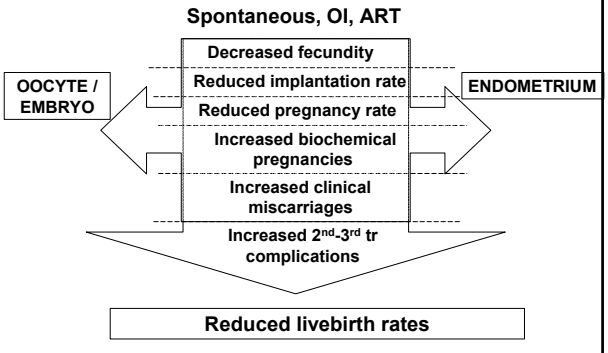
OR: 0.67 when BMI ≥ 27 kg/m^2

Lintsen et al, 2005 (n = 8457)

OR: 0.75 when BMI ≥ 30 kg/m^2

Fedorcsák et al, 2004 (n = 5019)

Obesity and reproductive outcome

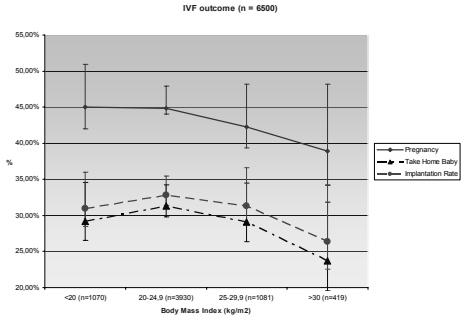


Obesity & oocyte/embryo quality after IVF

Obesity affects	*Obesity does not affect*
↓ No collected oocytes*	= No collected oocytes *Lashen et al, 1999; Nichols et al, 2003*
Poorer oocyte quality†	NS oocyte & embryo quality*
Less mature oocytes†	*Wang et al, 2000; Nichols et al, 2003; Metwally et al, 2007 (* >35 y)*
Lower fertilization rate‡	
Poorer embryo quality#	
Lower incidence of ET†,ɣ	NS incidence ET or No transferred embryos
Lower No transferred embryosɣ	*Lashen et al, 1999; Nichols et al, 2003*

* *Lewis et al, 1990; Crosignani et al, 1994; Wittemer et al, 2000; Fedorcsák et al,2000 & 2001; Spandorfer et al, 2004; Fedorcsák et al, 2004; Maheshwari et al, 2007*
† *Dokras et al, 2006;* ‡ *Krizanovská et al, 2002; van Swieten, 2005;* # *Carrell et al, 2001;* ɣ *Fedorcsák et al., 2004*

Obesity & oocyte/embryo quality after IVF



Bellver et al, 2008. In press

Obesity & oocyte/embryo quality after IVF

Obese group: ↑ Oligo-anovulation, years of infertility, FSH dose
= No oocytes & MII, Day ET, No ET, No cryopreserved, % BT

Correlations between fertilization rate, average embryo fragmentation, average number of cells 48 and 72 hours after fertilization and BMI

BMI	IVF/ICSI	
	r	p
Blastomeres number 48h	0.012	0.477
Blastomeres number 72h	0.011	0.505
Embryo fragmentation 48h	0.006	0.737
Embryo fragmentation 72h	0.007	0.693
Fertilization Rate	0.015	0.160

Bellver et al, 2008. In press

Associated male obesity

Reduction in semen quality

- **Significant reduction in sperm concentration (by 22%*) & total sperm count (by 24%*)**
- **Trend to impaired sperm morphology**
- **Significant ↓ testosterone, inhibin B & SHBG ↑ FAI & estradiol**

1558 Danish young men (mean age: 19 y) *Jensen et al, 2004*
72 Icelandic men (mean age: 37 y) *Magnusdottir et al, 2005*
274 Hungarian men (mean age: 26 y) *Koloszár et al, 2005*

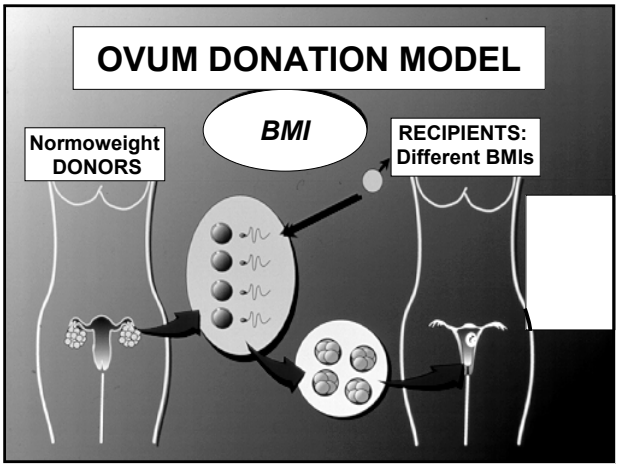
OR for infertility: 1.20 (overweight men)
1.36 (obese men)

26303 planned pregnancies
Nguyen et al, 2007

Subfecundity (TTP > 12 months): OR: 2.74 when both obese

64167 pregnant women *Ramlau-Hansen et al, 2007*

OVUM DONATION MODEL



Endometrium: Ovum donation

TABLE 1

Descriptive characteristics of the study group (n = 712).

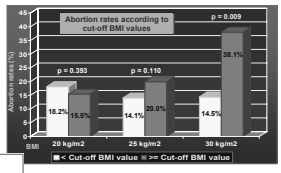
	BMI <20 (n = 92)	BMI = 20–24.9 (n = 398)	BMI = 25–29.9 (n = 172)	BMI ≥30 (n = 50)
Age of recipient (y)	38.0 ± 4.7	38.0 ± 5.3	39.2 ± 5.5	38.8 ± 5.4
No. of donated oocytes	9.7 ± 2.4	9.1 ± 2.6	9.4 ± 2.3	9.1 ± 1.9
Cycle of the donor	1.9 ± 1.0	1.8 ± 1.0	1.7 ± 0.9	1.8 ± 1.0
Use of GnRH agonist (%)	76 (82.6)	296 (74.4)	125 (72.7)	38 (76.0)
Days of estrogen therapy	30.6 ± 13.0	32.1 ± 13.8	31.3 ± 13.9	35.5 ± 13.0
Severe sperm pathology (%)	18 (19.6)	49 (12.3)	19 (11.1)	8 (16.0)
No. of ETs	2.9 ± 0.7	3.0 ± 0.6	3.1 ± 0.7	3.1 ± 0.6
Day of embryo development	3.2 ± 1.3	2.9 ± 1.1	3.0 ± 1.2	2.7 ± 1.0
Transferred blastocysts (%)	17 (18.5)	48 (12.1)	23 (13.4)	4 (8.0)
Implantation rate (%)	26.2	27.8	25.6	18.8
Pregnancy rate (%)	44 (47.8)	211 (53.0)	84 (48.8)	21 (42)
Spontaneous embryo reduction (%)*	2 (2.2)	12 (3.0)	3 (1.7)	1 (2.0)

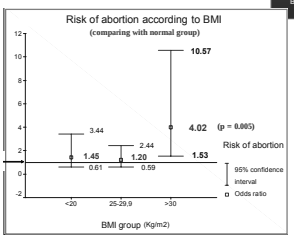
Note: Unless otherwise indicated, values are means ± SD. BMI = body mass index. *P* was not significant for all entries.
* All spontaneous embryo reductions were of one embryonic sac, except for two cases with reduction of two sacs (one in the BMI = 20–24.9 group, and the other in the BMI = 25–29.9 group).
Bellver. Obesity and spontaneous abortion. Fertil Steril 2003.

Bellver et al, Fertil Steril 2003; 79: 1136-40

Endometrium: Ovum donation

N = 712:
< 20 kg/m2 (n = 92; 12.9%)
20-24.9 kg/m2 (n = 398; 55.9%
25-29.9 kg/m2 (n = 172; 24.2%)
≥ 30 kg/m2 (n = 50; 7%)





Bellver et al, Fertil Steril 2003; 79: 1136-40

Endometrium: Ovum donation

n = 97, 1st cycles

TABLE 2

Recipient characteristics by BMI strata.

Variable	BMI strata: Underweight <20 (n = 7)	Normal ≥20–<25 (n = 52)	Overweight ≥25–<30 (n = 25)	Obese ≥30 (n = 12)	P value
Age (years)[a]	43.7 (28.2–48.9)	41.2 (29.4–43.0)	41.8 (21.6–50.7)	42.5 (30–50.7)	.38
Endometrial thickness (mm)[a]	8 (6–9)	10 (5–16)	10 (8–17)	10 (8–14)	.78
Embryos transferred[a]	3 (2–4)	3 (2–5)	3 (2–4)	3 (2–4)	.65
Embryo quality at embryo transfer[b]	1.0 ± 0.3	1.1 ± 0.6	0.9 ± 0.4	0.9 ± 0.4	.68
Blastomeres at embryo transfer[b]	5.6 ± 0.8	5.1 ± 1.2	5.5 ± 0.8	4.7 ± 1.1	.12
Embryos cryopreserved after embryo transfer[b]	4.8 ± 2.9	6.3 ± 5.0	7.8 ± 3.4	8.6 ± 5.6	.96
Implantation rate[c]	25 (8–67)	27 (19–36)	28 (21–34)	29 (9–49)	.31
Live birth rate/embryo transfer[c]	43 (8–93)	42 (28–56)	43 (32–55)	42 (9–74)	.78

[a] Values are median and range.
[b] Values are mean ± SD.
[c] Values are percentage and 95% CI.

