eshre

SCIENCE MOVING
PEOPLE
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New developments in the diagnosis and management of early pregnancy complications

Special Interest Group Early Pregnancy

3

27 June 2010
Rome, Italy

PRE-CONGRESS COURSE 3 – Table of contents

New developments in the diagnosis and management of early pregnancy complications

Organised by the Special Interest Group Early Pregnancy

ESHRE – European Society of Human Reproduction and Embryology

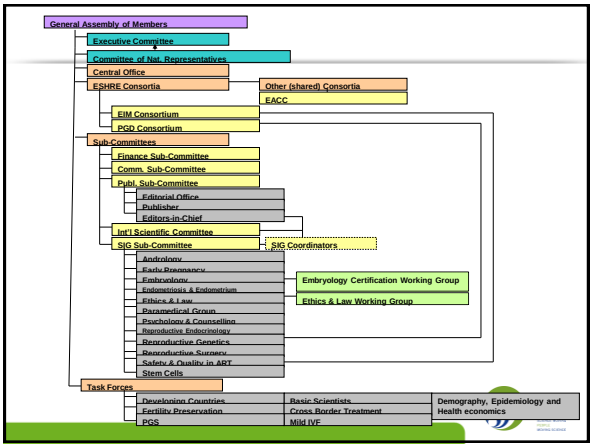
What is ESHRE?

ESHRE was founded in 1985 and its M**ission Statement** is to:

- promote interest in, and understanding of, reproductive science and medicine.
- facilitate research and dissemination of research findings in human reproduction and embryology to the general public, scientists, clinicians and patient associations.
- inform politicians and policy makers in Europe.
- promote improvements in clinical practice through educational activities
- develop and maintain data registries
- implement methods to improve safety and quality assurance

Executive Committee 2009/2011

Chairman	Luca Gianaroli	Italy
Chairman Elect	Anna Veiga	Spain
Past Chairman	Joep Geraedts	Netherlands
	Jean François Guérin	France
	Timur Gürgan	Turkey
	Ursula Eichenlaub-Ritter	Germany
	Antonis Makrigiannakis	Greece
	Miodrag Stojkovic	Serbia
	Anne-Maria Suikkari	Finland
	Carlos Plancha	Portugal
	Françoise Shenfield	United Kingdom
	Etienne Van den Abbeel	Belgium
	Heidi Van Ranst	Belgium
	Veljko Vlaisavljevic	Slovenia
	Søren Ziebe	Denmark



ESHRE Activities – Annual Meeting

- One of the most important events in reproductive science and medicine
- Steady increase in terms of attendance and of scientific recognition

Track record:

ESHRE 2008 – Barcelona: 7559 participants
ESHRE 2009 – Amsterdam: 8132 participants

Future meetings:

ESHRE 2010 – Rome, 27-30 June 2010
ESHRE 2011 – Stockholm, 3-6 July 2011

ESHRE Activities – Scientific Journals

Human Reproduction with impact factor 3.773

Human Reproduction Update with impact factor 7.590

Molecular Human Reproduction with impact factor 2.537

ESHRE Activities – Campus and Data Collection

- Educational Activities / Workshops
  - Meetings on dedicated topics are organised across Europe
  - Organised by the Special Interest Groups
  - Visit: www.eshre.eu under CALENDAR
- Data collection and monitoring
  - EIM data collection
  - PGD data collection
  - Cross border reproductive care survey

ESHRE Activities - Other

- Embryology Certification
- Guidelines & position papers
- News magazine “Focus on Reproduction”
- Web services:
  - RSS feeds for news in reproductive medicine / science
  - Find a member
  - ESHRE Community

ESHRE Membership (1/3)

- ESHRE represents over 5,300 members (infertility specialists, embryologists, geneticists, stem cell scientists, developmental biologists, technicians and nurses)
- Overall, the membership is distributed over 114 different countries, with 50% of members from Europe (EU). 11% come from the US, India and Australia.

ESHRE Membership (2/3)

	1 yr	3 yrs
Ordinary Member	€ 60	€ 180
Paramedical Member*	€ 30	€ 90
Student Member**	€ 30	N.A.

*Paramedical membership applies to support personnel working in a routine environment such as nurses and lab technicians.
**Student membership applies to undergraduate, graduate and medical students, residents and post-doctoral research trainees.

ESHRE Membership – Benefits (3/3)

1) Reduced registration fees for all ESHRE activities:

Annual Meeting	Ordinary	€ 480	(€ 720)
	Students/Paramedicals	€ 240	(€ 360)
Workshops	All members	€150	(€ 200)

2) Reduced subscription fees to all ESHRE journals – e.g. for Human Reproduction €191 (€ 573!)

3) ESHRE monthly e-newsletter

4) News Magazine "Focus on Reproduction" (3 issues p. a.)

5) Active participation in the Society's policy-making

Special Interest Groups (SIGs)

The SIGs reflect the scientific interests of the Society's membership and bring together members of the Society in sub-fields of common interest

- Andrology
- Early Pregnancy
- Embryology
- Endometriosis / Endometrium
- Ethics & Law
- Safety & Quality in ART
- Psychology & Counselling
- Reproductive Genetics
- Reproductive Surgery
- Stem Cells
- Reproductive Endocrinology

Task Forces

A task force is a unit established to work on a single defined task / activity

- Fertility Preservation in Severe Diseases
- Developing Countries and Infertility
- Cross Border Reproductive Care
- Reproduction and Society
- Basic Reproductive Science
- Fertility and Viral Diseases
- Management of Infertility Units
- PGS
- EU Tissues and Cells Directive

Annual Meeting

Rome, Italy 27 June to 30 June 2010

Pre-congress courses (27 June):

- PCC 1: Cross-border reproductive care: information and reflection
- PCC 2: From gametes to embryo: genetics and developmental biology
- PCC 3: New developments in the diagnosis and management of early pregnancy complications
- PCC 4: Basic course on environment and human male reproduction
- PCC 5: The lost art of ovulation induction
- PCC 6: Endometriosis: How new technologies may help
- PCC 7: NOTES and single access surgery
- PCC 8: Stem cells in reproductive medicine
- PCC 9: Current developments and their impact on counselling
- PCC 10: Patient-centred fertility care
- PCC 11: Fertility preservation in cancer disease
- PCC 12: ESHRE journals course for authors

Annual Meeting – Scientific Programme (1/2)

Rome, Italy 27 June to 30 June 2010

- Molecular timing in reproduction
- Rise and decline of the male
- Pluripotency
- Preventing maternal death
- Use and abuse of sperm in ART
- Live surgery
- Emerging technologies in the ART laboratory
- Debate: *Multiple natural cycle IVF versus single stimulated cycle and freezing*

Annual Meeting – Scientific Programme (2/2)

- Fertility preservation
- Congenital malformations
- ESHRE guidelines
- Data from the PGD Consortium
- European IVF Monitoring 2007
- Debate: *Selection of male/female gametes*
- Third party reproduction in the United States
- Debate: *Alternative Medicine, patients feeling in control?*
- Historical lecture: "Catholicism and human reproduction"

Certificate of attendance

1/ Please fill out the evaluation form during the campus

2/ After the campus you can retrieve your certificate of attendance at www.eshre.eu

3/ You need to enter the results of the evaluation form online

4/ Once the results are entered, you can print the certificate of attendance from the ESHRE website

5/ After the campus you will receive an email from ESHRE with the instructions

6/ You will have TWO WEEKS to print your certificate of attendance

Contact

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E-mail: info@eshre.eu

www.eshre.eu

PRE-CONGRESS COURSE 3 - Programme

New developments in the diagnosis and management of early pregnancy complications

Organised by the Special Interest Group Early Pregnancy

Course coordinators: Roy Farquharson (Coordinator, United Kingdom), Niek Exalto (The Netherlands), Ole Bjarne Christiansen (Denmark), Eric Jauniaux (France), Joson Horcajadas (Spain) , Mariette Goddijn (The Netherlands) and Marcin Rajewski (Poland)

Course description: New developments in the diagnosis and management of early pregnancy complications.

Target audience: Clinicians, scientists, nurse specialists and allied professionals

Scientific programme:

Session One – Treatment Intervention

09:00 – 10:30 Debate /Thrombophilia and Recurring Miscarriage: The role of Heparin
Pro: **Lesley Regan (United Kingdom)**
Con: **Carl Laskin (Canada)**
10:30 – 11:00 Coffee break

Session Two – Imaging Developments

11:00 – 11:45 Dating and growth in the first trimester – **Anne Pexsters (Belgium)**
11:45 – 12:30 3-D Virtual Reality imaging of early pregnancy - **Melek Rousian (The Netherlands)**
12:30 – 13:30 Lunch

Session Three – Diagnosis & Investigation

13:30 – 15:00 Debate / Should chromosome testing be selective?
Con: **Mary Stephenson (USA)**
Pro: **Mariette Goddijn (The Netherlands)**
15:00 – 15:30 Coffee break

Session Four – Pregnancy Outcome

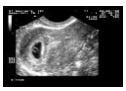
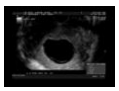
15:30 – 16:15 Gender specific immunological mechanism for recurrent pregnancy loss - **Henriette Svarre Nielsen (Denmark)**

Pre-congress course of Dr. Anne Pexsters

Disclosure of all commercial relationships or other activities that might be perceived as a potential conflict of interest

Pre-congress course early pregnancy
ESHRE Rome 2010

Dating and growth in the first trimester

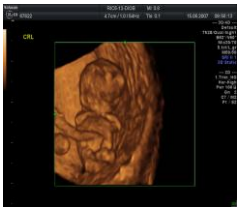
Dr. Anne Pexsters
Department of Obstetrics and Gynaecology
University Hospitals K.U.Leuven

