ESHRE Campus workshop

NEW TRENDS IN DIAGNOSIS AND MANAGEMENT OF EARLY PREGNANCY FAILURE

Poznan - Poland

15 to 16 December 2006
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Organisation

Organising Committee

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- Professor Eric Jauniaux - Past coordinator of the SIG
- Dr Ole Christiansen (DK) - Deputy of the SIG
- Dr Niek Exalto (NL) - Deputy of the SIG

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- T. Paszkowski, Lublin (PL)
- S. Quenby, Liverpool (UK)
- O. Sipak-Szmigiel, Szczecin (PL)
- P. Skalba, Katowice (PL)
- J. Skrzypczak, Poznan (PL)
Course description & objectives

The Poznan ESHRE Winter Symposium follows on from successful meetings held in Amsterdam and Liverpool. 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.
PROGRAM – 15 DECEMBER 2006

09.00: Registration and Welcome

Session 1: Introduction

09.30: Mechanisms of early pregnancy failure
N. Exalto, Hoofddorp (NL)

10.00: Cytogenetic aspects
M. Goddijn, Amsterdam (NL)

10.30: Early pregnancy clinics
R. Farquharson, Liverpool (UK)

11.00: Coffee Break

Session 2: Miscarriage

11.30: Expectant, medical or surgical treatment
P. Ankum, Amsterdam (NL)

12.00: Gestational trophoblastic disease
E. Jauniaux, London (UK)

12.30: Abnormalities in multiple pregnancy
G. Breborowicz, Poznan (PL)

13.00: Lunch Break

Session 3: Ectopic Pregnancy

14.00: Pregnancy of unknown location
E. Kirk, London (UK)

14.30: Ectopic pregnancy
M. Klimek, Kraków (PL)

15.00: Methotrexate in ectopic pregnancy
T. Paszkowski, Lublin (PL)

15.30: Coffee Break

Session 4: Recurrent miscarriage

16.00: ESHRE Guideline
O.B. Christiansen, Copenhagen (DK)

16.30: Recurrent miscarriage in Poznan
J. Skrzypczak, Poznan (PL)

17.00: Congenital uterine anomaly
J. Kotarski, Lublin (PL)
PROGRAM – 16 DECEMBER 2006

Session 5: Infection

09.00: Infection and early pregnancy loss
T. Niemiec, Warszawa (PL)

09.30: Sildenafil treatment of women with recurrent miscarriage. Influence on pregnancy outcome
M. Jerzak (PL)

10.00: Metformin treatment with PCOS and miscarriage
P. Skalba, Katowice (PL)

10.15: Immunogenetics and early pregnancy failure
O. Sipiak-Szmigiel, Szczecin (PL)

10.30: Coffee Break

Session 6: New Horizons

11.00: Failure of natural selection?
S. Quenby, Liverpool (UK)

11.30: Leukaemia Inhibiting Factor and implantation
M. Mikolajczyk, Poznan (PL)

12.00: Closing remarks and end of the meeting
Early Pregnancy Failure, a Review

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Summary

The European Society for Human Reproduction and Embryology’s Special Interest Group Early Pregnancy recently published a revised terminology for the description of early pregnancy events as well as evidence-based guidelines for the investigation and medical treatment of patients with recurrent miscarriage (RM). This review, designed for clinicians working in the field of human reproduction, starts with the updated glossary of terms essential for an accurate assessment and documentation of clinical events. The revised terminology is summarized in Table I. Many terms used in early pregnancy were old and had persisted before or since the introduction of ultrasound.

Keywords: Early pregnancy, nomenclature, recurrent miscarriage, diagnosis, treatment.

Introduction

Spontaneous miscarriage is the most common complication of human pregnancy. In the last decades the increasing use of high-resolution ultrasound and β human chorionic gonadotrophin (hCG) measurements, documented against the background of an accurate assessment of gestational age, has considerably improved our knowledge of the epidemiology and pathophysiology of early pregnancy events. Clinical assessment of all first-trimester losses, using an accurate description and classification, has become pivotal for the accurate evaluation of the different aetiologies, the parental grief process and for the counselling about therapeutic options for couples presenting with recurrent miscarriage (RM).

The European Society for Human reproduction and Embryology (ESHRE) recently recommended a revised terminology for the description of early pregnancy events (Farquharson et al 2005). The traditional grouping of all pregnancy losses before 24 weeks as “abortion”, for example, may have had pragmatic origins before the advent of ultrasound imaging 40 years ago, but it is poor in terms of definition and makes little clinical sense. The term abortion is also confusing for the parents as it also refers to the voluntary termination of pregnancy. Another example is the classical 1940s histopathological term, i.e. “blighted ovum” which should also be abandoned in view of the ultrasound findings showing that in the vast majority of these cases embryonic remnants can be found. The revised terminology was adopted recently by the Royal College of Obstetricians and Gynaecologists (RCOG 2006)

Over the last 50 years, couples presenting with RM have been offered an incredible range of therapeutic options resulting in an overwhelming amount of highly controversial data, variable results and medical disasters and scandals. Wide variations in patient selection criteria and treatment protocols, small size of most individual studies, poor stratification bias and matching of cases and controls have limited the translation of results into clinical practice. New randomized controlled trials
(RCTs) and meta-analysis have recently been published in the international literature. This has prompted the ESHRE’s Special Interest Group Early Pregnancy to provide an evidence-based guideline for the investigation and medical treatment of these patients (Jauniaux et al 2006).

The aim of the present review is to provide a brief summary of the revised early pregnancy terminology and investigation and treatment options especially for clinicians with special interest in human reproduction and fertility.

Normal embryonic development

The embryonic period starts at fertilization and occupies the first eight post-fertilization weeks, during which organogenesis takes place. Thereafter, the fetal period is characterized by growth. Embryologists prefer the term embryonic age and assess this by using 23 internationally recognized morphological stages (O’Rahilly and Muller, 2000). Clinicians, however, conventionally calculate it from the first day of the last normal menstrual period. The ultrasound crown-rump length measurement (CRL) has become the most accurate method (Pedersen, 1982) to confirm or calculate the “gestational age” of pregnancy, which is based on a theoretical ovulation date plus 2 weeks.

The terms egg and ovum, sometimes used in clinical publications, should be avoided because they have been used incorrectly for both an oocyte and an embryo as well (Rahilly, 1986). This author suggested that an egg should be reserved for a “nutritive object” only.

Similarly, the use of the term embryo versus fetus is confusing as infertility specialists use embryo in the pre-implantation period, whereas anatomists use embryo up until 8 weeks post-implantation. Gynaecologists and ultrasonographers acknowledge the “embryonic” period by referring to the presence of fetal heart action and fetal activity before the end of organogenesis. This evidence is vital for the parents, who see them as clear signs of life. Embryologists and ethicists, by contrast, continue to debate the meaning of an embryo but the general agreement is that an embryo should be synonymous to a pre-implantation fertilized oocyte up to the blastocyst stage. Although a clear distinction between embryonic and fetal periods is fundamental in teratology and epidemiology, we have to accept that modern terminology should reflect daily clinical practice whose description has changed in the last two decades and is more patient-centered. The term fetus should refer only to an ultrasound definition that includes fetal heart activity and/or a CRL >10mm.

The revised early pregnancy terminology is summarized in Table I.

Early pregnancy events

Historically, clinicians have grouped all pregnancy losses that occur at a gestational age before theoretical viability (24 weeks gestational age) under the umbrella of “abortion”. Possible pathophysiological mechanisms may be postulated and studied with the help of a more precise classification of pregnancy losses according to the gestational age at which they occur and a detailed description of the event. Biochemical pregnancy loss is a better description than trophoblast in regression or pre-clinical embryo loss. In the pre-clinical period (0-6 weeks gestational age), about 30% of all conceptions will fail to implant and an additional 30% do not survive shortly after implantation. This is considered to be due to a high number of abnormal embryos presenting with either chromosomal and/or gross morphological abnormalities (Macklon et al 2002). The absence of an identifiable pregnancy on ultrasound examination in combination with a positive urine or serum hCG pregnancy test is named a pregnancy of unknown location (Condous et al 2006). After ultrasound identification of pregnancy a miscarriage should be classified as early, before 12 weeks or late, after 12 weeks.