Wattanakumtornkul et al, Fertil Steril 2003; 80: 336-340

Endometrium: Ovum donation

n = 536

TABLE 1

Pregnancy outcome and BMI.

	≤20	21–25	26–29	≥30	P value
N	101	284	74	77	
Mean age (y)	40.2 ± 5.9	41.2 ± 1.6	40.4 ± 8.4	41.5 ± 6.9	.17
Implantation rate (%)	55.2	53.8	54.7	54.8	.90
Pregnancy rate (%)	71.3	73.2	74.3	74	.91
Loss rate (%)	25.0	24.5	10.9	29.8	.096
Mean no. of embryos	2.2 ± 0.002	2.3 ± 0.006	2.4 ± 0.003	2.3 ± 0.002	.43
% Blastocyst transferred	55.4[b]	60.6[b]	51.4[b]	59.7[b]	
% Day 3 transferred	44.6[b]	39.4[b]	48.6[b]	40.3[b]	

[a] vs. [b] P = .46.

TABLE 2

Pregnancy outcome and BMI analyzed by day of transfer.

	≤20		21–25		26–29		≥30		P value[a]	
	Blastocyst	D3	Blastocyst	D3	Blastocyst	D3	Blastocyst	D3	Blastocyst	D3
N	56	45	172	112	38	36	46	31		
Mean age (y)	39.9 ± 10.9	40.6 ± 12.8	41.2 ± 2.9	41.2 ± 3.3	41.7 ± 18.6	39.0 ± 13.6	41.9 ± 10.0	41.0 ± 22.6	.16	.10
Implantation rate (%)	67.0	40.6	63.8	38.5	67.0	41.5	68.1	34.9	.94	.81
Pregnancy rate (%)	80.3	60.0	80.2	58.9	84.2	63.9	80.4	58.1	.89	.98
Loss rate (%)	26.7	22.2	19.6	34.3	12.5	8.7	21.6	45.5	.48	.035
Mean no. of embryos transferred	2.0	2.5 ± 0.02	2.0	2.5 ± 0.03	2.0	2.8 ± 0.01	2.0	2.8 ± 0.01	.25	.28

[a] Significant, defined as P<.05 for ANOVA analyses and P<.005 for Bonferroni-corrected χ^2 analyses. D3 = day 3.

Styne-Gross et al, Fertil Steril 2005; 83: 1629-1634

Endometrium: Ovum donation

TABLE 1 n = 536

Pregnancy outcome and BMI.

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Pregnancy outcome and BMI analyzed by day of transfer.

	≤20		21–25		26–29		≥30		*P* value*	
	Blastocyst	D3	Blastocyst	D3	Blastocyst	D3	Blastocyst	D3	Blastocyst	D3
N	56	45	172	112	38	36	46	31		
Mean age (y)	39.9 ± 10.9	40.6 ± 12.8	41.2 ± 2.9	41.2 ± 3.3	41.7 ± 18.6	39.0 ± 13.6	41.9 ± 10.0	41.0 ± 22.6	.16	.10
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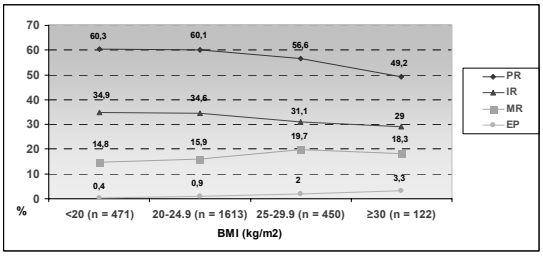
Loss rate (%)	26.7	22.2	19.6	34.3	12.5	8.7	21.6	45.0	.48	.035

Styne-Gross et al, Fertil Steril 2005; 83: 1629-1634

3.089 ovum donation cycles: global miscarriage → 17.6 %
< 45 y → 16.8 %

Soares et al. J Clin Endocrinol Metab 2005, 90: 4399-4404

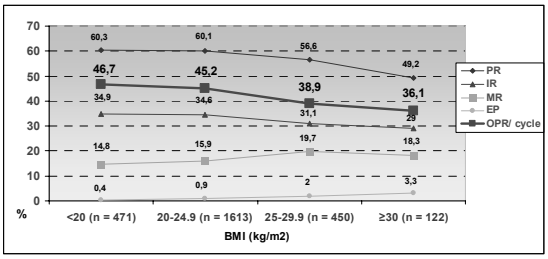
Endometrium: Ovum donation



n = 2656

Bellver J et al. Fertil Steril 2007, 88:446-51

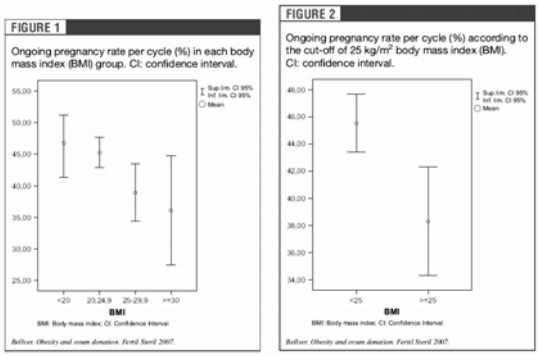
Endometrium: Ovum donation



n = 2656

Bellver J et al. Fertil Steril 2007, 88:446-51

Endometrium: Ovum donation



Bellver J et al. Fertil Steril 2007, 88:446-51

Conclusions

- Obesity affects reproductive performance

- Hyperandrogenism and abnormal secretion and action of insulin, leptin and ghrelin are the main pathophysiological features related to infertility

- Poor reproductive outcome in obese women *(before 2nd trimester of pregnancy)* could be mainly related to an impaired ovarian function, but the endometrium seems to play a role.

- Male obesity should be also considered

BEST TREATMENT = PREVENTION

Management of the obese infertile women before conception

Encourage weight loss
- low calorie diet
- sensible eating habits
- long-term compliance

Exercise
- appropriate for obese women
- reduces insulin resistance

Psychological
- behavior therapy

Appetite suppressants*
- appear to allow for weight loss
- side-effects
- need for follow-up

Surgery
- not usually recommended

Metformin?? / Preconception counselling

*** Sibutramine, Orlistat**

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Thank you

Ovarian Reserve
ART
Early Pregnancy Loss

ESHRE 2008
Barcelona

F.J. Broekmans, PhD, MD
Associate Professor
Division Reproductive Medicine
UMC Utrecht

Disclosure

Dr Frank J. Broekmans, MD, PhD
Member Advisory Board
Ferring Pharmaceuticals
The Netherlands

Learning Objectives

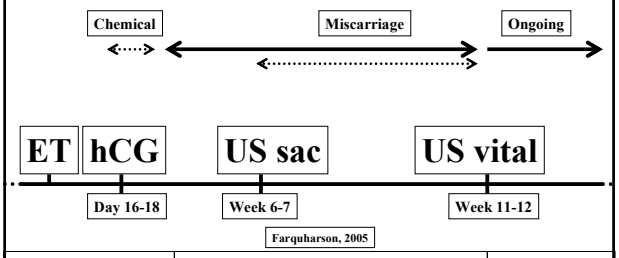
At the conclusion of this presentation the participant should be able to:

- Define and describe ovarian ageing in terms of quantity and quality changes
- Describe the role of ovarian ageing in the chance of pregnancy loss after ART
- Value the role of ovarian reserve testing in the management of the couple indicated for ART
- List the management options for preventing pregnancy loss after ART

Questions

- Does ART lead to increased rate of Pregnancy Loss
- What is Ovarian Reserve (OR)?
- Ovarian Reserve and Pregnancy Loss in ART: related?
- How can we assess Ovarian Reserve?
- OR Tests for Outcome prediction in ART: useful?
- Is Prevention of Pregnancy Loss feasible?
- Conclusions

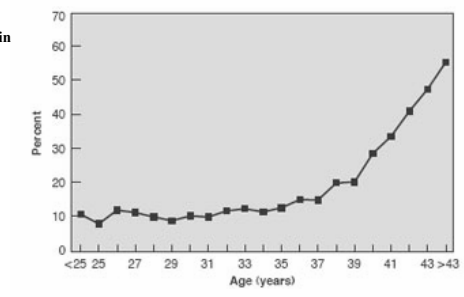
Definition Early Pregnancy Loss



Does ART lead to increased rate of Pregnancy Loss

Miscarriage Rate after ART in fresh non donor eggs - CDC data 2003

Same pattern as in spontaneous pregnancies



Does ART lead to increased rate of Pregnancy Loss

Early pregnancy loss increased in ART??