Learning objectives

- Knowledge of measurement pitfalls of routinely performed measurements in the first trimester of pregnancy: crown-rump length (CRL), gestational sac mean sac diameter (MSD) and yolk sac diameter (YSD)
- Terminology of first trimester events
- Importance of reference curves for CRL (dating) and MSD (prediction of miscarriage)
- Cross-sectional versus longitudinal analyzes of first trimester measurements
- Introduction of functional linear discriminant analysis (FLDA) as a new statistical and clinical tool to study first trimester growth of CRL and MSD
- Importance of a study on intra- and inter-observer variability of routinely performed first trimester measurements

Structures visible at early stages in the first trimester with high-resolution ultrasound

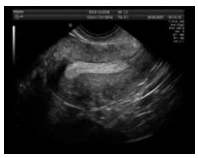
- Embryo (CRL measurement and FHR)
- Yolk sac (YS)
- Gestational sac (GS: MSD and GSV)



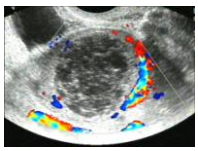
Reference: Bottomley C and Bourne T. Dating and growth in the first trimester. Best Practice and research Clin Obstet Gynaecol 2009

Landmarks < 28 days

- Empty uterus
- Thickened hyperechogenic endometrium

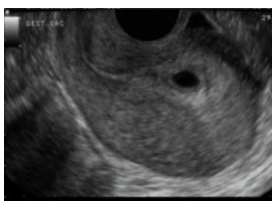


- Corpus luteum



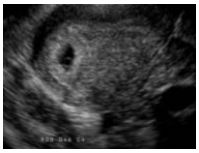
Landmarks 28-35 days

- Visible gestational sac

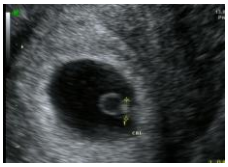


Landmarks > 35 days

- Visible yolk sac

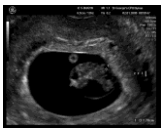


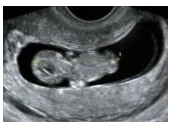
- Visible embryo



Landmarks > 49 days

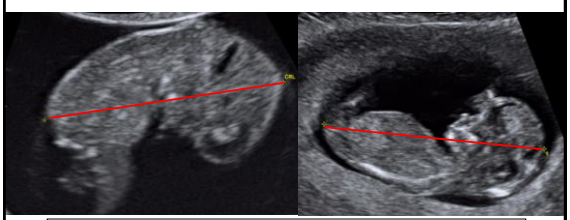
- Visible amniotic sac
- Cephalocaudal distinction from 52 days (single ventricle)
- Limb buds from 56 days
- Embryonic period complete from 70 days





Crown-rump length (CRL) measurement

8 weeks | **11 weeks**



At 8 weeks, the CRL measurement is the neck-rump length, whereas it is the actual crown-rump length at 11 weeks

Optimal time to date a pregnancy

When the measurement is most accurate and repeatable

- When the growth of the fetus is greatest
- With little biological variability
- But before the fetal movements and flexion or extension introduce further potential error

Suggested to be between 8 - 12/40

UK NICE guideline:

All pregnancies dated by USS at 11-14/40

Robinson H. British Medical Journal 1973 4, 28-31

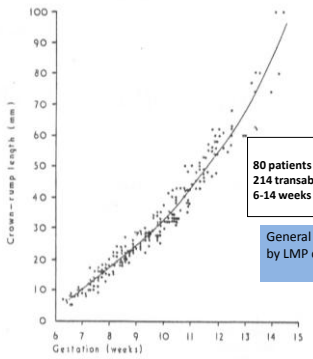


FIG. 5—Mean values and scatter of results of 214 measurements of fetal crown-rump lengths from six to 14 weeks of menstrual age.

80 patients
214 transabdominal US measurements
6-14 weeks

General overestimation of gestational age by LMP compared with USS

MacGregor et al Obstet Gynecol 1987

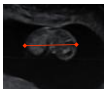
A new crown-rump length size curve based on over 3500 pregnancies

Anne Pexsters[1], Anneleen Daemen[2], Cecilia Bottomley[3], Dominique Van Schoubroeck[1], Luc De Catte[1], Bart De Moor[2], Thomas D'Hooghe[1], Christoph Lees[4], Dirk Timmerman[1] and Tom Bourne[1,5]

1. Department of Obstetrics and Gynaecology, University Hospitals, KU Leuven, Belgium
2. Department of Electrical Engineering (ESAT), Katholieke Universiteit Leuven, Belgium
3. Early Pregnancy and Gynaecological Ultrasound Unit, Department of Obstetrics and Gynaecology, St George's, University of London, UK
4. Division of Fetal-maternal Medicine, Addenbrookes Hospital, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge CB2 2QQ, UK
5. Imperial College London, Hammersmith Campus, Du Cane Road, London, UK

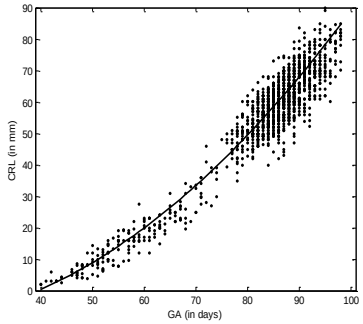
Retrospective database analysis between 2002 and 2008

- Total number of pregnancies: 6666
- Excluded: 2956 (uncertain dates, redated, infertility treatment, miscarriage, stillbirth, genetic or congenital abnormalities)
- Included: 3710 normal singleton pregnancies dated according to known and recorded last menstrual period (LMP) with confirmed viability at the time of the nuchal scan
- Predominantly transvaginal ultrasound below 10 weeks
- The gestational age (GA) ranged between 35 and 98 days
- Linear mixed-effects model in order to account for possible co-dependency of multiple CRL measurements in the same patient

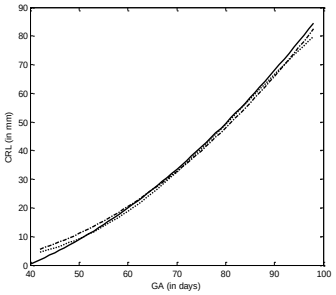


Reference: Bottomley C and Bourne T. Dating and growth in the first trimester. Best Practice and research Clin Obstet Gynaecol 2009

Our CRL curve validated on 1113 pregnancies (black *)



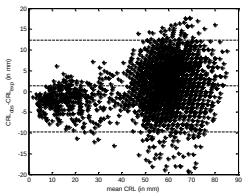
Comparison of the CRL curve (solid line) with the Robinson curve (dashdotted) and the Hadlock curve (dotted)



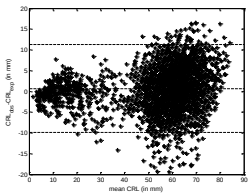
GA in weeks and days	CRL Robinson (in mm)	CRL (in mm)	Difference (in mm)	Difference (in days)
5+5	4.6	0.4	-4.2	**5.3**
6+5	8.8	6.1	-2.7	**2.9**
7+5	14.5	13.1	-1.4	1.3
8+5	21.6	21.2	-0.4	0.3
9+5	30.1	30.6	0.5	- 0.4
10+5	40.0	41.2	1.2	- 0.8
11+5	51.3	53.0	1.7	- 1.0
12+5	64.0	66.1	2.1	- 1.1
13+5	78.1	80.3	2.2	- 1.0

Comparison of the curves expressed by Bland-Altman plots

Robinson



Hadlock



Comparing the new CRL curve

with Robinson's:

- at 6 weeks: a difference in CRL of 3.7mm = 4 days underestimation by Robinson
- from 11 to 14 weeks: a difference in CRL from 0.9 to 1mm = 1 day overestimation by Robinson.

with Hadlock's:

- at 6 weeks: a difference in CRL of 2.7mm = 3 days underestimation by Hadlock.
- at 14 weeks: a difference in CRL of 4.8mm = 2 days overestimation by Hadlock.

At 9 weeks the curves are similar

Conclusions

- The greatest disparity between our curve and both Robinson's and Hadlock's is seen under 8 weeks gestation.
- The Bland Altman plots show at CRLs below 20 mm the majority of observed measurements are considerably lower than would be expected using either Robinson or Hadlock CRL curves.
- It is likely, given the sample size and the use of predominantly modern transvaginal ultrasound equipment, that our curve is more accurate at these relatively early gestations.
- Whilst a difference of several days may not seem clinically important in normal pregnancies, it is relevant for timing of first trimester screening and clinical decision making at the extremes of viability at around 24 weeks gestation, and when determining the appropriate time for post-term induction of labour.

Crown-rump length in genetically abnormal pregnancies compared to a reference CRL size curve of normal pregnancies

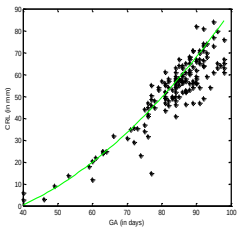
Anne Pexsters[1], Anneleen Daemen[2], Jean-Pierre Frijns[3], Cecilia Bottomley[4], Dominique Van Schoubroeck[1], Luc De Catte[1], Bart De Moor[2], Thomas D'Hooghe[1], Christoph Lees[5], Dirk Timmerman[1] and Tom Bourne[1,6]

1. Department of Obstetrics and Gynaecology, University Hospitals, KU Leuven, Belgium
2. Department of Electrical Engineering (ESAT), Katholieke Universiteit Leuven, Belgium
3. Department of Human Genetics, Katholieke Universiteit Leuven, Belgium
4. Early Pregnancy and Gynaecological Ultrasound Unit, Department of Obstetrics and Gynaecology, St George's, University of London, UK
5. Division of Fetal-maternal Medicine, Addenbrookes Hospital, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge CB2 2QQ, UK
6. Imperial College London, Hammersmith Campus, Du Cane Road, London, UK

Genetically abnormal pregnancies

Group	Number of scans
All	154
Trisomy 21	29
Trisomy 18	39
Trisomy 13	11
Triploidy	7
Others	68

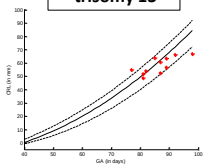
CRL curve with data points from the chromosomally abnormal pregnancies



