Clinical Aspects of Early Pregnancy Loss

Winter Symposium organised by the
ESHRE Special Interest Group Early Pregnancy

ESHRE Campus 2005

Liverpool, UK
9 and 10 December 2005

European Society of
Human Reproduction and Embryology

ESHRE Campus Course

CLINICAL ASPECTS OF EARLY PREGNANCY LOSS

Liverpool, United Kingdom

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General Information

Course description & target audience

The Liverpool ESHRE Winter Symposium follows on from successful meetings held in Amsterdam Medical Centre. The objective is to update delegates on recent significant advances in the theoretical basis for and clinical practice of early pregnancy loss.

The meeting is aimed at specialists, trainees and scientists in the disciplines of Gynaecology, Assisted Reproduction, Obstetrics, Genetics, Ultrasound, Haematology and ancillary professions.

Organizing Committee

Professor Eric Jauniaux (UK)
Coordinator of the Special Interest Group (SIG) Early Pregnancy
Dr Niek Exalto (NL)
Deputy and previous coordinator of the SIG
Dr Roy Farquharson (UK)
Deputy of the SIG and Local Organiser

Faculty

Dr John Aplin (Manchester, UK)
Professor Adam Balen (Leeds, UK)
Ms Ruth Bender Atik (Miscarriage Association, UK)
Dr Tom Bourne (London, UK)
Dr Feroza Dawood (Liverpool, UK)
Dr Ron Derksen (Utrecht, NL)
Dr Janine Elson (Sunderland,UK)
Dr Janesh Gupta (Birmingham, UK)
Dr Dharani Hapangama (Liverpool, UK)
Professor Eric Jauniaux (London, UK)
Dr Jemma Johns (London, UK)
Dr Pamela Loughna (Nottingham, UK)
Dr Ben Willem Mol (Amsterdam,NL)
Dr Thomas Philipp (Vienna, AT)
Dr Siobhan Quenby (Liverpool, UK)
Professor Lesley Regan (London, UK)
Dr Eric Steegers (Rotterdam, NL)
Professor Mary Stephenson (Chicago, USA)
Dr Mayumi Suguira-Ogasawara (Nagoya, Japan)

Scientific Program

9 December 2005

Morning sessions

09.00: Registration and Welcome

Session 1: Cytogenetic Aspects of Pregnancy Loss

09.30: Cytogenetic abnormalities of pregnancy loss in recurrent miscarriage
M. Stephenson (Chicago, USA)

10.00: Embryonic karyotype of abortuses in relation to the number of previous miscarriages
M. Suguira-Ogasawara (Nagoya, Japan)

10.30: The value of embryoscopy
Th. Philipp (Vienna, AT)

11.00: Coffee Break

Session 2: Implantation and Immunology

11.30: Immunotherapy and recurrent miscarriage: are we any wiser?
L. Regan (London, UK)

12.00: Endometrial natural killer cells and early pregnancy loss
S. Quenby (Liverpool, UK)

12.30: Implantation and endometrial receptivity
J. Aplin (Manchester, UK)

13.00: Lunch Break

Scientific Program

9 December 2005

Morning sessions

Session 3: Ultrasound

14.00: Polycystic ovarian disease and pregnancy loss: an overview
A. Balen (Leeds, UK)
14.20: Ultrasound uses and pitfalls
P. Loughna (Nottingham, UK)
14.40: Combined ultrasound/biochemical prediction of very early pregnancy loss
J. Elson (Sunderland,UK)
15.00: Aspects of gestational trophoblastic disease
E. Jauniaux (London, UK)

15.30: Tea Break

Session 4: Thrombophilia

16.00: Antiphospholipid syndrome and pregnancy loss: examining the evidence
R. Derksen (Utrecht, NL)
16.30: Homocysteine and pregnancy loss
E. Steegers (Rotterdam, NL)
17.00: Factor V gene polymorphism studies and fetal loss
F. Dawood (Liverpool, UK)

10 December 2005

Morning sessions

Session 5: Clinical

09.00: A new logistic regression model for predicting the outcome of pregnancies of unknown location
T. Bourne (London, UK)

09.30: Evidence based practice for management of early pregnancy loss
B.W. Mol (Amsterdam,NL)

10.00: Uterine anomalies and recurrent miscarriage
J. Gupta (Birmingham, UK)

10.30: Coffee Break

Session 6: Miscellaneous

11.00: Outcome of identified placental haematoma in early pregnancy
J. Johns (London, UK)

11.30: "That was my baby": caring for patients with pregnancy loss
R. Bender Atik (Miscarriage Association, UK)

12.00: Does endometriosis affect implantation?
D. Hapangama (Liverpool, UK)

12.30: Closing remarks and end of meeting

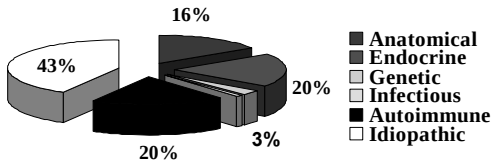
Recurrent Miscarriage: Cytogenetic Analyses of Miscarriage Specimens

Mary Stephenson, MD, MSc, FRCSC, FACOG
Professor of Obstetrics & Gynecology, Section of Reproductive Endocrinology and Infertility
Director, Recurrent Pregnancy Loss Program
University of Chicago

ESHRE Winter Symposium
December 2005

Recurrent Miscarriage (≥3 consecutive miscarriages <20 weeks)

Note: miscarriages with chromosome abnormalities excluded



Stephenson MD, Fertil Steril 1996

Classification of Miscarriage

Preclinical miscarriage: < 6 wks
- **Biochemical: demise <4 wks gestation +ve ßhCG only, U/S negative**
- **Anembryonic: demise between 4-5 wks empty gestational sac**
- **Yolk sac: demise between 5-6 wks gestationl sac with yolk sac**

Clinical miscarriage:	**6 - 20 wks**
Embryonic	**6 - 9 wks 6 days**
Fetal miscarriage	**10 - 19 wks 6 days**

Miscarriage in General Reproductive Population

Gestational Age	Risk of Miscarriage	Cytogenetic Abnormality
< 6 weeks	30-50%[1,2]	70%[5]
6-10 weeks	15%[3]	50%[6]
> 10 weeks	2-3%[4]	5%[4]

[1] Edmonds et al, 1982; [2] Wilcox et al, 1988; [3]Hassold and Chiu, 1985; [4] Simpson, 1990; [5] Ohno et al, 1991; [6] Jacobs et al, 1987

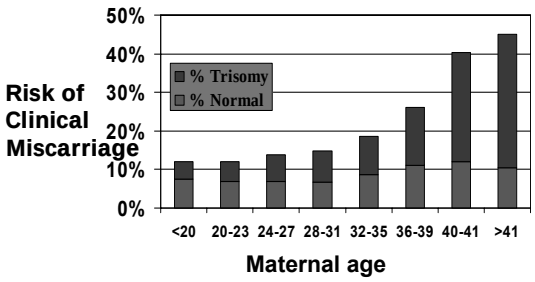
Miscarriage in General Reproductive Population: Cytogenetics

Jacobs et al, Human Genetics 1987

- Tabulation of seven studies
- N = 7,182 miscarriages
- 50% had a cytogenetical abnormality
  - 56% Trisomic (16, 21, 22, 15, 13)
  - 20% Polyploid
  - 18% Monosomy X
  - 4% Translocations
  - 2% Other

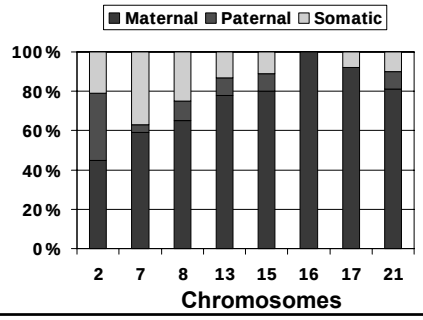
Impact of Maternal Age on Miscarriage

Hassold and Chiu, Hum Genet 1985



Origin of Trisomies

Jacobs and Hassold, 1980



Indications for Cytogenetic Analysis

- 2nd consecutive and all subsequent miscarriages <10 weeks
- Unexplained pregnancy loss >10 weeks
- Miscarriage following ART

Cytogenetic results obtained

Following D&C	91%	*P*<0.05
Expectant management	66%	

(*Stephenson et al, Human Reprod 2002)*

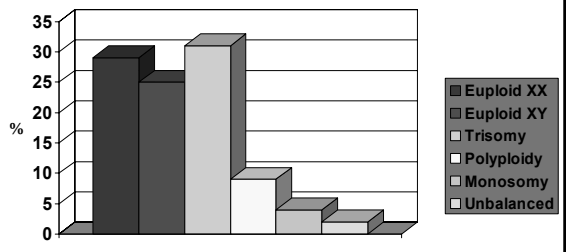
Note: Send the embryo, gestational sac and/or chorionic villi to Cytogenetics; otherwise maternal contamination becomes an issue.

Recurrent Miscarriage: Cytogenetic Questions

- Is there a recurrent miscarriage cohort who may have a higher risk of trisomic pregnancies?
  "Recurrent Trisomy"
- If a women with recurrent miscarriage has an euploid miscarriage, is it predictive of a subsequent euploid miscarriage?
  "Recurrent Euploidy"
- What is the likelihood of success in recurrent miscarriage carriers of a balanced chromosome rearrangement?
- Is IVF/PGD evidence-based therapy for recurrent miscarriage?

Recurrent Miscarriage: Increased rate of cytogenetic abnormalities?

420 miscarriages in 285 patients



Stephenson et al, Human Reprod 2002

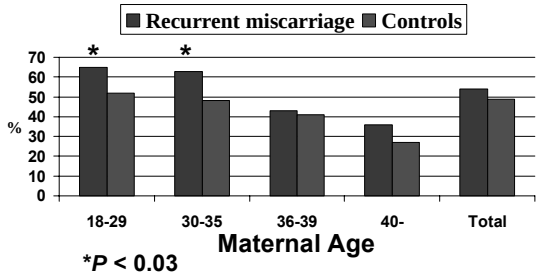
Recurrent miscarriage vs. general reproductive population

Stephenson et al, Human Reprod 2002
Jacobs et al, Human Genetics 1987

- **Similar distribution of trisomies**
  Recurrent miscarriage: 15, 16, 22, 21, 14, 13
  Controls: 16, 22, 21, 15, 13
- **Adjusted for maternal age:**
  No difference in the distribution of numeric chromosome abnormalities, i.e. trisomies, monosomy X, polyploidies.

Euploid Miscarriage and Age

***Stephenson et al, 2002* (n=420) vs.**
***Hassold and Chiu, 1985* (n=2,201)**



***P < 0.03**

Does "Recurrent Trisomy" or "Recurrent Euploidy" exist?

- Recurrent miscarriage patients (n=133)
- Cytogenetics of first 2 miscarriages analyzed (n=266)
- 665/867 (77%) of pregnancies ended in miscarriage
- Trisomic miscarriage followed by another trisomic miscarriage All ages: 60% (CI, 47-71%)
- Euploid miscarriage followed by another euploid miscarriage All ages: 69% (CI, 57-79%)

Stephenson et al, ESHRE abstract 2003

Likelihood: Age <36 Years (n=61)

		Miscarriage #2	
		Euploid	Aneuploid
Miscarriage #1	Euploid	35	5
	Aneuploid	11	10

P=0.002

Likelihood of "Recurrent Euploidy" 88% (95% CI, 73%-95%)

Likelihood of "Recurrent Aneuploidy" 48% (95% CI, 28%-68%)

Recurrent Miscarriage: Carriers of a Balanced Chromosome Rearrangement

Sugiura-Ogasawara et al, 2004

- Prospective cohort, nested case/control
- 100/1,284 (8%) couples with ≥2 miscarriages:
  - 58 reciprocal translocations
  - 11 Robertsonian translocations
  - 32 inversions (mostly chromosome 9)
- 49% live birth rate (n=90 pregnancies)
- Amniocentesis: 1 of 23 pregnancies had an unbalanced translocation

Recurrent Miscarriage: Carriers of a Balanced Chromosome Rearrangement

Goddijn et al, 2004

- Historic cohort and nested case-control study
- 41/1324 (3%) couples with ≥2 miscarriages
  - 26 reciprocal translocations
  - 5 inversions
  - 3 Robertsonian translocations and 3 others
- 70% live birth rate (n=43 pregnancies)
- Amniocentesis: 15 diploid, 11 balanced reciprocal translocations
  - No unbalanced ongoing pregnancies
- Is cytogenetic analyses of couples necessary?

Recurrent Miscarriage: Carriers of a Balanced Chromosome Rearrangement

Stephenson et al, Human Reprod 2006, in press

- Prospective cohort, nested case-control
- 51 of 1,893 (3%) couples of with ≥2 miscarriages
  - 28 reciprocal (15 female, 13 male)
  - 12 Robertsonian (9 female, 3 male)
  - 7 inversions and 4 other
- 215 pregnancies prior to evaluation:
  - 15% live birth rate
- 58 pregnancies subsequent to evaluation:
  - 71% live birth rate
- Amniocentesis: 7 diploid, 8 balanced chromosome rearrangements
  - No unbalanced ongoing pregnancies

Carrier of a Balanced Chromsome Rearrangement: Miscarriage Cytogenetics

	Euploid	Aneuploid/ Polyploid	Unbalanced
Recurrent Miscarriage + carrier (36 miscarriages)	33%	30%	36%
Recurrent Miscarriage (420 miscarriages)	54%	44%	2%
General population (7182 miscarriages)	52%	46%	6%

Stephenson et al, Human Reprod 2006, in press

IVF/PGD for Treatment of Unexplained Recurrent Miscarriage

Platteau et al, 2005

- **Prospective cohort study**
- **49 women with ≥3 miscarriages**
- **69 cycles**
- **Two blastomeres biopsied:**
  FISH for X, Y, 13, 18, 21, 16, 22

IVF/PGD: Aneuploidy Screening in Unexplained Recurrent Miscarriage

Platteau et al, 2005

	<37 years	≥37 years
Aneuploidy Rate	44%	67%
Pregnancy rate/ cycle started	26%	3%

Better pregnancy outcomes would have been obtained with TLC (Brigham et al, 1999)

Embryonic karyotype of abortuses in relation to the number of previous miscarriages

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Structured Abstract

Objective: To examine the frequency of chromosomal abnormalities in products of conception from patients with recurrent miscarriages in relation to the number of previous miscarriages.

Design: Retrospective analysis

Setting: Nagoya City University Medical Hospital

Patients: 1309 cases with a history of 2-20 consecutive first-trimester abortions.

Intervention: Chromosomal analysis performed on products of conception using a standard G-banding technique.

Main Outcome Measure: The frequencies of abnormal and normal embryonic karyotypes for each number of previous abortions were studied. The subsequent pregnancy outcome of patients whose previous miscarriages were karyotyped were studied along with the predictive value of karyotyping of previous miscarriages for subsequent miscarriages.

Results: The miscarriage rate increased with the number of previous spontaneous abortions. The frequency of abnormal embryonic karyotypes significantly decreased and that of normal embryonic karyotypes significantly increased with the number of previous abortions. 44 of 71 patients whose karyotypes were normal aborted subsequently and 23 of 60 patients whose karyotypes were abnormal aborted subsequently. Patients with a

previous normal embryonic karyotype aborted more frequently than those with an abnormal karyotype.

Conclusions: The frequency of normal embryonic karyotypes significantly increases with the number of previous abortions and a normal karyotype in a previous pregnancy is a predictor of subsequent miscarriage.

Key words: karyotype, recurrent miscarriage, immunotherapy, antiphospholipid antibody, abortus

Introduction

Cytogenetic evaluation of sporadic spontaneous abortions has shown that 50%-70% are chromosomally abnormal.1 It has been reported that there is a significantly increased risk of a chromosomally normal spontaneous abortion after a previous abortion with a normal karyotype.2 It seems to be common that normal karyotypes are associated with recurrent abortion,2, 3 but there is limited information about embryonic karyotype in series of patients suffering recurrent miscarriages.4-6 Stern et al. reported two studies concerning embryonic karyotypes in patients with recurrent abortions and found that no differences existed in the frequencies of abnormal karyotypes between single and recurrent aborters. However, previous studies only included a few cases with large numbers, 6 or more previous miscarriages, these being relatively rare.

Immunotherapy,7-9 prednisolone (PSL)-aspirin (ASA), heparin-aspirin therapy10-12 and high dose immunoglobulin (Ig)13, 14 are accepted worldwide as the most effective therapeutic approaches for recurrent miscarriages. If the treatment of other causes is successful, a normal embryonic karyotype would be expected to decrease with the number of previous miscarriages. We therefore studied this parameter in patients suffering 2-20 previous miscarriages who underwent immunological treatment.

Materials and Methods

Hysterosalpingography, chromosome analysis for both partners, immunologic tests for parameters such as natural killer activity15 and antiphospholipid antibodies (aPL; b2-glycoprotein I dependent anticardiolipin antibodies and lupus anticoagulant), blood tests for hyperthyroidism, diabetes mellitus, hyperprolactinemia and infections such as clamydia, were performed for 1309 patients with a history of 2-20 consecutive first-trimester abortions before subsequent pregnancy.

Patients with at least one kind of aPL were treated with ASA (40-81 mg/day), PSL (10-50 mg/day), heparin (10000 iu/ day) and/or Ig (5-20 g/day x 5 days).10-12

Those with a history of three or more miscarriages and with unexplained causes received treatments such as immunotherapy with x-irradiated paternal mononuclear cells7-9 and immunostimulation with a biological response modifier.16 Patients who had been treated with immunotherapy and failed, received treatments with Ig.13, 14

452 cases with a history of only 2 miscarriages received no medication.

All pregnancies were established between January 1986 and December 1997. The patients were admitted to Nagoya City University Hospital for rest for about 1 month at 4 weeks'

gestation to avoid possible external risk factors. Gestational age was calculated from basal body temperature charts. Ultrasonography was performed twice a week during pregnancy. D & C was carried out when miscarriages were diagnosed and the karyotypes of aborted conceptuses were ascertained using a standard G-banding technique, this parameter being financially supported by Nagoya City. Informed consent approved by the Institutional Review Board was obtained from all patients.

Similar analyses for 114 sporadic spontaneous abortions with no history of previous miscarriages were also performed (controls).

The miscarriage rate of subsequent pregnancies and the frequencies of abnormal and normal embryonic karyotypes with reference to the number of previous miscarriages were calculated. The subsequent pregnancy outcome of patients whose previous miscarriages were karyotyped and had no abnormal karyotype in either partner were studied and the predictive value of karyotyping of previous miscarriages for subsequent miscarriages were also studied.

Statistical Analysis

Data were analyzed by the Spearman's correlation coefficient using Stat View 4-0 and Fisher's exact probability using DA Stats on a Apple Macintosh computer. A significance level of $P < 0.05$ was applied for all tests.

Results

Of the 1309 cases 458 (35.0%) aborted and 234 of the aborted conceptuses (51.1%) could be karyotyped. Mean age increased with the number of previous abortions (Mean age 30.7Å}3.8, p=0.021). The miscarriage rate increased with the number of previous spontaneous abortions (p=0.0047). Miscarriage rate of patients with 6 or more previous abortions was over 50 %.

114 of the 234 (48.7 %) had normal and 120 (51.3 %) had abnormal chromosomes. The frequency of an abnormal embryonic karyotype significantly decreased with the number of previous abortions (p=0.013). While that of a normal embryonic karyotype significantly increased (p=0.011, Table 1). The similar results were found when cases with abnormal karyotypes in either partner were excluded.

27 of 114 sporadic abortions (23.7 %) analyzed had a normal karyotype. The incidence of karyotype normality in recurrent aborters was significantly higher than in controls (Table 2). The incidence of trisomy in sporadic abortions was significantly higher than in recurrent aborters.

44 of 71 patients whose karyotypes were normal miscarried subsequently as opposed to 23 of 60 patients with abnormal karyotypes. The patients with a previous normal embryonic karyotype miscarried significantly more frequently in subsequent pregnancies in our series of recurrent miscarriage cases (p=0.001).

Regarding aPL, 18 of 88 aPL-positive patients (20.5 %) miscarried in their subsequent

pregnancies. 4 of 10 patients (40 %) who were karyotyped had a normal embryonic karyotype (Table 3). There were no differences in incidence between aPL-positive patients and controls.

Discussion

It has been reported that abnormal chromosomes in either partner, antiphospholipid antibodies (aPL), uterine anomalies, luteal phase defects (LPD), diabetes mellitus and hyperthroidism cause recurrent miscarriages. A high prevalence of LPD in recurrent aborters have been meaning a cause. However, we recently found that pre-conceptional LPD is not predictive of subsequent pregnancy loss in patients with a history of two consecutive first trimester miscarriages.17 Also the abortion rate of patients with antinuclear antibodies is not significantly different from that without antinuclear and antiphospholipid antibodies.18 The evidence that diabetes mellitus, hyperthyroidism and uterine anomalies cause recurrent miscarriages is also controversial. Thus, the causes in many habitual aborters are unclear. An abnormal embryonic karyotype is one possible contributory factor but there have been few analyses of the percentage of abnormal and normal karyotypes of aborted concepti with reference to the number of previous miscarriages.

It has been reported that no differences exist in the frequencies of abnormal karyotypes between single and recurrent aborters.4, 5 In the present study of 1309 cases, however, the frequency significantly decreased with the number of previous abortions. This result provides support for the previous conjecture that a normal karyotype may predict subsequent normal karyotype abortions.2 Our study included severe cases with 10 or more previous miscarriages. Although few in number the proportion whose karyotypes were abnormal was low, suggesting the existence of unexplained causes for their miscarriages.

Cytogenetic evaluation of sporadic spontaneous abortions has shown that 50%-70% are chromosomally abnormal.1 The prevalence of miscarriage has been estimated to be between 10%-15% of all clinically recognized pregnancies.19 This means that about 5%-10.5% of all pregnancies result in sporadic abortions caused by chromosomal abnormalities. Ogasawara et al. "resubmit" No. 10

Estimated abnormal and normal embryonic karyotype rate were calculated (normal and abnormal karyotype rate times miscarriage rate) and shown in Figure 1. The abnormal karyotype rate did not change and the mean rate in each number of previous abortion was 18.3% and the normal karyotype rate also significantly increased with the number of previous miscarriages ($p=0.0063$).

The percentage of patients whose pregnancies were karyotyped was only 51.1 %, one reason for this low value being that materials were contaminated in tissue culture. We try to perform karyotyping as often as possible and this is financially supported by Nagoya City. Another reason why we could not perform karyotyping was that when miscarriages occurred on holidays or in the night when technical assistance is not available. There is a bias in this study because it concerns clinical data from 1986 to 1997.