Early pregnancy losses in 1999
in vitro fertilization and oocyte donation

Carlos Simón, M.D.,* Jose Landeras, M.D.,† Jose L. Zuzuarregui, Ph.D.,†
Julio Cesar Martín, Ph.D.,‡ José Remohí, M.D.,* and Antonio Pellicer, M.D.*

Not clearly…

Does ART lead to increased rate of Pregnancy Loss

This means that
1. ART itself is not likely to induce EPL
2. Chance of EPL mainly determined by gamete quality through the same routes as in spontaneous pregnancies

Questions

- Does ART lead to increased rate of Pregnancy Loss
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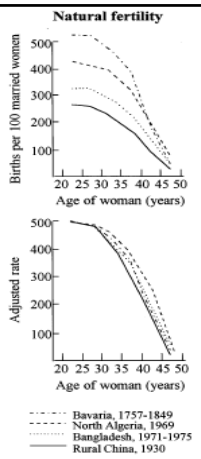
What is Ovarian Reserve

Fertility declines with female age = Reproductive ageing

Evidence from Natural Populations

O'Connor, 1999

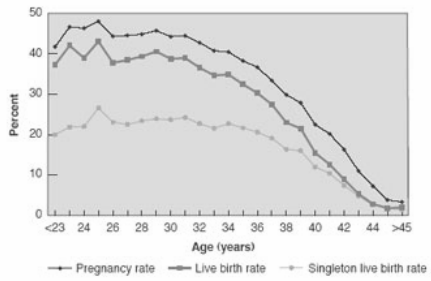


What is Ovarian Reserve

Also evident for ART Populations

2003 CDC ART report

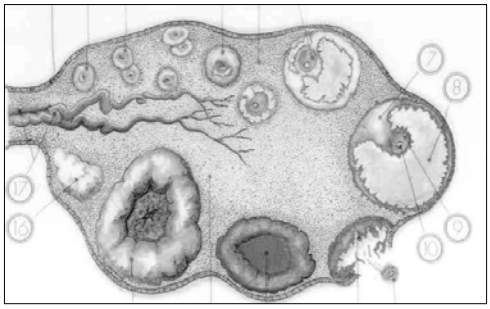
Fertility declines with female age = Reproductive ageing



What is Ovarian Reserve

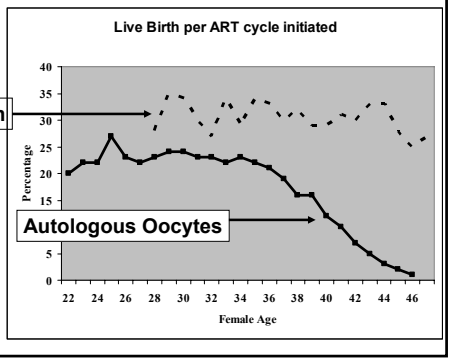
Te Velde, 2002

Reproductive ageing = Ovarian ageing



What is Ovarian Reserve

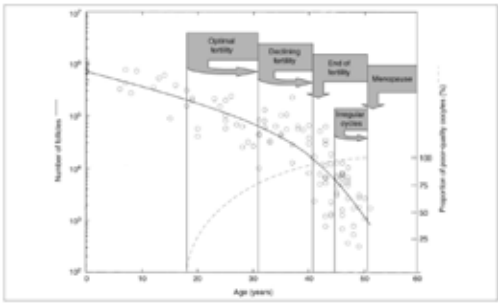
Reduced Uterine Reserve?
No evidence – CDC report 2003



What is Ovarian Reserve

Ovarian reserve

= Quantitative and qualitative decline of the follicle/oocyte pool



Faddy, 1995, De Bruijn, 2003, O'Connor, 2001

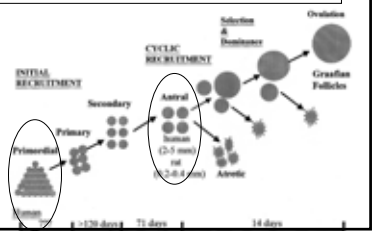
What is Ovarian Reserve

Ovarian Reserve - Quantity

= the size of the antral follicle cohort that is continuously present in the ovaries to be recruited by the late luteal FSH rise

This cohort is proportionally related the primordial follicle pool

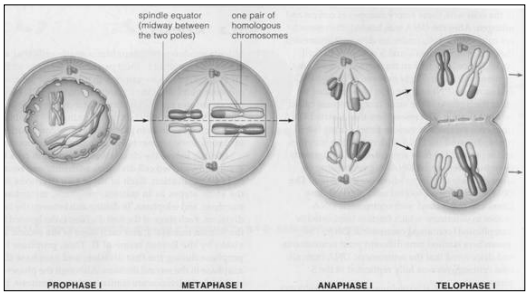
Quality????



What is Ovarian Reserve

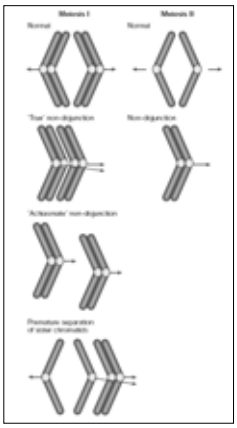
Ovarian Reserve - Quality

= determined by recombination errors in meiotic cell division of the oocyte



What is Ovarian Reserve

Meiotic
Non disjunction
=
Poor oocyte quality



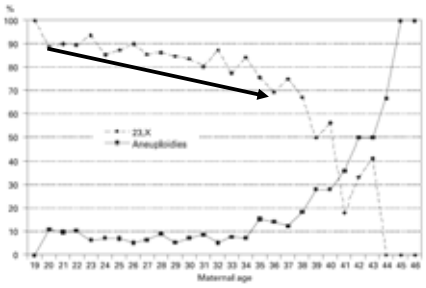
Hunt, 2001

What is Ovarian Reserve

Rate of haploidy and the global incidence of aneuploidy according to maternal age in a sample of 1,397 human oocyte II karyotypes.

Pellestor, CCI 2005

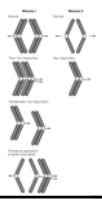
Resumed meiosis is increasingly defective with higher female age

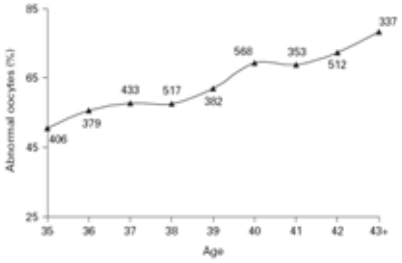


Increasing % aneuploid oocytes with higher female age

Meiose Errors
Chromatid Errors
Numeric Abn

Kuliev, CGR 2005





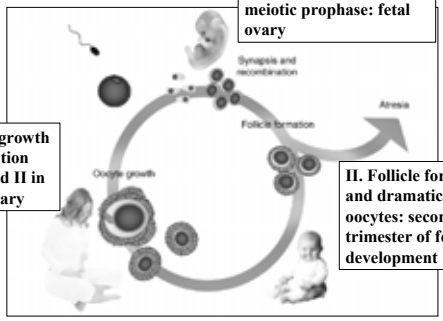
Mechanisms Aneuploidy

Production line theory
- First developed oocytes acquire the best quality in chromosome recombination
- First in first out…

Two hit theory
- Part of the developed oocytes already has defective recombination
- Another part develops non-disjunction during resumed meiosis through
  - accumulated damage oocyte
  - accumulated damage follicle/granulosa cells

The three vulnerable stages of oogenesis

I. Synapsis and recombination in the 1st meiotic prophase: fetal ovary

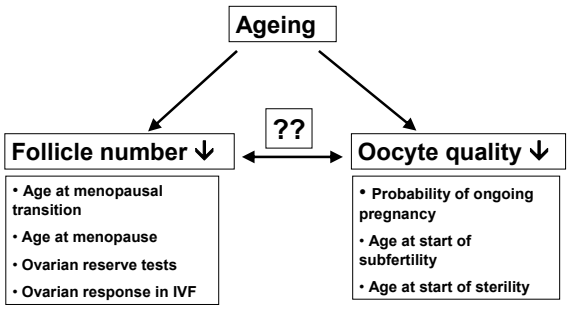


III. Oocyte growth and resumption meiosis I and II in the adult ovary

II. Follicle formation and dramatic loss of oocytes: second trimester of fetal development

Hunt, 2008

What is Ovarian Reserve? Follicle Number and Oocyte Quality



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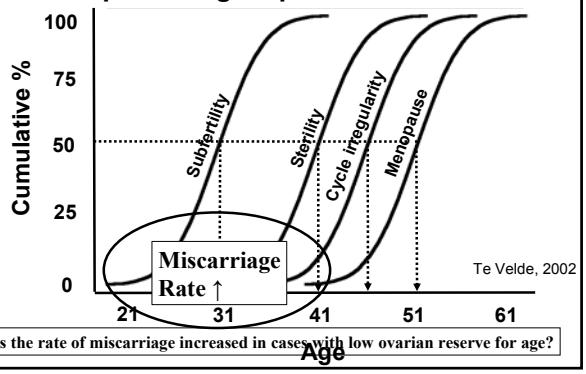
Ovarian Reserve and Pregnancy Loss: related?