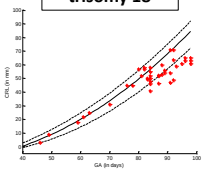
CRL: crown rump length
GA: gestational age

Comparison between trisomy 13 and 18 pregnancies and the CRL curve

trisomy 13



trisomy 18

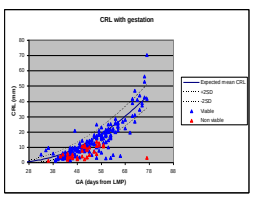


Conclusions

- There is a significantly smaller CRL in the overall population of genetically abnormal pregnancies when compared to a reference CRL size curve from normal pregnancies for our own population.
- A major factor in this is the difference seen in trisomy 18 pregnancies.
- Strengths of our study are that it reports earlier CRL measurements in the first trimester for genetically abnormal pregnancies than those currently available, and that we use an internally validated reference CRL size curve.
- When CRL is smaller than expected according to reported known LMP, a repeat scan should be organized.
- This underlines the importance of longitudinal data analysis, that have shown that growth rate is a better discriminator than a single CRL in these cases

Prospective investigation first trimester growth: prediction of miscarriage

Cross-sectional CRL data



Bottomley, C. et al. Hum. Reprod. 2009 24:278-283

Limitations

- Overlap between groups
- Only applies to women with known and certain menstrual dates
- Poor sensitivity as does not individualize *growth* for each individual
- Similar to late pregnancy: *growth* assessment needs to be made with interval USS assessment

Alternatives to cross-sectional approach

1) IVF pregnancies
2) Twin pregnancies

- Spontaneous reduction to singleton occurs in approximately 30% of twin pregnancies
- The normal twin can be used as control against which to assess growth of the slow growing twin

	Median discrepancy	Interquartile range	
Single embryonic demise	37.4%	10.7-64.3%	P=0.0012
Ongoing viable twins	5.9%	2.3-12.4%	

n = 57 (DCDA twins)
Bora et al 2009

3) Longitudinal approach

Longitudinal data analysis

- Consecutive women with a singleton pregnancy attending EPU who had at least two separate ultrasound scans confirming fetal heart pulsation.
- Outcomes were defined based on the outcome at the time of the 11-14 week (nuchal) scan:

Class 1	Viable ongoing pregnancy
Class 2	Miscarriage

Functional linear discriminant analysis (FLDA)

LDA aims to predict membership in two or more mutually exclusive groups from a set of predictors.
e.g. Ask whether we can predict whether a person will vote for Obama or McCain, from a knowledge of their age, their class, attitudes, values etc.
Maximum separability is aimed for between the two groups

FLDA

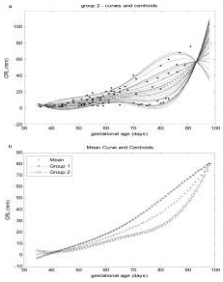
Extension of classical linear discriminant analysis where predictor variables are curves or functions (e.g. change in measurement over time).

Originally described to analyse changes in bone mineral density in the prediction of osteoporosis

James G, Hastie T (2001)
Journal of the Royal Statistical Society: Series B (Statistical Methodology) 63:533-550

Functional linear discriminant analysis: a new longitudinal approach to the assessment of embryonic growth

C. Bottomley, A. Daemen, F. Mukri, , A.T. Papageorghiou, E. Kirk, A. Pexsters, B. De Moor, Dirk Timmerman and Tom Bourne



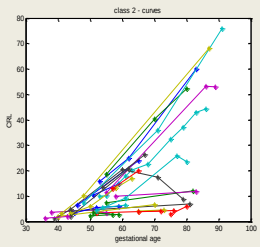
- Illustrative examples of the FLDA technique
- Group 1: 493 viable pregnancies
- Group 2: 28 miscarriages
- FLDA discriminates between normal and abnormal growth to predict miscarriage with high specificity. FLDA predicts miscarriage better than a single observation of a small CRL

Human Reproduction

Prediction of miscarriage

	TP	FN	TN	FP	Acc	Sens	Spec	PPV	NPV
FLDA	17	11	459	34	91.4	60.7	93.1	33.3	97.7
z-score (1st scan)	15	13	355	137	71.2	53.6	72.2	9.9	96.5

Rate of CRL growth in class2 (miscarriages)



- Small observed/expected CRL ratio in both aneuploid and euploid abortuses

Bessho (1995) Hum Reprod 10(10):2696-9

Late pregnancy outcome

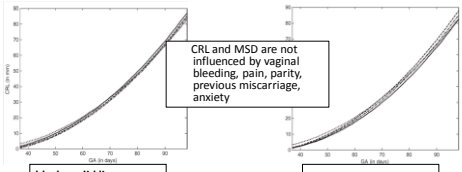
	Adverse outcome*
Predicted miscarriage (false positive)	27.6%
Predicted viable (true negative)	18.9%

*Adverse outcomes:

- Birthweight < 10th centile
- Preeclampsia
- Spontaneous preterm delivery before 37 weeks

Factors that can influence growth: rate of increase in CRL greater in black versus white and in more advanced maternal age

Ethnic background | **Maternal age**



CRL and MSD are not influenced by vaginal bleeding, pain, parity, previous miscarriage, anxiety

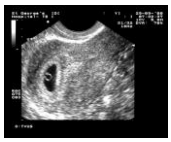
black= solid line
white= dashed line
Asian= dashdotted line
Robinson= dotted line

20 years= solid line
30 years= dashdotted line
40 years= dashed line
Robinson= dotted line

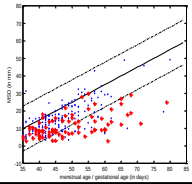
Bottomley, C. et al. Hum. Reprod. 2009 24:284-290

Human Reproduction

Gestational sac growth: functional linear discriminant analysis of mean sac diameter (MSD)



Gestational sac diameters are measured transvaginally in three orthogonal planes from the inner borders of the sac at the scans between 35 and 98 days. MSD is calculated as the average of the three diameter measurements



Reference curve by Hellman: MSD in viable (dots) and non-viable (stars) pregnancies
MSD growth seems to be linear in cross-sectional studies

Studies comparing gestational sac diameter with pregnancy outcome

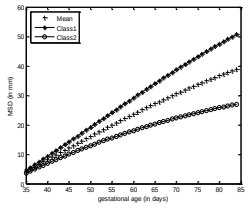
Variability in study populations and design

Jauniaux et al. The role of ultrasound imaging in diagnosing and investigating early pregnancy failure. Ultrasound Obstet Gynecol. 2005;25:613-624

FLDA of MSD growth discriminates between viable and non-viable pregnancies with high sensitivity (79%) and specificity (84%)

A. Pexsters, A. Daemen, C. Bottomley, Y. Abdallah, O. Naji, N. Raine-Fenning, B. De Moor, T. D'Hooghe, Dirk Timmerman and Tom Bourne

The final cohort included 281 women with a spontaneous singleton pregnancy which was either viable (n=199, 66%) or nonviable (n= 82, 27%) at twelve weeks



FLDA of mean sac diameter (MSD) performs better than maximum gestational sac diameter (GSmax) and gestational sac volume (GSvol)

	TP	FN	TN	FP	Mean accuracy (+std)	Mean sensitivity (+std)	Mean specificity (+std)	Mean PPV (+std)	Mean NPV (+std)
First scan	31	51	174	25	72.9	37.8	87.4	55.4	77.3
GS mean vs. GA	65	17	166	33	82.4 (0.5)	**79.3 (1.1)**	**83.6 (0.5)**	66.6 (0.8)	90.8 (0.5)
GS max vs. GA	65	17	158	41	79.4 (0.6)	79.3 (0.5)	79.5 (0.8)	61.4 (0.9)	90.3 (0.2)
GSvol vs. GA	69	13	142	57	75.1 (0.5)	84.5 (0.4)	71.3 (0.7)	54.2 (0.6)	92.0 (0.2)

TP, FN, TN, FP for FLDA = average values over 100 randomizations

None of the clinical parameters (history of previous miscarriage, uncertainty of dates) or symptoms (bleeding, pain, anxiety) studied with FLDA in the viable and non-viable group influenced MSD growth significantly

Conclusions

- FLDA of MSD growth can discriminate between pregnancies that turn out to be viable or non-viable at 12 weeks gestational age and therefore predict miscarriage with high specificity and high sensitivity.
- It performs better than one single MSD measurement as shown by the reference plot from Hellman in spontaneous pregnancies and confirmed by Rossavik in infertility patients.
- MSD performs better than GSmax or GSV, which show a lower specificity and therefore are less accurate in predicting miscarriage
- The sensitivity for MSD growth to predict miscarriage (79%) is much higher than the sensitivity for CRL growth (60.7%), which is probably due to the fact that most miscarriages show a stagnation in gestational sac growth at an early stage, whereas approximately half of the miscarriages do not show an early embryonic growth restriction
- None of the clinical parameters that were investigated (previous history of miscarriage, uncertainty of dates, pain, bleeding and anxiety) had a significant influence on MSD growth either in the viable or non-viable class, which values FLDA for MSD growth as a clinical tool.