The estimated abnormal karyotype rate did not change with the number of previous miscarriages and the mean rate was 18.3% in patients with recurrent miscarriages. Karyotype abnormalities can be speculated to happen to occur "spontaneously" even if either partner has no abnormal karyotype.1-3 "Accidents" must occur even with large

number of miscarriages. This means that about 20% of pregnancies in recurrent aborters result in miscarriages caused by abnormal embryonic karyotypes independent of the number of previous abortions. This suggests that the maximum success rate would be about 80 % were the treatment perfect.
With regard to aPL, a number of researchers have provided evidence of predictive value for a recurrent miscarriages using conventional ELISA methods, lupus anticoagulant and/ or b2glycoprotein I dependent anticardiolipin antibodies.20 In the 1990's ASA alone, or heparin combined with ASA and immunoglobulin have been considered very useful, but the most appropriate regimen has yet to be established.11, 12 Takakuwa et al. reported that the incidence of chromosomal abnormalities in anticardiolipin antibodies-positive recurrent aborters, though they were not treated, was 20 % (2 of 10 cases).21

In the present study, the abnormal karyotype rate of aPL-positive recurrent aborters was 60 % (6 of 10) and there were no differences between aPL-positive patients and controls. It is speculated that the treatment might be optimal for aPL-positive patients.

Immunotherapy with paternal mononuclear cells is frequent worldwide for recurrent aborters with unknown causes.7-9 However, the mechanisms underlying the beneficial effects are uncertain, and it is necessary to reconsider its true effectiveness. In 1994 a worldwide collaborative observational study revealed that allogenic leukocyte immunotherapy is effective for about 10 % of recurrent spontaneous abortion cases with unknown causes.9 The relatively low success rate is speculated to be due to the lack of allogenic parameters predicting subsequent pregnancy loss so that it is impossible to choose cases suitable for immunotherapy.

Immunoglobulin also is reported to be useful for treatment of patients with 5 or more miscarriages.13, 14 However, this approach is not only drastic but also expensive.

In the present study, the normal karyotype rate significantly increased with the number of previous miscarriages. However, cases with an apparently normal embryonic karyotype could have gene abnormalities such as the T/t complex.22 Patients with large number of miscarriages may have not been characterized by "accident" but rather by "inevitabilityÅh

Our patients with 3 or more previous miscarriages usually received medication in line with their wishes. It is clear that this introduced bias because only some patients were given ASA, IgG or immunotherapy. We should exclude patients with medication but there were insufficient such cases with 3 or more previous miscarriages. A normal karyotype increased in spite of medication. Such a tendency would be speculated to be enhanced if patients receive no medication. However, if the treatment were successful this would have increased the proportion of abnormality related abortions and therefore we believe that this study indeed has significance.

When the embryonic karyotype is normal after treatment of miscarriages, we should reconsider whether the therapy was appropriate and other causes of miscarriages in individuals experiencing 6 or more unexplained miscarriages.

Thus, the fact that the frequency in fact increased suggests that therapeutic approaches now accepted worldwide are not sufficiently efficacious or other causes of miscarriages such as gene abnormalities are responsible.

To close this report with another message, final goal of the treatment success rate for recurrent miscarriages may be estimated at around 80 % because the miscarriage rate caused by abnormal embryonic karyotypes is approximately 18 %.

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Table 1 Miscarriage and normal embrionic karyotype rates for treated recurrent miscarriage cases

Number of previous spontaneous abortions	%Normal karyotype rate*	% Miscarriage rate	Age (mean+SD)
2	36.4 (20 / 55)	23.2 (105 / 452)	29.4Å}3.8
3	41.0 (32 / 78)	32.4 (149 / 460)	30.6Å}3.6
4	44.7 (17 / 38)	37.0 (71 / 192)	31.4Å}3.9
5	61.1 (11 / 18)	48.7 (38 / 78)	32.5Å}3.6
6	71.4 (10 /14)	64.1 (25 / 39)	32.8Å}4.1
7	50.0 (4 / 8)	66.7 (16 / 24)	31.3Å}2.8
8	100.0 (7 / 7)	70.6 (12 / 17)	31.9Å}2.9
9	71.4 (5 / 7)	78.6 (11 / 14)	33.4Å}2.5
10-20	89.0 (8 / 9)	93.9 (31 / 33)	34.4Å}2.8

*The normal karyotype rate significantly decreased with the number of previous spontaneous abortions (p=0.011).

Table 2 Embrionic karyotype in 114 sporadic spontaneous abortions and 234 recurrent miscarriages

Embryonic karyotype	Sporadic spontaneous abortions	Recurrent miscarriages	Significance
Normal	27 (23.7 %)	114 (48.7 %)	p=0.000014
Abnormal	87 (72.3 %)	120 (51.3 %)	
Trisomy	63 (72.4 %)	63 (52.5 %)	p=0.0059
Double trisomy	0 (0 %)	7 (5.8 %)	p=0.024
Monosomy	5 (5.7 %)	5 (4.2 %)	NS
Triploidy	14 (16.1 %)	18 (15.0 %)	NS
Others	5 (5.7 %)	27 (22.5 %)	

Table 3 Karyotype rates for 88 treated recurrent miscarriage cases with antiphospholipid antibody

Success	70 cases (79.5%)
Abortion	18 cases (20.5%)
Abnormal karyotype	6 cases (60%)
Normal karyotype	4 cases (40%)

The incidence of karyotype abnormalities in antiphospholipid antibody-positive recurrent aborters was 60% and that of controls was 72.3% (No differences).

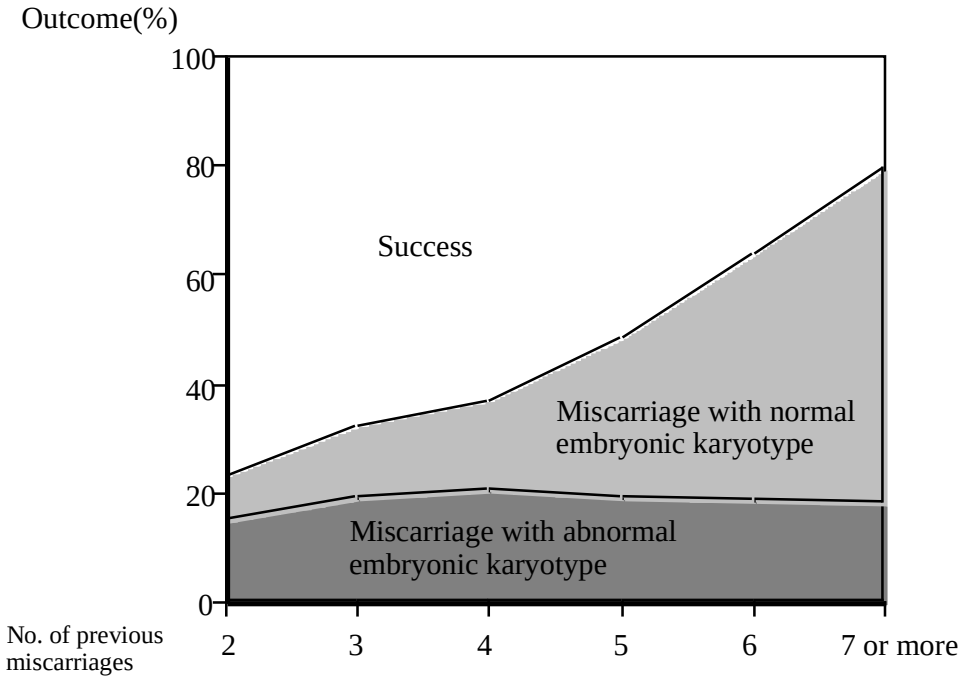

Figure 1**: Estimated miscarriage rate with normal and abnormal embryonic karyotypes if analyzed rate would be 100%.**
The normal karyotype rate significantly increased with the number of previous miscarriages. The abnormal karyotype rate did not change with the number of previous miscarriages.

NOTES

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Embryoscopic and cytogenetic analysis of 233 missed abortions: factors involved in the pathogenesis of developmental defects of early failed pregnancies

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BACKGROUND: While chromosomal abnormalities are often the cause of missed abortions, other defects could be involved, which might be screened for by transcervical embryoscopy. METHODS: A total of 272 patients with missed abortion underwent transcervical embryoscopy prior to dilatation and curettage, together with cytogenetic analysis of chorionic villi, using either standard G-banding cytogenetic techniques or comparative genomic hybridization in combination with flow cytometry analysis. RESULTS: Visualization of the embryo or early fetus (12 cases) was successful in 233 patients, and karyotyping in 221. Among 233 examined cases, 33 had normal external features, 71 were classified as growth-disorganized and 129 had either isolated or multiple defects, including holoprosencephaly, anencephaly, encephalocele, spina bifida, microcephaly, facial dysplasia, limb reduction defect, cleft hand, syndactyly, pseudosyndactly, polydactyly, various forms of cleft lip and an amniotic adhesion. Of the 165 cases with an abnormal karyotype, there were 46 grossly disorganized embryos, 98 multiple defects, six single defects and 15 morphologically normal cases. Of the 56 cases with a normal karyotype, there were 20 grossly disorganized embryos, 16 multiple defects, four single defects and 16 morphologically normal cases. CONCLUSIONS: A total of 75% of the cases with missed abortion had an abnormal karyotype, 18% had a morphological defect with a normal karyotype, while no embryonic or chromosomal abnormality could be diagnosed in 7% of the cases. Correlation of morphological and cytogenetic findings in spontaneous abortion specimens could provide valuable information for genetic counselling and prenatal care in future pregnancies in couples with a history of repeated pregnancy loss.

Key words: chromosome abnormalities/developmental defects/missed abortion

Introduction

Approximately 15% of all clinically recognized pregnancies are spontaneously aborted and ~60–70% of these are attributable to detectable chromosome abnormalities (Tariverdian and Paul, 1999).

Although the incidence of first trimester losses is high, spontaneous abortion material is often poorly described from a developmental perspective. More than one-half of early spontaneous abortion specimens contain no embryonic/fetal parts. If an embryo is present at all, it is often either severely damaged or fragmented (Kalousek, 1987). Transcervical embryoscopy in cases of missed abortion is a new technique that allows direct visualization of the dead embryo *in utero*, unaffected by the damage caused by either instrumental evacuation or spontaneous passage.

With respect to the various possible aetiological factors of developmental defects in early abortion specimens, cytogenetic analysis is an important component in the assessment of human malformation in early failed pregnancies. The detection of aneuploidy/polyploidy provides a causal explanation for the observed developmental defect and also indicates that the risk of recurrence of the observed developmental defect and chromosomal abnormality in these couples is not substantially increased (Warburton *et al*., 1987).

We have previously reported the detection of 48 growth-disorganized embryos in cases of embryoscopically examined missed abortion (Philipp and Kalousek, 2002). Ten selected cases of embryonic neural tube defect documented that the technique of embryoscopy offers the possibility of accurately diagnosing developmental defects in cases of early pregnancy loss (Philipp and Kalousek, 2001a,b,c).

The objective of this study was to estimate the frequency of a chromosomal abnormality or hitherto unexplained mechanism in the pathogenesis of external structural abnormalities of the

Table I. Summary of specimen morphology and karyotypic outcome in 233 missed abortions

Morphology	Total specimens		Total specimens successfully karyoytyped		Specimens with abnormal karyoytype	
	No.	%[a]	No.	%[b]	No.	%[c]
Normal	33	14.2	31	93.9	15	48.4
Growth disorganization	71	30.5	66	93.0	46	69.7
Combined defects	119	51.1	114	95.8	98	86.0
Isolated defects	10	4.3	10	100	6	60.0
Total	233	100	221	94.8	165	74.7

[a]Percentage of total number of specimens with that morphology.
[b]Percentage of each morphological category successfully karyotyped.
[c]Percentage of each morphological category with an abnormal karyotype.

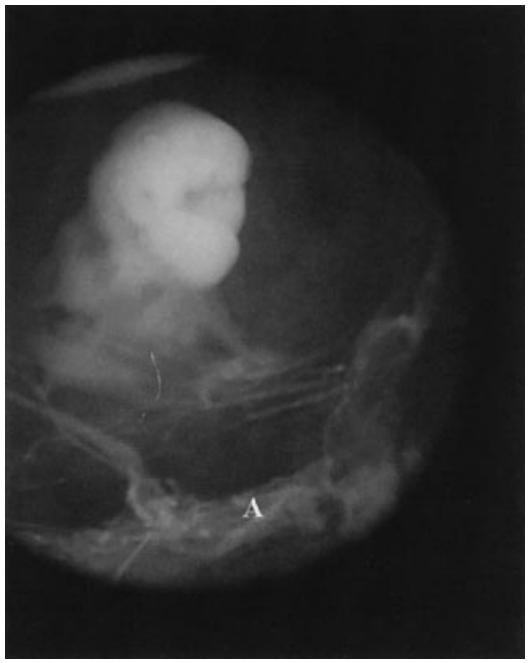


Figure 1. Close-up of a 4 mm crown–rump length growth-disorganized (GD) embryo. The GD 2 embryo showed no recognizable external structures after the amnion (A) was opened. Cytogenetically, trisomy 16 (47,XX,+16) was diagnosed.

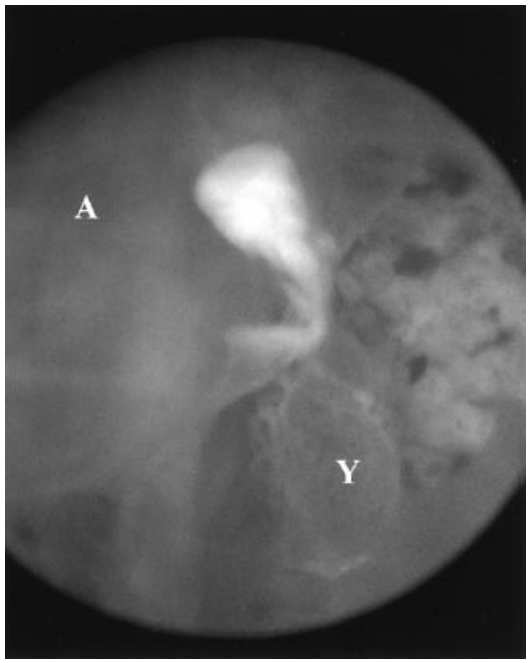


Figure 2. A growth-disorganized (GD) 2 embryo, with a crown–rump length of 6 mm, in the intact amniotic sac (A). The yolk sac (Y) is clearly discernible. A normal karyotype was diagnosed cytogenetically (46,XY).

first trimester conceptus. Indications for more extensive morphological examination of first trimester abortion specimens are discussed.

Materials and methods

A missed abortion was diagnosed in a total of 272 patients. The condition was established by sonography and the women were scheduled for elective dilatation and curettage (D&C) at the Danube Hospital, Vienna between April 1999 and September 2002. All of these cases were included in the present study which was approved by the ethics committee of the hospital. Informed consent for embryoscopy was obtained from all patients. The diagnosis of missed abortion was based on sonographic demonstration of an embryo or early fetus without cardiac activity on transvaginal ultrasonography (7.5 MHz transvaginal probe). The threshold separating embryos from fetuses was set at 30 mm crown–rump (CRL), which corresponds to ~8 completed weeks of development.

Accurate diagnosis of a specific defect present in an embryo or early fetus depends on correct evaluation of the developmental stage. The term gestational age, used in clinical terminology and ultrasound, was not used in this study of missed abortions, as most of these specimens were retained *in utero*. Instead, the term the developmental age (DA) was used. The actual DA was derived from the CRL, measured by ultrasonography, and from the developmental stage assessed by embryoscopy (Moore, 1993).

All patients were given general anaesthesia and placed in a dorsal lithotomy position. After careful dilatation of the cervix, the rigid hysteroscope (12° angle of view with both biopsy and irrigation working channel, Circon Ch 25–8 mm) was inserted transcervically into the uterine cavity and the implantation site of the pregnancy was visualized. Continuous normal saline flow was used throughout the procedure (pressure ranging from 40 to 120 mmHg) to clean the operative field. The chorion was opened with microscissors (CH 7–2 mm) and the embryo was initially viewed through the amnion. The amnion was then carefully opened using the microscissors to obtain a

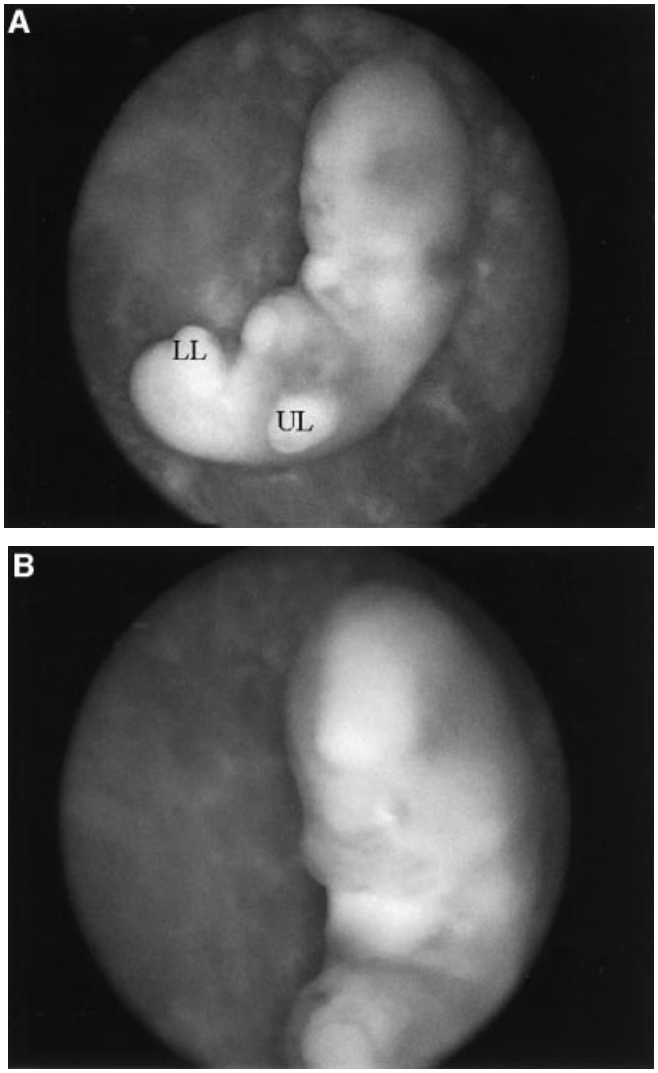


Figure 3. Lateral (**A**) and close-up of the face (**B**) of a trisomy 4 (47,XX,+4) growth-disorganized (GD) embryo. The GD4 embryo 11 mm in length shows a small head and a dysplastic face. There is evidence of upper (UL) and lower (LL) limb growth retardation relative to the crown–rump length.

detailed view of the embryo. A complete examination of the conceptus included visualization of the head, face, dorsal and ventral walls, limbs and umbilical cord. All procedures were viewed on a TV monitor by connecting a video camera (3-CCD Colour Camera, Circon Microdigital III) to the eyepiece of the endoscope, and were recorded for future analysis. Video-documentation of embryoscopically detected abnormalities helped investigators to cooperate with an experienced embryopathologist.

The embryoscopic findings were classified into four categories: (i) normal development; (ii) growth-disorganized embryos; (iii) specimens with multiple external defects; (iv) specimens with isolated external defects. Growth-disorganized embryos were further subdivided, based on their degree of disorganization (Poland *et al*., 1981).

After evacuation of the uterus, chorionic villi were separated from decidual contamination and blood clots, cultured and analysed cytogenetically using standard G-banding cytogenetic techniques. Comparative genomic hybridization in combination with flow cytometry analysis (CGH/FCM) of paraffin-embedded or frozen placental tissue was performed in 51 cases in which traditional cytogenetic analysis had failed to provide results (Lomax *et al*., 2000).

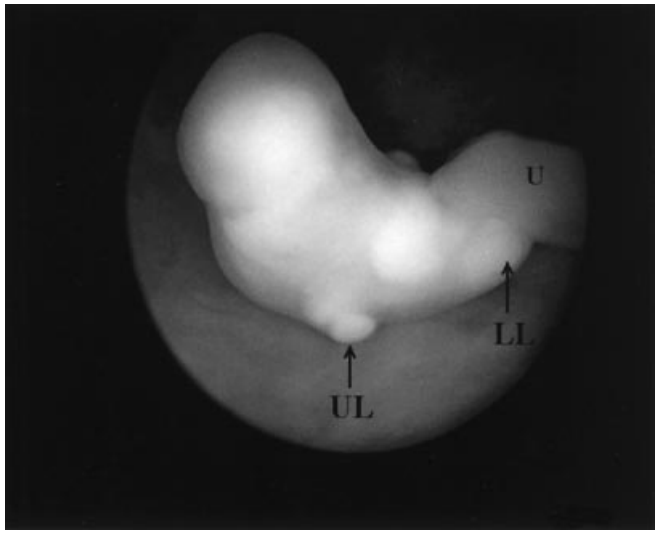


Figure 4. Lateral view of a growth-disorganized (GD) 4 embryo with a crown-rump length of 10 mm. Retarded upper (UL) and lower (LL) limb bud development is visible; no facial structures can be seen. U = umbilical cord. A normal karyotype was diagnosed cytogenetically (46,XY).

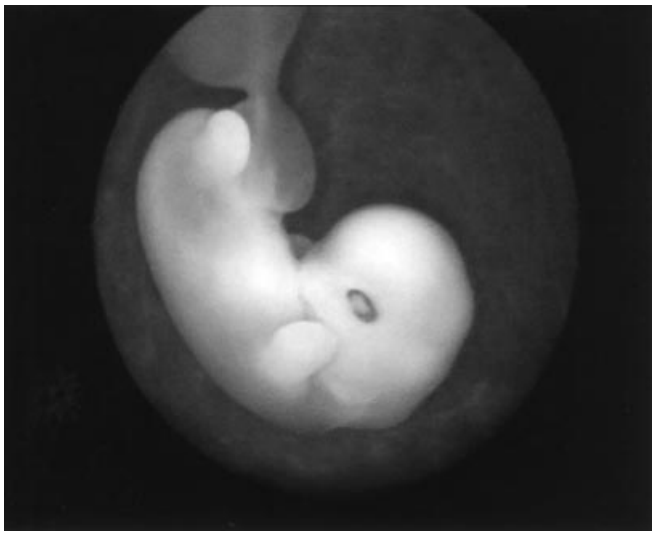


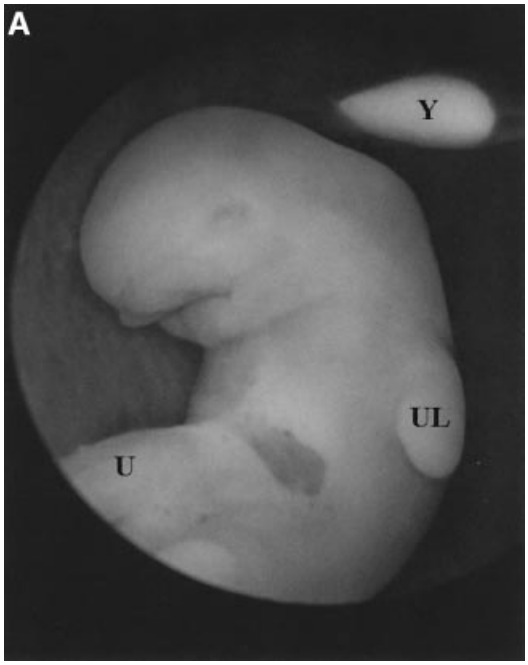
Figure 5. Lateral view of a microcephalic embryo 16 mm in length with fusion of the face to chest and retarded limb development. The karyotype showed tetraploidy (92,XXYY).