Ultrasound criteria

Ultrasound plays a major role in parental reassurance, where fetal cardiac activity is seen and is pivotal in the assessment of early pregnancy complications, such as vaginal bleeding (Jauniaux et al, 1999). Recent advice concludes that a diagnosis of an empty sac (previously named: blighted ovum or anembryonic pregnancy) should not be made if the sac diameter is less than 20 mm and viability should not be confirmed or refuted if the visible CRL is less than 6 mm, as only 65% of normal embryos at that stage will display cardiac activity (RCOG Guideline, 2006). Repeat transvaginal ultrasound examination after at least a week showing identical features and/or the presence of fetal bradycardia is strongly suggestive of impending miscarriage (Chittacharoen et al, 2004). The possibility of incorrect
dates should always be considered. In addition, it should be remembered that when the fetus has clearly
developed and the fetal heart activity is absent, the term “missed abortion”, originally introduced by
James Matthews Duncan in 1868, should, if used at all, be replaced by “delayed miscarriage” (Hutchon
and Cooper, 1997).

Epidemiology

Clinical miscarriage, occurring in 15-20% of all pregnancies, can be seen as a highly efficient
mechanism of selection, only allowing normal pregnancies to continue. The great majority occurs
early, before 12 weeks of gestation and fewer than 5% occur after identification of fetal heart activity
(Brigham et al., 1999). Second trimester loss, between 12 and 24 weeks, occurs less frequently and
constitutes <4% of pregnancy outcomes (Bricker and Farquharson 2002, Ugwumadu et al 2003).

Between 1 and 2% of fertile women will experience RM (Stirrat, 1990). The risk of recurrence
increases with the maternal age and the number of successive losses (Nybo Andersen et al 2000,
Brigham et al 1999) and the prognosis may be better for couples with prior live birth. The number of
previous miscarriages is an important covariate, which has to be taken into account when planning
therapeutic trials. The ideal trial should have stratification for the number of previous miscarriages,
with randomization between control and experimental treatments within each stratum. To date, such a
study has not been undertaken (Christiansen et al., 2005).

Although RMs have been directly associated with parental chromosomal anomalies, maternal
thrombophilic disorders and structural uterine anomalies and indirectly with maternal immune
dysfunction and endocrine abnormalities, the majority of RM cases are classified as idiopathic. On the
other hand, there is evidence that the endometrium in the humans may even be more receptive to
implantation in women with RM than in normal controls (Aplin et al 1996). Impairment of the
endometrial barrier function may allow “poor quality” embryos to implant and present later as
miscarriages (Quenby et al 2002). It may be an interesting hypothesis that these patients therefore are
presenting more often as RM rather than infertility.

A small number of non-randomised studies have reported that psychological support, i.e.
tender loving care in early pregnancy improves decreased miscarriage rates in women with unexplained
RM (Stray-Pedersen and Stray-Pedersen 1984) (Table II). Clifford et al. (1997) found that supportive
care in early pregnancy conferred a significant beneficial effect on pregnancy outcome with those who
attended the early pregnancy clinic having a 26% miscarriage rate in the next pregnancy compared to
51% for those who did not attend the clinic.

Histopathology

Whilst it is routine practice to send products of conception for histological examination,
mainly to exclude gestational trophoblastic disease, the usefulness of histopathological investigation of
placental tissue in RM on future pregnancy management for an individual couple remains to be
determined (Jauniaux and Burton, 2005). The inaccuracy of villous morphology and the limited clinical
significance of the finding of an aneuploidy in a sporadic miscarriage, has lead some authors to
conclude that the histological classification is a valueless clinical exercise (Fox, 1993). Recently, a new
classification has been introduced based on the finding that chorionic vascularization is reduced in
empty sacs and abnormal embryos as compared to normal embryos (Hakvoort et al., 2006).

Conseptus karyotype

The majority (90%) of karyotypically abnormal pregnancies m miscarry, whereas the majority of
karyotypically normal pregnancies continue. Miscarriage therefore can be seen as a process of natural
selection. The incidence of an abnormal karyotype in spontaneous miscarriage is about 60% (Macklon
et al., 2002). In RM, however, the embryo loss pattern is predominant in miscarriages with a normal
karyotype (Morikawa et al 2004). As a consequence, normal conceptus karyotype in a previous
pregnancy is a predictor of subsequent miscarriage (Ogasawara et al., 2000; Carp et al., 2001).

When stratified for maternal age, there is no difference in the distribution of cytogenetically
abnormal miscarriages in couples with RM compared with controls (Stephenson et al., 2002).

A precise morphologic and cytogenetic documentation of the products of conception is
essential in the management of RM patients. Without this information it is impossible to ascertain
whether the pregnancy loss is the result of treatment failure or a de novo chromosomal anomaly. The magnitude of the size of the treatment effect will be affected without correction for the aneuploidy factor (Christiansen et al., 2005).

Parental karyotype

The individual risk of a structural chromosomal abnormality in couples with RM is already increased after two miscarriages from below 0.5% to 2.2% (Braekeleer and Dao, 1990). At advanced maternal age however, the risk of parental carrier status is decreased. Low maternal age at second miscarriage, a history of three or more miscarriages, a history of two or more miscarriages in a brother or sister, and a history of 2 or more miscarriages in the parents of either partner all increase the probability of carrier status (Franssen et al., 2005). A probability of abnormal carrier status ≥ 2.2% is obtained when these four factors are combined (Table III).

Uterine malformations

Uterine anomalies are traditionally associated with RM. The prevalence is less than 5% (Homer et al., 2000). Traditionally, laparoscopy, hysterosalpingography and/or hysteroscopy have been used to diagnose these uterine malformations in women with RM. Ultrasound, in particular 3D ultrasound, has become an accurate, reproducible, non-invasive, out-patient method for the diagnosis of congenital uterine anomalies (Salim et al., 2003a). Using 3D ultrasound, it has been reported that women with a subseptate uterus have a higher incidence of first-trimester loss, whereas women with an arcuate uterus have a greater proportion of second-trimester loss and preterm delivery (Woelfer et al., 2001). A large comparative study of the ultrasound morphology of congenital anomalies in women with and without RM has shown no difference in the relative frequency of various anomalies between the two groups of women (Salim et al., 2003b). However, with both arcuate and subseptate uteri, the length of the remaining cavity was shorter and the size of the fundal distortion higher in the RM group. Although hysteroscopic resection of the septum is a relatively small operation, there is no evidence available that this procedure will improve pregnancy outcome in RM patients.

Fibroids

Although fibroids are mentioned traditionally as a possible cause for RM there is no conclusive literature about this subject. In an in vitro fertilization study intramural and/or submucous fibroids were associated with a significant lower implantation rate and a higher miscarriage rate (Gianaroli et al. 2005)

Endocrine abnormalities

Early epidemiological data have shown an association between RM and hypothyroidism or diabetes mellitus. Although current evidence indicates that treated hypothyroidism and well-controlled diabetes are not associated with RM (RCOG, 1998), thyroid function tests and HbA1c measurements are accurate and inexpensive and can still be considered as part of the evaluation of RM (Christiansen et al., 2005). Furthermore, low level of maternal thyroxin and poorly controlled maternal glucose levels in early pregnancy are associated with short and long-term consequences for fetal development and should therefore be diagnosed and treated before conception.