Intra- and inter-observer reliability of first trimester measurements

Anne Pexsters[1], Jan Luts[2], Dominique Van Schoubroeck[1], Cecilia Bottomley[3], Sabine Van Huffel[2], Thomas D'Hooghe[1], Christoph Lees[4], Dirk Timmerman[1] and Tom Bourne[1,5]

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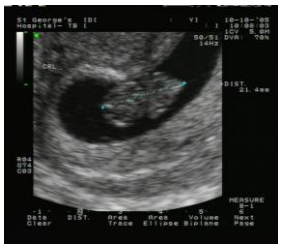
5. Imperial College London, Hammersmith Campus, Du Cane Road, London, UK

RCOG / RCR guidelines

Early pregnancy loss / delayed miscarriage

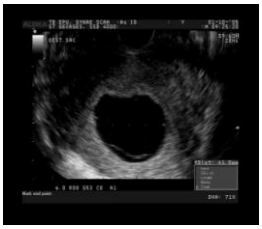


Intrauterine gestation sac with fetal pole of at least 6mm and no visible cardiac activity

(or <6mm without development of fetal cardiac activity over interval of at least 7 days)

Royal College of Radiologists, Royal College of Obstetricians and Gynaecologists. Guidance on Ultrasound Procedures in Early Pregnancy. London: RCR/RCOG; 1995. RCOG guidelines 2006

RCOG / RCR guidelines
Empty sac



Intrauterine gestation sac of mean sac diameter at least 20mm with no fetal structures visible

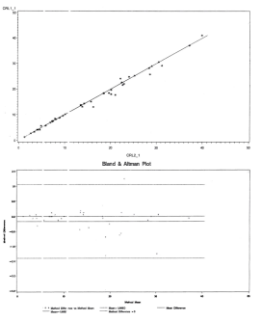
(or <20mm with no change over an interval of at least 7 days)

Royal College of Radiologists, Royal College of Obstetricians and Gynaecologists. Guidance on Ultrasound Procedures in Early Pregnancy. London: RCR/RCOG; 1995. RCOG guidelines 2006

Intra- and interobserver variability study of first trimester measurements between 6 and 9 weeks GA

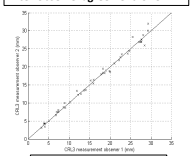
Intra-observer variability for CRL

- Preliminary analysis of 39 patients
- ICC 1,2,3: 0.99553
- In general good agreement of the measurements
- Variability increases for CRL > 15-20mm

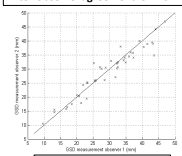


Inter-observer variability study of first trimester measurements between 6 and 9 weeks GA

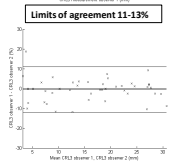
Inter-observer agreement for CRL



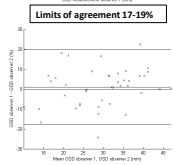
Inter-observer agreement for MSD



Limits of agreement 11-13%



Limits of agreement 17-19%



Expected variability in measurements for a second observer with a given measurement by one observer

CRL

CRL1 of first observer (mm)	95% PI for CRL1 of second observer (mm)
5	[4.5-5.6]
6	**[5.4-6.7]**
7	[6.3-7.9]
10	[8.9-11.2]
20	[17.9-22.4]
30	[26.7-33.5]

MSD

MSD of first observer (mm)	95% PI for MSD of second observer (mm)
17	[14.3-21.0]
18	[15.1-22.2]
19	[16.0-23.4]
20	**[16.8-24.5]**
21	[17.6-25.7]
22	[18.4-26.9]
23	[19.2-28.0]
24	[20.0-29.2]

Conclusion

- The variability of CRL measurements both for a single observer and between observers is smaller than the inter-observer variability for MSD measurements, and the variability will not influence the accuracy of dating
- Although small, these differences may have very significant clinical consequences when decision-making of viability is concerned
- Whatever single cutoff values may be used to define a miscarriage, great care must be taken when measurements approach the decision boundary
- We suggest that for any proposed cutoff value for CRL or MSD to define miscarriage, possible variations in measurement accuracy are taken into account before diagnosing miscarriage on the basis of one scan. Hence in the UK, an MSD of 20 mm to define miscarriage would become 24.5 mm to take into account possible measurement error
- In this way the risk of terminating wanted viable embryos should be minimized

Repeat scan

Present and future work

- Externally validate the new CRL curve and introduce it in clinical practice
- Prospective study on infertility patients with known dates of conception for longitudinal growth studies. Define a cut-off value for MSD above which growth is always viable
- Study of other parameters possibly influencing first trimester growth, such as BMI, maternal weight gain pattern, stress hormone respons

Thank you!

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Erasmus MC
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Three dimensional virtual reality imaging of early pregnancy

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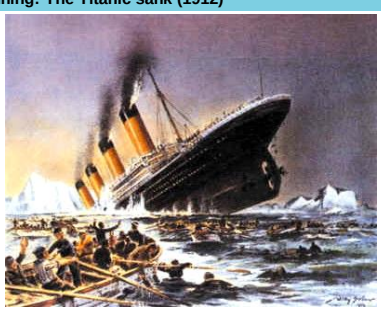
Learning objectives

- Historical overview ultrasound
- Advantages and disadvantages of conventional 3D ultrasound
- Virtual reality enables dept perception in 3D datasets
- Normal growth and development in virtual reality
- The effect of maternal age on embryonic growth and development
- Abnormal growth in miscarriage cases
- Abnormal development in cases with congenital anomalies
- Future research plans

The beginning: The Titanic sank (1912)



Richardson: sound could be used for the detection of icebergs

Fessenden: a successful demonstration in 1914

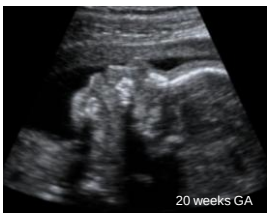
1957: First publication of a fetus, visualized with ultrasonography

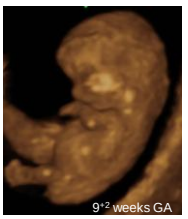
Ian Donald with the NE 4102

Ultrasound as a routine part of obstetric care

- Two dimensional (2D) obstetric ultrasonography
- Around 1990: introduction of three dimensional (3D) ultrasound

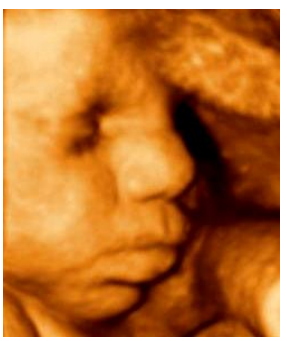
International Radiology Congress in 1989





Advantages 3D ultrasound: general

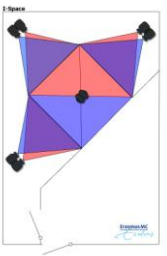
- Extra information, in particular details of external structures
- Additional value: abnormalities of these external structures
- Better understanding by parents: improved counseling



Problems with 3D ultrasound

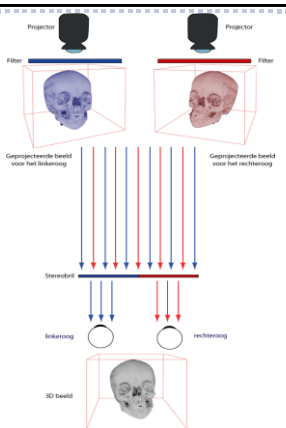
- With 3D (and 4D) ultrasound the third dimension is not used to its fullest
- Evaluation is performed from either paper or a computer screen, i.e. a 2D medium which does not allow depth perception
- I-Space virtual reality system can be used to investigate the benefits of the third dimension in the evaluation of 3D ultrasound datasets

The Erasmus MC I-Space



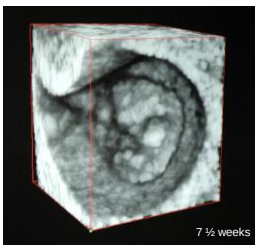
- Projection on three walls and the floor by 8 different projectors
- 'Hologram' created by V-Scope
- Depth perception by *stereoscopic imaging*

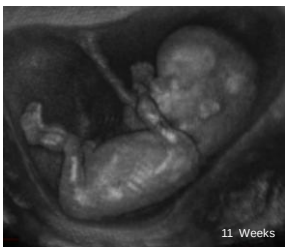
Koning et al. 2009



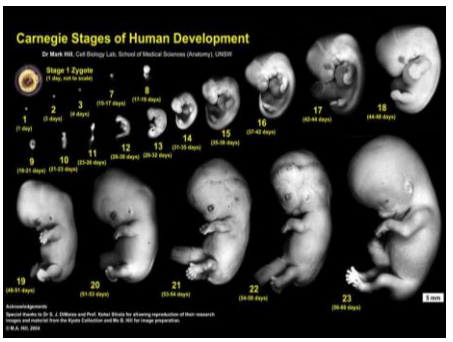
I-Space in Prenatal Medicine

Growth and development in early pregnancy





Morphology

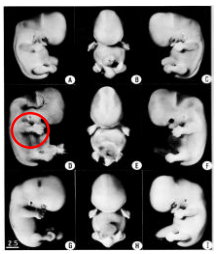


Carnegie stages in the I-Space: Methods

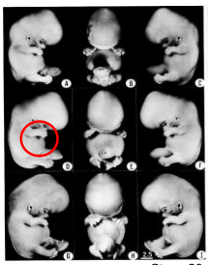
- IVF/ICSI pregnancies
- Carnegie stages:
  external morphological characteristics
- Greatest lenght (CRL) in the I-Space
- Age based on date of oocyte retrieval compared with classical data on embryology

Verwoerd-Dikkeboom et al. 2008

Results



Stage 19



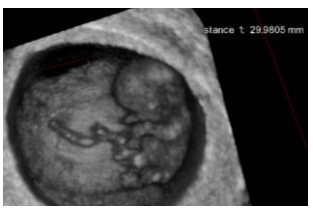
Stage 20

Verwoerd-Dikkeboom et al. 2008

Measuring the third dimension: reproducibility

- Interclass correlation coefficient (ICC)
- ICC > 0,90 ➔ very good agreement

3D vs I-Space	ICC > 0.96
3D intra- en interobserver	ICC > 0,96
I-Space intra- en interobserver	ICC > 0,98

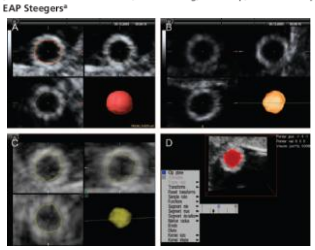


Verwoerd-Dikkeboom et al. 2008

Volume measurements: accuracy and reliability

Early pregnancy volume measurements: validation of ultrasound techniques and new perspectives

M Rousian,[a] CM Verwoerd-Dikkeboom,[a] AHJ Koning,[b] WC Hop,[c] PJ van der Spek,[b] N Exalto,[a] EAP Steegers[a]