Results

The procedure of embryoscopy required an average of 10 min (range 3–25). A complete anatomical survey was possible in 233 cases.

In 15 cases the embryonic structure could not be identified after the chorion had been opened and in 24 cases a complete evaluation of the embryo was not possible because the investigator's vision was obscured. The causes were bleeding, a tight amniotic sac, or a short umbilical cord closely attaching the embryo to the chorionic plate and therefore hindering the examination.

Table I provides a general description of 233 studied cases. Fifteen of these were early fetuses, with a CRL of >30 mm



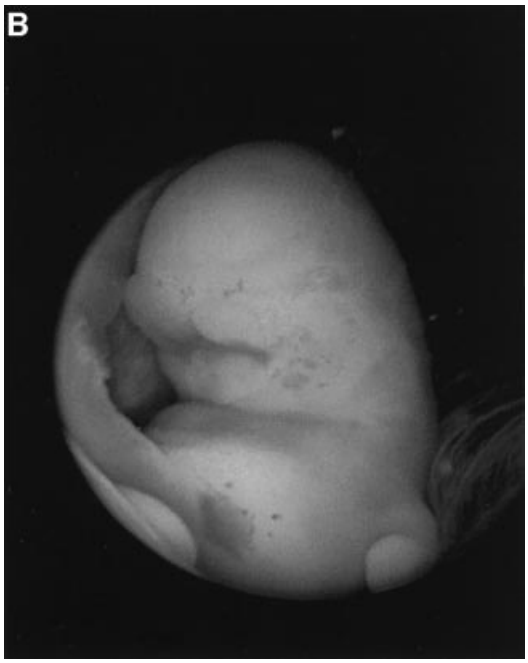
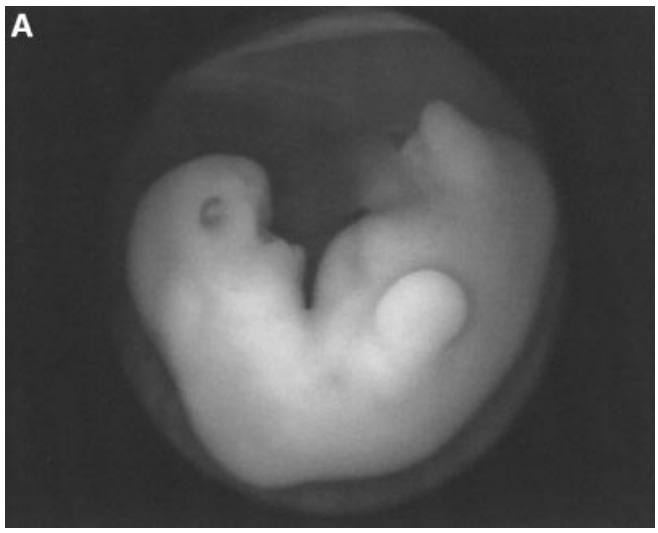


Figure 6. Anterolateral view (**A**) and close-up (**B**) of a trisomy 22 (47,XX,+22) embryo. External developmental defects of the 14 mm embryo are severe microcephaly, facial dysplasia, retarded upper (UL) and lower limb (LL) development. The dark area close to the umbilical cord (U) is due to necrosis. Y = yolk sac.



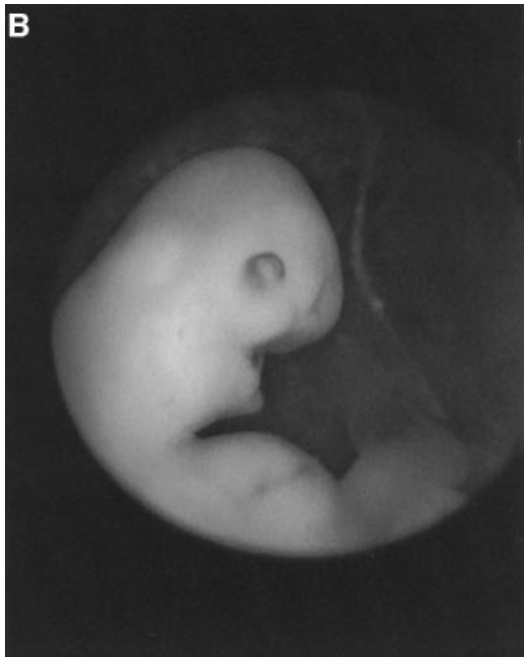


Figure 7. (Case 18, Table III). Lateral view (**A**) and close-up (**B**) of a microcephalic embryo with a crown–rump length (CRL) length of 14 mm. A dysplastic face is visible. Limbs are paddle-shaped, indicating retarded development relative to CRL. Chromosome analysis revealed a normal (46,XY) karyotype.

(range 32–57). Table I shows that no external abnormalities were found in 33 cases (14%), whereas abnormal development was seen in 200 (86%) missed abortions. Among the abnormal cases, embryonic growth disorganization (GD2–4) was reported in 71 cases. GD2 embryos showed embryonic tissue 3–5 mm in length. These conceptuses had no recognizable external embryonic landmarks and no retinal pigment (Figure 1 and Figure 2). GD3 embryos were ⩽10 mm long, lacked limb buds but retinal pigment was often present. A cephalic and caudal pole could be distinguished. GD4 embryos had a CRL of >10 mm with a discernible head, trunk and limb buds. The limb buds showed marked retardation in development and the development of the facial structures was highly abnormal (Figures 3 and 4).

A total of 119 cases showed no disorganization of development, but had severe combined developmental defects such as: (i) fusion of the face to the chest in combination with microcephaly and retarded limb development (13 cases) (Figure 5), (ii) severe microcephaly, facial dysgenesis, retarded limb development and often a short umbilical cord (41 cases) (Figures 6 and 7), (iii) microcephaly and retarded limb

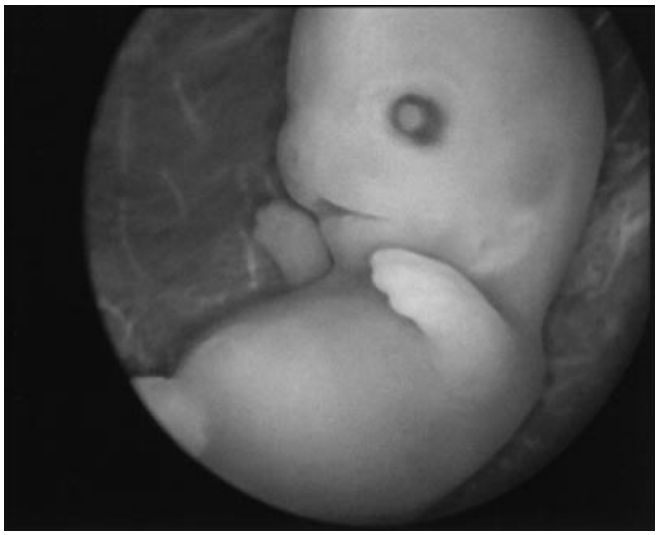


Figure 8. Anterolateral view of a microcephalic 45,X embryo with a crown–rump length of 25 mm. Distinct grooves are formed between the fingers, but the digits are not separated and the upper limbs are not bent at the elbows, indicating retarded development for an embryo of this size.

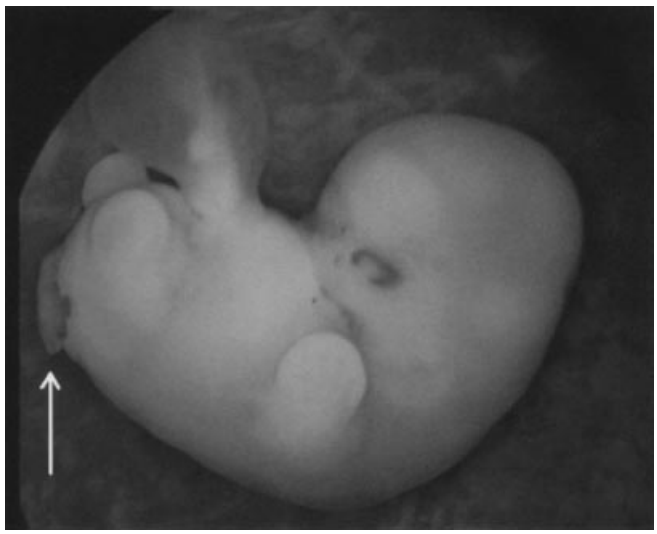


Figure 9. Lateral view of a triploid embryo (69,XXY) 15 mm in length. A large neural tube defect involving the lumbosacral area (arrow) is present. There is evidence of upper limb growth retardation relative to the crown–rump length. The face is fused to the abdominal wall. The dark brown area in the frontal region is due to necrosis. Herniation of the mid-gut into the umbilical cord is still physiological at this stage of development.

development (32 cases) (Figure 8) and (iv) specific developmental defects similar to those seen in fetuses or newborns (30 cases) (Figures 9, Figure 10 and Figure 11). These specific defects were all associated with other developmental defects such as microcephaly, facial dysgenesis, delayed limb development and face-to-chest fusion, and included holoprosencephaly (one case), anencephaly (two cases), encephalocele (10 cases), spina bifida (10 cases), various forms of cleft lip (three cases), limb reduction defect (two cases), cleft hand (one case) and an amniotic adhesion (one case).

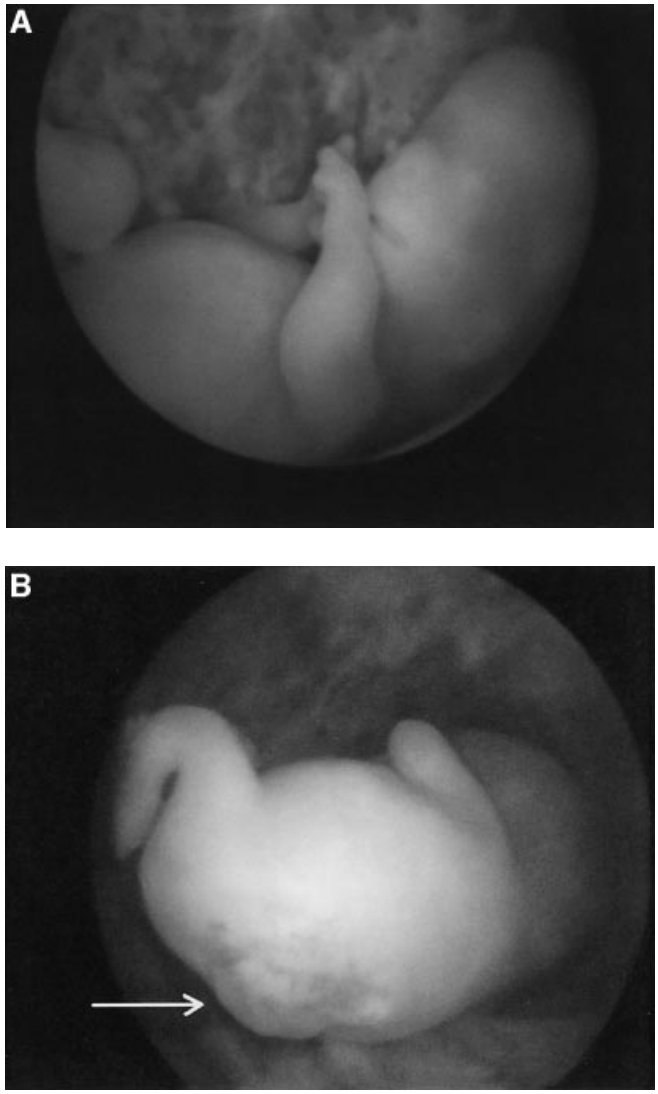


Figure 10. (Case 8, Table III.) Lateral (**A**) and posterior view (**B**) of an embryo with a crown–rump length of 28 mm. Note the absence of normally developed eyes of the microcephalic embryo (**A**). A spina bifida involving the lumbar area (arrow) is present (**B**). The karyotype was normal (46,XY).

In three cases amniotic bands caused combined defects which were discernible on embryoscopy. The spectrum of defects seen in one embryo and two early fetuses with amniotic band syndrome included constrictions of the digits, pseudosyndactyly due to wrapping of fingers and toes, umbilical cord stricture, gastroschisis and omphalocele.

Ten specimens had isolated developmental defects (Figure 12) including anencephaly (one case), microcephaly (two cases), polydactyly (one case), limb reduction defect (one case) and retarded development of the limbs (five cases).

Of the 233 cases studied on embryoscopy, a successful cytogenetic evaluation was performed in 221 cases (95%; Table I). A total of 165 (75%) specimens were abnormal, of which 101 (61%) were trisomic, 37 (22%) monosomic X, 19 (12%) polyploid and eight (5%) were structural chromosome anomalies. Trisomies for all chromosomes with the exception

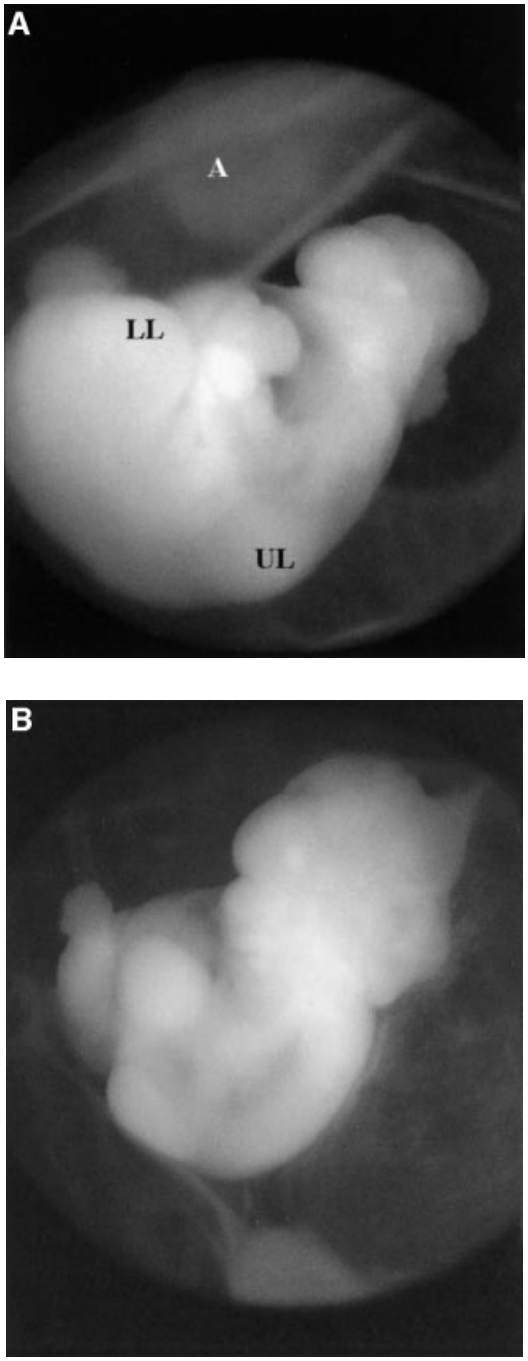


Figure 11. Caudo-lateral (**A**) and lateral (**B**) view of a trisomy 7 (47,XX,+7) embryo with anencephaly. The exposed brain tissue of the 8 mm long embryo is still intact. Upper (UL) and lower limb (LL) development appears to be retarded in relation to the size of the embryo. 'A' marks remnants of the amniotic membrane.

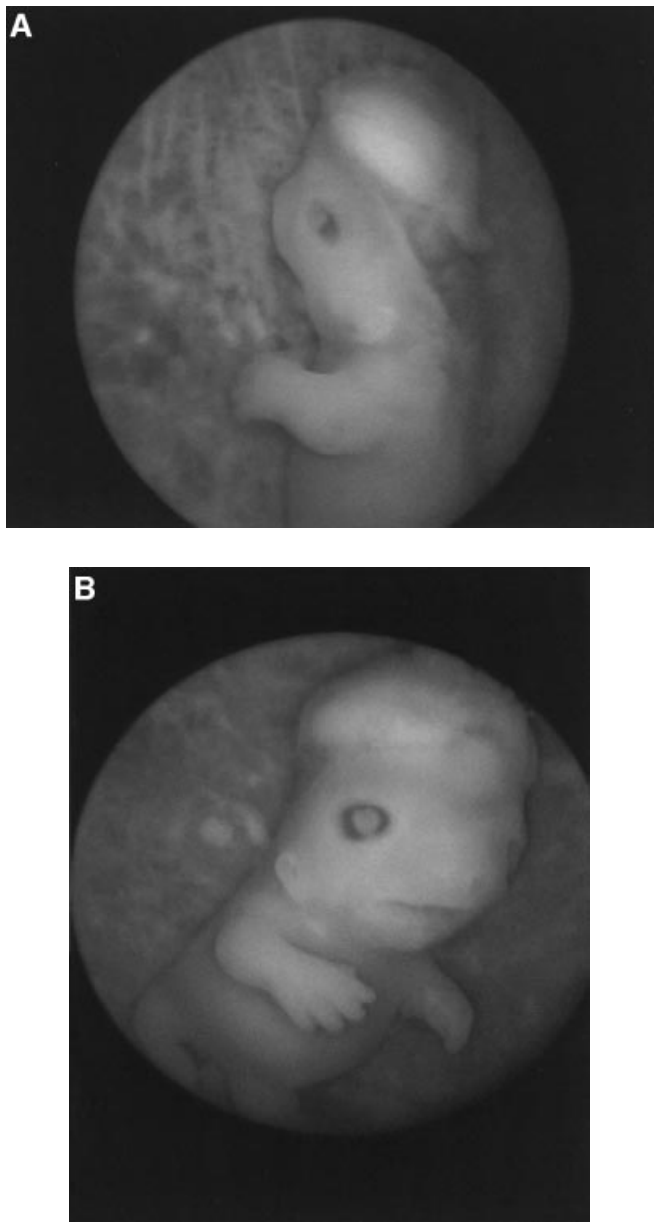


Figure 12. (Case 17, Table III.) Close-ups of an embryo with cranio-rachischisis, 22 mm in length. Lateral (**A**) view of the upper portion shows the extent of the lesion, leaving a mass of proliferating neural tissue over the cranial structures (**B**). A normal karyotype was diagnosed cytogenetically (46,XX).

of chromosomes 1, 5 and 19 were observed. The most common trisomy was 15 (17 cases), followed by trisomies 16 (16 cases), 21 (15 cases), 22 (14 cases), 14 (seven cases), 13 (five cases), 8 (five cases) and 9 (five cases). Correlations of morphology and specific cytogenetic findings are shown in Table II.

The highest rate of chromosome anomalies was found in the category of 119 conceptuses with combined developmental defects. A successful cytogenetic evaluation in this subgroup was performed in 114 cases. Chromosomal abnormalities were found in 98 cases (86%; Table I). Specific cytogenetic findings among abortuses with severe combined developmental defects are listed in Table II.

Among the 71 grossly disorganized embryos, 66 could be analysed cytogenetically. Of these, 46 (70%; Table I) were cytogenetically abnormal; the data are shown in Table II.

The lowest rate of chromosomal abnormality was found in phenotypically normal specimens and in specimens with isolated defects (see Tables I and II). Of 33 cases with normal external features, 31 could be analysed cytogenetically. Cytogenetic results showed abnormality in 15/31 (48%) cases

Table II. Summary of cytogenetic findings among 33 cases with normal external features, 71 growth-disorganized embryos, 119 specimens with severe combined developmental defects and 10 cases with isolated developmental defects

Karyotype	No external embryonic abnormalities	Growth disorganization	Combined developmental defects	Isolated developmental defect
XX/XY	16	20	16	4
Trisomy 2		1		
Trisomy 3		1		
Trisomy 4		3	1	
Trisomy 6		1		
Trisomy 7			1	
Trisomy 8		4	1	
Trisomy 9			5	
Trisomy 10		1		
Trisomy 11		1		
Trisomy 12		3		
Trisomy 13	1		3	1
Trisomy 14		1	6	
Trisomy 15		1	16	
Trisomy 16		16		
Trisomy 17		1		
Trisomy 18	1			
Trisomy 20	1	1		
Trisomy 21	9		6	
Trisomy 22		7	7	
Triploidy	1	3	11	1
Tetraploidy			3	
45,X			33	4
Structural defect	2	1	5	
No cytogenetic results available	2	5	5	
Total	33	71	119	10

in this subgroup. Six of 10 specimens with isolated defects showed chromosomal abnormalities (60%).

Discussion

The morphological features of a consecutive series of 233 missed abortions are described in this report.

Of 165 cases with an abnormal karyotype, 150 (91%) showed abnormal development (46 GD embryos, 98 multiple defects, six single defects) and in 15 cases no external embryonic abnormalities could be detected on embryoscopy. The grossly abnormal development documented by embryoscopy in the majority of these aneuploid specimens suggests a severe disturbance in their early development and shows that early stages of human development are particularly vulnerable to genetic disorders.

Of the 56 cases with a normal karyotype, no external embryonic abnormalities could be detected in 16 cases, whereas amniotic bands (cases 2, 10, 13; Table III) interfered with normal embryonic development in three cases.

Thus, there were 37 cases (20 growth disorganized embryos, 13 specimens with multiple developmental defects and four cases with isolated defects) with an apparently normal karyotype and a maldevelopment similar to that resulting from aneuploid syndromes, without the diagnosis of a specific pathogenetic mechanism. Table III provides a detailed morphological description of 13 cases with combined defects (cases 1, 4, 6–9, 11, 12, 14, 15, 18–20) and four specimens with isolated defects (cases 3, 5, 16, 17) and an apparently normal karyotype.

Embryonic development is a precisely choreographed event of programmed developmental steps, requiring many genes to regulate growth and morphogenesis. The grossly abnormal development documented by embryoscopy in these cases with apparently normal chromosomes was as severe as that resulting from an aneuploidy. They might have been due to genetic lesions that prevent normal embryogenesis and are undetectable by the techniques used in the present study.

These factors are usually not considered to be aetiologically related to early pregnancy loss, as there has been a tendency in the past to assume that if no laboratory test confirms the presence of a genetic disorder, one should search for non-genetic causes.

Embryoscopy in cases of missed abortion might reveal subtle morphological abnormalities undetectable by ultrasound (Blaas, 1999) and expand the diagnostic spectrum used for the evaluation of reproductive loss. This technique could establish a highly characterized cohort of abortion specimens with apparently normal chromosomes as a starting point for further detailed genetic studies. Such studies are needed to reach a better understanding of embryopathogenesis and, consequently, of early pregnancy loss itself.