A high miscarriage risk in patients with polycystic ovarian syndrome (PCOS) seemed to be related to high levels of luteinizing hormone (LH), although the success rate did not improve after LH suppression (Clifford et al., 1996). Obesity is associated with a statistically significant increased risk of first-trimester and recurrent miscarriage (odds ratios 1.2 and 3.5, 95% CI 1.01-1.46 and 1.03-12.01, respectively) (Lashen et al., 2004). Obesity certainly has a wider impact on women’s health, and several studies have shown that the association between PCOS and RM could be secondary to the association between obesity and miscarriage (Fedorcsak et al., 2000; Bellver et al., 2003). Weight loss should be considered as a first option for women who are infertile and overweight (Clark et al., 1998), and there is little doubt that the same concept applies to women with RM. Several publications are suggesting a beneficial effect of metformine in PCOS patients resulting in a reduction of first-trimester miscarriage (Palomba et al., 2005a; Palomba et al 2005b; Tacher and Jackson, 2006). Although the findings are interesting it has to be mentioned that none of the studies meets the proper criteria for a well-designed RCT.
Other endocrinologic disorders, including high androgen levels, hyperprolactinemia and, luteal phase defects have been associated with RM. Current evidence suggests that like for hypothyroidism and diabetes, infertility is more likely a problem than pregnancy loss. A recent systematic review has found no evidence to support the routine use of progesterone in the first trimester to prevent miscarriage (Oates-Whitehead et al., 2005). Carmichael et al. (2005) have recently reported that maternal intake of progestins in early pregnancy is associated with an increased risk of hypospadia in the male offspring (Odds ratio 3.7, 95% CI 2.3-6.0).

Thrombophilia

Acquired maternal thrombophilia is a well-recognized cause of RM. All women with a history of three or more early pregnancy losses, i.e. before 10 weeks, or one or more unexplained deaths at ≥10 weeks of a morphologically normal fetus or one or more premature births at ≤34 weeks with severe preeclampsia or placental insufficiency, should be offered a testing for lupus anticoagulant and anticardiolipin antibodies, known collectively as antiphospholipid antibodies, to exclude an antiphospholipid syndrome (APS) (Wilson et al., 1999). Aspirin and/or heparins have become routine treatment for women with APS and a history of RM on the basis of limited evidence. Although it was reported in a RCT that the combination of low-dose aspirin and heparin was superior to aspirine alone (Kutteh, 1996; Rai et al., 1997), recent studies reported that aspirin as well as heparin as a stand-alone therapy are associated with similar live birth rates (Farquharson et al., 2002; Derksen et al., 2004; Empson et al., 2005). However, so far, no trials have compared the use of heparin and aspirin to placebo or no treatment in APS-positive RM patients.

The data on the use of anticoagulants for the treatment of RM in women without APS is too limited to recommend their routine use within this context (Di Nisio et al., 2005).

An increased incidence of early and recurrent fetal loss has also been suggested in women with inherited thrombophilia including Factor V Leiden deficiency, activated protein C resistance, prothrombin G20210A and protein S deficiency (Rey et al., 2003, Dawood et al., 2003), however, other authors have found no association between maternal thrombophilia and pregnancy loss < 10 weeks of gestation (Roque et al., 2004). Some studies reported a decreased risk of miscarriage in women with inherited thrombophilia (Carp et al., 2002; van Dunne et al., 2005) whereas one study reported that multiple genetic thrombophilic mutations in either partner seem to increase the risk of miscarriage in a subsequent pregnancy (Jivrai et al., 2006). Larger epidemiological studies are clearly needed to justify testing couple with RM for inherited thrombophilia in routine clinical practice (Robertson et al., 2005). As a consequence, in these patients thrombo-prophylaxis is only indicated to prevent thrombosis in case of a high risk and not for improvement of the pregnancy outcome, unless Factor V Leiden mutations are associated with other thrombophilic mutations.

Hyperhomocysteinaemia

High level of homocysteine (hyperhomocysteinaemia) can be associated with RM (Nelen et al., 2000). Among the genetic causes of this condition, a common one is polymorphism at position 677 in the methyl tetrahydrofolate reductase gene, which in the homozygous form leads to a thermolabile enzyme variant (Makris, 2000). Within this context, low plasma folate levels have been associated with an increased risk of first-trimester miscarriage (George et al., 2002). Investigation for the above condition remains technically difficult and should not be performed outside a specific clinical context.

Immunology

An excessive maternal immune response against paternal antigens resulting in abnormal immune cells and cytokines production has and is still thought to be one of the causes of RM (Laird et al., 2003). Until now there is no scientific basis for introduction of peripheral blood natural killer (NK) cells testing or cytokines peripheral level measurements into routine practice (Moffet et al., 2004; Wold and Arici, 2005; Rai et al., 2005)

Recent data have shown that a high number of uterine NK cells is found in the endometrium of women with RM and this could be reduced by therapy (Quenby et al., 2005). However, prospective trials are needed to evaluate the possible use of this finding and currently endometrial sampling should only be offered to women within the context of research programs.
The reported association between RM and deficiency of mannin-binding lectin, a serum protein involved in the immune response, is another subject of research programs (Kruse et al., 2002).

The use of intravenous immunoglobulin (IVIG), anti-tumor necrosis factor-α, glucocorticoids or cellular therapies in order to prevent or reduce an “excessive immune response” and/or abrogate maternal-fetal incompatibility in women with RM remains controversial. IVIG might be of benefit to women with unexplained RM (Christiansen et al., 2004) but within this context, its use should only occur as part of a RCT. IVIG could be more efficacious in women presenting with secondary RM or repeated second-trimester intrauterine fetal deaths (Christiansen and Nielsen, 2005).

The majority of trials using cellular treatment for RM have failed to find any beneficial effect. The most common of the cellular treatments are transfusions of paternal leucocytes before conception. So far, meta-analyses have shown no significant benefit of paternal leucocytes, third-party donor leucocytes or trophoblast membranes on pregnancy outcome compared to placebo (Porter et al., 2006); however, in the three small trials that tested third-party donor leucocytes, the pooled odds ratio for live birth was 1.39 (95% CI 0.68-2.82) in the treatment group compared with the placebo group emphasising that this treatment should be further tested in RCTs.

Infections

Infections with bacteria, viruses or parasites can all interfere with early pregnancy development, but none seems to be a significant cause of RM (Simpson et al., 1996). TORCH screen is therefore of limited value in the investigation of RM outside an acute infectious episode (Li et al., 2002).

Teratology

A lot of information is available about environmental toxins. The association between miscarriage and ionizing radiation, organic solvents, alcohol, mercury and lead is confirmed whilst an association to caffeine, hyperthermia and cigarette smoking is suspected (Gardella and Hill, 2000).

Vitamin supplementation has been advocated in the context of an association between poor dietary intake of vitamins and an increase risk of miscarriage. A recent meta-analysis has shown that taking vitamin supplements, alone or in combination with other vitamins, before conception or in early pregnancy does not change the risk of early or late miscarriage (Rumbold et al., 2005). Currently, the data on individual vitamin supplementation in women with RM are insufficient to perform any meaningful analyses.