Variation in age at menopause and preceding "reproductive events"



Is the rate of miscarriage increased in cases with low ovarian reserve for age?

Ovarian Reserve and Pregnancy Loss: related?

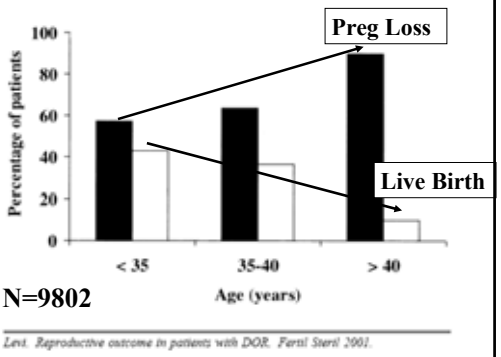
Is the rate of miscarriage increased in cases with low ovarian reserve for age?

Is the rate of diminished ovarian reserve increased in repeated early pregnancy loss

Is the rate of diminished ovarian reserve increased in cases with aneuploid early pregnancy loss

Ovarian Reserve and Pregnancy Loss: related?

Age effect alone

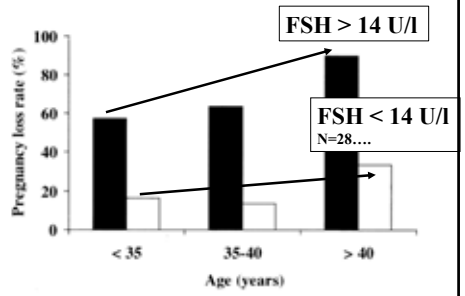


N=9802

Levi. Reproductive outcome in patients with DOR. Fertil Steril 2001.

Ovarian Reserve and Pregnancy Loss: related?

Effect of female age and basal FSH = OR marker



N=9802

Levi. Reproductive outcome in patients with DOR. Fertil Steril 2001.

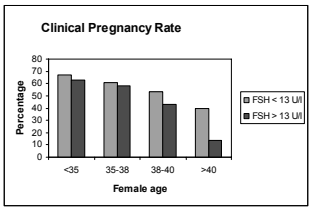
Ovarian Reserve and Pregnancy Loss: related?

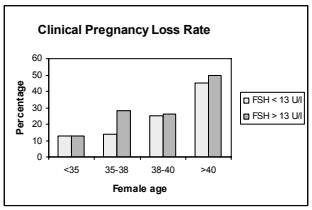
Ovarian Reserve Status (basal FSH) within age categories only predicts CP rate in > 40 women

But fails to predict pregnancy loss after sac identification week 6-7

Luna, 2007

N=2708



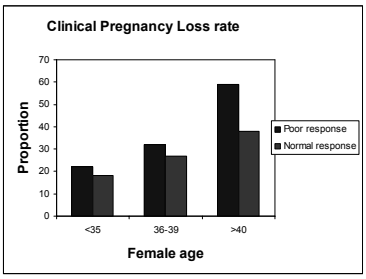


Ovarian Reserve and Pregnancy Loss: related?

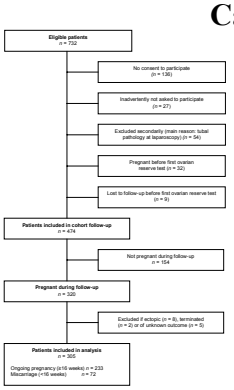
Ovarian Reserve Status (Poor Response) within age categories only predicts clinical pregnancy loss after sac identification week 6-7 in women over 40 years

Kumbak, 2008

N=2160



Ovarian Reserve and Pregnancy Loss: related?



Cases in Infertility work-up
N=305 pregnancies
Spontaneous
IUI
ART

Does any ORT predict occurrence of miscarriage?

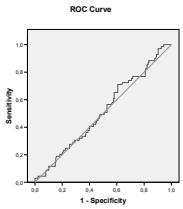
Haadsma, submitted

Ovarian Reserve and Pregnancy Loss: related?

Discrimination of live birth and miscarriage.

After adjustment for female age, not any ORT has any discriminatory capacity…

OR Tests
- **FSH**
- **AFC**
- **InhibinB**
- **CCCT**



Haadsma, submitted

Ovarian Reserve and Pregnancy Loss: related?

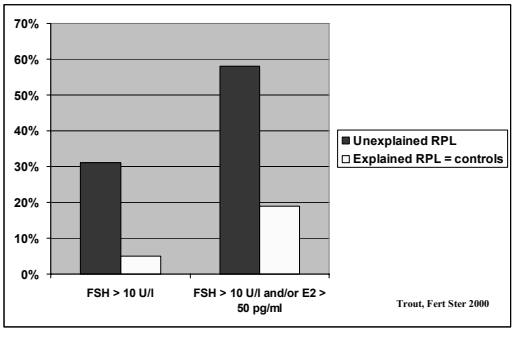
In women with Diminished Ovarian Reserve: higher chance of aneuploidy?

DOR = FSH↑ or poor response
7 chromosome FISH
Weghofer, FS 2007

	DOR patients (N=20)	Controls (N=20)	
Age (y)	34.6 ± 3.7	34.6 ± 3.7	NS
Weight (lb)	132.4 ± 29.3	144.0 ± 33.7	NS
Basal FSH (mU/mL)	7.1 ± 2.6	5.5 ± 1.9	.04
Basal E_2 (pg/mL)	55 ± 39	42 ± 15	NS
Peak E_2 (pg/mL)	2,171 ± 1,156	2,984 ± 1,721	NS
Days of stimulation	10.3 ± 1.7	10.0 ± 1.9	NS
Ampoules of gonadotropins used	59.8 ± 22.0	36.0 ± 11.4	.001
No. of oocytes retrieved	10.0 ± 6.4	13.0 ± 7.1	NS
Aneuploid embryos (%)	52.6	52.2	NS
No. of embryos transferred	1.1 ± 0.9	1.6 ± 0.9	NS
Clinical PR per ET (%)	43	47	NS
Miscarriage (%)	50	13	NS
Delivery rate per ET (%)	21	41	NS
Babies' birth weight (lb)	7.9 ± 0.6	7.3 ± 1.3	NS

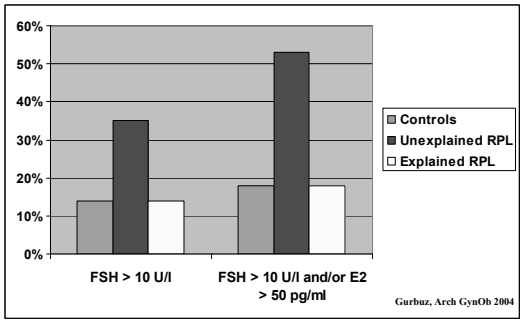
Ovarian Reserve and Pregnancy Loss: related?

In recurrent Miscarriage e.c.i. more often reduced ovarian reserve….



Ovarian Reserve and Pregnancy Loss: related?

In recurrent unexplained miscarriage more cases with diminished ovarian reserve



Ovarian Reserve and Pregnancy Loss: related?

In recurrent miscarriage IVF-PGS does not yield more aneuploidy..

- 9 chromosomes FISH
- Recurrent Miscarriage in under and over 35 years af age group
- Controls regular PGS group with ≤ 1 miscarriage

Chromosome abnormalities in RM and comparison groups after PGD.

Group	RM group (y) <35	RM group (y) ≥35	Comparison group (≥35 y)
Analyzed	241	409	1,295
% Normal	43	33	32
% Aneuploid	28	34	38
% Other abnormal[a]	29	33	30

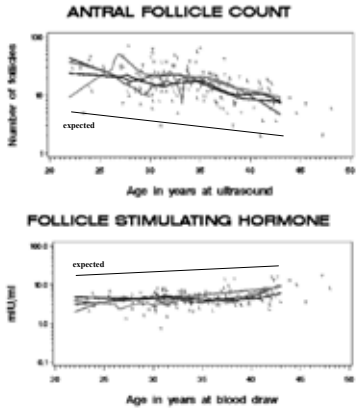
[a] Polyploidy, haploidy, complex abnormal, and extensive mosaics (if the embryo was reanalyzed).

Munné. PGD improves recurrent miscarriage outcome. Fertil Steril 2005.

Ovarian Reserve and Pregnancy Loss: related?