Whether embryoscopy and cytogenetic studies should be offered to all women with missed abortion is debatable. This policy has the advantage of providing comprehensive aetiological data, but has the disadvantage of requiring an invasive

Table III. Summary of embryoscopic and clinical data of 16 specimens with severe combined developmental defects, and four embryos with isolated developmental defects and an apparently normal karyotype

Case no.	CRL[a] (mm)	Karyotype	Description	Maternal age (years)	Parity	Spontaneous abortions
1[b]	19	46,XX	Macerated microcephalic embryo with retarded limb development, mid-line brownish pigmentation in the frontal region, umbilical cord cyst	28	1	–
2[c]	35	46,XX	Early fetus with amnion adhesion at the tip of the nose, strands of amnion wrapped around the terminal phalanges of both feet	30	2	–
3	26	46,XY	Macerated microcephalic embryo with no other apparent abnormalities	40	–	1
4	16	46,XY	Macerated embryo, severe microcephaly, facial dysplasia, absence of cervical flexion, retarded limb development, bilateral cleft lip	37	3	–
5	23	46,XY	Macerated well–preserved embryo with generalized oedema, severe microcephaly and an unusually large physiological umbilical hernia	38	1	–
6	10	46,XY	Microcephalic embryo closely attached to the amnion, fusion face to the chest, retarded limb development	24	–	–
7	10	46,XX	Macerated embryo, severe microcephaly, facial dysplasia, retarded limb development	32	–	–
8	28	46,XY	Microcephalic embryo with no eyes, large open neural tube defect of the lumbar spine	35	–	2
9	17	46,XX	Microcephalic embryo with a dysplastic face and retarded limb development	29	3	–
10	21	46,XX	Fine amniotic bands wrapping the digits of both hands, umbilical cord stricture, gastroschisis	17	–	–
11	21	46,XY	Microcephaly, parietal encephalocele, limb reduction defect affecting all limbs	23	–	1
12	20	46,XX	Macerated microcephalic embryo with a dysplastic midface and a large frontal encephalocele	35	–	–
13	39	46,XX	Early fetus with a large omphalocele, strands of amnion wrapped around the terminal phalanges of the right hand, constricting band around the umbilical cord	20	1	–
14	16	46,XY	Microcephaly, fusion of the face to the chest, retarded limb development	30	–	–
15	12	46,XX	Microcephaly, fusion of the face to the chest, retarded limb development	29	–	–
16	24	46,XY	Transverse limb reduction defect affecting digit IV of both hands	28	–	1
17	22	46,XX	Anencephaly with spinal rachischisis	34	3	–
18	14	46,XY	Macerated embryo, severe microcephaly, facial dysplasia, retarded limb development	31	1	1
19	11	46,XX	Microcephalic embryo with retarded limb development and a large neural tube defect involving the lumbosacral area	37	–	–
20	16	46,XX	Microcephaly, fusion of the face to the chest, retarded limb development	24	–	1

[a]Crown–rump length.
[b]Also reported in Philipp and Kalousek (2001c).
[c]Also reported in Philipp and Kalousek (2001a).

procedure and of inducing extra costs for the management of a condition with a low risk of recurrence.

However, a detailed embryoscopic examination of the dead embryo is likely to be useful in couples who have experienced recurrent abortion. In such cases, chromosome analysis is generally recommended (Wolf and Horger, 1995), and an elevated risk of birth defects in subsequent pregnancies was recorded (Khoury and Erickson, 1993).Therefore, transcervical embryoscopy could be indicated prior to D&C or medically induced abortion (Blanch *et al*., 1998; Lelaider *et al*., 1993).

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Abnormal embryonic development diagnosed embryoscopically in early intrauterine deaths after in vitro fertilization: a preliminary report of 23 cases

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Objective: To provide data about the phenotypic appearance of the embryo of early failed pregnancies after IVF.

Design: Clinical prospective descriptive study.

Setting: Tertiary care center.

Patient(s): Twenty-three women who had conceived by IVF and had a missed abortion before 12 weeks of gestation.

Intervention(s): Embryoscopic examination of the embryo before curettage. Cytogenetic analysis of the chorionic villi by standard G-banding cytogenetic techniques or by comparative genomic hybridization in combination with flow cytometry analysis.

Main Outcome Measure(s): Embryonic phenotype and karyotype were determined.

Result(s): Twenty-one of 23 IVF embryos showed structural defects on embryoscopic examination. Seventeen of 23 specimens had a chromosomal abnormality. The majority were numerical aberrations such as monosomy X (2 cases). Trisomies for chromosomes 18 (one case), 16 (three cases), 15 (one case), 14 (two cases), 13 (one case), 12 (one case), 11 (one case), 10 (one case), 9 (one case), 8 (one case), and 3 (one case) were observed. A structural chromosome anomaly leading to a chromosomal trisomy was observed in one case. Aneuploidy explained the grossly abnormal embryonic development documented by embryoscopy in 15 of 21 cases.

Conclusion(s): Aneuploidy is the major factor affecting normal embryonic development in missed abortions after IVF. Further investigation is needed to elucidate mechanisms that might prevent normal embryogenesis but evade detection by the cytogenetic techniques used in the present study. (Fertil Steril® 2004;82:1337–42. ©2004 by American Society for Reproductive Medicine.)

Key Words: Abnormal embryonic development, chromosome abnormalities, in vitro fertilization, missed abortion, transcervical embryoscopy

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About 20% of clinically recognized pregnancies are aborted, the majority of these being early spontaneous pregnancy losses before 12 weeks of gestation (1). Although the incidence of clinical abortions after IVF is equally high (2, 3), little is known about whether embryonic maldevelopment is a contributing factor for embryonic loss after IVF. Morphological studies of the dead embryo are difficult to perform. On account of its minute size, the embryo is rarely available in spontaneous abortion specimens. Macerated embryos are especially fragile, and mechanical trauma, occurring either during spontaneous passage or instrumental evacuation of the uterus, leads to further destruction and subsequent loss of the embryonic parts. Transcervical embryoscopy in cases of missed abortion (4) is a technique that allows direct visualization of the dead embryo in utero, unaffected by the damage caused by either instrumental evacuation or spontaneous passage.

In the present study, localized and systemic defects diagnosed embryoscopically in the embryonic morphogenesis of 23 missed abortions

resulting from IVF are described. The findings are supplemented by the results of cytogenetic analysis in all cases. Factors that might influence a positive outcome of pregnancy after IVF and possible implications for future preimplantation genetic diagnosis (PGD) protocols for aneuploidy screening in patients undergoing IVF treatment are discussed.

MATERIALS AND METHODS

The study population included 23 women who had conceived by IVF and had suffered a first-trimester missed abortion. Only abortuses with ultrasonographic evidence of a dead embryo were included in this study. The 23 patients had been transferred from an IVF center for detailed embryoscopic and cytogenetic evaluation of the dead embryo to the Danube Hospital (Vienna, Austria), and anembryonic sacs had been excluded. The diagnosis of missed abortion was based on demonstration of an embryo without cardiac activity by means of transvaginal ultrasonography (7.5-MHz transvaginal probe).

The study was approved by the ethics committee of the hospital, and informed consent for embryoscopy was obtained from the patients. Embryoscopy and subsequent curettage were performed under intravenous general anesthesia by an obstetrician specialized in endoscopic techniques. The patient was placed in a dorsal lithotomy position, and a speculum cleansed with Betadine solution was inserted into the vagina. After careful dilatation of the cervix, and before curettage was performed, a rigid hysteroscope (12° angle of view with both the biopsy and the irrigation working channel, Circon Ch 25: 8 mm) was passed transcervically into the uterine cavity. A continuous normal saline flow was used throughout the procedure (pressure, 40–120 mm Hg) to help distend and clean and thus provide a clear view. The chorion was opened with microscissors (CH 7: 2 mm), and the embryo was first viewed through the amnion. The amnion was then carefully opened with microscissors, in most cases, to obtain a detailed view of the embryo. All procedures were viewed on a television monitor by connecting a video camera (STORZ, tricam SL, Karl Storz, Tuttlingen, Germany) to the eyepiece of the endoscope and were recorded for later analysis.

The embryoscopic findings were classified into three categories: [1] embryos showing normal development, [2] growth-disorganized (GD) embryos, and [3] embryos with isolated or combined external defects.

Growth-disorganized embryos were further subdivided according to their degree of disorganization (5). The first category of growth disorganization, empty sac or anembryonic sac (GD1), was excluded, because our study was limited to abortuses with ultrasonographic evidence of a dead embryo. Embryos in the second category of growth disorganization, GD2, showed embryonic tissue 3 to 5 mm in length. These conceptuses had no recognizable external embryonic landmarks and no retinal pigment.

Embryos categorized as GD3 were ≤10 mm long. They lacked limb buds, but retinal pigment was often present. A cephalic and caudal pole could be identified.

Embryos categorized as GD4 were not observed in this study. These embryos have a crown-rump length of >10 mm, with a discernible head, trunk, and limb buds. The limb buds show markedly retarded development, and the facial structures are usually highly abnormal.

The diagnosis of localized and systemic defects in embryonic morphogenesis was made by experienced embryopathologists.

Karyotyping was attempted in all cases. Chorionic villi were obtained either by curettage (18 cases) or by direct chorion biopsies (5 cases). One woman had a bichorionic, biamniotic twin pregnancy with early intrauterine death of both embryos; the two chorionic sacs were biopsied separately (6). The chorionic villi were placed in normal saline and carefully dissected. Samples from the curettage material were freed from decidual cells and blood and were washed two times in normal saline. The chorionic villi were placed in culture medium (Chang Medium C; Irvine Scientific, Santa Ana, CA) and immediately forwarded to the cytogenetic laboratory for further processing. Subsequently the tissue was cultured and analyzed cytogenetically, using standard G-banding cytogenetic techniques. Comparative genomic hybridization in combination with flow cytometry analysis was performed in three cases in which the traditional cytogenetic analysis failed to provide results (7).

RESULTS

The mean age of the 23 women experiencing missed abortion after IVF was 35.5 years (range, 29–42 years). Fifteen patients had a history of at least two previous IVF failures. Table 1 summarizes the embryonic and cytogenetic findings and the clinical data obtained in these cases.

An embryo could be visualized in 22 cases. In one case, evaluation of the embryo was not possible because the investigator's vision was obscured by bleeding at the site. The collective included one set of twins, yielding a total of 23 embryos that could be subjected to a complete anatomic survey. Both twin embryos exhibited embryonic growth disorganization and had a normal karyotype (46,XY).

Table 1 shows that no external embryonic abnormalities could be detected in two (9%) cases, whereas abnormally developed embryos were seen in 21 (91%) missed abortions resulting from IVF.

Among the abnormal embryos, embryonic GD was detected in 11 embryoscopies. Nine GD2 conceptuses showed no recognizable external embryonic landmarks and no retinal pigment (Figs. 1 and 2). Two GD3 lacked limb buds, but

TABLE 1

Summary of embryoscopic and cytogenetic findings identified in 23 patients with missed abortions in pregnancy by IVF.

Case	Maternal age (y)	Crown–rump length (mm)	Morphology	Karyotype
1	37	5	Normal embryo with an upper limb bud and a prominent tail	46,XY
2	38	10	Normal embryo	47,XX,+18
3[a]	32	10	Macerated embryo in a tight amniotic sac with severe microcephaly, facial dysplasia, and retarded limb development	46,XX
4	35	3	GD2	47,XX,+10
5[a]	33	18	Macerated, microcephalic embryo with retarded limb development	45,X
6	36	18	Generalized degeneration, microcephaly with facial dysplasia, retarded limb development, umbilical cord cyst	47,XX,+14
7[a]	37	23	Macerated microcephalic embryo with incomplete separation of the digits, indicating retarded limb development for an embryo of this size	45,X
8	40	3	GD2	47,XX,+12
9	38	6	GD3	47,XX,+14
10	29	22	Microcephalic embryo with a median cleft lip and an appendicular sixth digital ray postaxially; the axes of the arms were at right angles to the body but the upper limbs were not bent at the elbows, indicating retarded limb development for an embryo of this size	46,XX,−14,+t(13q;14q)
11	41	12	Microcephalic embryo with retarded limb development	47,XY,+9
12	35	5	GD2	47,XY,+11
13	32	8	GD2	46,XX
14	35	17	Macerated embryo, severe microcephaly, facial dysplasia, retarded limb development	47,XY,+15
15	30	16	Microcephaly, fusion of the face to the chest, retarded limb development	46,XY
16	42	15	Macerated microcephalic embryo with face-to-chest fusion, brownish pigmentation in the thoracic region, retarded limb development	47,XY,+13
17	40	3	GD2	46,XY
18[b]	28	4	Dichorionic twin pregnancy; both embryos consisted of embryonic tissue showing no external embryonic landmarks and no retinal pigment (GD2)	46,XY
		4		
19[b]	37	8	GD3	47,XX,+16
20[b]	37	3	GD2	47,XY,+3
21[b]	36	5	GD2	47,XY,+16
22	33	4	Not available	47,XY,+8
23[b]	35	4	Abnormal embryo directly attached to the amnion, cephalic end with visible forebrain prominence and nonpigmented eye spot, tail present, no limb buds seen	47,XX,+16

[a] Were examined by comparative genomic hybridization–flow cytometry analysis.

[b] Direct chorionic biopsies were performed.

Philipp. Morphology of early intrauterine deaths after IVF. Fertil Steril 2004.

retinal pigment was present. A cephalic and caudal pole could be identified.

Ten cases had multiple local developmental defects, including central nervous system defects (microcephaly), facial dysplasia, facial cleft, fused mouth, and retarded limb development (Figs. 3 and 4). Microcephalic embryos were seen on embryoscopy with a poorly developed cranium showing a loss of normal vascular markings. In particular, the frontal area lost the usual bulge expected for embryos of this size.

In three embryos, the brachial arch and midface structures seemed to be poorly developed on embryoscopic examination; the endoscopic appearance was suggestive of facial dysplasia.

Of the 23 cases studied, a successful cytogenetic evaluation was performed in all cases. Chromosomal abnormalities were found in 17 (74%) of 23 missed abortions. The majority were numerical aberrations such as monosomy X (two cases). Trisomies for chromosomes 18 (1 case), 16 (three cases), 15 (one case), 14 (two cases), 13 (one case), 12 (one case), 11 (one case), 10 (one case), 9 (one case), 8 (one case), and 3 (one case) were observed. A structural chromosome anomaly leading to chromosomal trisomy was observed in one case. Parental karyo-

FIGURE 1

Case 20. Close up of a trisomy 3 (47,XY,+3) IVF embryo. The GD2 embryo, 3 mm in length, showed no recognizable external structures after the amnion had been opened.

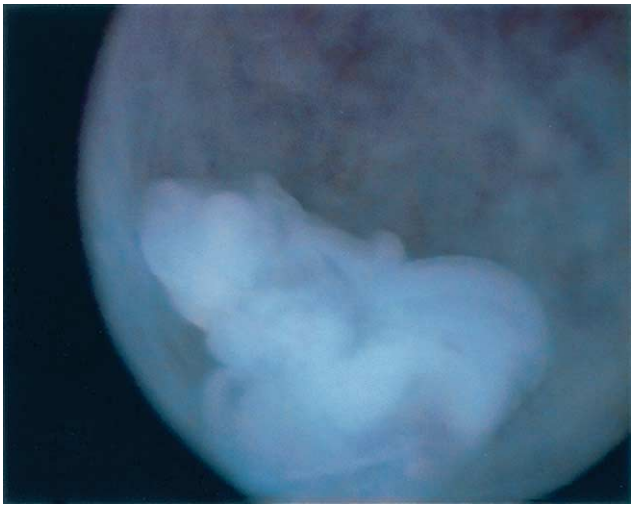

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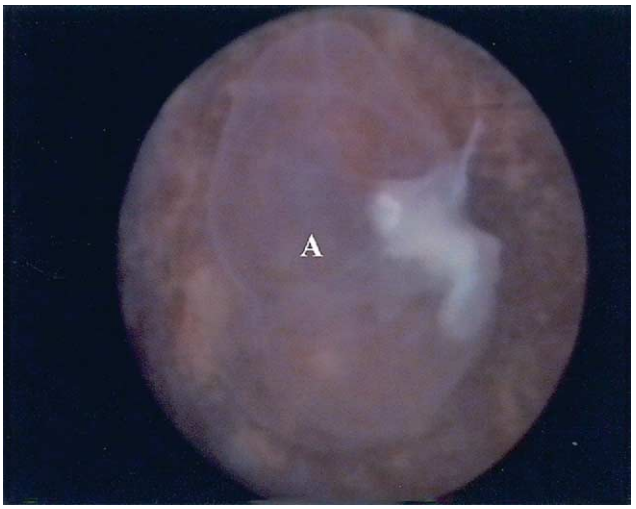
FIGURE 2

Case 13. Intact amniotic sac (A) containing a growth-disorganized IVF embryo 8 mm in length. The GD2 embryo is directly attached to the amnion. An apparently normal karyotype was diagnosed cytogenetically (46,XX).



Philipp. Morphology of early intrauterine deaths after IVF. Fertil Steril 2004.

typing established de novo origin of the chromosomal anomaly.

DISCUSSION

The morphologic features of 23 early intrauterine deaths resulting from IVF are described in this report. Twenty-one (11 GD embryos and 10 multiple defects) of 23 embryos showed abnormal development, whereas 2 cases revealed no external embryonic abnormalities on embryoscopy.

Chromosomal aneuploidy was identified cytogenetically in 17 of 23 missed abortions and explained the grossly abnormal embryonic development documented by embryoscopy in 15 of 21 embryos studied (cases 4–12, 14, 16, 19–21, and 23; Table 1), suggesting that embryonic aneuploidy is the major factor affecting normal embryo development in missed abortions after IVF.

The chromosome abnormalities observed in this study were confined to missed abortions that originated de novo after IVF. The majority of the abnormalities are lethal (e.g., trisomies 3, 10–12, 14–16) or have an estimated prenatal survival ranging between 1% (trisomy 8, 9 monosomy X), 3% (trisomy 13), and 5% (trisomy 18). Although the study was limited to abortuses with ultrasonographic evidence of death and excluded anembryonic sacs, it is interesting to note that abnormal embryonic formation could be documented embryoscopically, even among lethal trisomies.

In vitro fertilization allows PGD of aneuploidy. Fluorescent in situ hybridization probes for up to nine chromosomes (X, Y, 13–16, 18, 21, and 22) have been integrated in assisted reproduction programs to reduce the likelihood of spontaneous abortion after IVF (8).

It is interesting to note that 6 of 15 trisomies (trisomies 3, 8–12) observed in our small series are usually not tested in PGD protocols using fluorescent in situ hybridization analysis with chromosome-specific probes. These trisomies are considered rare among early abortion specimens, their frequency ranging between 0.2%–1% (trisomy 3, 11, 12) and 2%–4% (trisomies 8–10) (9).

Comparative genomic hybridization is a molecular technique that simultaneously evaluates all chromosomes from a single cell and allows aneuploidy involving any chromosome to be ruled out before implantation (10, 11). Although new molecular genetic techniques such as comparative genomic hybridization might signify a marked advancement in future PGD protocols by enhancing the proportion of embryos that have the full potential of completing their development to term and being transferred to the mother, the technique might have certain limitations.

In this series, six grossly abnormal embryos had an apparently normal karyotype. At present, our knowledge about the mechanism leading to abnormal embryonic development with a normal karyotype is limited. Specimens with a grossly abnormal embryonic development and apparently normal chromosomes have not been investigated so far, because intact embryonic specimens resulting from IVF with a known karyotype are rarely available.

FIGURE 3

Case 16. Close-up lateral (**a**) and anterolateral (**b**) view of a macerated trisomy 13 (47,XY,+13) embryo after the amniotic membrane (*A*) had been opened. External developmental defects of the 15-mm-long embryo resulting from IVF are severe microcephaly, fusion of the face to the chest, and retarded limb development. The dark brown areas in the thoracic region are due to maceration; umbilical cord (*U*).

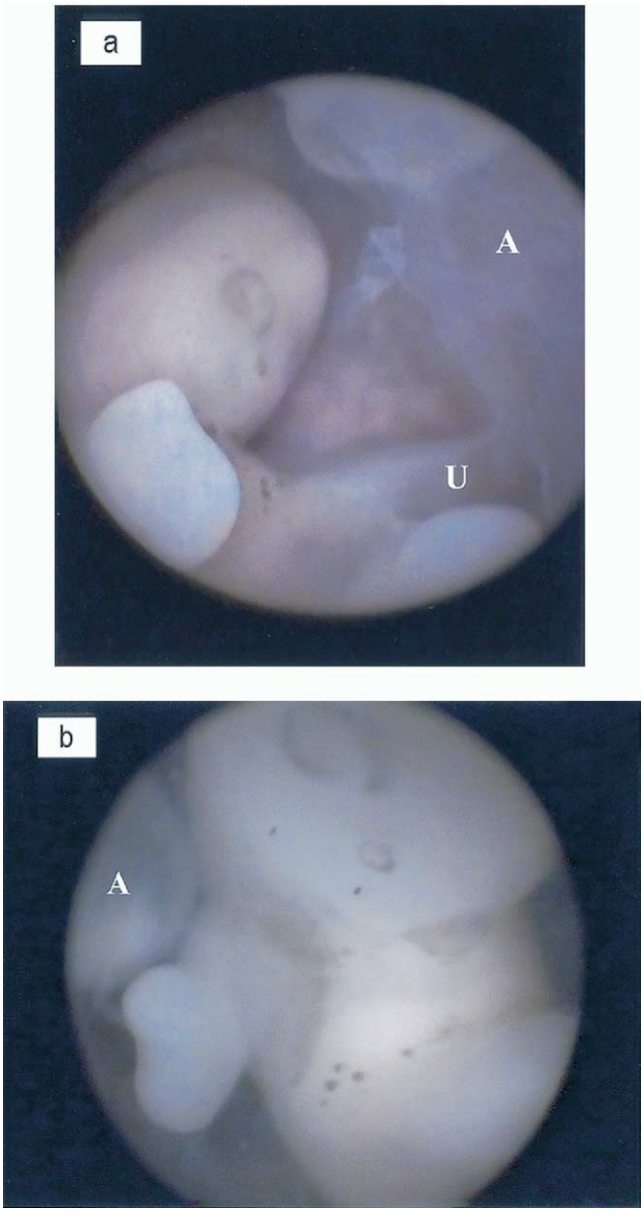


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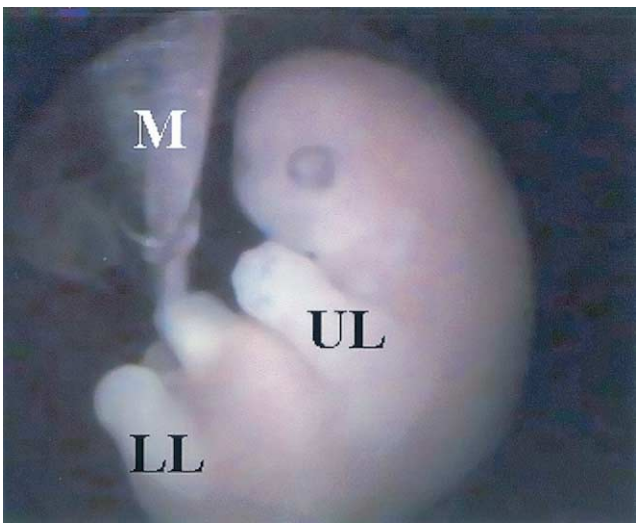
FIGURE 4

Case 6. Lateral view of a macerated, microcephalic trisomy 14 (47,XX,+14) embryo. Upper (*UL*) and lower (*LL*) limbs of the 18-mm-long embryo resulting from IVF show hand and foot plate development. Digital rays are beginning to be apparent on the hands but are not notched, indicating retarded development for an embryo of this size. *M* marks the microforceps.