References

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Makris M (200) Hyperhomocysteinemia and thrombosis. Cl Lab Haemat 22: 133-143


Table I. Early pregnancy terms and events. *LMP* last menstrual period, *CRL* crown-rump length, *PUL* pregnancy of unknown location

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<th>Avoid</th>
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<td>Oocyte</td>
<td>CRL &lt; 10 mm</td>
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<td>CRL ≥ 10 mm and/or fetal heart activity</td>
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<td>Preclinical abortion</td>
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<tr>
<td></td>
<td>prothrombin G20210A</td>
<td></td>
</tr>
<tr>
<td>Hyperhomocysteinaemia</td>
<td>homocysteine</td>
<td>folic acid</td>
</tr>
<tr>
<td></td>
<td>folic acid</td>
<td></td>
</tr>
<tr>
<td>Abnormal Immune response</td>
<td>NK cells in endometrium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MBL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Life style</td>
<td>history</td>
<td></td>
</tr>
<tr>
<td>Teratology</td>
<td>history</td>
<td></td>
</tr>
</tbody>
</table>
Table III: Probability of carrier status in couples with two or more miscarriages according to the multivariable logistic regression model (Modified from Franssen et al 2005)

<table>
<thead>
<tr>
<th>Maternal age at second miscarriage</th>
<th>(RM\textsubscript{parents}) +</th>
<th>(RM\textsubscript{parents}) -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥3 misc.</td>
<td>2 misc.</td>
</tr>
<tr>
<td>&lt; 23 years</td>
<td>(RM\textsubscript{bs}) +</td>
<td>10.2%</td>
</tr>
<tr>
<td></td>
<td>(RM\textsubscript{bs}) -</td>
<td>5.7%</td>
</tr>
<tr>
<td>23-33 years</td>
<td>(RM\textsubscript{bs}) +</td>
<td>10.0%</td>
</tr>
<tr>
<td></td>
<td>(RM\textsubscript{bs}) -</td>
<td>5.7%</td>
</tr>
<tr>
<td>34-36 years</td>
<td>(RM\textsubscript{bs}) +</td>
<td>5.8%</td>
</tr>
<tr>
<td></td>
<td>(RM\textsubscript{bs}) -</td>
<td>3.2%</td>
</tr>
<tr>
<td>37-38- years</td>
<td>(RM\textsubscript{bs}) +</td>
<td>4.0%</td>
</tr>
<tr>
<td></td>
<td>(RM\textsubscript{bs}) -</td>
<td>2.2%</td>
</tr>
<tr>
<td>≥ 39 years</td>
<td>(RM\textsubscript{bs}) +</td>
<td>1.8%</td>
</tr>
<tr>
<td></td>
<td>(RM\textsubscript{bs}) -</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

Grey area: couples with a probability of carrier status <2.2%. Karyotyping can be withheld in these couples.

RM\textsubscript{bs} = a history of ≥2 miscarriages in a brother or sister of either partner, RM\textsubscript{parents} = a history of ≥2 miscarriages in parents of either partner; ≥3 misc. = a history of ≥3 miscarriages in the couple, 2 misc. = a history of ≥2 miscarriages in the couple.
Cytogenetic aspects of Early Pregnancy Failure
a clinical point of view

M. Goddijn
Center for Reproductive Medicine
Academic Medical Center, Amsterdam

ESHRE wintercourse, Poznan
15 december 2006

Cytogenetic aspects of
Early Pregnancy Failure

- (Sporadic) Miscarriage
- Recurrent Miscarriage
- Ectopic Pregnancy
- Interventions

Fetal Karyotype
sporadic miscarriage

<table>
<thead>
<tr>
<th>Review</th>
<th>Miscarriage n</th>
<th>Abnormal n (%)</th>
<th>Num chrom abn n (% of abn)</th>
<th>Sex chrom abn n (% of abn)</th>
<th>Other chrom abn n (% of abn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simpson 1987</td>
<td>5318</td>
<td>2606 (51)</td>
<td>2564 (96)</td>
<td>1617 (60)</td>
<td>510 (19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>trisomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>501 (19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>monosomy X 457 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>80 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goddijn 2000</td>
<td>4596</td>
<td>2310 (46)</td>
<td>2005 (44)</td>
<td>1216 (52)</td>
<td>481 (21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>monosomy X 308 (13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>132 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>162 (6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fetal Karyotype
sporadic miscarriage

- fetal karyotype in 50% abnormal
- In recent studies: a slightly higher percentage of structural chromosome abnormalities and other chromosome abnormalities is found

Fetal Genotype – advanced techniques
sporadic miscarriage

- Various techniques used: FISH, CGH, FCM, array CGH
- Less failures.
- Additional chromosome abnormalities (numerical chromosome abnormalities/ subtle deletions/ mosaics) in:
  - culture failures
  - culture negative results
- The definite role of these techniques needs to be established.


Maternal age
sporadic miscarriage

- Graph showing correlation between maternal age and miscarriage rates.

Hassold Nature Reviews Genetics 2; 280-291 (2001)
Mean age of women at birth 1st child

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean Age of women at birth of 1st child (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>36.5</td>
</tr>
<tr>
<td>2001</td>
<td>35.5</td>
</tr>
<tr>
<td>2002</td>
<td>35.0</td>
</tr>
</tbody>
</table>

Fetal Karyotype

<table>
<thead>
<tr>
<th>Year</th>
<th>abnormal karyotype n (%)</th>
<th>normal karyotype n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992</td>
<td>40 (43)</td>
<td>54 (57)</td>
</tr>
<tr>
<td>2000</td>
<td>114 (49)</td>
<td>120 (51)</td>
</tr>
<tr>
<td>2001</td>
<td>89 (71)</td>
<td>36 (29)</td>
</tr>
<tr>
<td>2002</td>
<td>225 (54)</td>
<td>195 (46)</td>
</tr>
</tbody>
</table>

Parental chromosome analysis in The Netherlands

- Increase in annual number of chromosome analyses
  - 1992: 1298 couples
  - 2000: 2362 couples

- Decrease in incidence of carrier status
  - 1992: 6.8%
  - 2000: 3.6%
Risk of carrier status
recurrent miscarriage

- General population: 0.7%
- After one miscarriage: 2.2%
- After two miscarriages: 4.8%
- After three miscarriages: 5.2%

Identification of risk factors in 279 carrier couples and 428 non-carrier couples

Structural chromosome abnormalities
recurrent miscarriage

- Reciprocal translocations: 61%
- Paracentric inversions: 8%
- Other: 7%
- Pericentric inversions: 8%
- Robertsonian translocations: 16%

Univariable logistic regression (p ≤ 0.20)
recurrent miscarriage

- Maternal age
  at first miscarriage, at second miscarriage, at chromosome analysis
- General history
  radiotherapy
- Obstetrical history
  at least one healthy child, at least one ectopic pregnancy
- Number of miscarriages
  3 vs 2 miscarriages, 3 and 4 vs 2 miscarriages
- Family history
  miscarriages brothers/ sisters, miscarriages parents, antenatal DES exposure
Multivariable logistic regression

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>OR</th>
<th>CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at second miscarriage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 23 years</td>
<td>6.2</td>
<td>1.8 - 21.4</td>
<td>.04</td>
</tr>
<tr>
<td>23 - 34 years</td>
<td>6.1</td>
<td>1.8 - 21.0</td>
<td>.04</td>
</tr>
<tr>
<td>34 - 37 years</td>
<td>3.3</td>
<td>0.7 - 16.1</td>
<td>.25</td>
</tr>
<tr>
<td>37 - 39 years</td>
<td>2.3</td>
<td>0.4 - 12.0</td>
<td>.33</td>
</tr>
<tr>
<td>&gt; 39 years</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>≥ 3 miscarriages (of the couple)</td>
<td>1.4</td>
<td>1.0 - 2.1</td>
<td>.05</td>
</tr>
<tr>
<td>≥ 2 miscarriages parents</td>
<td>1.4</td>
<td>0.9 - 2.2</td>
<td>.10</td>
</tr>
<tr>
<td>≥ 2 miscarriages brother/sister</td>
<td>1.9</td>
<td>1.5 - 2.4</td>
<td>.05</td>
</tr>
</tbody>
</table>

Franssen BMJ 2005

Risk of carrier status

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 23 years</td>
<td>10.2%</td>
<td>7.3%</td>
<td>7.3%</td>
</tr>
<tr>
<td>(Miscbrother/sister)</td>
<td>5.7%</td>
<td>4.0%</td>
<td>4.1%</td>
</tr>
<tr>
<td>≥ 3 miscarriages (of the couple)</td>
<td>16.0%</td>
<td>7.2%</td>
<td>7.2%</td>
</tr>
<tr>
<td>≥ 2 miscarriages parents</td>
<td>5.1%</td>
<td>4.0%</td>
<td>4.0%</td>
</tr>
<tr>
<td>≥ 2 miscarriages brother/sister</td>
<td>2.8%</td>
<td>2.2%</td>
<td>2.2%</td>
</tr>
<tr>
<td>≥ 39 years</td>
<td>5.9%</td>
<td>4.1%</td>
<td>4.1%</td>
</tr>
<tr>
<td>&lt; 23 years</td>
<td>3.2%</td>
<td>2.2%</td>
<td>2.2%</td>
</tr>
<tr>
<td>≥ 3 miscarriages (of the couple)</td>
<td>4.9%</td>
<td>2.8%</td>
<td>2.8%</td>
</tr>
<tr>
<td>≥ 2 miscarriages parents</td>
<td>2.8%</td>
<td>1.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td>≥ 2 miscarriages brother/sister</td>
<td>2.2%</td>
<td>1.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td>≥ 39 years</td>
<td>1.9%</td>
<td>1.2%</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