Follicle pool and aneuploidy:

Cases with chromosomally abnormal fetal losses RED+GREEN have the SAME follicle count and FSH level as live birth cases DARKBLUE



Kline, 2004

Ovarian Reserve and Pregnancy Loss: related?

No clear differences in ovarian reserve test results between Chromosomally *normal and abnormal* pregnancies

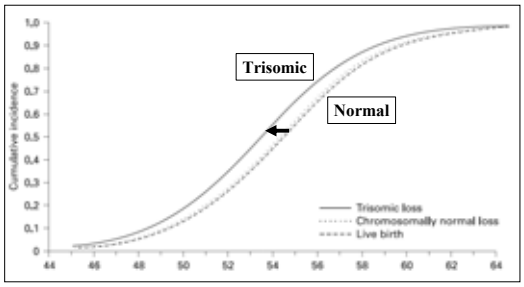
	Abnormal		Normal	
	Loss with trisomy	Loss with other chromosome abnormality	Chromosomally normal loss	Livebirth
Number of women	54	24	21	65
Follicle count (day 5–7)	14.5	17.0	20.1	13.4
FSH (mIU/ml; day 1–4)	4.4	4.2	3.8	4.6
Inhibin B (pg/ml; day 1–4)	71.5	70.8	90.9	64.7
Estradiol (pg/ml; day 1–4)	35.2	31.2	43.4	38.5

Warburton , 2005

Ovarian Reserve and Pregnancy Loss: related?

Age at menopause may be 0.9 years earlier for women with trisomic pregnancies than for women without trisomic pregnancies.

Warburton , 2005



Ovarian Reserve and Pregnancy Loss: related?

The proof is not there

It may be hard to demonstrate the effect of diminished ovarian reserve on top of the strong effect of female age….

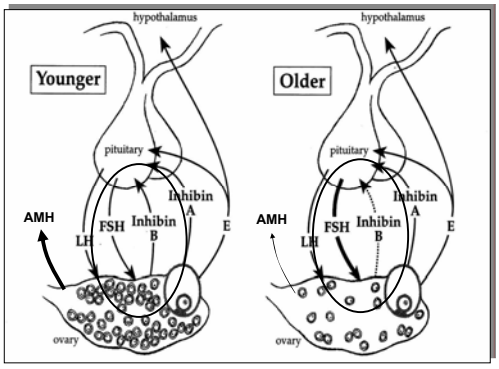
Questions

- **What is Ovarian Reserve (OR)?**
- **Ovarian Reserve and Pregnancy Loss in ART: related?**
- **How can we assess Ovarian Reserve?**
- **OR Tests for Outcome prediction in ART: useful?**
- **Is Prevention of Pregnancy Loss feasible?**
- **Conclusions**

Ovarian reserve tests

- **Basal Hormones**
  FSH, inhibin-B, Anti-Müllerian Hormone (AMH), oestradiol
- **Sonographic parameters**
  Antral follicle count (AFC), Ovarian volume, Ovarian vascular flow
- **Challenge tests**
  Clomiphene citrate, GnRH and FSH
- **Combinations** of tests
- **Repeating** tests in subsequent cycles

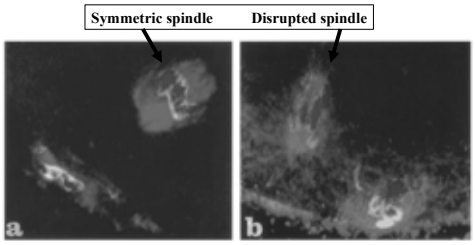
Ovarian Reserve assessment = quantity



Soules, 1998

How can we assess Ovarian Reserve?

Ovarian Reserve - Quality



Not through direct methods

How can we assess Ovarian Reserve?
By quantity assessment

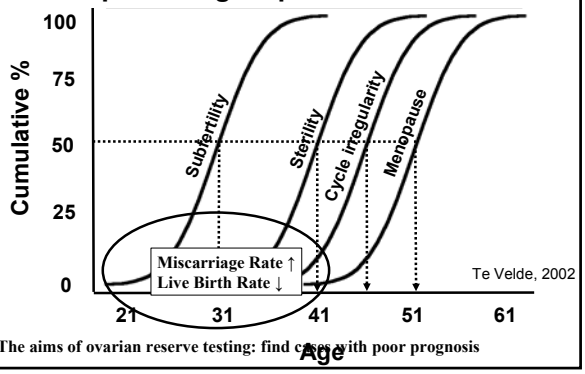
But quantity and quality are not necessarily directly related

Questions

- **Does ART lead to increased rate of Pregnancy Loss**
- **What is Ovarian Reserve (OR)?**
- **Ovarian Reserve and Pregnancy Loss in ART: related?**
- **How can we assess Ovarian Reserve?**
- **OR Tests for Outcome prediction in ART: useful?**
- **Is Prevention of Pregnancy Loss feasible?**
- **Conclusions**

OR Tests for outcome prediction in ART: useful?

Variation in age at menopause and preceding "reproductive events"



The aims of ovarian reserve testing: find cases with poor prognosis

OR Tests for outcome prediction in ART: useful?

= cases with high chance of EPL or Non Implantation. And then what do?..

Initiate treatment in time
- In subfertile couples with otherwise good prognosis

Adapt treatment in IVF/ICSI indicated couples
- hormonal stimulation
- type of stimulation protocol
- apply embryo selection

Refuse treatment in IVF/ICSI indicated couples
- very poor chance of pregnancy (< 5% per cycle)

OR Tests for outcome prediction in ART: useful?

Most studies evaluate outcomes poor response and pregnancy after ART, but do not specifically study EPL…..

OR Tests for outcome prediction in ART: useful?

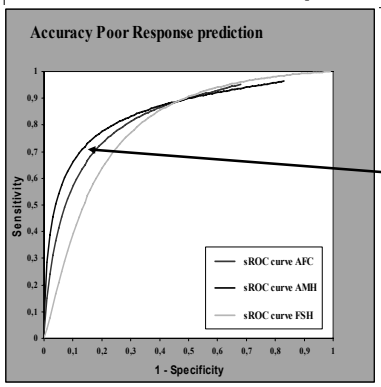
Systematic review Meta-analysis

- Accuracy of the test from aggregate analysis
- Clinical value from pre --- post test probability at several cut offs with good likelihood ratio
- Clinical value from consequences of abnormal test for treatment and false positive rate

Broekmans et al, Hum Reprod Update , 2006

OR Tests for outcome prediction in ART: useful?

Prediction of Poor Response



At the best cut off:
Sens 70%
FPRate 10%
LR+ 7

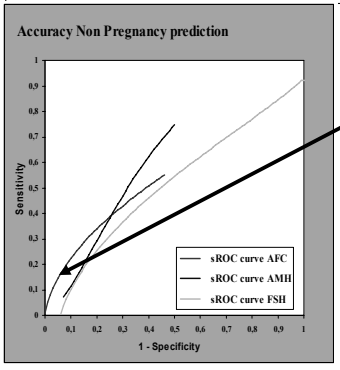
Quite good

Broekmans et al.

Human Reproduction Update 2006;12:685-718

OR Tests for outcome prediction in ART: useful?

Prediction of Pregnancy



At extreme cut off:
FPrate 5%
Sens 15%
LRpos 3
% pos tests 2%

Is Poor…..

OR Tests for outcome prediction in ART: useful?

Prediction Poor Response
Individualize dose FSH?

- Yes: an individual stimulation dose based on a model with AFC, Ovarian volume, Ovarian flow, female Age and Smoking resulted in higher pregnancy rates compared to a standard dose (Popovic-Todorovic et al. Hum Reprod 2003).
- No: predicted poor responders based on AFC did not have better pregnancy rates with higher compared to normal doses (Klinkert et al. Hum Reprod 2005).

ORT and pregnancy failure

Predictor	AUC-ROC	p value	cut-off	Sens	Spec	PPV	LR
Miscarriage							
Basal FSH	**0.56**	**0.3**					
Female age	**0.43**	**0.4**					
AFC	**0.65**	**0.04**	**7 fo**	**71**	**63**	**56**	**1.9**
Biochemical							
Basal FSH	**0.53**	**0.3**					
Female age	**0.41**	**0.7**					
AFC	**0.68**	**0.02**	**7 fo**	**79**	**60**	**42**	**2.0**

Elter, 2005

Poor test accuracy

ORT and pregnancy failure

Predictor	cut-off	Sens	Spec	PPV	NPV	LR+
Preg Loss < 7 wk						
AMH	**14 pMol/l**	**45**	**71**	**30**	**17**	**1.6**

Poor test accuracy

Lekamge, 2007

OR Tests for outcome prediction in ART: useful?