Rousian et al. 2009

Volume measurements: accuracy and reliability

Table 3. Evaluation of reliability of the yolk sac volume measurements

Technique	n	Mean difference* (cm^3)	95% CI of mean difference (cm^3)	Limits of agreement** (cm^3)	ICC	95% CI of ICC
VOCAL 30° vs VOCAL 15°	24	−0.001	−0.003 to 0.003	−0.004 to 0.002	0.996	0.990–0.998
VOCAL 30° vs Inversion mode	24	−0.004	−0.013 to 0.001	−0.010 to 0.003	0.963	0.715–0.989
VOCAL 30° vs SonoAVC	20	0.002	−0.008 to 0.012	−0.007 to 0.012	0.958	0.884–0.984
VOCAL 30° vs V-Scope	24	−0.000	−0.004 to 0.004	−0.005 to 0.004	0.992	0.981–0.996
Inversion mode vs SonoAVC	20	0.006	−0.003 to 0.020	−0.004 to 0.016	0.909	0.340–0.975
Inversion mode vs V-Scope	24	0.003	−0.004 to 0.016	−0.006 to 0.012	0.943	0.777–0.981
SonoAVC vs V-Scope	20	−0.002	−0.012 to 0.006	−0.012 to 0.007	0.956	0.879–0.983

n is the number of yolk sacs that could be compared.
*Mean difference is calculated as technique 1 minus technique 2.
**Limits of agreement are calculated as mean difference ± 1.96SD.

ESHRE 27 june 2010

Rousian et al. 2009

Embryonic volume measurements

Aim

Evaluation of embryonic body volumes in first trimester pregnancies using a virtual reality application

Patients

- 50 IVF/ICSI and spontaneous pregnancies recruited
- 8 patients excluded

Ultrasound

- Weekly scanned between 5^{+5} and 12^{+6} weeks of gestation
- 180 3D ultrasound scans performed
- 88 ultrasound scans were transferred to the I-Space

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Methods II

V-Scope

- 88 ultrasound scans
- CRL measurements with trace application [1]
- Segmentation algorithm measured the volumes
- Post processing tools are available

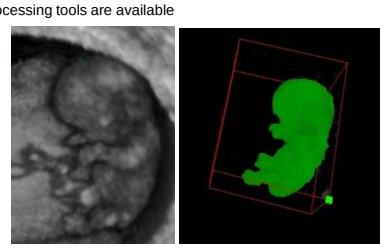

Image of a volume measurement (9^{+5} weeks)

1. *Verwoerd-Dikkeboom et al. 2008*

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Methods III

Analysis

- Repeated measurements ANOVA
- Interobserver agreement established by Intraclass Correlation Coefficient (>0.90 represents good agreement)

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Results I

Characteristic	Mean or Median	SD or Range
Embryonic Volume (mm^3) (median/range)	2214	14 – 29877
GA (days) FV (mean/SD)	66	11
CRL (mm) FV (median/range)	25.7	3.0 – 68.0
Birth weight (g) (mean/SD)	3346	588
GA delivery (weeks plus days) (mean/SD)	39^{+2}	10 days

Rousian et al. revision 2010

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Results II

Mean embryonic volume estimations (mm^3)

Interobserver agreement: ICC of 0.99

CRL (mm)	Embryonic Volume (mm^3)
5	22
10	147
15	443
20	967
25	1 778
30	2 921
35	4 443
40	6 391
45	8 806
50	11 731
55	15 206
60	19 270
65	23 960

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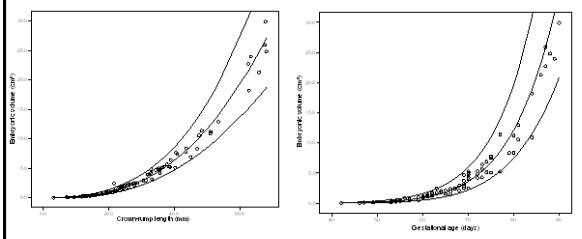
Results III: Embryonic volume versus CRL and GA



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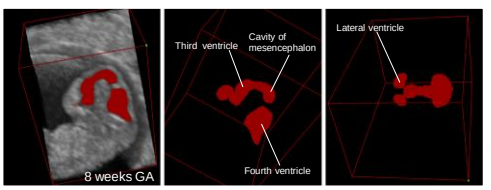
Brain ventricle development using virtual reality

- Automated
- Total brain ventricle volume
- Use of depth perception: easy



Rousian et al. In progress 2010

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Aim

To evaluate the embryonic brain ventricle development & volumetry using a virtual reality application

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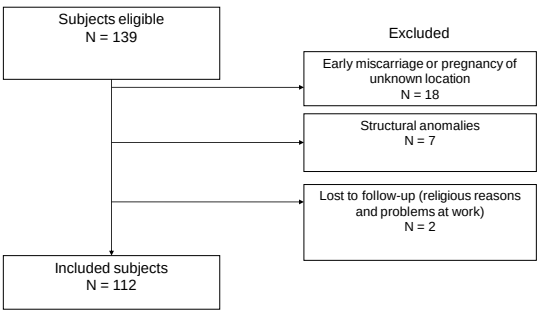
Material and methods

- Study design — Prospective cohort study
- Inclusion criteria — Singleton, confirmed viability, no fetal abnormalities
- Study moment — Weekly; between 7^{+0} and 10^{+6} weeks' gestational age
- Materials — 3D ultrasound and the I-Space
- Measurements — CRL and total brain ventricle volume
- Analysis — Descriptive statistics in SPSS; repeated measurements ANOVA using SAS

Results I

Study population



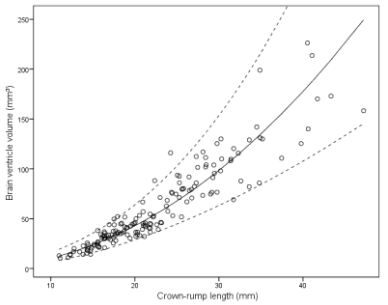
Results II

Maternal and ultrasound characteristics

	N (%)	MEDIAN	RANGE
Maternal age (years)	112 (100%)	31.5	19 – 42
Gestational age (days)	422 (100%)	61	51 - 76
Crown-rump length (mm)	399 (90%)	20.9	11.0 – 47.2
Embryonic volume (mm^3)	324 (53%)	973.1	11 – 6540.0

Brain ventricle volume versus crown-rump length

161 brain ventricle volume measurements



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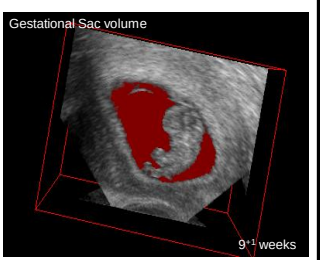
Measurements of other structures

- Gestational sac measurements
- Yolk sac volume
- Early cerebellum development
- Fysiological herniation volume



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General conclusion

- I-Space is a valuable and reliable tool to visualise and quantify embryonic growth and development
- Morphology, biometry and volumetry can be studied
- New area to study the relationship between embryonic growth, development and morphology and second and third trimester pregnancy complications

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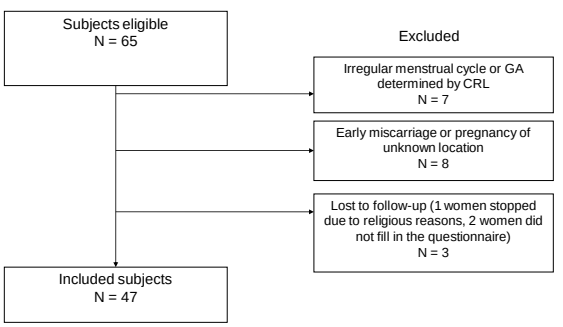
Influences on embryonic growth

- Growth processes essential during embryogenesis
- Nutrition, especially folate
  Timmermans et al. Br J Nutr 2009
- Life-style factors: smoking, drinking
  Mook-Kanamori et al. JAMA 2010
- Maternal characteristics: Ethnicity and maternal age
  Bottomley et al. Human Reproduction 2009
- Recurrent miscarriages
  Mantoni et al. BJOG 1982
- Chromosomal abnormalities
  Bahado-Singh et al. Am J Obstet Gynecol 1997
- Growth restriction
  Bukowski et al. BMJ 2007

Aim, material and methods

Aim	**To evaluate embryonic growth and the influences of maternal age**
Study design	Prospective periconceptional cohort study
Inclusion criteria	Singleton, confirmed viability, no fetal abnormalities, regular menstrual cycle
Study moment	Weekly; 6-7 weeks until 12 weeks
Materials	General questionnaires, 3D ultrasound and I-Space
Analysis	Linear mixed model using SAS Covariates: fertility treatment, parity, recurrent miscarriages, folic acid intake, medication use and smoking

Results study population



Results maternal and ultrasound characteristics

	N (%)	MEDIAN	RANGE
Maternal age (years)	47 (100%)	32.7	18.9 – 39.9
Gestational age (days)	284 (100%)	69	42 - 90
Crown-rump length (mm)	256 (90%)	28.7	4.6 – 77.2
Embryonic volume (mm^3)	151 (53%)	1 646.7	7.1 – 21 700.0

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Rousian et al. In progress 2010

Results

- Women > 36 years → 1.9% greater CRL measurement (ns.), adjusted for parity
- Women > 36 years → 26.1% greater EV measurement (p=0.0034)

Conclusion: Maternal age ≥ 36 may affect first trimester embryonic growth

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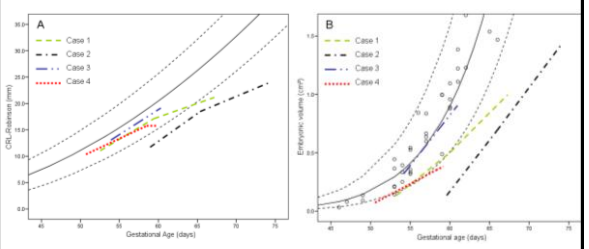
Abnormalities in the I-Space (I)

CRL of the patients plotted in the Robinson growth chart

Embryonic volume of the patients plotted in our growth chart



Rousian et al. Submitted 2010

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Therefore…..