Risk of carrier status < 2.2%  Risk of carrier status < 3%

Franssen BMJ 2005

Successful reproductive outcome (accumulated)

<table>
<thead>
<tr>
<th></th>
<th>Carriers n = 247</th>
<th>Controls n = 409</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st pregnancy</td>
<td>148 (60%)</td>
<td>220 (58%)</td>
</tr>
<tr>
<td>2nd pregnancy</td>
<td>173 (70%)</td>
<td>324 (79%)</td>
</tr>
<tr>
<td>3rd pregnancy</td>
<td>194 (79%)</td>
<td>332 (81%)</td>
</tr>
<tr>
<td>4th pregnancy</td>
<td>205 (81%)</td>
<td>339 (83%)</td>
</tr>
<tr>
<td>Total follow-up</td>
<td>205 (83%)</td>
<td>344 (84%)</td>
</tr>
</tbody>
</table>

*p < 0.05

Franssen BMJ 2006
Adverse reproductive outcome
recurrent miscarriage - carrier couples

<table>
<thead>
<tr>
<th></th>
<th>Carriers n = 239</th>
<th>% unbalanced of total no of pregnancies n = 550</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of terminated pregnancies</td>
<td>6</td>
<td>2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>No. of children with congenital abnormalities</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>No. of post-partum deceased children</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>No. of stillbirths</td>
<td>3</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

* after PND

Ectopic Pregnancy

The etiology of 30% of cases remains unknown
Bouyer Am J Epidemiol 2003

Suggested other factors
ectopic pregnancy

- underreporting of PID (chlamydia)
- hormonal disturbances (E2/ Prog)
- fetal chromosome abnormalities

association between EP and: high maternal age
recurrent miscarriage

Cytogenetic findings
éctopic pregnancy

- Type of chromosome abnormalities comparable with those in miscarriage tissue
- Percentage chromosome abnormalities is low when expective management is applied

Interventions

- Recurrent miscarriage of unknown cause ➔ IVF/PGS
- Recurrent miscarriage / carrier status of structural chromosome abnormality ➔ IVF/PGD
Interventions: PGS or PGD

Interventions

<table>
<thead>
<tr>
<th>PGS</th>
<th>PGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>IVF population</td>
</tr>
<tr>
<td>Advanced maternal age</td>
<td>M. Huntington</td>
</tr>
<tr>
<td>Repeated IVF failure</td>
<td>translocations</td>
</tr>
<tr>
<td>Recurrent miscarriage</td>
<td>Prevention of the birth of a seriously handicapped child</td>
</tr>
<tr>
<td>Testicular sperm extraction</td>
<td></td>
</tr>
<tr>
<td>Aim</td>
<td>Reduce aneuploidies</td>
</tr>
<tr>
<td>Technique</td>
<td>FISH / CGH</td>
</tr>
<tr>
<td>Chromosomes</td>
<td>X, Y, 13, 16, 18, 21, 22</td>
</tr>
</tbody>
</table>

IVF-PGS in RM eci – the evidence

- > 600 cycles reported\(^1,2,3,4,5,6\)
- Promising results:
  - Live birth rates: 20 – 42% / ET
- Lower live birth rates in ≥34 year:
  - Live birth rate: 6 – 34% / ET
- ET not possible: 11-28% of cycles

\(^1\) Pellicer 1999, \(^2\) Rubio 2003, \(^3\) Wilding 2004, \(^4\) Rubio 2005, \(^5\) Munne 2005, \(^6\) Platteau 2005
IVF-PGS in RM eci – the evidence

- Proper control group lacks in PGS data
- No RCT’s reported comparing IVF-PGS with spontaneous cycles
- High spontaneous chance on ongoing pregnancy

1Twisk, Cochrane Review 2006, 2Bingham 1999

PGD in RM translocation carriers - the evidence

- IVF/PGD is feasible
- > 100 cycles reported
- Live birth rate/ ET: 22-23%
- ESHRE-PGD data: low pregnancy rates in translocation carriers but RM subgroup not identified
- No RCT’s available


Summary I

- In sporadic miscarriage and RM a high number of fetal chromosome abnormalities exist
- More risk factors for carrier status exist than just the number of miscarriages:
  - Maternal age at second miscarriage
  - RM in the parents
  - RM in a brother or a sister
- A multivariable model more accurately predicts the risk of carrier status in couples with two or more miscarriages.
Summary II

- Selective chromosome analysis in couples with two or more miscarriages might reduce the costs.
- Chromosome abnormalities do not play an important role in the etiology of vital ectopic pregnancies.
- No proven therapeutic interventions are available for cytogenetic abnormalities in (recurrent) miscarriage.
Acknowledgment

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R. Hochstenbach (UMC Utrecht)
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C.H. Wouters (Erasmus MC)
K.B.M. Hansson (LUMC)

ZonMW
Early Pregnancy Units

**Aim:**
- All women with early pregnancy problems will have prompt access to a dedicated Early Pregnancy Assessment Unit (EPU) that provides efficient management, patient counselling and access to appropriate information. At all times women will be supported in making informed choices about their care and management.

**EPU Setting Standards 2006**

- **Auditable Standards**
  - The EPU should benchmark all audited activity against published series and adopt all evidence based practice recommendations for diagnosis and management of early pregnancy problems.
  - Patient satisfaction surveys of Early Pregnancy Unit service.
  - Ruptured ectopic pregnancy number and failed diagnosis rate per year following opportunity for diagnosis in EPU.
  - False diagnosis rate for fetal loss in early pregnancy ultrasound assessment.
  - Audit of patient choice and uptake rates of medical/surgical/conservative management of miscarriage and ectopic pregnancy and their complication rates.
  - Assessment of training and educational opportunities for EPU staff including medical trainees and nurses.
  - Audit of pregnancy of unknown location (PUL) outcome following EPU assessment and benchmarking against peer review published standards.
  - Appropriate use of anti-D prophylaxis.
  - Analysis of visit numbers to establish confirmed diagnosis.
  - Audit of anti-chlamydial treatment uptake, compliance and provision following pregnancy loss.
  - Standards of documentation.
What does an EPU do?

- Evidence based practice standards
- Provision of accurate patient information
- Pregnancy of unknown location
- Ectopic pregnancy (CEMACH)
- Viability assessment
- Initiate relevant treatment
- Management planning
- Target milestone of antenatal booking

Where is the pregnancy?
Typical EPU presentation – sound familiar?

- Day 1
  - 5 weeks pregnant
  - 3 day history PV spotting
  - Intermittent lower abdo pain
  - Threatened miscarriage

- Day 2
  - Reattended with increasing pain
  - Tender LIF
  - Left adnexal mass
  - Cervical excitation tenderness present
  - Ectopic pregnancy

HCG changes in normal pregnancy

- Mean (SE) serum concentrations of human chorionic gonadotrophin
  (adapted from Braunstein et al 1976)

Placental Haematoma in early pregnancy
Appendix 1 - Basic Diagnostic Algorithm

USS – TAS / TVS

- 'Pregnancy of Known Location'
- 'Pregnancy of Unknown Location'

Intrauterine pregnancy

- Ectopic pregnancy
  - Viable IUP
  - Non-viable IUP
  - Resolved PUL
  - 'Uncertain viability'
  - Rescan in 7-10 days

Diagnostic algorithm for 'PUL'

Basic diagnostic algorithm for early pregnancy loss

USS – TAS / TVS

- 'Pregnancy of Known Location'
- 'Pregnancy of Unknown Location'

Intrauterine pregnancy

- Ectopic pregnancy
  - Viable IUP
  - Non-viable IUP

Diagnostic algorithm for 'PUL'

Basic diagnostic algorithm for early pregnancy loss
Transabdominal Cerclage -- tying the knot anterior.