No, as…

- Prediction of poor response does not clearly alter treatment
- Prediction of non pregnancy is inaccurate and will hardly lead to refusal of treatment
- If pregnant, ORTs hardly add information

Questions

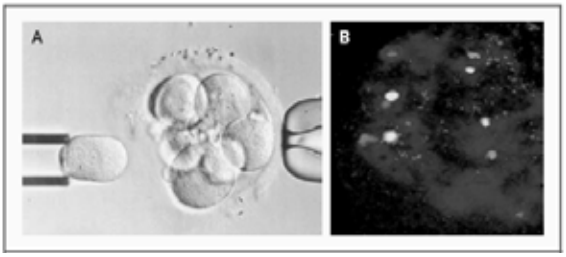
- **Does ART lead to increased rate of Pregnancy Loss**
- **What is Ovarian Reserve (OR)?**
- **Ovarian Reserve and Pregnancy Loss in ART: related?**
- **How can we assess Ovarian Reserve?**
- **OR Tests for Outcome prediction in ART: useful?**
- **Is Prevention of Pregnancy Loss feasible?**
- **Conclusions**

Is Prevention of Pregnancy Loss feasible?

- **Blastocyst Culture**
- **Pre Implantation Genetic Screening for Aneuploidy**
- **or…**

Is Prevention of Pregnancy Loss feasible?

Embryo Selection
PreImplantation Genetic Screening
PGS: Hunting for aneuploidy



Is Prevention of Pregnancy Loss feasible?

Current value of preimplantation genetic aneuploidy screening in IVF

P.Donoso[1,4], C.Staessen[1,2], B.C.J.M.Fauser[3] and P.Devroey[1]

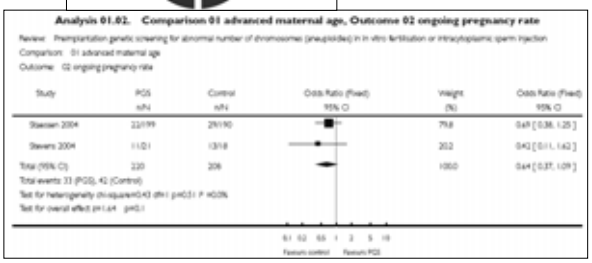
"Although, to date, multiple studies have addressed this issue, contradictory results have been encountered. As a result, the effectiveness of aneuploidy screening remains to be established. Moreover, childoutcome studies documenting the safety of this procedure are needed."

HRU, 2007

Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection (Review)

2007

No benefit of PGS in diminished ovarian reserve cases. Even harm by biopsy possible…



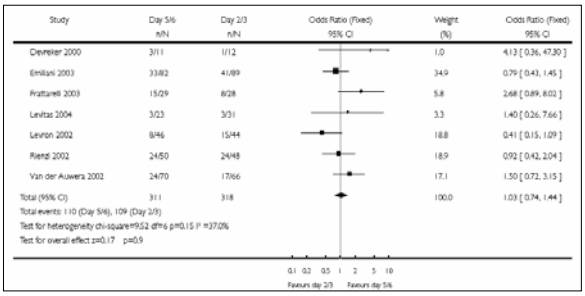
Role of Blastocyste culture

Day five replacement may offer improved delivery rates in young patients.
Papanikolaou, NEJM 2006

Variable	Single Blastocyst-Stage Embryo Transferred (N=175)	Single Cleavage-Stage Embryo Transferred (N=176)	Relative Risk (95% CI)*	P Value
	% (no.)			
Rate/patient randomly assigned to treatment				
Pregnancy†	41.7 (73)	33.5 (59)	1.23 (0.95–1.63)	0.11
Clinical pregnancy	33.1 (58)	23.3 (41)	1.42 (1.01–2.00)	0.04
Ongoing pregnancy	33.1 (58)	21.6 (38)	1.54 (1.08–2.18)	0.02
Pregnancy loss‡				
Ectopic pregnancy	1.4 (1)	1.7 (1)		
1st Trimester	19.2 (14)	33.9 (20)	0.57 (0.31–1.02)	0.07
2nd Trimester	2.7 (2)§	0		
Delivery	32.0 (56)	21.6 (38)	1.48 (1.04–2.11)	0.03
Multiple births	0	5 (2)	0.14 (0.01–2.77)	0.16

Blastocyste Transfer – Controversial

No difference in Live birth rate per couple in Cochrane review
Blake, Cochrane 2005



Application of PGS for aneuploidy results in lower rates of pregnancy loss (undefined)… but the rate of ongoing pregnancy per initiated cycle may remain unaltered…due to many cycles with no embryo available…

Pregnancy loss rates in the general IVF population and PGD for 8 chromosomes.

	Age 35–40 y	Age >40 y
IVF population[a]	19.0% (n = 7682)[c]	40.6% (n = 1024)[d]
PGD group[b]	14.1% (54/382)[c]	22.2% (40/180)[d]
Subgroup RPL	13.7% (7/51)	
Subgroup no RPL	14.5% (48/331)	

Munne, 2006

Is Prevention of Pregnancy Loss feasible?

Pregnancy loss, much like ongoing pregnancy, is almost exclusively determined by

female age (Hourvitz, 2006, Winter, 2002, Lambers, 2007

Prevention therefore may lie in early treatment OR early family building…

Conclusions

- ART itself does not elicit pregnancies with a higher rate of EPL after female age correction
- Ovarian Reserve is in fact an oocyte quality problem
- In cases with reduced Ovarian Reserve for age the rate of Pregnancy Loss is not consistently elevated

Conclusions

- Quantitative Ovarian Reserve can be adequately assessed
- But OR Tests for Outcome prediction in ART are not to be recommended as screening tool
- Prevention of Pregnancy Loss seems currently not feasible..

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Soules MR, Battaglia DE, and Klein NA (1998) Inhibin and reproductive aging in women. Am J Human Biol, 30, 193-204.

te Velde ER and Pearson PL (2002) The variability of female reproductive ageing. Hum Reprod Update, 8, 141-154.

Endometrial gene expression during ART implantation window

24th Annual Meeting of ESHRE
Barcelona – 2008
Pre-congress course Early Pregnancy

Course title: Pregnancy after ART on behalf of SIGEP

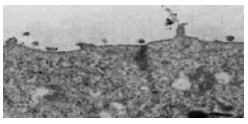
Dr. José A. Horcajadas, PhD
Molecular Biology Group Leader
Fundación IVI (FIVI)-Instituto Universitario IVI (IUIVI)
and University of Valencia (Spain)

LEARNING OBJECTIVES

- (1) To define endometrial receptivity.
- (2) To describe the different gene expression profiles between the window of implantation (WOI) in natural and controlled ovarian stimulation (COS) cycles.
- (3) To understand the application of the new technologies for the development ovarian stimulation treatments protocols.

Receptive endometrium features

- » **Morphological markers**
- » **Biochemical markers**
- » **Gene expression pattern**



GENE EXPRESSION PROFILING OF HUMAN ENDOMETRIAL RECEPTIVITY ON DAYS LH+2 VS LH+7 BY MICROARRAYS

EXPERIMENTAL DESIGN

FIVE WOMEN

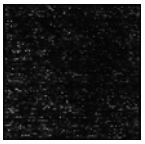
TWO ENDOMETRIAL BYOPSIES

LH+2

LH+7

RNA ISOLATION

AFFIMETRIX HG-U95A 12,686 genes



MICROARRAY

Caucasian
Fertile women with normal cycles
23-39 years
body mass index:19-25 kg/m2

Riesewijk et al. Molec Human Reprod 9, 253-64, 2003

Genes regulated during human endometrial receptivity

	Up at LH+7	Down at LH+7
Strong (>10)	22	5
Medium (5-10)	47	12
Weak (3-5)	84	41
	153	58

Results (>3.0 fc in 4 out of 5)

DESIGNS TO ANALYZE HUMAN ENDOMETRIAL RECEPTIVITY

Kao et al. 2002
11 SAMPLES
RNA NOT POOLED
Proliferative phase VS Receptive phase
AFFIMETRIX HG-U95A
156 Up / 377 Down — Fold >2.0
(Endocrinology)

Carson et al. 2002
6 SAMPLES
RNA POOLED
LH+(2-4) VS LH+(7-9)
AFFIMETRIX HG-U95A
323 Up / 370 Down — Fold >2.0
(Mol Human Reprod)

Borthwick et al. 2003
10 SAMPLES
RNA POOLED
Proliferative 9-11 VS LH+(6-8)
AFFIMETRIX HG-U95A-E
90 Up / 46 Down — Fold >2.0
(Mol Human Reprod)

Riesewijk et al. 2003
10 SAMPLES
RNA NOT POOLED
LH+2 VS LH+7
AFFIMETRIX HG-U95A
153 Up / 58 Down — Fold >3.0
(Mol Human Reprod)

Mirkin et al. 2005
8 SAMPLES
RNA NOT POOLED
Early luteal 16 VS Mid luteal 21
AFFIMETRIX HG-U95A
49 Up / 58 Down — Fold >2.0
(Human Reprod)