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It is interesting to note that embryonic maldevelopment documented embryoscopically in these cases with apparently normal chromosomes was as severe as that resulting from the lethal aneuploid syndromes listed above. This preliminary report supports the idea that genetic lesions, undetectable by the cytogenetic techniques used in the present study, might have prevented normal embryogenesis in these cases (12).

The grossly abnormal development observed on embryoscopy in six embryos with an apparently normal karyotype in our small series indicates that such investigations are necessary and might assist investigators in answering specific questions from parents concerning the probable cause of early intrauterine death of greatly desired IVF pregnancies.

Embryoscopy in cases of missed abortion spots subtle morphologic abnormalities and thus permits better monitoring of early pregnancy loss after IVF. The technique might be a central component for further detailed genetic studies to specifically identify submicroscopic deletions or duplications of specific chromosomes preventing normal embryogenesis. If we are correct in hypothesizing that submicroscopic chromosomal imbalances containing genes required for survival exist in chromosomally normal abortions with developmental defects, the finding might serve as a foundation for future PGD protocols for aneuploidy screening. This could improve the outcome of assisted reproductive procedures by further enhancing the proportion of embryos that have the full potential of completing their development to term and being transferred to the mother.

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Imunotherapy and recurrent miscarriage: are we any wiser?

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NOTES

Endometrial natural killer cells and early pregnancy loss

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Recurrent Miscarriage

- 3 consecutive pregnancy losses prior to 20 weeks gestation
- 50% no known cause
- ?endometrial factor

uNK cells

- CD56+, CD16-, CD3-
- Different from peripheral NK cells
  - CD56+, CD16+, some CD3+
- Materno-fetal interaction in early pregnancy
  - Most numerous early pregnancy
  - Adjacent to fetal trophoblast
  - Express receptors that recognise trophoblast antigens
- Express glucocorticoid receptors
  - Henderson et al., 2003
- Function unknown

Background

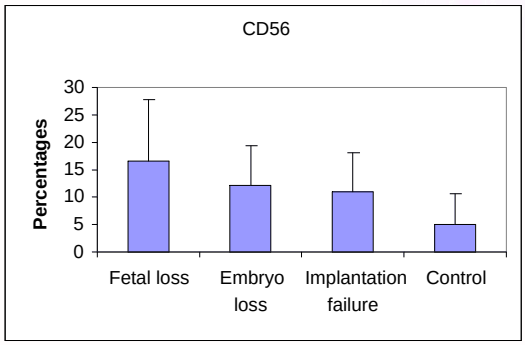
- uNK cells more numerous in idiopathic RM
  - Quenby et al, 1999; Clifford et al, 1999
- More uNK cells in RM women predicted further miscarriage
  - Quenby et al 1999

Are the uNK cells in RM active?

NK cell receptors that may recognize the class I HLA of trophoblast.

Trophoblast	uNK cells
HLA-E	CD94/NKG2
HLA-C	KIRs
HLA-G	ILT-2 (+ILT-4)
	KIR2DL4

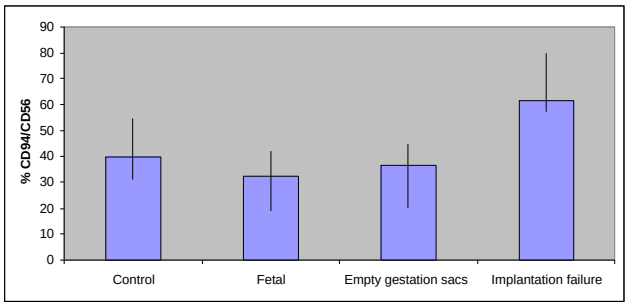
CD56+/stromal cell in reproductive failure



Killer inhibitory receptor/CD56+ cell in reproductive failure

Killer inhibitory receptor/CD56+ cell in reproductive failure

Killer activatory receptor/CD56+ cell in reproductive failure



Case History

17 consecutive miscarriages
No Cause found
Most NK cells in study n=40
Preconceptual prednisolone 5mgs
Two further miscarriages
Higher dose (prednisolone 20mg)
Live Birth aged 42
- (IUGR 32/40)

Alive and well age 2 years
- Quenby et al., 2004

Was this a chance occurrence or did prednisolone reduce the uNK cells?

(Quenby et al., 2005 F&S)

Methods

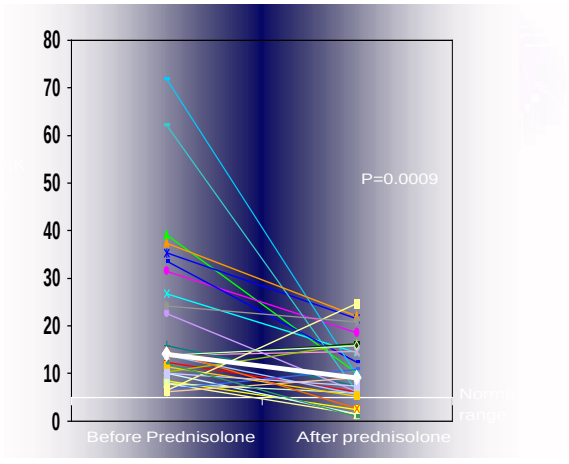
110 contacted clinic
Following publicity from all over UK
85 with RM
3-22 previous miscarriages
Lining of the womb sampled
day 21
Counted 5000 cells to find % uNK
29 took prednisolone
Repeated sample

Patients

	Controls n=18	RM uNK <5% n=43	RM uNK >5% n=32
Age Mean Range	 37 24-41	 35 20-46	 39 32-49
Miscarriages Mean Range	 0	 6 3-20	 6 3-22
Women who had a previous live birth % number	 100 18	 28 12	 16 5
% CD56+			

Methods-2

- 29 took prednisolone
  - 20mgs day1-day21
- Endometrial biopsy repeated
  - 10mgs prednisolone 3 days
  - 5mgs prednisolone 4 days



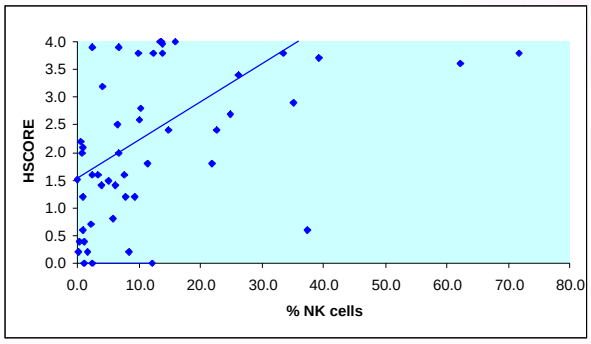
Observational data

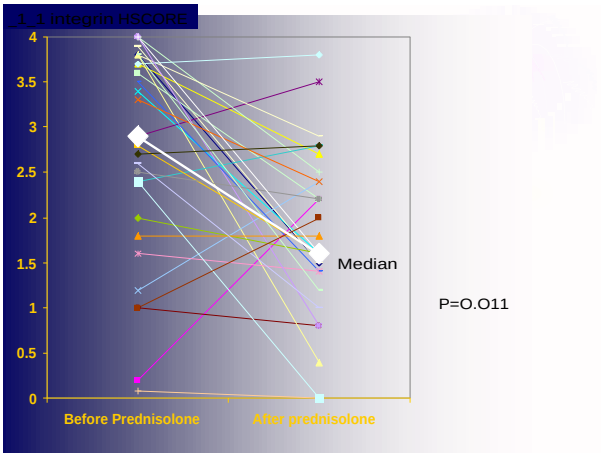
- Six successful pregnancies
  - Another IUGR 32/40 OK
- 3 miscarriages
- Other trouble conceiving
  - Side effects
- Plan to give in early pregnancy
  - uNK most active then

Adhesion molecules

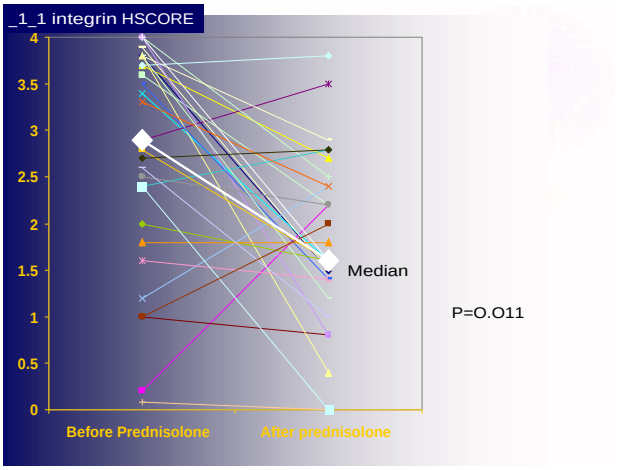
- Integrins help attachment of embryo to luminal surface endometrium
  - $\alpha 1\beta 1$, $\alpha 4\beta 1$, $\alpha v\beta 3$
- Maximal expressed in implantation window
- Lower in infertile women

_1_1 integrin and uNK cell



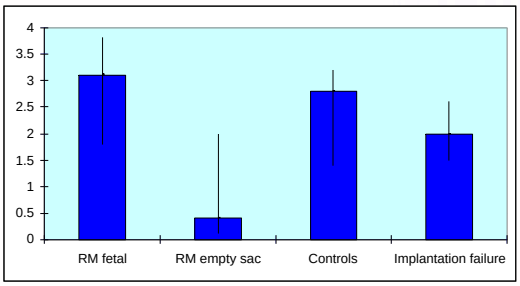


P=O.O11

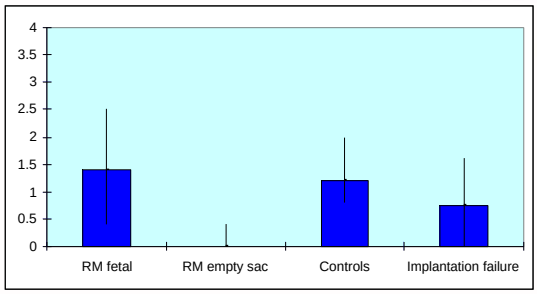


P=O.O11

_1_1 on glandular epithelium HSCORE medians and IQ ranges



_1_1 on luminal epithelium
HSCORE medians and IQ ranges



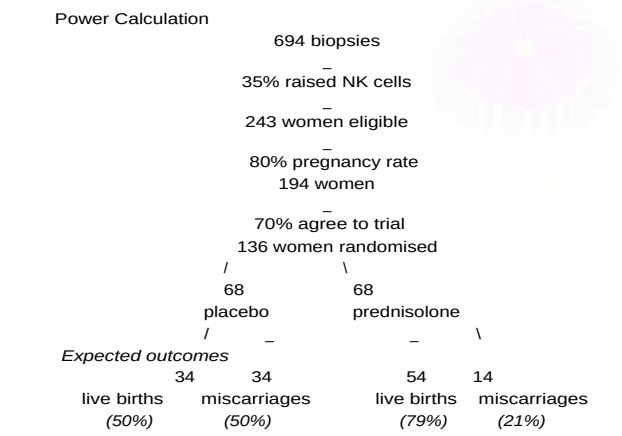
Uterine NK

- **Increased numbers of RM patients**
  - **Quenby et al, 1999; Clifford et al, 1999**
- **more numerous in chromosomally abnormal miscarriages than in chromosomally normal miscarriages**
  - **Yamamoto *et al,* 1999, Quack et al., 2001**
- **may facilitate the implantation of abnormal blastocysts**

Conclusions

- Prednisolone reduced number uNK cells
- Does this reduction lead to more live births?
- Need randomised controlled trial before introducing prednisolone as treatment

A randomised controlled trial of prednisolone for women with recurrent miscarriage and high levels of uNK cells in the endometrium.



Inclusion

- >2consecutive idiopathic first trimester miscarriages
- >5% of endometrial cells CD56+,
- Age 20 - 40.

Exclusion

- Known cause for pregnancy losses;
  - APS
  - parental balanced translocation,
  - uterine anomaly
    - subseptate uterus, cervical weakness
  - known thrombophilia
- Contraindications to steroid therapy;
  - hypertension, diabetes, mental health problems, obesity BMI>25.

Outcome measures

- *Primary*
  - Live birth rate
- *Secondary*
  - Miscarriages
    - First/ Second trimester losses
    - Karyotype of miscarried pregnancies
  - Still births
  - Obstetric complications
    - IUGR, Pre-eclampsia, abruption,
    - gestation at delivery
  - Fetal abnormality
  - Side effects of steroids

DATA ANALYSIS

- Intention to treat basis.
- A Chi squared test
  - live birth rate between prednisolone and placebo groups.
  - $P<0.05$ will be considered to be significant.

Acknowledgements

- LWH funded
- Patients
- **Miscarriage clinic team**
  - **R Farquharson**
    - **C Kalumbi, R Kaur, K Moore,**
    - **Ann Marie Hughes**
- **Laboratory staff**
  - **M Bates, G Vince**
    - **M Anim-Somuah,**

Implantation and endometrial receptivity

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NOTES

Polycystic ovarian disease and pregnancy loss: an overview

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- **1. Polycystic ovary syndrome**
- **2. PCOS and miscarriage: is there an increased incidence?**
- **3. Can risk of miscarriage be reduced?**

The Rotterdam ESHRE/ASRM Consensus Group
Revised 2003 Diagnostic Criteria for PCOS

2 out of 3 criteria required

Oligo- and/or anovulation

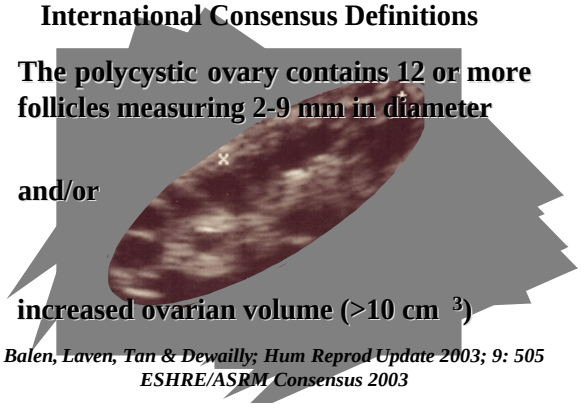
Hyperandrogenism (clinical and/or biochemical)

Polycystic ovaries

Exclusion of other aetiologies

Human Reproduction 2004; 19: 41-47. Fertility & Sterility, 2004; 81: 19-25.

Ultrasound Assessment of the Polycystic Ovary: International Consensus Definitions



The polycystic ovary contains 12 or more follicles measuring 2-9 mm in diameter

and/or

increased ovarian volume (>10 cm ^{3})

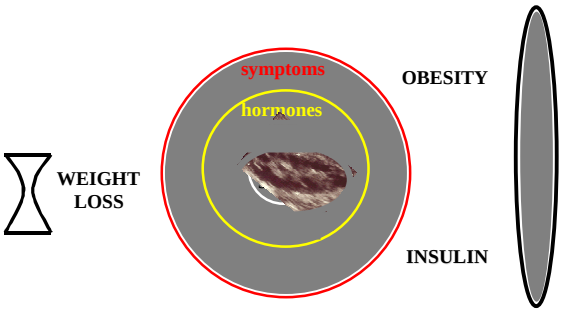
Balen, Laven, Tan & Dewailly; Hum Reprod Update 2003; 9: 505
ESHRE/ASRM Consensus 2003

Heterogeneity in 1741 women with PCOS

Obesity	**40%**
Amenorrhoea	**20%**
Oligomenorrhoea	**50%**
Hyperandrogenism	**66%**
Elevated total testosterone	**33%**
Elevated LH	**40%**

Balen et al, Hum Reprod 1995; 10: 2107

1741 Women with PCOS



Balen et al Hum Reprod 1995; 10: 2107

Menstrual Cycle Abnormalities:

Greater if overweight

Inter-cycle length correlates with degree ofhyperinsulinaemia

Conway et al., Clin Endo 1993; 30: 459

PCOS and hyperinsulinaemia

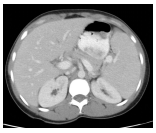
- **Hyperinsulinaemia in 30% slim and 75% obese PCOS**
- **Insulin resistance out of proportion to obesity in PCOS:**
  increased truncal abdominal fat,
  even if BMI normal

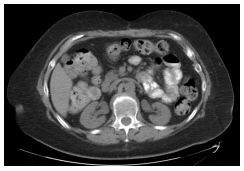
Kirchengast et al F&S 2004;81:539

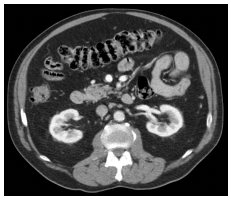
Obesity:

BMI – WHO criteria (overweight 25-30, obese > 30 kg/m^2)

Waist Circumference > 88 cm







- **PCOS and miscarriage:**
- **is there an increased incidence?**

Prevalence of miscarriage in PCOS

Mean prevalence – 43%

Range - 25-65%

Glueck et al, 2002	***206/319 (65%)***
Jakubovicz et al, 2002	***13/31 (41%)***
Wang et al, 2002	***93/373 (25%)***

Possible causes of increased miscarriage risk in PCOS

- **Obesity, Hyperinsulinemia**
- **High LH**
- **High PAI-1**
- **Poor egg and/or endometrial quality**

PCOS and anovulatory infertility

Risk of infertility correlates with

rising BMI (particularly > 30 kg/m^2)
rising serum LH concentration

Balen et al Hum Reprod 1995; 10:2107

Current Understanding of Polycystic Ovary Syndrome

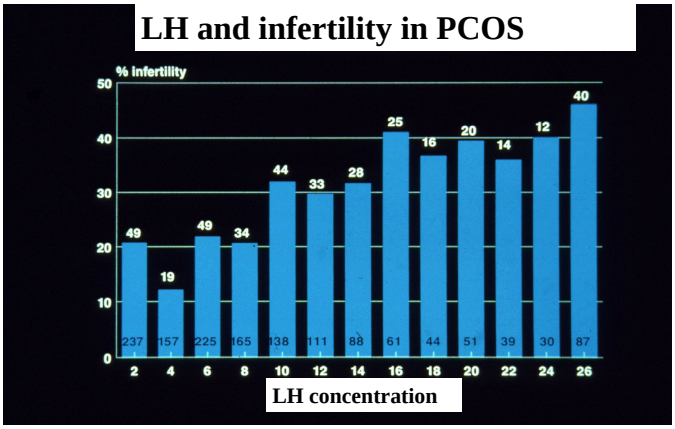
- **LH**

Hypersecretion of LH in PCOS

Associated with:

- **reduced chance conception – spontaneous and stimulated cycles**
- **increased risk of miscarriage**

LH and infertility in PCOS



Balen et al Hum Rep '95;10:2107

High LH?

Natural cycles (n = 193)

LH	> 10	< 10 iu/l
Miscarriage	65%	12%

Regan et al, 1990

High LH

Pulsatile GnRH therapy for PCOS (n=54)

	LH (IU/l)
Normal pregnancy	9.6
Early pregnancy loss	17.9

Homburg et al, 1988

Clomiphene citrate (n = 128)

miscarriage rate 23.6% - LH higher

Kousta et al, Hum Reprod Update 1997; 3: 359

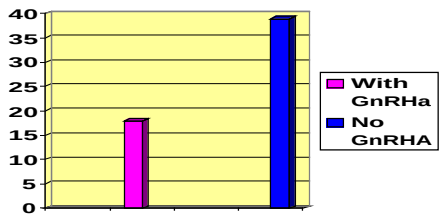
Low dose gonadotrophin therapy (n=100)

high LH - higher miscarriage rate

Hamilton-Fairley et al, 1992

Treatment with GnRH agonists in ovulation induction and IVF

% Miscarriage rate



Balen et al, 1993, Homburg et al, 1993

Laparoscopic ovarian drilling

- **Fall in LH is main endocrine change.**
- **Miscarriage rate of 14%**
  Abdel Gadir, 1990; Balen, 1994; Armar,1993
- **Meta-analysis – surgical treatment of PCOS**
  Miscarriage rate – 15.9% of 1076 pregnancies.
  Campo, 1998

•Obesity

Obesity

- **Obesity → EPL ↑**
  Wang et al, 2002; Hamilton-Fairley et al, 1992
- **PCOS → Obesity ↑**
  Pasquali et al, 1997; Solomon 1999
- **PCOS → EPL ↑**
  Glueck et al, 2002; Homburg et al, 1993;Balen et al, 1993

PCOS or Obesity ?

- **1018 patients treated with ART (37% PCOS)**
- **EPL - PCOS 25%,**
  - normal ovaries 18%
- **Multivariate logistic regression–**
  Higher risk of EPL in PCOS likely due to high prevalence of obesity

Wang et al, 2001

Treatment of Obesity

- **67 patients, BMI > 30, infertility > 2 years**
- **Lifestyle change programme, 6 months**
- **Before loss of weight –**
  75% spontaneous abortions
- **After losing mean 10.2kg body weight**
  52 pregnancies–
  18% spontaneous abortions

Clark et al, 1998

Hyperinsulinemia

Hyperinsulinemia associated with

- **Obesity**
- **High plasminogen activator inhibitor activity (PAI) = hypofibrinolysis**

Glueck et al, 1999

Plasminogen activator inhibitor (PAI-1)

- **Glycoprotein**
- **Potent inhibitor of fibrinolysis**
- **Elevated in PCOS, hyperinsulinemia**
- **High levels are risk factor for EPL in PCOS**

Insulin resistance and recurrent pregnancy loss (RPL)

- **74 women with RPL vs**
  74 women with no RPL and live infants
- **Matched for age, race, BMI**
- **Insulin resistance:**
  RPL – 27%
  controls – 9.5%

Craig et al, 2002

Treatment of hyperinsulinemia

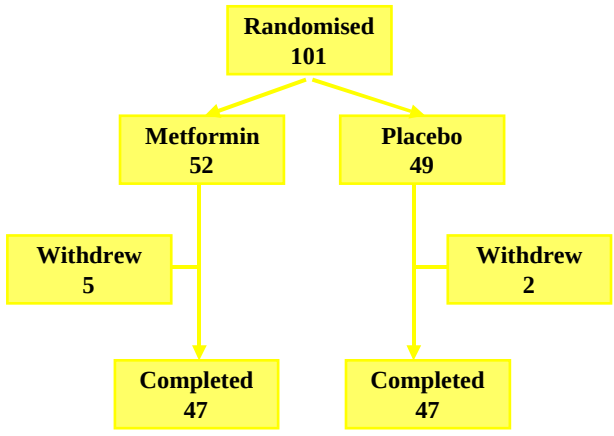
Metformin –

- **Reduces insulin, androgen, LH**
- **Reduces waist-hip ratio**
- **Reduces PAI concentrations**

Treatment of hyperinsulinemia

Egg or Endometrium?