Activities in Progress

- Dissemination of Protocol
  Revised Nomenclature for Early Pregnancy Events
  (Hum Rep 2005, 20, 3008-11)

- Evidence Based Practice
  Guidelines for Investigation and treatment for recurring miscarriage
  (Hum Rep 2006, e-pub, in press)

- Improving visibility of Early Pregnancy Unit network

- Website enhancement (earlypregnancy.com)

Future Activities

- ESHRE Winter Symposium Poznan, PL
  15th and 16th December 2006
  New Trends in diagnosis and management of early pregnancy failure

- ESHRE annual scientific meeting Lyon, FR
  June 2007  Joint PCC with Psychology and Counselling plus Keynote Speaker nominations

- ESHRE Winter symposium jointly with SIGEP and SIG Rep Endo (Nick Macklon Co-ordinator)
  December 2007 ?Utrecht, NL

- ESHRE annual meeting Barcelona, ES 2008
New Ideas

• ESHRE Guidelines for **best practice** to be taken up in EU countries especially new member states

• **Standard setting** for audit purposes to allow departmental benchmarking between units and countries

• **Collaboration** with frontier research enabling fast track access to peer review & presentation

• **Occlusion Trial** EU RCT of vaginal/abdominal cerclage in early pregnancy

ESHRE meeting 2008

Speculation or Anticipation?

• “There are events in the womb of time, as yet, undelivered “ Othello, W.Shakespeare
Management of first trimester miscarriage

Dr W.M. Ankum

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1100 DE Amsterdam
The Netherlands
Email: w.m.ankum@amc.uva.nl

Introduction
From a biological perspective, mankind has been quite successful in inhabiting the globe, resulting in overpopulation as being one of the major challenges of our times. In contrast to the booming success as a species, the efficiency of human reproduction on the individual level is rather poor. Of all successfully fertilized ova, only about 30% will result in a living child. Approximately 60% do not even reach the stage of a clinically recognised pregnancy: so-called “occult” miscarriages which result from immediate demise or failed implantation. Another 10-15% of conceptuses are bound to miscarry after the missed period in the first trimester.1,2,3 This paper summarises current knowledge on the epidemiology, diagnosis and treatment of first trimester miscarriages, with some emphasis on the author’s personal experiences.

Epidemiology
First trimester miscarriage is a common event that will be experienced by about 25% of all women during their reproductive career. The vast majority of first trimester miscarriages are sporadic events, half of which being accounted for by cytogenetic abnormalities, i.e. numerical or structural chromosomal anomalies and mosaicism as demonstrated by classical techniques. With improving cytogenetic techniques, the unexplained other half might also turn out to represent hitherto unrecognised more subtle chromosomal anomalies.
In daily practice it is generally acknowledged that parental cytogenetic screening is unnecessary in sporadic miscarriages, and should be restricted to couples with recurrent miscarriages, a topic beyond the scope of this paper.
Because the occurrence of miscarriages is a highly age dependent phenomenon, incidence rates vary widely from 10% for women aged 20-24 years, to a staggering 90-100% for those between 45 and 50 years of age.4 Increased age is not only associated with an increased risk of Down-syndrome, as is generally known and acknowledged, but also predisposes for other chromosomal abnormalities which explain the increased risk of (repeated) miscarriages in these women.5,6

Natural course and clinical findings
Vaginal bleeding is the first clinical symptom of an impending miscarriage in the majority of cases. This symptom is by no means specific, since about 50% will prove to be viable pregnancies on sonographic examination and progress without serious consequences. In these women, the exact origin of bleeding usually remains unknown. When other causes, especially cervical Chlamydial infection, cervical carcinoma or a bleeding ectropion- have been ruled out, this type of bleeding may be attributed to the process of placental invasion of the endometrium. Straightforward evidence to substantiate this origin, though plausible from a biological viewpoint, is lacking.
Sooner or later, women with first trimester miscarriages will experience uterine cramps as an accompanying symptom. During the process of expulsion which follows – i.e. the actual miscarriage or abortion- vaginal bleeding increases and blood clots may be lost as well. The cervix gradually dilates to allow passage of the non-vital pregnancy, which usually is passed as a complete gestational sac, which can easily be distinguished from accompanying blood clots. Whenever the miscarriage is complete, pain and bleeding decrease promptly to the level of a regular menstrual period.8
Some bleeding may persist for several weeks, and is followed by a normal period some 4-7 weeks after the actual miscarriage.\textsuperscript{9} In case of an incomplete miscarriage, where a portion of the gestational sac is retained in the uterus, persistent cramping pain and excessive bleeding should indicate the need for surgical evacuation, rather than mere sonographic findings.

Knowledge about the natural course of miscarriages is important whenever expectant management is aimed for. If patients have not been counseled properly beforehand about what to expect, they may easily be alarmed by the natural course of events and end up undergoing unnecessary surgical evacuation in the final stages of the process. Unawareness of these matters might easily render the entire experience into a disillusion for both patient and physician.

Another problem arises when a spontaneous miscarriage simply doesn’t happen within a reasonable period of time. In our experience, this situation occurs in about half of the cases. After two weeks of waiting in vain, even well motivated women lose their faith, change their minds, and tend to ask for surgical intervention. These matters also should be addressed when counseling women about treatment options.

**Diagnostic management**

Clinical characteristics of women presenting with first trimester bleeding are of little value in correctly diagnosing miscarriages and cannot be relied upon in daily practice.\textsuperscript{10} The only exception is the presence of an expelled gestational sac on vaginal examination. This is a highly specific finding, but because of its rarity (4%), sensitivity is low.\textsuperscript{11,12} There is no doubt that transvaginal sonography is the most reliable tool in the diagnosis of first trimester miscarriage at present. Sonographic equipment and expertise, therefore, are absolute prerequisites for any unit providing care for women with first trimester pregnancies and its complications.

The most constant sonographic findings indicative of a miscarriage are those of an empty gestational sac, where no yolk sac and no embryonic pole are present, or the finding of an embryo or fetus without cardiac activity. There are caveats in the interpretation of these findings, and both need further specification. Firstly, a gestational sac can only be called empty with acceptable certainty whenever its mean diameter exceeds 15 mm. Secondly, the absence of fetal cardiac activity can only be diagnosed with certainty in case of a fetal crown-rump length exceeding 5 mm. If these criteria are not met, the pregnancy may turn out to be vital when sonography is repeated after a week.\textsuperscript{13} Some authors have advocated the additional use of serum progesterone measurements in differentiating between viable and non-viable pregnancies in these cases with encouraging results.\textsuperscript{14}
The usefulness of a single progesterone measurement without further sonographic evaluation, however, is limited. Apart from sonographic observations indicative of a miscarriage or a vital pregnancy, another sonographic finding deserves attention, namely when sonography fails to show any signs of an intra-uterine gestation. In the absence of a clear history of a recent spontaneous miscarriage, this finding is suggestive of an ectopic pregnancy. Since, obviously, the absence of a gestational sac also complies with the non-pregnant state, a pregnancy test should be done straightforward to rule out this embarrassing possibility. Many ectopic pregnancies are easily detected by transvaginal sonography and should be looked for carefully. A gestational sac outside the uterus is a very specific finding, but some free fluid in the cul-de-sac and an ectopic mass are less reliable. The addition of serum hCG measurements is useful when sonography fails to identify an intra-uterine gestation, or whenever no ectopic pregnancy is detected or findings are dubious, i.e. in case of a “Pregnancy of Unknown Location” (PUL). In these circumstances, hCG levels >1500-2000, or plateauing hCG concentrations at a lower level on follow-up, are indicative of ectopic pregnancy. Both serum hCG and progesterone measurements may be used in monitoring the expectant management of self-limiting ectopic pregnancies which resolve spontaneously in the majority of cases, without any need to perform a laparoscopy or uterine curettage for either diagnostic or therapeutic purposes.