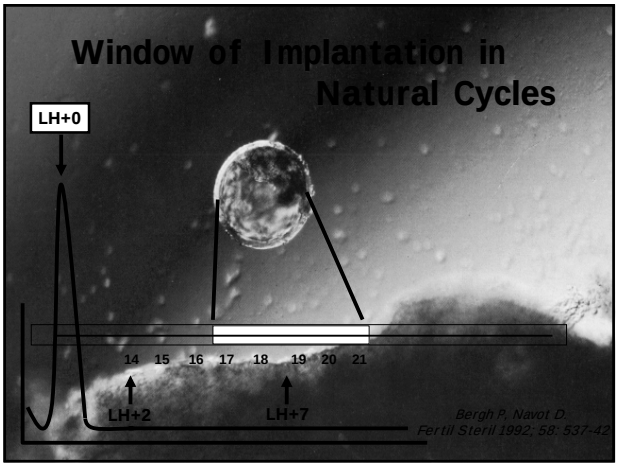
CONSENSUS GENES: FOLD CHANGE >3,0

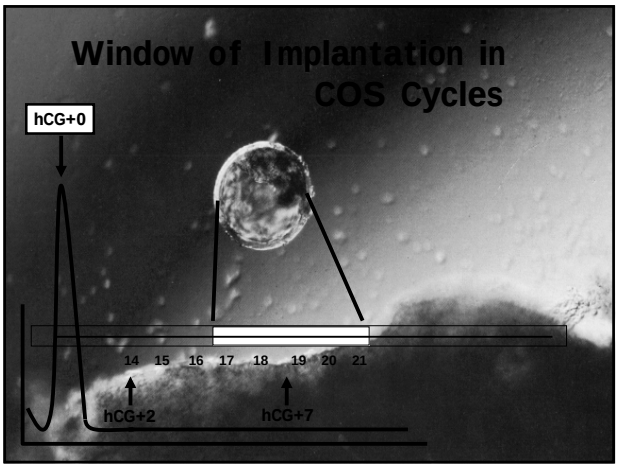
Accesion number (Function)	Gene name	Riesewijk	Kao	Carson	Borthwick
UP-REGULATED GENES PRESENT IN THE FOUR WORKS					
AF052124 (Structural protein)	Osteopontin	✓	✓	✓	✓
J02611 (Trasporter)	Apolipoprotein D	✓	✓	✓	✓
AB020315 (Signalling)	Dickkopf/DKK1 (hdkk-1)	✓	✓	✓	✓
UP-REGULATED GENES PRESENT IN THREE OUT OF FOUR WORKS					
J04129 (Secretory protein)	Placental protein-14/Glycodelin	✓	✓		✓
M31516 (Immunomodulator)	Decay accelerating factor for complement (CD55, Cromer blood group system)	✓	✓		✓
M84526 (Complement protein)	Adipsin/complement factor D	✓	✓		✓
M55543 (GTP-Binding protein)	Guanylate binding protein 2, interferon-inducible	✓		✓	✓
AB000712 (Receptor)	Claudin 4/CEP-R	✓	✓	✓	
AA420624 (Signalling)	Monoamine oxidase A (MAOA)	✓	✓		✓
M60974 (Regulatory protein)	Growth arrest and DNA-damage-inducible protein (gadd45)	✓	✓		✓
AB002365 (Cell death factor)	Nip2	✓		✓	✓
TOTAL GENES ANALYZED		153	60	120	85
DOWN-REGULATED GENES PRESENT IN THE FOUR WORKS					
U79299 (Secretory protein)	Olfactomedin-related ER localized protein	✓	✓	✓	✓
TOTAL GENES ANALYZED		58	87	153	40

Horcajadas et al. (2004) J. Reprod. Immunol. 63:41-49

THE IMPACT OF COS IN ENDOMETRIAL RECEPTIVITY

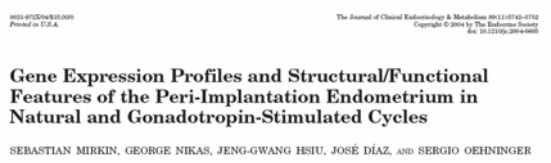
- In high responders to gonadotrophins, supraphysiological levels of E2 on the day of hCG administration, are deleterious to embryonic implantation (Simón et al., 1995, 1998, 2003; Pellicer et al., 1996)
- Low doses of E2 maintain the uterus in a receptive state, high doses cause it to become refractory in mice (Ma et al., 2003, PNAS).
- Uterine receptivity is diminished during COS used for IVF compared to natural cycles (Paulson et al., 2000). The endometrium is histologically advanced.





STUDIES OF THE GENE EXPRESSION PROFILE OF THE ENDOMETRIUM UNDER COS

- **Gene expression profile of the endometrium during the WOI in women under treatment with agonists and different doses of antagonist and in comparison to natural cycle**



Gene Expression Profiles and Structural/Functional Features of the Peri-Implantation Endometrium in Natural and Gonadotropin-Stimulated Cycles

SEBASTIAN MIRKIN, GEORGE NIKAS, JENG-GWANG HSIU, JOSÉ DÍAZ, AND SERGIO OEHNINGER

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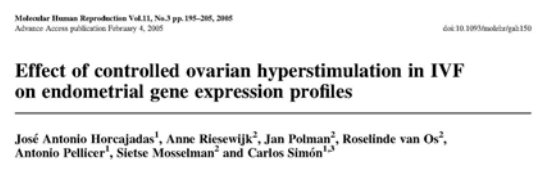

Molecular Human Reproduction Vol.11, No.3 pp.195–205, 2005
Advance Access publication February 4, 2005

Effect of controlled ovarian hyperstimulation in IVF on endometrial gene expression profiles

José Antonio Horcajadas[1], Anne Riesewijk[2], Jan Polman[2], Roselinde van Os[2], Antonio Pellicer[1], Sietse Mosselman[2] and Carlos Simón[1,3]

STUDIES OF THE GENE EXPRESSION PROFILE OF THE ENDOMETRIUM UNDER COS

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Human Reproduction Vol.20, No.12 pp. 3318–3327, 2005 doi:10.1093/humrep/dei243
Advance Access publication August 5, 2005.

Similar endometrial development in oocyte donors treated with either high- or standard-dose GnRH antagonist compared to treatment with a GnRH agonist or in natural cycles

C.Simon[1,2,6], J.Oberyé[3], J.Bellver[2], C.Vidal[2], E.Bosch[2], J.A.Horcajadas[1], C.Murphy[5], S.Adams[5], A.Riesewijk[4], B.Mannaerts[3] and A.Pellicer[1,2]

COMPARISON OF THE DIFFERENT STIMULATION PROTOCOLS

			Window of implantation genes	
	Regimen/direction of regulation†	N° of genes	Typically upregulated (n = 894)	Typically downregulated (n = 504)
	Leuprolide (agonist)			
	Up	281	9	115
	Down	277	227	0
	Ganirelix 0.25 mg/day (antagonist)			
	Up	22	0	4
	Down	69	46	0
	Ganirelix 2 mg/day (antagonist)			
	Up	88	0	7
	Down	24	15	1
	Buserelin long protocol (agonist)			
	Up	22	3	4
	Down	100	76	2

GENE EXPRESSION PROFILING OF WINDOW OF IMPLANTATION IN NATURAL AND STIMULATED CYCLES

EXPERIMENTAL DESIGN

50 WOMEN

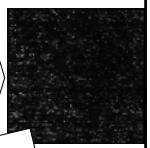
Caucasian
Fertile women with normal cycles
23-39 years
body mass index:19-25 kg/m2

FIVE ENDOMETRIAL BYOPSIES AT EACH TIME POINT

LH Day 1 2 3 4 5 6 7 8 9

hCG Day 1 2 3 4 5 6 7 8 9

AFFIMETRIX HG-133A >22,000 genes



GeneSpring 7.0
GEPAS

MICROARRAY

HISTOLOGICAL CONTROL: BLIND DATING BY NOYES CRITERIA

PCA OF THE ENDOMETRIAL BIOPSIES

FROM LH+1 TO LH+9 AND hCG+1 TO hCG+9

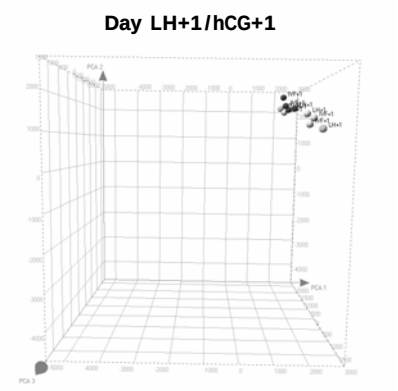
- **Principal Component Analysis (PCA) integrates the gene expression data of thousand of genes randomly selected to establish relationships between samples.**

- **This analysis allows to distribute the endometrial samples in a three dimensional space according to their gene expression profile.**

- **Those samples with similar gene expression patterns cluster together in this type of analysis.**