The use of metformin for women with PCOS undergoing IVF treatment



Outcomes (Metformin v Placebo)

	•MET	•PLA	•p
•No of embryo transferred	•2	•2	•0.695
•Average embryo score	•17	•16	•0.259
•Positive preg. rate per cycle (%)	•48.1	•34.7	•0.245
•Positive preg. rate per transfer (%)	•55.6	•40.5	•0.233
•Clinical preg. rate per cycle (%) (beyond 12 weeks)	•35.5	•16.3	•0.023
•Clinical preg. rate per transfer (%) (beyond 12 weeks)	•44.4	•19.1	•0.022

Conclusions

- **Women who have PCOS have higher rates of miscarriage than women with normal ovaries**
- **Obesity, high LH, hyperinsulinemia and high concentrations of PAI may all be involved**
- **Treatment to reduce weight, LH and insulin levels may improve the miscarriage rate**

Treatment with metformin

- **Without metformin – 319 pregnancies**
  Live births 34%
  Early pregnancy loss 65%
- **With metformin - 328 pregnancies**
  Live births 80%
  Early pregnancy loss 20%

Uses patients as retrospective controls …

Glueck et al, 2002

Ultrasound uses and pitfalls

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NOTES

Combined ultrasound/biochemical prediction of very early pregnancy loss

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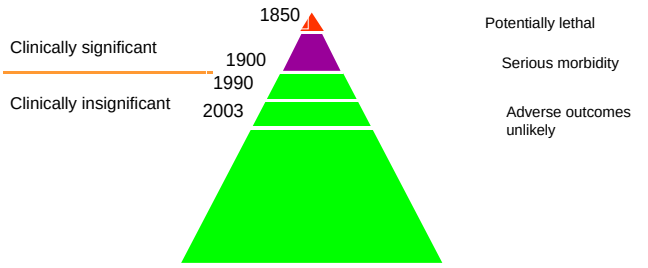
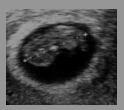
Early Pregnancy Loss



Guidelines to establish embryonic death

Ultrasound findings	*Action*
Gestational sac >20 mm with no embryo or yolk sac	Repeat scan in one week
Crown-rump length >10 mm with no heart action	Repeat scan in one week
Gestational sac <15 mm or crown-rump length <10 mm	Repeat scan in two weeks

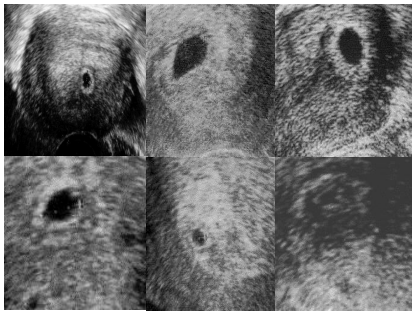


Hatley et al 1995

Models for prediction of early pregnancy viability

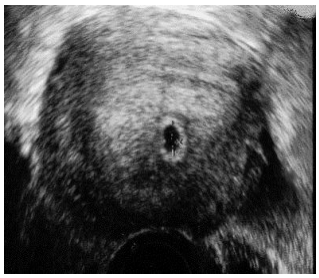
- Logistic Regression Models
- Decision Tree analysis

Which one is normal?



Small Sac –prediction of viability

- Prospective observational study
- 50 women, bleeding
- MSD <16mm No FP
- Multiple parameters
  - Maternal age
  - Menstrual age
  - MGSD
  - hCG
  - Sonographic age



Logistic Regression Model
Small sacs & bleeding

Probability of miscarriage = $1/(1+e^{-z})$

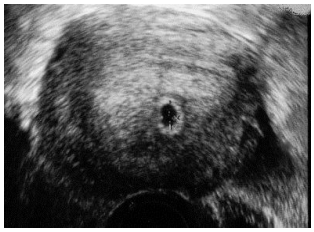
Where z =
(-0.9808 + 3.5457x *SD score mean gestational sac diameter)*

Sensitivity 81% (95% CI 0.64-0.93)
Specificity 89% (95% CI 0.65-0.99)

Falco 2003

Small sac - prediction of viability

- 200 women with mean sac diameter <20mm
- Multiple parameters recorded at initial visit



Elson et al 2003

Small sac
Results

Variable	*Viable pregnancies N= 118*	*Non-viable pregnancies N= 82*	*P*
Maternal age (yrs)	29.3 (6.2)	32.3 (7.4)	<0.01
Gestational age (days)	42.8 (9.8)	59.8 (16.2)	<0.01
Vaginal bleeding (%)	34.7	76.8	<0.01
Gestational sac diameter (mm)	6.8 (4.2-8.3)	10.7 (6.0-15.8)	<0.01
Progesterone (nmol/l)	84 (62 – 109)	31 (19 – 41)	<0.01
β-hCG (IU/l)	3974 (1661-8638)	3556 (1000-11083)	>0.05

Logistic regression model
Small sacs spontaneous conception

Probability of viability = $1/(1+e^{-z})$

Where z = (6.091 x *ln progesterone*) - (0.159 x *sac diameter*) - (0.1640x *maternal age*) – 17.435

Sensitivity 99.2% (95% CI 95.8 – 99.97)
Specificity 70.7% (95% CI 61.3 – 78.9)

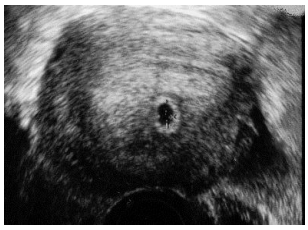
Elson 2003

Small Sacs Models Performance

	Sensitivity (%)	Specificity (%)
LR original data	99.2 (95% CI 95.8 – 99.97)	70.7 (95% CI 61.3 – 78.9)
LR prospective	98.1 (95% CI 94.45 – 100)	63.8 (95% CI 50.9 – 77.54)

Small sac in ART patients- prediction of viability

- 49 women with an intrauterine gestation sac on scan
- Blood sample taken for measurements of serum hCG and progesterone
- Follow up scans were arranged at 7 weeks gestation



Small sac ART
Results

Variable	*Normal pregnancy N = 34*	*Miscarriage N = 15*	*p*
Maternal age (yrs)	31.6 (4.2)	34.4 (2.6)	<0.05
Gestational age (days)	19.6 (3.25)	20 (3.3)	>0.05
Vaginal bleeding (%)	5	33	<0.05
Sac diameter (mm)	7.09 (4)	4.8 (2)	<0.05
β-hCG (IU/l)	2117 (1275-3490)	706 (438-2480)	<0.01
Progesterone (nmol/l)	84.4 (42.2-123)	45.5 (34.6-74)	<0.01

N =49

Logistic regression model
Small sacs ART

Probability of viability = $1/(1+e^{-z})$

Where z = (3.445 x ln progesterone) + (1.994 x sac diameter) - (3.94 x maternal age) – (10.524 x bleeding) – (7.82 x gestational age)+ 5.079

Sensitivity 100%
Specificity 73%

Elson et al 2005

Conclusions

- Progesterone significant marker predicting viability spontaneous and ART pregnancies
- More work done level appropriate for ART patients
- Question the need to supplement with progesterone for pregnancy maintenance

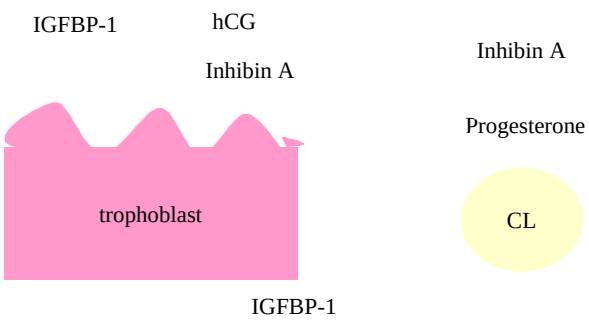
Inhibin A

- Glycoprotein 32KDa
- Produced by corpus luteum and synctiotrophoblast. Unsure which is dominant source
- Short half life 45 minutes
- Acts via GnRH affect placental hCG production
- Animal sources suggested maintains steroid production from corpus luteum

IGFBP-1

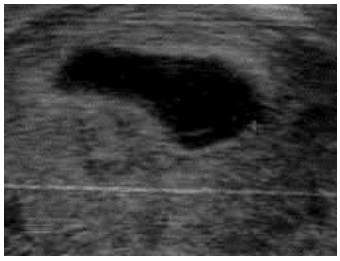
- Major decidualised endometrial glycoprotein
- pIGFBP-1 and npIGFBP-1
- Acts as maternal restraint to invading trophoblast
- Over expression results in disorders of abnormal placentation : IUGR, PET
- Regulated by progesterone

Early Pregnancy Loss Biochemistry

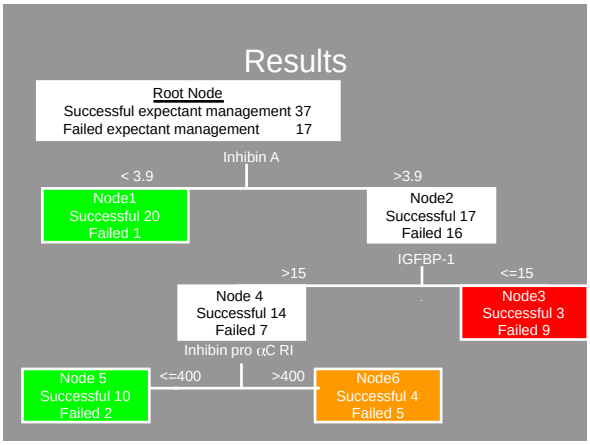


Outcome of conservative management of miscarriage

- 54 women with diagnosed miscarriage
- Serum hCG, progesterone, 17-OH progesterone, inhibin A, inhibin pro α C RI and IGFBP 1



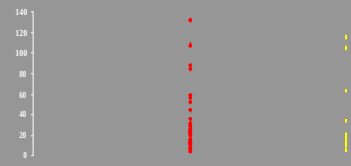
Results



Conclusions

- Biochemical markers could assist in the prediction of outcome of expectant management of miscarriage
- Low inhibin A and high IGFBP1 most useful in assessing the likely outcome in individual cases

IGFBP-1 in ART



Viable Miscarriage

P>0.05

Elson et al 2005

New Hypothesis?

- Low progesterone leads to tissue rejection
- Action tissue rejection stimulates IGFBP-1 further preventing further invasion cytotrophoblast causing miscarriage ?
- Embryonic signalling nonviability causes rise IGFBP-1?
- Answer lie npIGFBP-1 v pIGFBP-1?

Models for prediction of early pregnancy viability

- ➳
  - Multiple markers
  - Reduce diagnostic error
  - Numerical probability/ flow chart
  - Dynamics of markers
- ➳
  - LR computer
  - Similar population

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Davor Jurkovic & team EPAGU
Rehan Salim
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Aspects of gestational trophoblastic disease

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Routine histopathological examination in sporadic miscarriage has generated a lot of debate and controversy mainly because of the inaccuracy of histological criteria in identifying the cause of an early pregnancy failure. It is well established that more than 50% of sporadic miscarriages are associated with a chromosomal defect of the conceptus and that the incidence of chromosome abnormality increases with increasing maternal age and decreasing gestational age[1,2]. Most of these abnormalities are numerical chromosomal abnormalities and less than 10% are caused by structural abnormalities or other genetic mechanisms[3]. The overall recurrence risk of numerical chromosomal abnormalities is low and the risk of live born trisomy following an aneuploid early pregnancy failure is around 1%[4]. Within this context the role of routine histology of sporadic miscarriage is limited, however, a molar pregnancy is a condition, which needs to be detected because of the potential long-term risk to the mother. A method of determining which cases are more likely to need follow-up may help improve diagnostic accuracy.

The estimated incidence of partial mole (PHM) is one in 700 pregnancies whereas the incidence of complete mole (CHM) is around one in 1500-2000 pregnancies[5,6]. The vast majority of CHM and PHM abort spontaneously during the first three to four months of pregnancy resulting in an incidence of molar placenta of one in every 41 miscarriages[5]. Following uterine evacuation approximately 10-20% of women with a CHM develop persistent gestational trophoblastic disease (GTD)[7]. The incidence of this complication after a PHM ranges between 0.5 and 11%[7,8,9,10] and is almost certainly underestimated since many early PHM will escape diagnosis.

Placental molar changes can now be detected from the third month of pregnancy by ultrasound which typically reveals a uterine cavity filled with multiple sonolucent areas of varying size and shape (“snow storm appearance”) without associated embryonic or fetal structures in the case of CHM[11]. In the case of a PHM, the early ultrasound diagnosis may be more difficult because the placental changes may be limited to a few molar villi and/or an increase in placental thickness[12-13]. Histological examination of early products of conception will identify about 60-70% of molar pregnancies[14].The distinction between CHM and PHM was made in the late 1970s on the basis of gross morphological, histological and cytogenetic criteria in second and third trimester pregnancies[15,16]. The complete or classical hydatidiform mole has been defined as a conceptus with a placenta showing generalized swelling of the villi and diffuse trophoblastic hyperplasia, in the absence of an ascertainable fetus[17]. The partial hydatidiform mole has been characterized by focal trophoblastic hyperplasia with focal villous hydrops and identifiable embryonic or fetal tissue. The clinico-pathologic picture of the two molar syndromes overlap to a degree since both the phenotype and natural history of the partial mole seem to represent a mild, bland version of those of the complete mole[18].

We have recently prospectively evaluated the role of ultrasound examination in combination with serum hCG in screening for molar changes in women diagnosed with a first trimester miscarriage[19]. All women with suspected molar pregnancy on transvaginal ultrasound were recommended surgical evacuation, at which tissue was sent for histological examination plus karyotype if possible. All cases of molar pregnancy diagnosed histologically were examined and cross referenced with cases diagnosed on ultrasound and with the supplementary report from the regional referral centre. Fifty-one cases of suspected molar pregnancy were referred to the regional centre for further histological opinion and follow-up, five cases were subsequently excluded from the final analysis when the diagnosis was confirmed as hydropic abortion (HA). In 33 cases, a molar pregnancy was suspected at the initial scan. Of these 26 (78.8%) were confirmed on histology resulting in a 56% detection rate using ultrasound alone. In 15 cases, pre-operative serum hCG results were available, of which nine were greater than two multiples of the median (MoM).

The diagnosis of both complete (CHM) and partial moles (PHM) in first trimester miscarriages is more difficult because both ultrasound and histological appearances are less pronounced that later in pregnancy. Serum hCG is significantly higher in both CHM and PHM and in conjunction with transvaginal ultrasound may provide the screening test required to reduce the need for routine histopathological examination. Morphological features, including villous size and proliferative activity of trophoblast, change with gestation and need to be taken into account when examining specimens of varying gestations. Difficulties arise when determining between PHM, CHM and hydropic miscarriage particularly when there is prolonged post-mortem retention in utero in missed miscarriage for example[14] and where there are focal hydropic changes found in aneuploidies. It has been suggested that PHM in the first trimester are frequently missed on ultrasound and that pathological examination should remain the mainstay of diagnosis[9]. The debate surrounding whether or not tissues obtained after evacuation of the uterus should be sent for routine histological examination has been long and is still unresolved[4]. Routine histological examination of products of conception is expensive and time consuming and the histological features of molar pregnancy are also different in the first trimester. Typical ultrasound features may or may not be present, with the diagnosis of PHM often being difficult even in later gestations, presenting with fetal growth restriction or subtle placental changes.

Pre-evacuation hCG levels may be a useful adjunct to histology in first trimester spontaneous miscarriages, in particular in cases with unusual ultrasound appearances[18]. Nine of our 13 molar pregnancies in which a pre-operative hCG was available demonstrated an hCG of 2 to 10.8 MoM. The two hydropic abortions had very low hCG levels. In three cases of partial mole, where the MoM were low, there was a significant discrepancy between gestational age from the date of the last menstrual period (LMP) and the dates suggested by the ultrasound scan. This would suggest prolonged post-mortem retention and trophoblast degeneration, explaining the low serum hCG. Ongoing CHM are associated with beta hCG levels of 10-200 MoM, PHM with levels of 10-60 MoM[13]. In the case of CHM, the typical ultrasound features in association with a high hCG are diagnostic of molar pregnancy even before histological diagnosis to confirm this[11]. With the use of high resolution ultrasound early in pregnancy in combination with early determination of hCG levels, diagnostic accuracy could be improved on the strength of the absence of fetal tissue and abnormally high hCG level. Caution should be exercised, however, in cases where there is a significant discrepancy between LMP and US dates occurs as demonstrated above. .Karyotype or ploidy determination could be a useful adjunct to diagnosis in difficult cases, but are not useful as first line diagnostic tools as they are

expensive. DNA ploidy can be useful in problem cases to determine between PHM and CHM and is cheaper and faster than karyotype[9], but can also be associated with misclassification, particularly if maternal tissue is present.

The routine use of histopathology in the diagnosis of molar pregnancy is significantly more expensive than the cost of a single serum hCG. The examination of products of conception costs approximately £40 per patient in our hospital whereas the cost of a single serum hCG is approximately £8. There are an estimated 200 000 miscarriages in the UK per annum. If it is assumed that 70% of these will undergo ERPC and therefore routine histopathology the cost to the NHS would be approximately £5.6 million per annum. The use of serum hCG as a screening tool to identify those women at risk could result in a five fold reduction in cost, with a significantly smaller number of cases requiring additional histopathological examination and follow up.

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Antiphospholipid syndrome and pregnancy loss: examining the evidence

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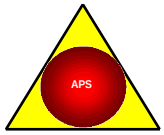
Antiphospholipid syndrome (APS)

Persistent presence of aPL

PLUS

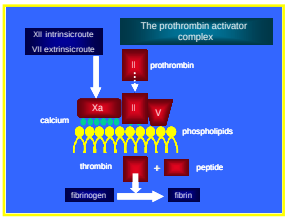


- Thrombosis
  - Venous vessels
  - Arterial vessels
  - Large vessels
  - Small vessels
- Pregnancy morbidity

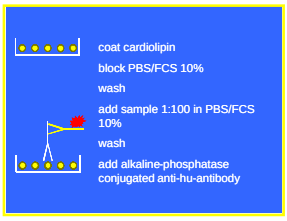
- Thrombocytopenia
- Livedo reticularis
- Heart valve lesions
- Movement disorders
- Nephropathy

Antiphospholipid antibodies (aPL)

Lupus anticoagulant (LAC)



Anticardiolipin antibodies (aCL)



Anti-$_{2}$ glycoprotein 1 antibodies

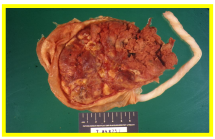
Persistent presence of LAC and/or medium or high levels aCL

+

- ≥ 3 consecutive unexplained losses < 10 weeks gestation
- ≥ 1 unexplained fetal deaths with normal morphology > 10 weeks gestation
- ≥ 1 premature birth < 34 weeks gestation because of severe preeclampsia or placental insufficiency

Obstetric APS

Non-thrombotic



Thrombotic

aPL

Thrombotic

Direct activation of platelets, endothelial cells, monocytes

Interference with humoral coagulation (a.o. Protein C system)

Indirect activation of platelets, endothelial cells, monocytes and coagulation via **complement activation**

Non-thrombotic

Influence on implantation and placentation by effects on apoptosis and mitosis

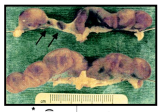
BALB/c mouse

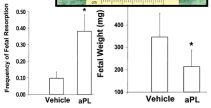
Jane Salmon et al.
J Exp Med 2002; 2: 211
JCI 2003; 112: 1644

Days 8 and 12 of pregnancy

Hu aPL-IgG intraperitoneal

Sacrified day 15

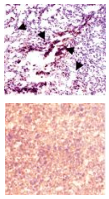




Investigate decidua in uteri removed 90 min after injection

With hu aPL-Ig (not controls)

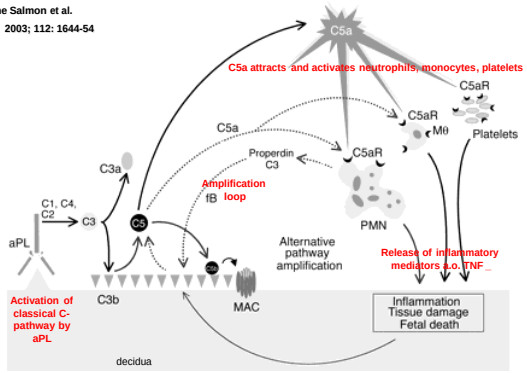
- Cellular infiltrates
- Focal necrosis
- Clumping, apoptosis and clusters of neutrophils
- Deposition of Ig
- Deposition of C3



Hu-IgG

HYPOTHESIS
C-activation is essential for hu-aPL associated fetal loss

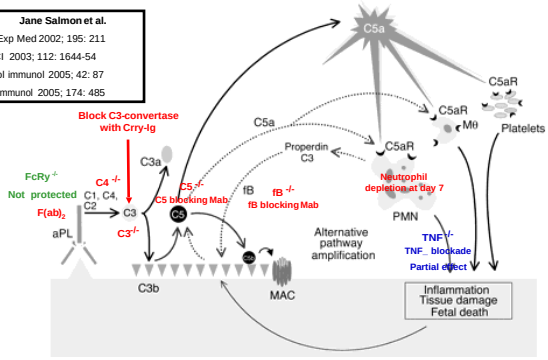
Jane Salmon et al.
JCI 2003; 112: 1644-54

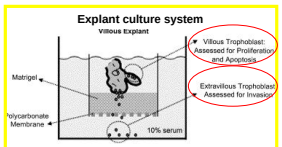


HYPOTHESIS -basis-

Situations that abrogate effects of hu-IgaPL

Jane Salmon et al.
J Exp Med 2002; 195: 211
JCI 2003; 112: 1644-54
Mol immunol 2005; 42: 87
J Immunol 2005; 174: 485





Culture of placental tissue in 24-well dishes. Assessment of trophoblast function in villous and extravillous sites.

et al. Am J Obstet Gynecol 2004

With hu- LAC-positive samples

- More apoptosis (TUNEL, M30 formation, DNA laddering)
- Less poliferation of cytotrophoblast (decreased villous trophoblast proliferation)
- Less extravillous trophoblast invasion through matrigel

LAC → **apoptosis_ mitosis_** → **Poor differentiation cytotrophoblast** → **No proper invasive potential**

Detrimental effects on the trophoblast layer and thus the whole placenta and the developing fetus

Study group	prevalence (%) aCL	LAC
• Normal controls	1-6	<2
• Women with recurrent pr. loss	10-20	5-10
• SLE	25-40	10-30

aPL & pregnancy
- different populations -

apparently healthy women
0-2 early losses; < 3% fetal loss
no SLE; no thrombotic history

high-risk groups
poor obsteric history; high % fetal loss
32-74 % SLE; 12-41 % thrombotic history

healthy women with obstetric APS
2-3 consecutive early losses; rarely fetal loss
no SLE; no thrombotic history

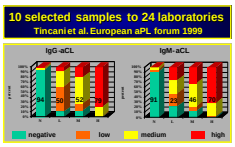
Problems with the literature

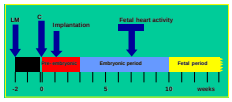
Relevant aCL levels?

LAC defined according to official guidelines?