- Useful in differentiation between early normal and abnormal growth

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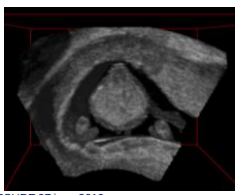
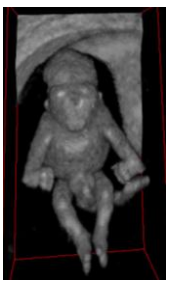
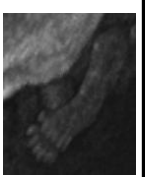
Abnormalities in the I-Space (I): Trisomy 18 (12w2d GA)

- Exencephalos
- Absent Radius
- Spina Bifida
- Omphalocele
- SUA
- Polydactyly







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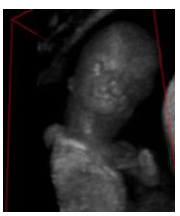
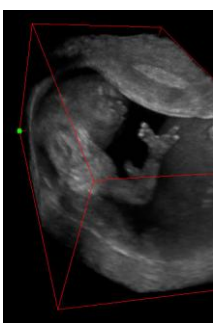
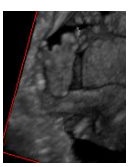
Abnormalities in the I-Space (II): EEC syndrome (12w5d GA)

Ectrodactyly-ectodermal dysplasia-cleft syndrome (AD)

- Split hand and foot
- Bilateral OFS







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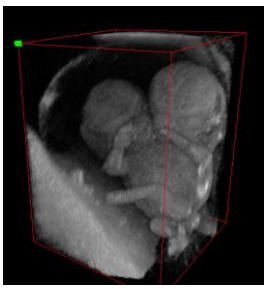
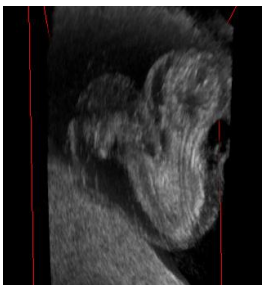
Abnormalities in the I-Space (III): Conjoined twin (11w6d GA)

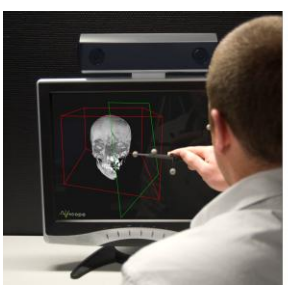


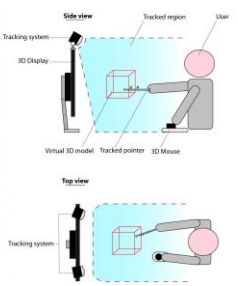


Future research

- First trimester growth measurements in relation to outcome
- Influence of other maternal and environmental factors
- Abnormal development
- Comparison between 2D and real 3D (virtual reality)
- Desktop system

Desktop system





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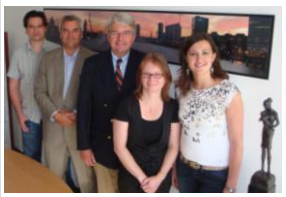

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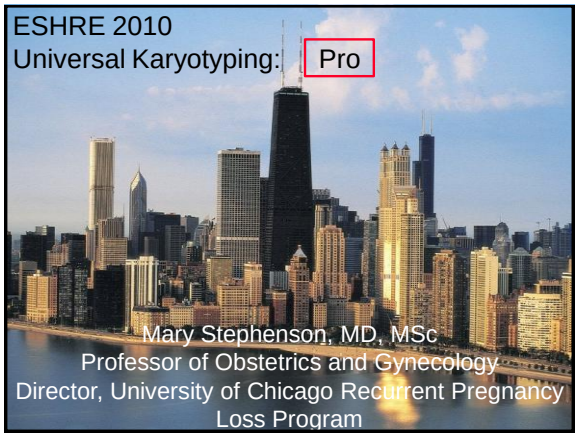


Cohort of interest

- Recurrent miscarriage:
  - ≥3 consecutive miscarriage (ESHRE 2006)
  - ≥3 pregnancy losses (RCOG 2003)

In this debate:

- Recurrent early pregnancy loss (REPL):
  - ≥2 miscarriages <10 wks (Stephenson, 2008)

*Biochemical miscarriages excluded in discussion

Karyotype

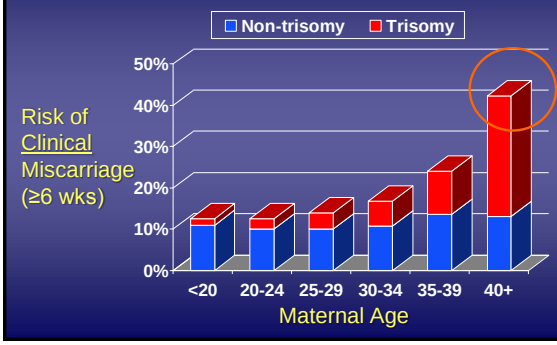
Risk of Miscarriage in the General Population

Gestational Age	Risk of Miscarriage	Chromosome errors
Preclinical (< 6 wks)	30-50%[1,2]	70%[5]
Clinical (6 to <10 wks)	15%[3]	50%[3]
Fetal (≥ 10 wks)	2-3%[4]	5%[4]

[1]Edmonds et al. 1982; [2]Wilcox et al. 1988; [3]Jacobs et al. 1987; [4]Simpson, 1990; [5]Ohno et al. 1991

Clinical Miscarriage and Advancing Maternal Age

Hassold and Chiu, Hum Genet 1985



When to evaluate?

- ACOG Practice Bulletin (2001): Classically, 3 but consider after 2 consecutive miscarriages
- Consecutive vs. non-consecutive: Is there a difference?
  Van den Boogaard et al, Hum Reprod epub
- Presently, maternal/paternal factors are the focus of evaluation in couples with REPL
  → need to shift our focus to the evaluation of prior miscarriages

Why evaluate the miscarriage?

- Evidence of causality
  - ✓ Numeric chromosome errors: trisomy, monosomy, polyploidy
  - ✓ Unbalanced translocations
  - ✓ Major developmental anomalies
  - ✓ Infection
  - ✓ Thrombotic features
  - ✓ Immunological features
  - ✓ Miscarriage genes: To be determined…

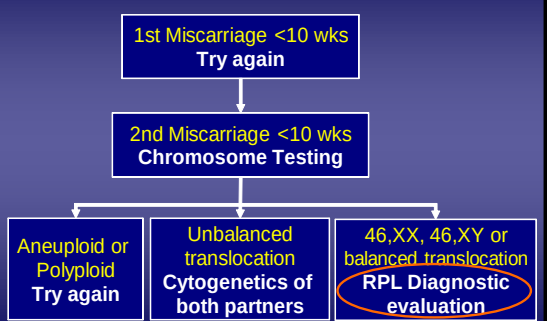
Numeric chromosome errors

- "Explained" miscarriage
- Random event: Risk of subsequent miscarriage generally not increased
  Warburton et al, ; Stephenson ESHRE
- Useful information for patient:
  - ✓ Understands why the miscarriage occurred
  - ✓ Informed decision whether to try again
- Useful information for clinician:
  - ✓ No further evaluation required

Translocations

- Unbalanced translocation: "Explained" miscarriage
- Balanced translocation: "Unexplained" miscarriage,
  → Indication to evaluated other factors
- Miscarriage with translocation: Indication for cytogenetic analysis of both partners
  → Inherited or random event?
- Useful information for patient:
  - ✓ Unbalanced translocation: Understands why the miscarriage occurred
  - ✓ Informed decision whether to try again

With accurate chromosome testing…



Stephenson, 2008

Selective Chromosome Testing: Against

- Universal chromosome testing of the miscarriage:
  With the second and all subsequent miscarriages
- Universal chromosome testing of couple:
  With a miscarriage found to have a translocation
- Why?
  - ✓ >50% of miscarriages are due to chromosome errors
  - ✓ Identifies RPL couples with an increased risk of further euploid miscarriages → RPL evaluation
  - ✓ Patient: Emotional and financial benefits

Universal Karyotyping: *con*

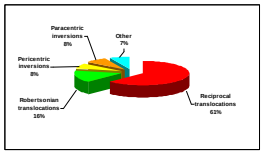
Mariëtte Goddijn
Center for Reproductive Medicine

Universiteit van Amsterdam

amc center for reproductive medicine

Background: incidence carrier status RM

- General population: 0.7%
- After 1 miscarriage: 2.2%
- After 2 miscarriages: 4.8%
- After 3 miscarriages: 5.2%



Category "other": deletions and duplications

Hook Ann Hum Genet 1989
De Braekeleer Hum Reprod 1990

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Chances of carrier status: healthcare problem

- **Increase in annual number of chromosome analyses**
  - **1992: 1298 couples**
  - **2000: 2362 couples**
- **Decrease in incidence of carrier status**
  - **1992: 6.8%**
  - **2000: 3.6%**

Time consuming diagnostic procedure
High costs
Low detection rate of carriers

Annuals of Postnatal Cytogenetic Analysis in The Netherlands 1992 - 2001

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Selective karyotyping model

Maternal age at second miscarriage		(RM$_{parents}$) +		(RM$_{parents}$) -	
		≥3 misc.	2 misc.	≥3 misc.	2 misc.
< 23 years	(RM$_{fem}$) +	10.2%	7.3%	7.3%	5.2%
	(RM$_{fem}$) -	5.7%	4.0%	4.1%	2.8%
23-34 years	(RM$_{fem}$) +	10.0%	7.2%	7.2%	5.1%
	(RM$_{fem}$) -	5.7%	4.0%	4.0%	2.8%
34-37 years	(RM$_{fem}$) +	5.8%	4.1%	4.1%	2.9%
	(RM$_{fem}$) -	3.2%	2.2%	2.2%	1.6%
37-39 years	(RM$_{fem}$) +	4.0%	2.8%	2.8%	2.0%
	(RM$_{fem}$) -	2.2%	1.5%	1.5%	1.1%
≥ 39 years	(RM$_{fem}$) +	1.8%	1.2%	1.3%	0.9%
	(RM$_{fem}$) -	1.0%	0.7%	0.7%	0.5%