Therapeutic management

At present, three different treatment options are being used in managing first trimester miscarriages: i.e. expectant, surgical, and medical management. Expectant management, i.e. awaiting the natural course as described earlier, represents the oldest type of managing first trimester miscarriages. Obviously, expectant management originates from a time when no other options were available, circumstances which still apply for many less privileged parts of the world. During the first half of the 20th century, surgical evacuation became the preferred treatment option for managing miscarriages in many parts of the Western world. This strategy was prompted by the high incidence of septicaemia and mortality in cases of retained products of conception. Undoubtedly, many of these complicated cases resulted from criminal attempts to terminate unwanted pregnancies, rather than being complications of spontaneous miscarriages. Expectant management remained in use, albeit modestly, in some Western societies, especially those with medical systems where general practitioners were routinely involved in providing obstetric care.

During the last decade, the expectant management of spontaneous miscarriages has seen a revival in many Western countries which earlier had abolished its use. Ironically, again terminations of pregnancies played a pivotal role in this process of change. The renewed interest in non-surgical management of miscarriages has been systematically reviewed. According to this study, aspiration curettage results in the highest complete evacuation rate in comparison to non-surgical management options. Medical management (i.e. misoprostol administered orally or vaginally) reduces the need for curettage by 81%-99%, whereas expectant management reduces this need by 28-94%, depending on whether miscarriages were either incomplete or those with a gestational sac still being present. The vast majority of incomplete miscarriages were managed safely without the need for additional surgical interventions. The incidence of pelvic inflammatory disease as a complication of treatment was evenly distributed among women undergoing curettage and those managed non-invasively. In studies comparing medical with expectant management, misoprostol was more effective in reaching complete evacuation of the uterus, at the cost, however, of minor gastro-intestinal side effects and an increased need of analgesics. These findings were confirmed in a later paper comparing expectant and medical treatment in a randomised placebo controlled trial. In that study, a regimen of daily 600 µg misoprostol administered vaginally up to two doses, had similar side effects compared to placebo. Recently the MIST trial, a large randomised study compared all three available options: expectant, medical -800 µg misoprostol vaginally-, and surgical management. No difference was found in infection rates between the three (2-3%), nor in the need for blood transfusions (0-1%). More women undergoing expectant management (50%) than those treated medically (38%) needed a curettage. The risk of unplanned hospital admission was highest with expectantly management. Despite this, the net societal costs were lowest in the expectantly managed group at £1086, versus £1410 in the medical group, and £1585 in the surgery group.
An interesting delayed management option has also been reported recently; in a randomised trial comparing the vaginal administration of misoprostol with curettage after a week of failed expectant management, the non-invasive strategy was found to be more cost-effective.\(^{32,33}\) In the absence of clinically relevant differences in safety, non-invasive treatment modalities can now be offered with confidence to women with first trimester miscarriages who wish to avoid surgery. This is important, since freedom of treatment choice improves quality of life in these unfortunate women.\(^{34,35}\)

References

GTD: CLASSIFICATION

- CLASSICAL HYDATIDIFORM MOLE (CHM)
  - SINGLETON CHM
  - CHM IN A MULTIPLE PREGNANCY
- PARTIAL HYDATIDIFORM MOLE (PHM)
- INVASIVE CHM
- PLACENTAL SITE TROPHOBLASTIC TUMOR
- CHORIOCARCINOMA
- PERSISTENT TROPHOBLASTIC TUMOR
DEFINITION: HYDATIDIFORM (MOLAR) VILLOUS TISSUE

THE MOLAR VESICLES ARE SECONDARY TO AN ABNORMAL DEVELOPMENT OF THE VILLOUS VASCULATURE \(\Rightarrow\) EDEMA (HYDROPS).

!!!!!! NON SPECIFIC !!!!!!!

+ **TROPHOBLASTIC HYPERPLASIA**

COMPLETE HYDATIDIFORM MOLE (CHM)

©GENERALIZED TROPHOBLASTIC HYPERPLASIA AND VILLOUS EDEMA \(\Rightarrow\) BUNCH OF GRAPES. NO FETUS. (ancient egypt: BC)

ORIGIN OF CHM

Empty egg or with an inactivated genome

46 chromosomes (usually 46,XX) entirely of paternal origin
**CHM: SEMIOLOGY**

- UTERINE ENLARGEMENT (50%)
- VAGINAL BLEEDING (90%)
- HYPEREMESIS (20%)
- ANAEMIA
- HIGH MS-hCG 10-200 MoM
- MORE COMMON <20 & >40 YEARS OF AGE

1: 1000-1500/Deliveries (& 1% OF EPF)

**TWIN/TRIPLET CHM**

- MULTIPLE CONCEPTION COMBINING A CHM & A NORMAL PLACENTA + FETUS
- 1/22000-100000 PREGNANCIES
- MS-hCG: 50-1000 MoM
- VAGINAL BLEEDING (90%)
- THECA LUTEIN CYST (25%)
- PIH (25%), PL (25%), IUD (>10%)

**PARTIAL HYDATIDIFORM MOLE (PHM)**

Szulman et al., AJOG, 1978

©LOCALIZED TROPHOBLASTIC HYPERPLASIA AND VILLOUS EDEMA. FETUS OR FETAL REMNANTS.
PATHOGENESIS OF PHM

TRIPLOIDY type I
Partial Mole
(PHM)

TRIPLOIDY type II
non-molar small placenta

PHM: SEMIOLOGY

- UTERINE ENLARGEMENT (10%)
- VAGINAL BLEEDING (20%)
- HYPEREMESIS (5%)
- MULTICYSTIC OVARIIES (10%)
- HIGH MShCG 10-60MoM

1% OF PREGNANCIES & 2% OF EPF

PHM: CLASSIFICATON

TRUE PHM
- TRIPLOID (90%)
- TETRAPLOID
- DIPLOID
- MOSAICS

PSUEDO PHM (NO GTD)
- IUD CHANGES
- MESENCHYMAL DYSPLASIA
- TRISOMY
PERSISTENT TROPHOBLASTIC TUMOR (PTT)

- CHM: 10-20%
- PHM: 0.1-3%

PTT AFTER TWIN PREGNANCY COMBINING CHM & NORMAL PREGNANCY = 50-60%

2% OF CHM PROGRESS TO CHORIOCARCINOMA

CHORIOCARCINOMA AFTER PHM

Seckl et al. Lancet, 2000

- 3000 patients with PHM (London) =>
- 15 required chemotherapy for persisting GTD.
- 3 (triploid PHM) transformed into choriocarcinoma.

GTD
ULTRASOUND DIAGNOSIS
**CHM: US DIAGNOSIS**

- Snowstorm appearance
- Theca-lutein cysts (30%?)
- No embryo/fetus
- Low uterine RI/PI
- High uterine PSV

**TWIN/TRIPLET CHM: US DIAGNOSIS**

Jauniaux, & Nicolaides, Ultrasound in Obstet Gynecol, 1997

Fibroid 75% MSHCG = 70MoM

Correct US diagnosis is around 70%

**PHM: US DIAGNOSIS**

Jauniaux, Ultrasound in Obstet Gynecol, 1998

- Swiss cheese appearance > 9 weeks.
- Placentomegaly
- Abnormal fetus (FGR & defects) if triploid PHM
**MID-TRIMESTER DIANDRY TRIPLOIDY**

PHM placenta, moderate symmetric FGR

MShCG MoM > 5 MoM at 12-20 weeks

---

**INVASIVE MOLE**

Jauniaux, Ultrasound in Obstet Gynecol, 1998

- Rare complication of CHM.
- Focal echogenic heterogeneous myometrial lesion containing fluid-filled cavities
- Contains villous tissue (ACCRETA)

---

**PTT: CDI vs ANGIOGRAPHY**

Jauniaux, Ultrasound in Obstet Gynecol, 1998

- TVUS is as accurate with no morbidity.
- Serial MShCG is the gold standard for diagnosis and monitoring.
- CDI: lacunae + nodules + A-V shunts (Persist).
GTD

SCREENING
(First Trimester)

TRIPLOID PHM: 10-14 WKS
Jauniaux et al., AJOG, 1997

FIRST TRIMESTER US DIAGNOSIS OF
CHM (retrospective)

Lazarus et al JUM 1992 (n= 21)
Mean GA= 10.5 weeks. Correct US
diag in 57% (No TL cyst).