Obstetric APS

Different definitions of pregnancy loss





Pregnancy loss
- pre-embryonic
- embryonic
- fetal

aPL in low-risk pregnancies (apparently healthy women)

first author	year	women (n)	gestational age (weeks) at aPL test	aPL + (n)	aPL + (%)	live birth rate aPL+	live birth rate aPL-
Pattison	1983	933	0	18	2	83	98
Lockwood	1989	737	16	16	2	62	91
Lynch	1994	389	13	95	24	84	93
Yasuda	1994	860	9	60	7	72	90

Comments

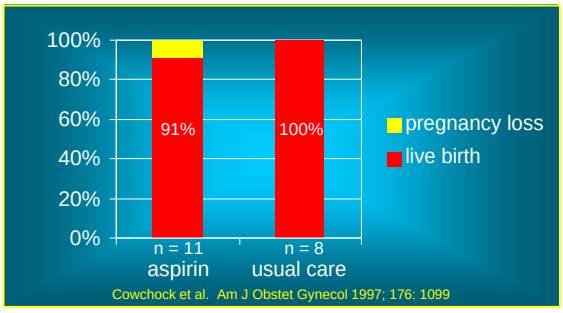
aPL status based on results with a single sample
aPL definitions very heterogeneous

Conclusions

Prevalence of aPL is low in healthy women
With aPL higher rates of pregnancy loss
No indication for screening or treatment

aPL in low-risk pregnancies: no indication for treatment

19 healthy women with aPL and 0-1 spontaneous abortion
Randomisation: aspirin or usual care



Cowchock et al. Am J Obstet Gynecol 1997; 176: 1099

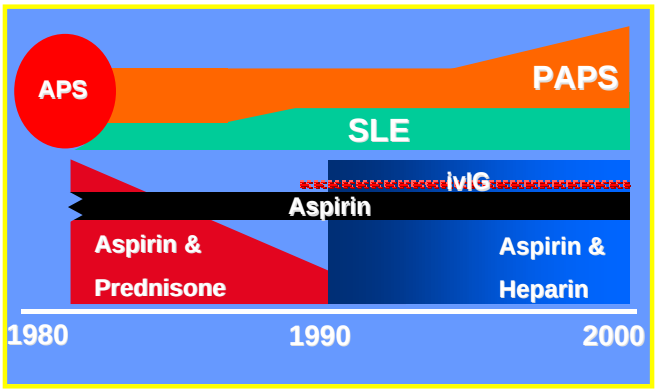
Since 1983 almost every patient with obstetric APS had pharmacologic treatment

6 pregnant women with LAC
4/6 SLE; 6/6 ANA pos.
all 14 previous histories ended with fetal loss (50% > 20 weeks)

R/ prednisone 40-60 mg/day
aspirin 75 mg/day

outcome:
live infant 5/6 (4<37 weeks)
1 fetal loss 16 weeks

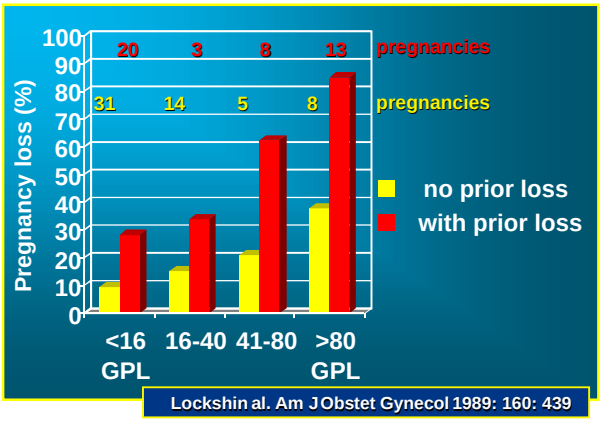
Lubbe et al. Lancet 1983



high-risk groups

first author	year	study type	pregnancies (n)	women (n)	live birth rate (%) related to treatment: any	H	H+A	H+A+γglob
Lockshin	1989	retro	30	25	33	-	-	-
Rosove	1990	retro	15	14	-	93	-	-
Branch	1992	retro	82	54	63	-	-	-
Lima	1996	retro	60	47	70	-	-	-
Branch	2000	rando	16	16	-	-	100	100

Criticism
often > 1 pregnancy/patient included
Up to 31 % primigravida's included
Impossible to compare treatment strategies



Lockshin al. Am J Obstet Gynecol 1989: 160: 439

16 aPL women with poor obstetric prognosis

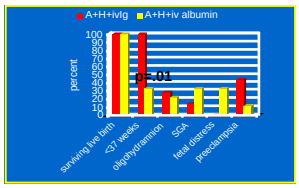
Randomisation:

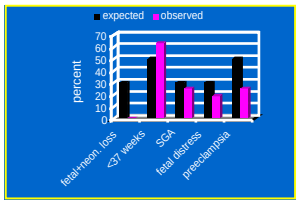
Aspirin, heparin, albumin iv

versus

Aspirin, heparin, high dose iv-IG

With both strategies much better results than was expected





Branch DW et al. Am J Obstet Gynecol 2000: 182: 122

Healthy women with obstetric APS

first author	year	study type (n)	pregnancies = women (n)	live birth rate (%) related to treatment no R/	A	A+H	P+A
Cowchock	1992	rando	20	-	-	75	75
Silver	1993	rando	34	-	100	-	100
Balasch	1993	pro/o	18		91		
Rai	1995	pro/o	20	10			
Kutteh	1996	C/NR	50	-	44	80	-
Kutteh	1996	C/NR	50	-	-	80/76*	-
Tulppala	1997	rando	12	17	17	-	-
Laskin	1997	rando	88	52	-	-	60
Rai	1997	rando	90	-	42	71	-
Backos	1999	pro/o	150	-	-	71	-
Pattison	2000	rando	40	85	80	-	-
Farquharson	2002	rando	98		72	78	-

rando= randomized; pro/o= prospecrive/observational; C/NR = controlled/non-randomized;
*= high/low dose heparin

aPL related recurrent embryonic loss
- without therapy poor prognosis -

20 women WITH aPL
4 consecutive miscarriages
32 years

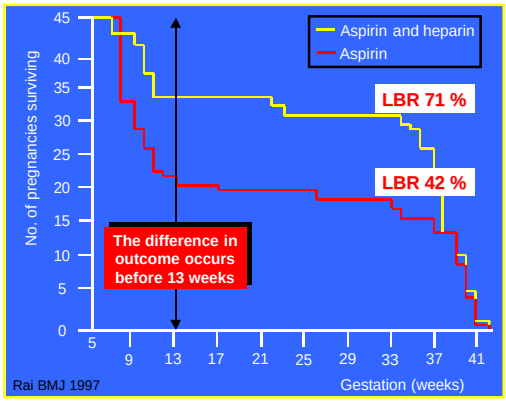
DECLINED MEDICAL TREATMENT

90 % loss
(94 % 1st trimester)

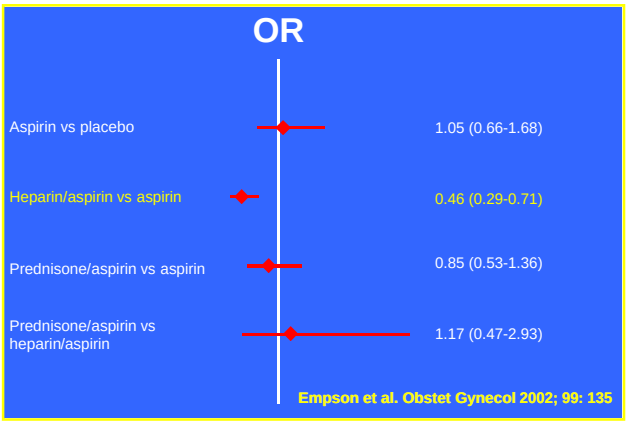
100 women WITHOUT aPL
4 consecutive miscarriages
33 years

34 % loss
(97 % 1st trimester)

Rai et al. HumReprod 1995; 10: 3301



Meta-analysis of RR for pregnancy loss with interventions in APS



Meta-analysis Empson et al. Obstet Gynecol 2002; 99: 135

Aspirin vs. Placebo or usual care (OR 1.05)

first author	year	patients (n)	
Cowchock	1997	11 vs 8	Patients did not have obstetric aps
Tulppala	1997	6 vs 6	Counts blighted ovum and ectopic pregnancy as losses
Pattison	2000	20 vs 20	

Aspirin vs. Heparin+aspirin (OR 0.46)

first author	year	patients (n)	
Kutteh	1996	25 vs 25	Non-randomized study
Rai	1997	45 vs 45	< 45% live birth rates with aspirin

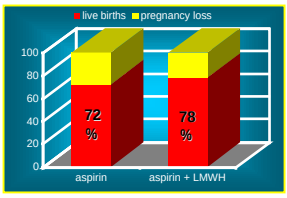
Multicenter randomized trial

recurrent miscarriage

47 aspirin

51 aspirin + LMWH

result: NS



FarquarsonR. Obstet Gynecol 2002

Comparison of the important trials

	Kutteh 1996	Rai 1997	Farquharson 2002
live birth rate (%)			
aspirin	44	42	72
aspirin + heparin	80	71	78
patients			
total	50	90	98
LAC (%)	0	91	42
GPL Units	≥27	≥5	≥9
MPL Units	≥ 23	≥3	≥5
start medication			
aspirin	preconception	pos. pr. test	<12 weeks
heparin	pos. pr. test	pos. fetal heart activity	pos. fetal heart activity
heparin	unfractionated adjusted	unfractionated fixed	LMWH fixed

Supportive care alone results in over 85% live birth rate in women with unexplained recurrent miscarriage

Stray-Pedersen et al. Am J Obstet Gynecol 1984; 148: 140
Liddell et al. Aust NZJ Obstet Gynecol 1991; 31: 320
Clifford et al. Hum Reprod 1997; 12 : 140

- **Frequent antenatal visits**
- **Continuity of care-providers**
- **Liberal admission policy**
- **Multidisciplinary approach with AID**
- **Obstetric ultrasound every 2 to 4 weeks**
- **From 16 weeks on (at least) monthly Doppler velocimetry of umbilical arteries**
- **From 24 weeks on (at least) weekly cardiotocography**
- **From 26-130 weeks on weekly non-stress testing**

A rationale for heparin and aspirin other than anticoagulation

Hu-aPL Ig mice model

- Unfractionated heparin and LMWH, even at subanticoagulant doses, NOT hirudin or Fondaparinux (specific Xa inhibitor), given from day 7-13 prevented complement activation and protected mice from aPL-induced pregnancy complications. Binding aPL-decidua persists.
- Via inhibition of classical pathway inhibition. Enhanced effects by blockade at multiple points in the C-cascade

Girardi et al. Nature Med 2004; 10: 1222

A rationale for heparin and aspirin other than anticoagulation

Explant culture system

Am J Obstet Gynecol 2004; 191: 2125

- Unfractionated heparin attenuated LAC-induced apoptosis and facilitated trophoblast invasion via reduction of apoptosis
- Via interaction with _2GP-1?, heparin-binding epidermal growth factor (HB-EGF)? or fibroblast groth factor 4 (FGF4)?

Am J Obstet Gynecol 2005; 192: 23

- Both unfractionated heparin (25 IU/ml) and aspirin (3 _g/ml) attenuate trophoblast apoptosis

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Editorial

Nutrient-gene interactions in early pregnancy: a vascular hypothesis

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Abstract

It is hypothesized that the following periconceptional and early pregnancy nutrient-gene interactions link vascular-related reproductive complications and cardiovascular diseases in adulthood: (1) Maternal and paternal genetically controlled nutrient status affects the quality of gametes and fertilization capacity; (2) The embryonic genetic constitution, derived from both parents, and the maternal genetically controlled nutrient environment determine embryogenesis and fetal growth; (3) Trophoblast invasion of decidua and spiral arteries is driven by genes derived from both parents as well as by maternal nutritional factors; (4) Angiogenesis, vasculogenesis and vascular function are dependent on the genetic constitution of the embryo, derived from both parents, and the maternal genetically controlled nutritional environment.

Early intra-uterine programming of vessels may concern the same (in)dependent determinants of vascular-related complications during pregnancy and cardiovascular diseases in later life.

Keywords: Embryogenesis; Placental development; Congenital malformations; Preeclampsia

1. Introduction

Abnormal placental development and vascularisation in early pregnancy causes a substantial part of perinatal and maternal damage, for example 8–14% of spontaneous miscarriage and 6–20% of hypertensive disorders. Many birth defects are also related at least in part to vascular disruptions in embryonic tissues [1], and adult cardiovascular disease associated with low birthweight as a proxy for intra-uterine nutritional deprivation [2]. We suggest that periconceptional and early pregnancy nutrient-gene interactions, especially those related to folate, link vascular-related reproductive complications and cardiovascular diseases in adulthood.

2. Nourishment in pregnancy

Tissue-specific nutritional status is partly determined by genes and partly by exogenous factors, such as food intake and life style factors, and partly with endogenous determinants involved in nutrient metabolization and transfer. Growth requires nutrition, which is provided by genetically controlled metabolic and endocrine adaptations. It is likely that nutritional deficiencies affect fertilization capacity and early embryonic development. Malnourishment during folliculogenesis, a period characterized by follicular angiogenesis and transcription of genetic information into proteins, results in deceased oocyte quality [3]. The pre- and early post-implantation period represent a period of rapid growth and differentiation of the morula and blastocyst. Before implantation nutrient requirements are provided by oviductal fluid [4], during implantation nutrients are transported across the trophectoderm, the extra-embryonic coelom and primary yolk sac (histiotrophic nutrition), and after implantation the embryonic vascular system is used. Vessel formation begins with blood island formation in the yolk sac on day 17. They consist of haemoblasts differentiating into bloodcells, and endothelial cells which develop into blood-vessel endothelium. These vessels form a vascular network which vascularize the yolk sac, connective stalk and chorionic villi invading the spiral arteries (haematrophic nutrition). On day 18 vasculogenesis starts in the embryonic mesoderm, in which angioblasts develop into cords and form the initial embryonic circulatory system [5]. After 9 weeks amenorrhea the regression of the yolk sac starts and trophoblastic plugs, that obliterate the tip of the utero-placental arteries, gradually disappear. As a result a continuous blood

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flow in the intervillous space of the placenta allows the exchange of nutrients and oxygen [6].

3. Hypothesis

We postulate that interactions between embryonic genes, derived from both parents, and the maternal nutritional status, in particular that of folate, affect vascular-related reproduction processes from fertilization throughout embryogenesis and pregnancy. These interactions also contribute to the risk of cardiovascular diseases in adulthood. Folate is essential for DNA synthesis and remethylation of homocysteine into methionine. *S*-Adenosylmethionine is an important methylgroupdonor for cellular metabolism and regulation of the expression of certain developmental genes. Folate deficiency leads to hyperhomocysteinemia and is due to a reduced intake, bioavailability, functional aberrations in methylene tetrahydrofolate (MTHFR) or methionine-synthase reductase (MTRR) genes, or enhanced needs. This can result in aberrant DNA synthesis and inhibition of DNA methyltransferase by *S*-adenosylhomocysteine resulting in compromised cellgrowth and meiotic nondysjunction. The toxicity of hyperhomocysteinemia has been shown on neural crest and endothelial cells [7,8]. Moreover, homocysteine promotes the growth of vascular smooth muscle cells as a link to atherosclerosis [9].

The following nutrient-gene interactions can be identified:

1. The maternal and paternal genetically controlled nutrient status affects the quality of gametes and fertilization capacity. Folate, present in follicular fluid and seminal plasma, may influence the quality of follicles, oocytes and semen. This is supported by the significantly increased sperm count after folic acid and zincsulphate intervention [10]. Aneuploidy associated with ovarian hyperstimulation may be due to follicular folate depletion leading to chromosomal segregation and methylation disorders. Otherwise chronic nutritional deprivation including that of folate, in utero and/or during postnatal life, may affect oocyte pool, quality, fertilization capacity and the occurrence of aneuploidy. The associations between polymorphism's in MTHFR and MTRR genes and the increasing likelihood of meiotic nondisjunctions, such as in Down syndrome, support this hypothesis [11].
2. The embryonic genetic constitution, derived from both parents, and the maternal genetically controlled nutrient environment determine embryogenesis and fetal growth. A diminished embryonic folate status, due to MTHFR and MTRR polymorphisms and/or a compromised maternal folate status due to the same polymorphism's and interactions with exogenous and endogenous determinants, are risk factors for neural tube and congenital heart defects [12,13]. We hypothesize that folate deficiency and mild hyperhomocysteinemia detrimentally affect the precise control of embryonic cellular processes such as migration, differentiation, proliferation, apoptosis and intracellular signaling. Moreover, the disbalanced folate, homocysteine and NO-status may disturb embryonic vasculogenesis, through which the delivery and clearance of these and other nutrients is compromised [14].
3. The trophoblast invasion of decidua and spiral arteries is driven by genes derived from both parents as well as by maternal nutritional factors. Moreover, nutrients in maternal blood are essential to counteract the oxidative stress in the intervillous space and trophoblast. Thus, nutrient shortages will affect trophoblast function and invasion and may contribute to spontaneous abortion, preeclampsia and fetal growth restriction. Folate deficiency could be one of such factors detrimentally affecting vasculogenesis of the yolk sac, embryonic tissues and placenta. Trophoblast apoptosis is a possible mechanism involved. Increased apoptosis in trophoblastic cells cultured in folate-free medium [15], hyperhomocysteinemia during early pregnancy prior to the development in preeclampsia [16], and increased placental apoptosis [17] in preeclampsia are herewith in line.
4. Angiogenesis, vasculogenesis and vascular function are dependent on the genetic constitution of the embryo, derived from both parents, and the maternal genetically controlled nutritional environment. We hypothesize that the early intra-uterine programming of vessels concerns the same (in)dependent determinants of vascular-related complications during pregnancy and cardiovascular diseases in later life. Folate and hyperhomocysteinemia are related to congenital heart diseases, carotid artery wall thickness and cardiovascular diseases [18,19]. Therefore, hyperhomocysteinemia could be the link between congenital heart disease in the offspring and maternal cardiovascular diseases in later life. The induction of endothelial dysfunction in the embryo as well as in the adult, partially due to a mechanism involving reactive oxygen species induced by hyperhomocysteinemia, could be an underlying mechanism [14]. This is supported by the higher prevalence of chronic hypertension in mothers with congenital heart disease offspring and fits with the hypothesis of intra-uterine programming of cardiovascular diseases [13].

4. Conclusion

Angiogenesis and vasculogenesis are fundamental features of reproduction [20]. Fetal growth and cardiovascular diseases in adulthood seem to be related. We consider nutrient-gene interactions, in particular that of folate, to contribute to congenital defects, vascular-related pregnancy complications and cardiovascular diseases in later life. Therefore, strategies should be developed, evaluated and implemented to prevent these complications and diseases in the next generation.

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Factor V gene polymorphism studies and fetal loss

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(NO TEXT RECEIVED)

NOTES

A new logistic regression model for predicting the outcome of pregnancies of unknown location

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(NO TEXT RECEIVED)

NOTES

Evidence based practice for management of early pregnancy loss

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Aims

- To demonstrate that classification of evidence in levels is misleading
- (or at least should be differentiated in therapeutic and non-therapeutic issues)
- To provide funded recommendations on what to do in recurrent miscarriage

ESHRE Winter symposium• december 10th 2005

Evidence-based medicine is the judicious and conscientious use of current best evidence from medical care research for making medical decisions.

ESHRE Winter symposium – december 10th 2005

Evidence-based medicine (EBM)
is the integration of
best research evidence with
clinical expertise and patient
values.

Pubmed-search

- "Abortion, Habitual"[MeSH]
  - 4527 hits
- Limits
  - Randomized clinical trial 83 hits
  - Meta-analysis 23 hits
  - Review 631 hits

Royal College of Obstetricians and Gynaecologists

Setting standards to improve women's health

Guideline No. 17
Revised May 2003

THE INVESTIGATION AND TREATMENT OF COUPLES WITH RECURRENT MISCARRIAGE

1. Purpose and scope

Recurrent miscarriage is defined as the loss of three or more pregnancies. Recurrent miscarriage is a heterogeneous condition that has many possible causes; more than one contributory factor may underlie the recurrent pregnancy losses.

The art of medicine consists of amusing the patient while nature cures the disease

ESHRE Winter symposium – december 10th 2005

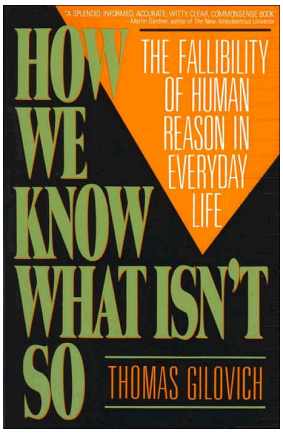

Table 2.1 page 13

ESHRE Winter symposium – december 10th 2005

Human Reproduction vol.14 no.11 pp.2868–2871, 1999

A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage

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Recurrent miscarriage is a difficult clinical problem occurring in ~1–2% of fertile women. Following investigation, most cases fail to reveal an identifiable cause and are therefore classified as idiopathic. The aim of this study was to identify important gestational milestones for pregnancy success prediction in women following idiopathic recurrent miscarriage. A total of 325 consecutive patients with idiopathic recurrent miscarriage was involved in a prospective longitudinal observational study. Patients were identified from a miscarriage database of 716 patients. Preconceptual presentation and investigation excluded patients from the study sample with known associations of recurrent pregnancy loss, such as antiphoshollipid syndrome, oligo-

of management of these patients is empirically based upon tender loving care and emotional support.

In the absence of predicted success rates with idiopathic recurrent miscarriage, the clinician is at a disadvantage in the miscarriage clinic setting, where the most commonly posed question concerns the chance of future pregnancy success. Previous population studies are small, and few have documented sufficient patient numbers to generate confidence with clinical prediction of future pregnancy outcome, in terms of success or failure. The effect of emotional support, supplemented by ultrasound in early pregnancy gives 'success rates' of between 70 and 80% (Stray-Pedersen and Stray-Pedersen, 1984; Liddell *et al.*, 1991; Clifford *et al.*, 1997). As important as an overall success rate, however, is the significance of each gestational milestone attained in the first trimester, which has not been previously determined.

In this large prospective study, an attempt has been made to identify important gestational milestones for women presenting

Assessment of a Prognostic Study

- Was an inception cohort assembled?
- Was complete follow-up achieved?
- Was outcome assessment blinded?
- Were different prognostic profiles taken into account?

Was an inception cohort assembled?

- History of two or three consecutive miscarriages
- Exclude known associations of recurrent pregnancy loss (APL, oligomenorrhea, cervical weakness, abnormal chromsomes)
- No clear description of work-up

Was outcome assessment blinded?

Was outcome assessment blinded?

- Standardized clinical protocol
- Transvaginal sonography
- Assessment of viability at 12 weeks
- Succesfull outcome: survival beyond 24 weeks

Was complete follow-up achieved?

- 716 consecutive patients in database
  - 325 had 'idiopathic recurrent miscarriage'
  - 23 lost to follow-up
  - 76 no further pregnancy
  - 2 ectopics, 2 terminations of pregnancy
- 222 with 'at risk' for miscarriage
  - 90% were seen < 8 weeks, 98% < 10 weeks

Differences in prognosis

- Results were stratified for age and number of previous miscarriages

Table III. Predicted percentage success rate of subsequent pregnancy according to age and previous miscarriage history

Age (years)	Number of previous miscarriages			
	2	3	4	5
20	**92 (86–98)**	**90 (83–97)**	**88 (79–96)**	85 (74–96)
25	**89 (82–95)**	**86 (79–93)**	**82 (75–91)**	79 (68–90)
30	**84 (77–90)**	**80 (74–86)**	**76 (69–83)**	71 (61–81)
35	**77 (69–85)**	**73 (66–80)**	**68 (60–75)**	62 (51–74)
40	69 (57–82)	64 (52–76)	58 (45–71)	52 (37–67)
45	60 (41–79)	54 (35–72)	48 (29–67)	42 (22–62)

Values are percentages with 95% confidence intervals (CI) shown in parentheses. Where the CI <20%, the values are shown in bold print.