Franssen BMJ 2005
Jauniaux; Guideline ESHRE 2006
Guideline NVOG 2007

Validation other cohorts ongoing

Chance of a healthy child (follow up) literature

Study	No of couples	No of live births n (% per couple)	No of miscarriages n (% per couple)	No of viable unbalanced offspring
*(a) reproductive outcome in **the first** pregnancy after parental chromosome analysis (including couples with failure to conceive)*				
Carp *et al.* (2004)	99	33 (33%)	40 (40%)	None reported
Franssen *et al.* (2006)	247	148 (60%)	91 (37%)	0
Stephenson *et al.* (2006)	52	29 (56%)	11 (21%)	0
Sugiura-Ogasawara *et al.* (2008)	71	39 (55%)	22 (31%)	0
*(b) reproductive outcome of **all** pregnancies after parental chromosome analysis*				
Franssen *et al.* (2006)	247	205 (83%)*	120 (49%)**	4 2 at PND 2 live births
Stephenson *et al.* (2006)	51	33 (65%)*	11 (22%)**	0

In 469 carrier cases (>780 pregnancies),
only 4 x unbalanced offspring reported

Reproductive outcome

	Carriers *n = 247*	Controls *n = 409*
Failure to conceive	8 (3%)	19 (5%)
Miscarriage	120 (49%)	122 (30%)*
Terminated pregnancy	6 (2%)	8 (2%)
Ectopic pregnancy	3 (1%)	13 (3%)
Stillbirth	3 (1%)	6 (2%)
Post-partum deceased child	1 (0.4%)	4 (1%)
Ill/ handicapped child	2 (1%)	11 (3%)
Healthy child	205 (83%)	344 (84%)

** p < 0.001*

Franssen BMJ 2006

Chances of unbalanced outcome- literature

- Liveborn children general population : 0.06%
- Liveborn children in our total RM screening population: 5/25012 0.02%
- *De novo* unbalanced chromosome abnormalities:
  - at PND ± 50%
  - liveborn ± 20%

Jacobs J Med Genet 1992, Hook Am J Hum Genet 1984
Daniel Am J Med Genet 1989
Franssen BMJ 2006 (additional calculations)

PGD in carrier couples with RM

Does PGD contributes to the chance of a healthy child?

	No of studies	No of couples	Started cycles	No of live births n (% per couple)	No of miscarriages n (% per couple)
Natural conception					
First pregnancy after natural conception	4	468	NA	249 (53%)	156 (33%)
All pregnancies after natural conception*	2	298	NA	238 (80%)	131 (44%)
PGD	21	126	133	44 (35%)	6 (5%)

No RCT's or comparative studies available
No higher chance of a healthy child after PGD
PGD might reduce miscarriage rate

Carp 2004, Stephenson 2006, Franssen 2006, Sugiura Ogasawara 2008, 21 PGD studies: see Franssen subm

Rationale fetal karyotyping

- Offers an explanation for the loss
- Supposed to give prognostic information
- After a trisomy the prognosis seems favorable
- A maternal cause of pregnancy is excluded

Summary and key messages

- RM carriers have a high chance of a healthy child
- RM carriers have a low chance of a handicapped child
- The number of congenital malformations in carriers equals the number of congenital malformations in non-carriers
- RM carriers refrain more often from further childhood
- Data are insufficient that PGD improves live birth rates in couples with RM carriers
- Fetal karyotyping in individual patients does not add to the knowledge of both patient and doctor; only in research setting more fetal karyotypes are needed
- No more parental karyotyping, PGD, fetal karyotyping in couples with recurrent miscarriage
- Counselling and reassurance are essential

Sex specific immunological mechanisms for pregnancy complications

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I have no commercial/financial or other conflicts of interest

Learning objectives

To understand the scientific data supporting sex specific immunological mechanisms in:

- secondary recurrent miscarriage
- recurrent placental abruption
- stillbirth and preterm births in the background populations
- To advocate collaborative translational studies to further increase the understanding of immunological mechanisms in early pregnancy

Take home message

BIG BROTHER
IS KILLING YOU

Outlines for the next 44 minutes

- Definitions

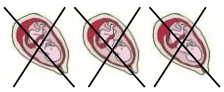
The influence of sex of prior children in

- Secondary recurrent miscarriage
- Recurrent placental abruption
- Possible underlying immunological mechanisms

The influence of sex of prior children on outcomes of subsequent pregnancies in the background population

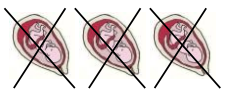
Recurrent Miscarriage

1% of women



. . . Primary (65%)

PRM



. . . Secondary (35%)

SRM

What causes RM

50% of cases are possibly explained by

- Chromosomal abnormalities in the couple or fetus
- Abnormal uterine anatomy
- Irregular periods
- Presence of lupus anticoagulant

- the remaining 50% are defined as unexplained

Indications for immunological background SRM

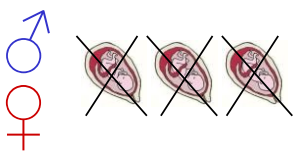
- Obviously, no genetic or chromosomal constitutions preventing the birth of a child
- Transfer of fetal cells greatest in last part of pregnancy
- Miscarriages from SRM have lower frequency of chromosomal abnormalities than PRM
- Immunological high responder allele HLA-DR3 more common in SRM than PRM

Huppertz et al 2006
Adams et al 2007
Kruse et al 2004

Indications for sex specific mechanisms in SRM

Sex of child prior to miscarriages and chance of live birth following the miscarriages

N=182



Live birth:

58%

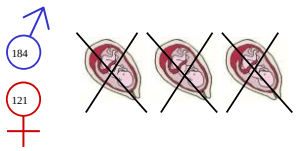
… Hazard ratio:0.59
p=0.02

76%

Christiansen et al 2004

Sex of child prior to miscarriages and chance of live birth following the miscarriages

N=305



Live birth:

56%

… OR: 0.37 p=0.0001

78%

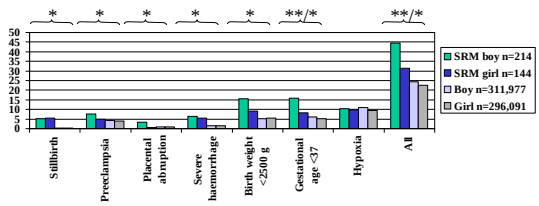
Nielsen et al 2008

Sex ratio in birth prior and subsequent to SRM

	SRM	Controls	p
Prior to SRM compared to primi-para (n=358/n=608,068)	1.49	1.05	0.001
Subsequent to SRM compared to secondi-para (n=213/510,264)	0.76	1.06	0.02
p	<0.0001	0.89	

Nielsen et al 2010

Obstetric complications prior to the series of miscarriages

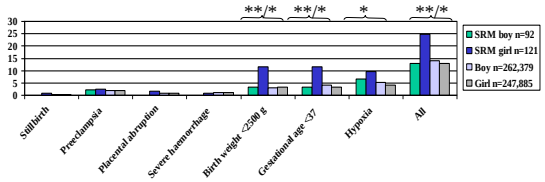


* Significant difference between SRM patients and controls

** Significant difference between SRM with boy compared to girl

Nielsen et al 2010

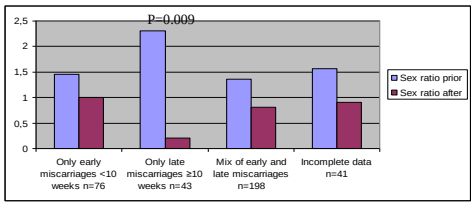
Obstetric complications following the series of miscarriages



* Significant difference between SRM patients and controls
** Significant difference between SRM with boy compared to girl

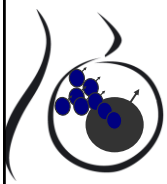
Nielsen et al 2010

Sex ratio according to gestation of miscarriages



Nielsen et al 2010

Sex specific immunity in normal pregnancy



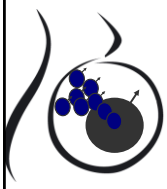
Maternal cellular anti-HY immunity can be detected up to 22 years after the birth of a boy

HY-antigenes are presented to the immune system by HLA alleles for

6 genes ⇨ **10 HY-antigenes**

James 2003
Verdijk et al 2004
Piper et al 2007

Sex specific immunity in transplantation



H-Y specific T-cell responses primed in male fetus pregnancies are held responsible for increased graft versus host disease in male recipients of female stem cells

HY antibodies in recipients of stem cell grafts correlates with GvHD

Gratwohl et al 2001

Miklos et al 2005

Hypothesis

Abberant or non-tolerated

maternal immune reactions against

male-specific (H-Y) antigens

primed in first pregnancy are

responsible for subsequent adverse pregnancy outcomes

HY-restricting HLA and pregnancy outcome in SRM

class I presenting HY-antigens:

HLA-A1, -A2, -A33, -B7, -B8, -B52, -B60

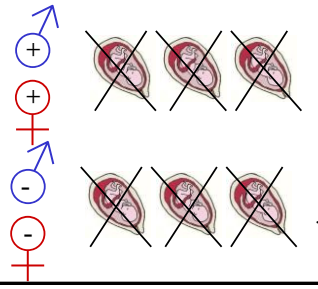
NO IMPACT ON PREGNANCY PROGNOSIS

Nielsen HS et al 2009

Maternal HY-restricting HLA class II and pregnancy outcome in SRM

HLA-class II presenting HY-antigens : HLA-DQ5, -DRB1*15, -DRB3*0301

Maternal H-Y restricting HLA class II



Live birth:

44%

. . . OR:0.17 p=0.0001

82%

66%

. . . OR:1.0 p=0.9

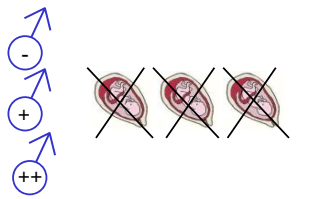
69%

Nielsen HS et al 2009

HY-restricting HLA class II and pregnancy outcome in SRM

HLA-class II presenting HY-antigens : HLA-DQ5, -DRB1*15, -DRB3*0301

Maternal H-Y restricting HLA class II



Live birth

. . . **66%**

. . . **47% OR:0.46, p=0.02**

. . . **29% OR:0.21, p=0.02**

Nielsen HS et al 2009

HY-restricting HLA of child born prior to miscarriage

HLA-class II presenting HY-antigens : HLA-DQ5, -DRB1*15, -DRB3*0301

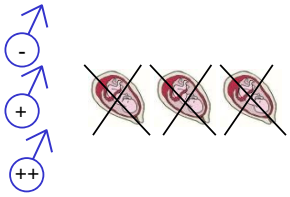
203 children – 178 mother subsequently pregnant

Nielsen HS et al 2009

HY-restricting HLA of child born prior to miscarriage

HLA-class II presenting HY-antigens : HLA-DQ5, -DRB1*15, -DRB3*0301

Child H-Y restricting HLA class II



Live birth:

62%

- - - 56% OR:0.77, p=0.54

22% OR:0.18, p=0.05

Nielsen HS et al 2009

HY-restricting HLA of child born prior to miscarriage

HLA-class II presenting HY-antigens : HLA-DQ5, -DRB1*15, -DRB3*0301

Live birth:

- - - 83% OR:2.59, p=0.29

mothers negative, n=12

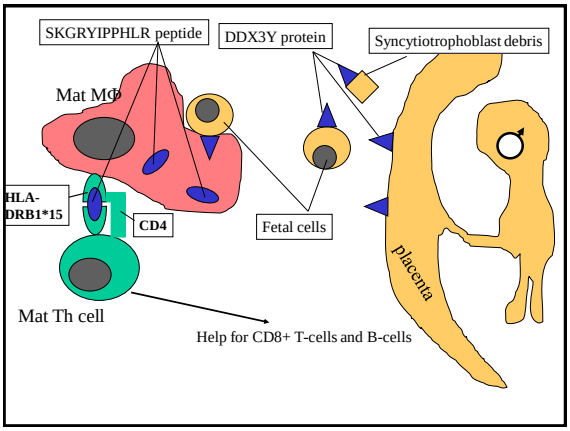
Nielsen HS et al 2009

Maternal HY-restricting HLA class II

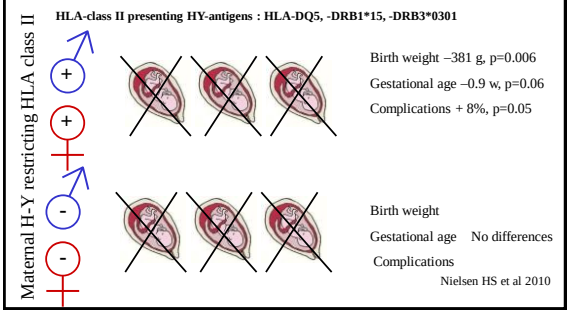
reduces the chance of a successful pregnancy in patients with recurrent pregnancy losses subsequent to a boy

Nielsen HS et al 2009

it is indicated that fetal HY antigens are presented to the maternal immune system by the indirect pathway – HY antigens are taken up and processed by maternal macrophages and presented to maternal CD4 positive T lymphocytes



Maternal HY-restricting HLA class II and obstetric complications after SRM



H-Y antibodies and pregnancy outcome in SRM

Is presented in a poster at ESHRE 2010

Main findings:

More SRM patients than controls are H-Y antibody positive, 46% vs 19%, p=0.004

Presence of these antibodies in early pregnancy is associated with low male: female ratio in survivng neonates 12% vs 42%, p=0.05

Boys compared to girls prior to the miscarriages in SRM

- reduced chance of a subsequent live birth
- screwed sex-ratios
- high frequency of obstetric complications
- only when + maternal HY-restricting HLA class II
- + maternal HY-restricting HLA class II also associated with obstetric complications in surviving pregnancies

Indications of sex specific mechanisms in recurrent placental abruption

Nielsen HS et al 2007

Patients and controls

881 patients with recurrent pregnancy losses

8 patients with severe (fetal death) recurrent placental abruption
Total: 22 abruptions, 18 fatal for the fetus, 15 (68%) male fetuses
7 patients with firstborn boys

Controls: 37 control women with 2 boys and no obstetric problems

Nielsen HS et al 2007

HY-presenting HLA class II haplotypes:

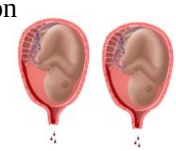
Patients: 9/14	64%
Controls:21/74	28%, p=0.009

Homozygocity for HY-presenting HLA class II haplotypes:

Patients: 3 of 7	43%
Controls:2 of 37	5%, p=0.02

Nielsen HS et al 2007

Maternal immunological responses against HY-antigens might play a role in fatal recurrent placental abruption



Nielsen HS et al 2007

Indications for sex specific mechanisms in perinatal complications in the background population

Sex of prior children and obstetric complications in subsequent pregnancies

- Cohort: All women giving birth to their first singleton 1980-1998 in Denmark
- Follow- up in the National Birth Registry - 2004
- Birth weight and stillbirth among later born children in relation to sex of preceding sibs

Birth weight related to sex of prior children

Boy with one older brother	-29g	Girl with one older brother	-17g	P=0.0001
Boy with two older brothers	-38g	Girl with two older brothers	-21g	

The differences are smaller or disappear if paternity changes

Nielsen HS et al 2007

Magnus et al 1985

Sex of prior children and risk of stillbirth in subsequent pregnancies

- 558,314 2nd-5th children
- 0.5% stillborn
- Risk of stillbirth increases if preceded by boys compared to girls, RR: 1.12 (1.02- 1.23), p=0.02
- SMALL RISK – BUT BOYS ARE A COMMON EXPOSURE

Nielsen HS et al 2010

Sex of prior children and risk of preterm births in subsequent pregnancies

- The Danish and the Swedish National birth registry 1980-2003
- Risk of preterm second birth according to sex of first child
- Included second borns : DK: n=393,686 S: n = 607,400
- 3.9% preterm
- Risk of preterm birth increases if preceded by boys compared to girls, Hazard ratio: 1.10 (1.07-1.13)

Mortensen et al 2010

Preceding brothers and a twin brother

Reduces life time reproductive success in subsequent siblings in pre-industrial Finns

Richard et al 2007

Lummaa et al 2007

Prior birth of boy(s)

- Reduces birth weight of subsequent children
- Increases the risk of subsequent stillbirth and preterm births
- Decreases the chance of reproductive success in subsequent siblings

Conclusions

- Prior birth of boy(s) are associated with secondary recurrent miscarriage, recurrent severe placental abruption and perinatal complications in the background population
- Maternal H-Y restricting HLA class II is associated with outcome in SRM
- H-Y antibodies more frequent and associated with the sex of children born after SRM

Perspectives

- The combination of information from epidemiologic and immunogenetic studies are an optimal approach for getting insight in the pathophysiology of secondaray recurrent miscarriage, recurrent placental abruption
- Other cohorts and larger studies are needed – colaborative studies!

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Mark your calendar for the upcoming ESHRE campus workshops!

- **Basic Genetics for ART Practitioners**
  organised by the SIG Reproductive Genetics
  16 April 2010 - Porto, Portugal

- **Array technologies to apprehend developmental competence and endometrial receptivity: limits and possibilities**
  organised by the Task Force Basic Science in Reproduction
  22 April 2010 - Brussels, Belgium

- **The management of infertility – training workshop for junior doctors, paramedicals and embryologists**
  organised by the SIG Reproductive Endocrinology, SIG Embryology and the Paramedical Group
  26-27 May 2010 - Kiev, Ukraine

- **Preimplantation genetic diagnosis: a celebration of 20 years**
  organised by the SIG Reproductive Genetics
  1 July 2010 - Rome, Italy

- **EIM 10 years' celebration meeting**
  organised by the European IVF Monitoring Consortium
  11 September 2010 - Munich, Germany

- **The determinants of a successful pregnancy**
  organised by the SIGS Reproductive Surgery, Early Pregnancy and Reproductive Endocrinology
  24-25 September 2010 - Dubrovnik, Croatia

- **Basic training workshop for paramedics working in reproductive health**
  organised by the Paramedical Group
  6-8 October 2010 - Valencia, Spain

- **Forgotten knowledge about gamete physiology and its impact on embryo quality**
  organised by the SIG Embryology
  9-10 October 2010 - Lisbon, Portugal

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(see "Calendar")

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Keep an eye on our calendar section for more information on

Upcoming events

- **Female and male surgery in human reproductive medicine**
  8-9 October 2010 - Treviso, Italy
- **Promoting excellence in clinical research: from idea to publication**
  5-6 November 2010 - Thessaloniki, Greece
- **"Update on pluripotent stem cells (hESC and iPS)" and hands on course on "Derivation and culture of pluripotent stem cells"**
  8-12 November 2010 - Valencia, Spain
- **Women's health aspects of PCOS (excluding infertility)**
  18 November 2010 - Amsterdam, The Netherlands
- **Endoscopy in reproductive medicine**
  24-26 November 2010 - Leuven, Belgium
- **Fertility and Cancer**
  25-26 November 2010 - Bologna, Italy
- **The maternal-embryonic interface**
  2-3 December 2010 - Valencia, Spain
- **GnHR agonist for triggering of final oocyte maturation – time for a paradigm shift**
  3 December 2010 - Madrid, Spain
- **Raising competence in psychosocial care**
  3-4 December 2010 - Amsterdam, The Netherlands

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