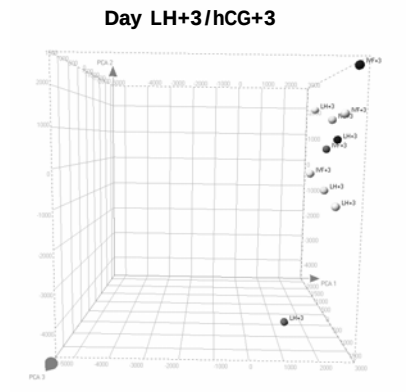
PRINCIPAL COMPONENT ANALYSIS (PCA)

Day LH+1/hCG+1



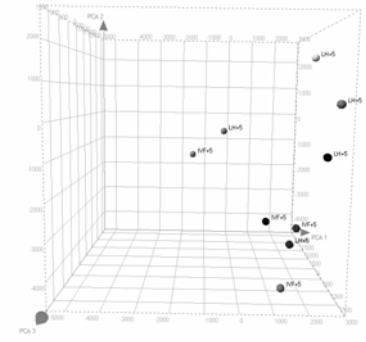
PRINCIPAL COMPONENT ANALYSIS (PCA)

Day LH+3/hCG+3



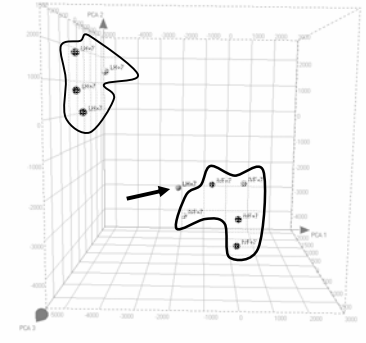
PRINCIPAL COMPONENT ANALYSIS (PCA)

Day LH+5/hCG+5



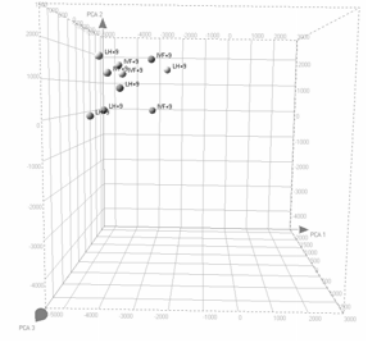
PRINCIPAL COMPONENT ANALYSIS (PCA)

Day LH+7/hCG+7



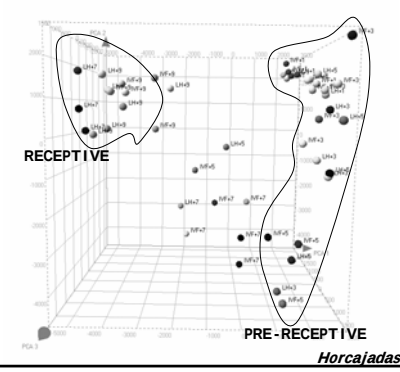
PRINCIPAL COMPONENT ANALYSIS (PCA)

Day LH+9/hCG+9



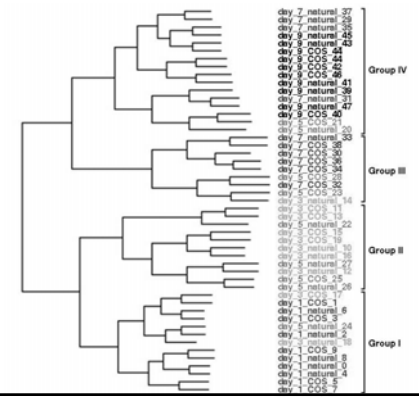
PRINCIPAL COMPONENT ANALYSIS (PCA)

Natural/LH vs IVF across the WOI

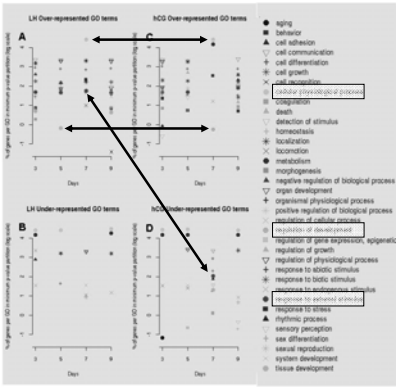


Horcajadas et al., 2008

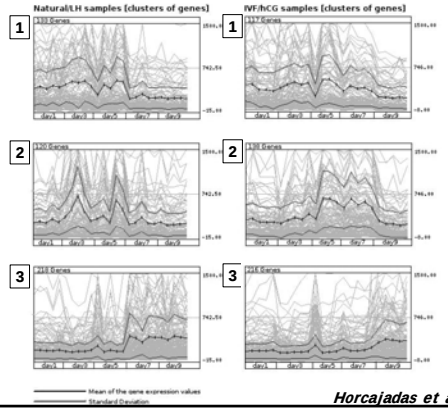
SAMPLE CLUSTERING BY SOTA ALGORITHM



BIOLOGICAL PROCESSES FROM DAY LH+1 TO LH+9 COMPARED TO COS CYCLES

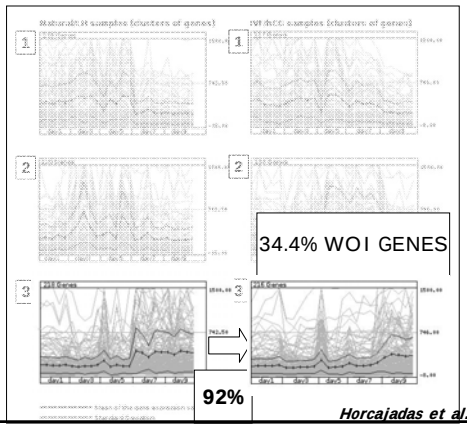


GENE CLUSTERING IN NATURAL AND COS CYCLES



Horcajadas et al., 2008

GENE CLUSTERING IN NATURAL AND COS CYCLES

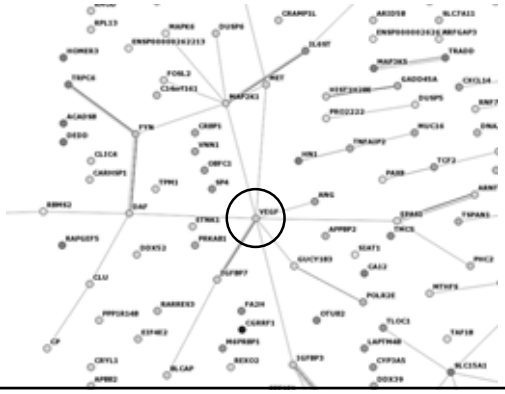


Horcajadas et al., 2008

BIOLOGICAL PROCESSES OF THE 203 DELAYED GENES

BIOLOGICAL TERM	Count	%	PValue
taxis	5	2,67%	0,0437137
cell motility	7	3,74%	0,04103273
→ blood vessel development	4	2,14%	0,03996548
negative regulation of physiological process	12	6,42%	0,09769689
transport	41	21,93%	0,02625948
→ positive regulation of apoptosis	6	3,21%	0,02985379
locomotory behavior	5	2,67%	0,04919509
phosphate metabolism	16	8,56%	0,07933242
negative regulation of biological process	15	8,02%	0,03312411
locomotion	7	3,74%	0,04103273
cell death	11	5,88%	0,09003195
localization of cell	7	3,74%	0,04103273
localization	48	25,67%	0,00371126
fructose 6-phosphate metabolism	2	1,07%	0,04132375
organic acid metabolism	10	5,35%	0,08394103
carboxylic acid metabolism	10	5,35%	0,08237153
chemotaxis	5	2,67%	0,0437137
behavior	6	3,21%	0,05876496
positive regulation of programmed cell death	6	3,21%	0,03071029
negative regulation of cellular process	13	6,95%	0,07576561
phosphorus metabolism	16	8,56%	0,07933242
negative regulation of cellular physiological	12	6,42%	0,08059537
cellular physiological process	126	67,38%	0,05501213
development	27	14,44%	0,0787654
→ angiogenesis	4	2,14%	0,03590782
vasculature development	4	2,14%	0,03996548
response to stress	19	10,16%	0,04424593
negative regulation of cell proliferation	5	2,67%	0,080454
death	11	5,88%	0,09312635
response to chemical stimulus	9	4,81%	0,05742527
→ cell proliferation	13	6,95%	0,0123572
establishment of localization	47	25,13%	0,00571022

BIOLOGICAL CONECTIONS AMONG THE 203 DELAYED GENES



CONCLUSIONS (I)

-There is a high number of genes, with a define pattern, involved in endometrial receptivity (WOI genes)

-There is a high number of WOI genes that are aberrantly expressed in stimulated cycles at the time of implantation (LH+7 in natural cycles and hCG+7 in COS cycles)

- Microarray technology is a good tool for analyzing gene expression profile of the endometrium at the time of implantation to compare optimal versus non optimal conditions (infertility or subfertility)

CONCLUSIONS (II)

-These data are useful for both, to improve the stimulated cycles in IVF and also to increase our knowledge in the physiology of the implantation process