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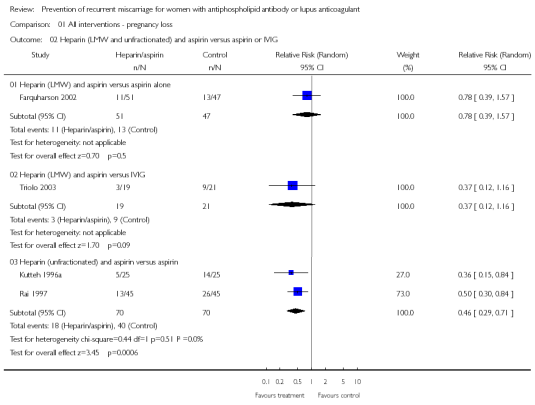
Values are percentages with 95% confidence intervals (CI) shown in parentheses. Where the CI <20%, the values are shown in bold print.

Level of Evidence IV

Strength of recommendation C

Fig. 2. Comparison 01. All interventions - pregnancy loss

01.02 Heparin (LMW and unfractionated) and aspirin versus aspirin or IVIG



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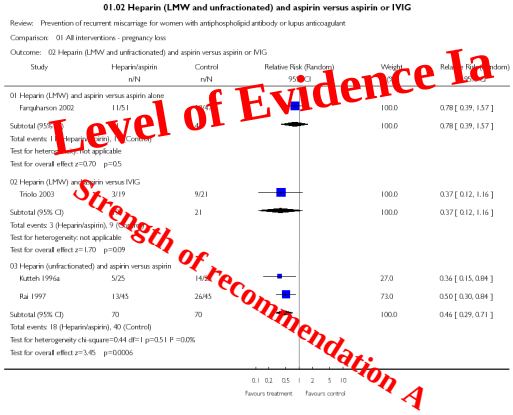

Level of Evidence Ia

Strength of recommendation A

Study	Rai 1997
Methods	Single centre, non-blinded, non-placebo controlled RCT.
Participants	Inclusion criteria: 1) >/= 3 consecutive miscarriages. 2) +ve APL antibody on at least 2 occasions > 8 weeks apart determined by ACL IgG > 5 GPL units or ACL IGM > 3 MPL units or a positive LA (APTT, dRVVT ratio>/= 1.1 confirmed by platelet neutralisation - decrease of >/= 10% of ratio). Exclusion criteria: 1) Previous thromboembolism. 2) SLE.

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The prevalence of persistently positive tests for LA was 9.6% and for immunoglobulin G (IgG) and immunoglobulin M (IgM) ACA was 3.3 and 2.2% respectively.

Hum Reprod. 1995 Aug;10(8):2001-5.

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The prevalence of persistently positive tests for LA was 9.6% and for immunoglobulin G (IgG) and immunoglobulin M (IgM) ACA was 3.3 and 2.2% respectively.

Repeat testing, after an interval of at least 8 weeks, demonstrated that only 65.7% of LA positive, 36.6% IgG ACA positive and 36.0% IgM ACA positive women on initial testing had a second positive test result.

Hum Reprod. 1995 Aug;10(8):2001-5.

Guidelines

Despite statements on the need for repeated testing with 8 weeks intervals and the treatment effect of heparin, no clear statements are given on when to test for APS

Age
Number of miscarriages

Level of Evidence Ia???

[Treatment outcome in women suffering from recurrent miscarriages and antiphospholipid syndrome]

[Article in Polish]

Malinowski A, Dynski MA, Maciolek-Blewniewska G, Glowacka E, Pawlowski T, Babula G

Kliniki Ginekologii Operacyjnej i Endoskopowej Instytutu Centrum Zdrowia Matki Polki w Lodzi.

OBJECTIVE: To evaluate the outcomes of treatment in patients suffering from recurrent spontaneous abortion and antiphospholipid syndr
METHODS: 148 observed women suffering from recurrent abortion with presence of lupus anticoagulant antibodies (LA) and/or high mc
antibodies (ACA) have been divided randomly into followed three treated groups: I--56 patients treated by low-dose of acetylsalicylic ac
treated by low molecular weight heparin (applied in dose of 20 g daily); III--53 patients treated by LDA and low molecular weight hepari
been affirmed that coincidental application of low-dose of acetylsalicylic acid and low molecular weight heparin statistically more often inc
pregnancy in comparison with application of low molecular weight heparin or acetylsalicylic acid alone. In the group where only low-dose
success of pregnancy equaled 89.3%, in the group where only low molecular weight heparin was applied the successful pregnancy equale
acetylsalicylic acid and low molecular weight heparin being applied together the successful pregnancy equaled 92.5%. In has simultaneous
pregnancy loss is statistically higher in the women suffering from isolated occurrence of lupus anticoagulant antibodies (21.2%) in compari
occurrence of anticardiolipin antibodies (6.7%) and anticardiolipin antibodies with lupus anticoagulant antibodies simultaneously. CONCL
of low-doses of acetylsalicylic acid and low molecular weight heparin seems to be the best solution in patients suffering from recurrent spo

Genetic causes

RCOG:
All couples with recurrent miscarriage should have peripheral blood karyotyping performed.

NVOG:
Peripheral blood karyotyping should be offered after two miscarriages

Genetic causes

Selective chromosome analysis in couples with two or more miscarriages: case-control study

Maureen T M Franssen, Johanna C Korevaar, Nico J Leschot, Patrick M M Bossuyt, Alida C Knegt, Klasien B J Gerssen-Schoorl, Cokkie H Wouters, Kerstin B M Hansson, Ron Hochstenbach, Kamlesh Madan, Fulco van der Veen, Mariette Goddijn

Abstract

Objective To identify additional factors, such as maternal age or factors related to previous reproductive outcome or family history, and the corresponding probability of carrying a chromosome abnormality in couples with two or more miscarriages.
Design Nested case-control study.
Setting Six centres for clinical genetics in the Netherlands.
Participants Couples referred for chromosome analysis after two or more miscarriages in 1992-2000; 279 carrier couples were marked as cases, and 428 non-carrier couples served as controls.
Main outcome measures Independent factors influencing the probability of carrier status and the corresponding probability of carrier status.
Results Four factors influencing the probability of carrier status could be identified: maternal age at second miscarriage, a history of three or more miscarriages, a history of two or more miscarriages in a brother or sister of either partner, and a history of two or more miscarriages in the parents of either

Obstetricians and Gynaecologists recommends chromosome analysis after three miscarriages, whereas the American College of Obstetricians and Gynecologists and the Dutch Society of Obstetrics and Gynaecology recommend chromosome analysis after two miscarriages.[1–3]

These guidelines are based on the fact that the probability of carrier status is increased after two or three miscarriages. Whether this probability is also modified by maternal age or by factors related to previous reproductive outcome or family history is not known. If it is, the possibility of withholding chromosome analysis from couples with a low probability of carrier status could be considered. We aimed to identify additional factors influencing the probability of carrier status in couples with two or more miscarriages and to calculate the associated probability of carrier status for every combination of these factors.

Methods

Patients

Nested case-control study

Population 12.000 couples having chromosome analysis for ≥2 miscarriages.

Carrier couples (cases) and non-carrier controls

Exposure:

- maternal age
- number of miscarriage
- history of two or more miscarriages in a brother or sister of either partner,
- history of two or more miscarriages in the parents of either partner.

Table 3 Factors influencing probability of carrier status after multivariate logistic regression analysis (P≤0.10)*

Covariates	Odds ratio (95% CI)	P value
Maternal age (years) at second miscarriage:		
<23	6.2 (1.1 to 34.3)	0.04
23-33	6.1 (1.3 to 27.7)	0.02
34-36	3.3 (0.7 to 16.1)	0.13
37-38	2.3 (0.4 to 12.0)	0.33
≥39	1.0	–
3 *v* ≥2 miscarriages	1.4 (1.0 to 2.1)	0.05
≥2 miscarriages in a brother or sister	1.9 (1.1 to 3.2)	0.02
≥2 miscarriages in parents	1.4 (0.9 to 2.2)	0.10

*Limited to 528 couples with complete data.

Table 4 Probability of carrier status in couples with two or more miscarriages, according to multivariate logistic regression model*. Values are percentages

Maternal age (years) at second miscarriage	(RM_m)	($RM_{parents}$) +		($RM_{parents}$) –	
		≥3 misc	2 misc	≥3 misc	2 misc
<23	+	10.2	7.3	7.3	5.2
	–	5.7	4.0	4.1	2.8
23-33	+	10.0	7.2	7.2	5.1
	–	5.7	4.0	4.0	2.8
34-36	+	5.8	4.1	4.1	2.9
	–	3.2	2.2	2.2	1.6†
37-38	+	4.0	2.8	2.8	2.0†
	–	2.2	1.5†	1.5†	1.1†
≥39	+	1.8†	1.2†	1.3†	0.9†
	–	1.0†	0.7†	0.7†	0.5†

RM_m=history of ≥2 miscarriages in a brother or sister of either partner; $RM_{parents}$=history of ≥2 miscarriages in parents of either partner; ≥3 misc=history of ≥3 miscarriages in couple; 2 misc=history of ≥2 miscarriages in couple.
*Limited to 528 couples with complete data.
†Couples with probability of carrier status <2.2%.
Intercept based on the total population = –5.388.

Prevalence of Uterine Malformations

Uterine malformations are identified in 15% of RPL cases.

Type	Cases	Percent of Anomalies
Arcuate	255	18
Septate	486	35
Bicornuate	362	26
Unicornuate	134	10
Didelphys	114	8
Agenesis	40	3

Summary of nine prevalence reports
Grimbizis et al 2001. Hum Reprod Update 7:161-74
Adapted from John Collins

Untreated Uterine Malformations

Type	Conceptions	Live Births (%)
Arcuate	241	66
Septate	499	50
Bicornuate	627	55
Unicornuate	260	54
Didelphys	152	56
Agenesis		

Summary of nine prevalence reports
Grimbizis et al 2001. Hum Reprod Update 7:161-74
Adapted from John Collins

Surgical Procedures for Septate Uterus

Year	Patients	Metroplasty	Births (%)	Reference
1990	73	abdominal	70	Candiani et al. BJOG 1990; 97:613-7.
1997	116	none	67	Heinonen. JAAG Lapar 1997; 4:311-7.
	32	abdominal	87	
	20	hysteroscopic	91	
2001	72	"	46	Venturoli ea. Arch GO 2002;266:157-9.
2002	59	"	80	Saygili-Yilmaz Arch GO 2003;268:289.
2002	322	"	83	Grimbizis ea 2001. HRUpdate 7:161-74

Summary of nine prevalence reports
Grimbizis et al 2001. Hum Reprod Update 7:161-74
Adapted from John Collins

Conclusions

- Spontaneous cure rate > 50%
- Randomised studies, if present, do not have a non-intervention arm
- Although patients with APS should be treated with heparin, it is not clear who should be treated for APS

Conclusions

- Genetic testing should depend on age, number of miscarriages and family history
- The levels of evidence are misleading in the assessment of prognostic/ diagnostic studies


Comparable groups?


Randomisation


to the U.S. Constitution

Uterine anomalities and recurrent miscarriage

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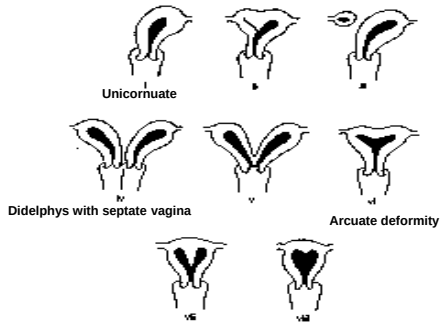
Prevalance

Rate of uterine anomalies in UK and estimates in other populations vary between 3-5% (Acien, 1997)

Can be as high as 43% from high risk populations with infertility and recurrent pregnancy loss

Uterine malformations have been associated with recurrent miscarriage, second trimester abortion, late fetal loss, preterm labour, malpresentation and increased incidence of caesarean section

Types of Abnormalities



Outcome of pregnancy

Fetal survival rates in untreated uterine anomalies

Type of uterine anomaly	Acien, 1993 N = 176 Fetal survival rate (%)	Heinonen *et al*, 1982 N = 182 Fetal survival rate (%)
Unicornuate	71	40
Didelphys	72	64
Bicornuate	44	64
Septate	59	86
Subseptate	69	89

Types of surgical interventions for uterine anomalies

Abdominal wedge resection
(Jones / Strassman procedure)
Excision of septum
(Tomkin's procedure)
Hysteroscopic excision
- shorter inpatient stay
- reduced operating time
- avoidance of laparotomy
- possibility of vaginal delivery

Reproductive Performance

Septate uterus is only type amenable to hysteroscopic surgical correction

HSG can only demonstrate bicornuate or septate uterus

Diagnostic hysteroscopy and laparoscopy can distinguish between the two

Reproductive Performance

Didelphic Uteri

- **23-43% fetal wastage rates**
- **57% successful pregnancy without any treament**
- **Similar success rates (60%) with unicornuate uteri**

Semmens 1962
Musich & Behrman 1978

Reproductive Performance

Septate Uteri

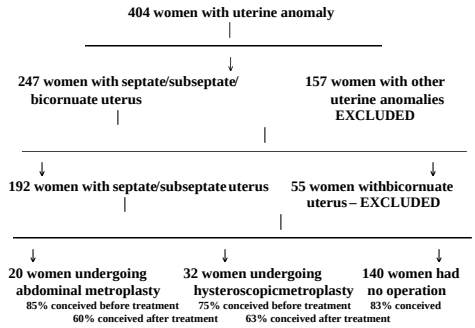
- **10 year retrospective study from Italian multicentre data reporting experience**
- **973 women**
- **Hysteroscopic metroplasty**
- **Pregnancy rates "good"**
- **Hysteroscopic surgery safe and effective in pregnancy rates and outcome**

Colacurci *et al* 1998

Infertility and hysteroscopic metroplasty

Colacurci *et al* 1996 and Daly *et al* 1989 separately looked at the role of metroplasty in infertility and demonstrated that conception rates were not different to the general infertile population after hysteroscopic metroplasty and that metroplasty did not 'cure' infertility.

Summary of critical appraisal of comparison of women with abdominal or hysteroscopic metroplasty or no treatment (Heinonen, 1997)

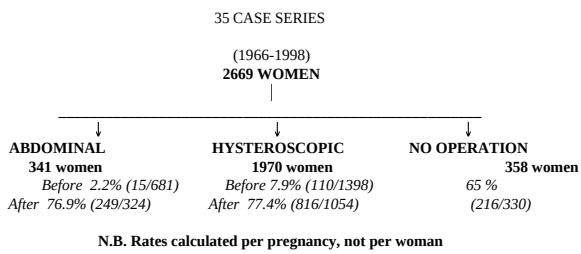


The group was heterogenous, containing women with both recurrent and late pregnancy loss and with infertility

87% of these women had another attributed cause for their failure to conceive

This study showed that surgery reduced conception rates and did not produce a significant improval in pregnancy rate or fetal survival

Summary of evidence available on fetal survival rates from case series on abdominal and hysteroscopic metroplasty in septate uterus.



N.B. Rates calculated per pregnancy, not per woman

Reproductive Performance

Proposed Theories for Miscarriage

- **Cervical Incompetence** ✗
- **Inadequate Vascularisation**
- **Volume Defect of Uterine cavity**

Reproductive Performance

Inadequate Vascularisation

Histologic study shows that septum has:

- **Less connective tissue**
- **More muscle fibres**
- **More vessels than uterine wall**

Dabirashrafi *et al* 1995

Reproductive Performance

Marked increased volume may occur after excision of septum

Wedge resection following abdominal metroplasty markedly reduces uterine volume

Fetal survival is same in both procedures

Conclusions

- Counsel patients to make informed decisions
- Abdominal metroplasty has been replaced by hysteroscopic metroplasty in the treatment of septate and subseptate uterus in the last decade
- Evidence supporting hysteroscopic metroplasty is of poor quality on critical appraisal
- Metroplasty in recurrent abortion and infertility may reduce conception rates
- Other causes for pregnancy loss and infertility should be excluded before embarking on surgery for uterine anomaly i.e. Causes of infertility or miscarriage may be inherent rather than related to uterine anomaly
- Until a multi-centre Randomised Controlled Trial is conducted to address the efficacy of surgery on uterine anomaly, more harm than good may be done by performing hysteroscopic surgery

Outcomes after threatened miscarriage and placental haematomas in early pregnancy

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Introduction

Threatened Miscarriage, defined as vaginal bleeding before 24 weeks gestation occurs in 15-20% of ongoing pregnancies1. It is the commonest reason for emergency gynaecology GP referrals in the UK. Threatened miscarriage in the first trimester has been associated with many adverse pregnancy outcomes. If not associated with immediate fetal loss, threatened miscarriage has been linked to fetal loss later in the pregnancy1;2 as well as abruption3-5, fetal growth restriction (FGR), pre-term labour (PTL)3-5, preterm pre-labour rupture of the membranes (PPROM)4, pre-eclampsia (PET)4 and low birth weight6 (LBW). A frequent ultrasonographic finding in women with bleeding in the first trimester is the presence of subchorionic bleeding or intrauterine haematoma (IUH). These IUH are present in approximately 18% of women with threatened miscarriage7;8 and there has been much debate over the resolution and clinical significance of their presence on ultrasound. The incidence of miscarriage has been variably reported and ranges from 4-33 % depending on the gestation at presentation9. Intrauterine hematoma have been shown to be associated with an increased incidence of pre-term labour and low birth weight6, but an association with complications such as PPROM, fetal growth restriction and pregnancy-induced hypertension is still debated6;9-13.

If the link between first trimester threatened miscarriage and PET, FGR, PPROM and subsequent PTL is confirmed, it will have major implications for health care resources and provision. Identification of women at risk remains the only strategy for reducing the incidence of premature delivery and if 15-20% of ongoing pregnancies are complicated by threatened miscarriage, a large number of potentially high risk women are going unidentified. Many maternal serum markers have been investigated in attempts to predict the outcome of pregnancy in the first trimester and in particular the likelihood of subsequent miscarriage, with varying degrees of success. The relationship between first trimester serum markers and later pregnancy complications is uncertain14;15 and associations have been made between low levels and FGR14;16 and pregnancy induced hypertension (PIH).

Objectives

The objectives of this prospective study were to investigate the link between threatened miscarriage and placental haematoma and adverse pregnancy outcome, and also to determine if levels of inhibin A, activin A, follistatin, hCG and PAPP-A in women who present with first trimester threatened miscarriage are related to pregnancy outcome.

Materials and Methods

We identified women with a clinical diagnosis of threatened miscarriage who were referred to the Early Pregnancy Unit of a large London teaching hospital by their GP or from the A&E department between April 2003 and March 2004 and followed them prospectively until the outcome of the pregnancy was known.

Results

The first trimester miscarriage rate, after confirmation of viability in the threatened miscarriage group was 9.3%. Compared with controls, women presenting with threatened miscarriage were more likely to deliver prematurely (RR 2.29, 95%CI 1.4-4.6) and this was most likely to be between 34 and 37 weeks. They were also more likely to have pre-term pre-labour rupture of the membranes (RR 3.72, 95% CI 1.2-11.2).

When gestation at maximum hematoma volumes for each pregnancy were compared, those that ended with first trimester miscarriage reached maximum volume significantly earlier than term births (p=0.001). Pregnancies ending in pre-term birth reached maximum hematoma volume significantly later than term births (p=0.00).

Inhibin A levels were significantly lower in cases of threatened miscarriage that ended in first trimester miscarriage, when compared with both pre-term and term labours (p=0.04 and p=0.0007 respectively). The levels were also lower in the miscarriage cases than controls (p=0.02). Activin A levels were lower in the cases that ended with first trimester miscarriage than pre-term (p=0.018) and control cases (p=0.012) but not for term births. The trend of the hCG levels were similar to that of inhibin A. The levels were significantly lower in cases of threatened miscarriage that subsequently ended in a first trimester miscarriage when compared with pre-term (p=0.017) or term deliveries (p=0.0001). hCG levels were significantly higher when all cases of threatened miscarriage were combined and compared with the control pregnancies (p=0.0009) with pre-term and term births having significantly higher hCG levels than the control pregnancies individually (p=0.032 and p=0.0001 respectively). PAPP-A levels in the threatened miscarriage group were lower in pregnancies ending in first trimester miscarriage (p=0.033) when compared with term births.

Overall, oestradiol levels were significantly lower in the threatened miscarriage group when compared with the controls (p=0.02) and with those that went on to both a pre-term and a term birth (p=0.014 and p=0.0001 respectively). Progesterone levels were lower in the cases of threatened miscarriage that went on to miscarry when compared with pregnancies that delivered at term (p=0.03).

Logistic regression analysis of inhibin A and hCG MoM's found that inhibin A in isolation provided the best predictor for miscarriage in the first trimester miscarriage after threatened miscarriage.

Discussion

Women with threatened miscarriage in the first trimester are at increased risk of premature delivery and this risk factor should be taken into consideration when deciding upon antenatal surveillance and management of their pregnancies. In this study, inhibin A alone was found

to be highly predictive of first trimester miscarriage, without the addition of other markers and its potential for use with other markers such as ultrasound parameters and demographic features requires further investigation. Development of interventions, such as progesterone and antioxidant supplementation require further investigation, however identification of women at risk would allow such interventions to be implemented from an early gestation.

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Coping with pregnancy loss

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Human aspects of pregnancy loss

- Experiences and feelings
- Wants and needs
- Meeting those needs
- Meeting your needs

The Miscarriage Association

Acknowledging pregnancy loss

Individuality of experience

- This woman / couple
- This pregnancy / this time

Possible responses

- Relief
- Regret
- Acceptance & moving on

Common responses (1)

- Shock
- Loss & grief
- Confusion & powerlessness
- Anxiety & fear
- Anger .....

Common responses (2)

- Seeking explanations
- Guilt & self-blame
- Isolation & loneliness
- Jealousy
- Loss of confidence

Wants and needs

Everyone differs, but …

- Respect
- To be heard
- Acknowledgement of feelings
- Information

Meeting those needs (1)

You can't meet them all, but …

- Respect for the patient, partner and baby
- Listening
- Acknowledging feelings
- Providing information …..

Meeting those needs (2)

- Explaining process and events
- Meeting physical needs
- Sensitive terminology
- Language and culture

Your feelings & needs

- Coping with patients' responses
- Your own feelings

Meeting those needs

- Support for yourself
  - in the workplace
  - external sources
- Time out

The Miscarriage Association

A national UK charity:

- **offering support**
- **providing information**
- **raising awareness**
- **promoting good practice**

The Miscarriage Association

Acknowledging pregnancy loss

Does endometriosis affect implantation?

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