Lindholm & Flam Acta OG Scand
1999 (n= 75)
Mean GA= 12.4 weeks. Correct US
diag in 84%.

Benson et al UOG 2000 (n= 24)
Mean GA= 8.7 weeks. Correct US
diag in 71%. 
EARLY US DIAGNOSIS OF PHM

- Lindholm & Flam (Acta OG Scand 1999) n= 60
  Mean GA at the time of US= 14.3 weeks
  Correct US diagnosis in only 30% of the cases.

SCREENING FOR GTD IN EPL

- ULTRASOUND FEATURES.
- MShCG (8£).
- FLOW CYTOMETRY OR FISH OR CYTOGENETIC (More expensive).
- HISTOPATHOLOGY (FOR ALL ????)
  => 40£/patient (200,000 mis in UK=> 8 M £/ year)

EPL: CYTOGENETIC ABNORMALITIES

- CA are found in:
  50-60% of all EPL
- Triploidy + Tetraploidy= 21%
  => Risk of PTD
TRIPLOID MISCARRIAGE

Zaragoza et al., Am J Hum Genet, 2000

- Most triploid miscarriages are diandric (paternal).
- No PHM triploidies are digynic but digynic triploidies are associated with early embryonic arrest => Retention.
- Only 60% of triploid EPL present with molar changes.

SCREENING FOR GTD IN EPL:

UCLH STUDY 1998-2004 (Johns et al., UOG, April, 2005)

- Compared TVS with Histopath in women with suspected CHM or PHM on TVS in case of miscarriage.
- UCLH EPU over 5 years (n= 7563).
- 2768 miscarriages: 556 incomplete (20%)
  1550 complete (56%)
  662 missed (24%)
- 852 ERPC.

SCREENING FOR GTD IN EPL:

UCLH STUDY 1998-2004 (Johns et al., UOG, April, 2005)

- 51 cases diagnosed by histopath & referred to Charing Cross (5 were hydropic abortion).
- 33 (10 CHM & 13 PHM) were suspected by TVS of which 22 were confirmed by histopath (DR= 56%)
- MShCG was available in 15 cases => 9 were elevated (all were CMH or PHM).
HISTOPATHOLOGY & EPL: Conclusions

• VALUES – SCREENING FOR GTD.
  - Investigation of the pathophysiology.
  - Epidemiology.
• PITFALL => VILLOUS CHANGES IN EPL ARE SECONDARY TO DEGENERATION FOLLOWING HYPEROXIA AND RETENTION IN UTERO (unrelated to the etiology of the EPL). Jauniaux & Burton, Placenta, 2005.

SCREENING FOR GTD IN EARLY PREGNANCY: Conclusions

• MOLAR TRANSFORMATION IS PROGRESSIVE AND NON-SPECIFIC.
• DIFFERENTIAL DIAGNOSIS OF GTTs AND OF PSEUDO-MOLES USING MShCG AND US IMAGING (IS CURRENTLY INVESTIGATED PROGRESSIVELY).
Pregnancies of Unknown Location (PULs)

Emma Kirk
Early Pregnancy Unit,
St. George’s Hospital, London

Positive Pregnancy Test

TVS

90%

10%

Intra-Uterine Pregnancy (IUP)
Ectopic Pregnancy (EP)

PUL

IUP Failing EP

PUL

– Positive pregnancy test
– No pregnancy visualised on scan
Pregnancies of Unknown Location (PULs)

- Is not interchangeable term with ectopic pregnancy (EP)

- Rate of PULs is related to quality of scanning, i.e. high sensitivity for the detection of EP using TVS associated with a low PUL rate
Management Considerations

1. Safety
2. Surgical intervention
3. Use of mathematical models
4. Time to diagnosis

1. Safety

- The majority of PULs are not ectopic pregnancies
- Expectant management has been well documented
- Outpatient management has been demonstrated to be safe and not associated with any serious adverse outcomes


2. Surgical Intervention

- Laparoscopy
- Curettage

- The combination of a positive pregnancy test and the absence of an IUP on TVS is an accepted indication for laparoscopy
- Serial measurements of hCG and progesterone, TVS and uterine curettage have been combined into various diagnostic algorithms when a pregnancy cannot be seen on TVS
Discriminatory zone
- a level of hCG above which an intra-uterine pregnancy should be visualised on USS

Concept introduced in 1980, with relation to transabdominal USS (TAS). Kadar et al., 1981

EP likely if serum hCG >6500 IU/L and an IUP could not be seen on TAS. Probability > 95%.

With the introduction of high resolution TVS the discriminatory zone has decreased.

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Diagnostic accuracy of varying discriminatory zones for the prediction of ectopic pregnancy in women with a pregnancy of unknown location

---

Objectives
- To evaluate the use of varying discriminatory zones of serum hCG > 1000 U/L, 1500 U/L and 2000 U/L in diagnosing EPs in women with a PUL

Results
- 5544 consecutive women attending the Early Pregnancy Unit
- 527 (9.5%) cases with PUL analyzed
PUL Results

hCG > 1000 U/L

- 78% of ectopics missed
- 10 laparoscopies to detect one ectopic
- 4x laparoscopy IUP
- 5x laparoscopy failing PUL

2. Surgical Intervention

PUL Results

hCG > 1500 U/L

- 81% of ectopics missed
- 5 laparoscopies to detect one ectopic

2. Surgical Intervention

PUL Results

hCG > 2000 U/L

- 86% of ectopics missed
- 5 laparoscopies to detect one ectopic
2. Surgical Intervention

Varying discriminatory zones

<table>
<thead>
<tr>
<th>hCG Level</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1000 U/L</td>
<td>21.7%</td>
<td>87.3%</td>
</tr>
<tr>
<td>&gt;1500 U/L</td>
<td>15.2%</td>
<td>93.4%</td>
</tr>
<tr>
<td>&gt;2000 U/L</td>
<td>10.9%</td>
<td>95.2%</td>
</tr>
</tbody>
</table>

Conclusions

- Varying the discriminatory zone does not significantly improve the detection of EP in a PUL population
- A single serum hCG, when used in a scanning based centre, is not only potentially falsely reassuring but unhelpful in excluding the presence of an EP
- Laparoscopy should not be a first line investigation in the asymptomatic woman with a PUL

Curettage

- In many centres the diagnosis of an EP in a PUL population is based on documenting that there is no IUP
- Uterine curettage is used as a diagnostic tool to differentiate an EP from miscarriage
- In these circumstances an endometrial biopsy that does not contain chorionic villi is considered to be diagnostic of an EP
Curettage

There is no role for uterine curettage in the contemporary diagnostic workup of women with a pregnancy of unknown location


Objectives

- To generate and evaluate a new protocol that defined non-viability in the PUL population and ensured no viable IUP would be interrupted if curettage performed
- To evaluate published biochemical criteria that define non-viability in a PUL population to establish if these criteria could result in inadvertent termination of pregnancy

Methods

- 4 protocols compared:
  - Protocol 1:
    - Model developed retrospectively so that no cases of viable IUP would undergo curettage
    - Tested prospectively
  - Protocol 2:
    - Defined non-viability as hCG ratio <1.66 Kadar & Romero, 1988
  - Protocol 3:
    - Uterine curettage when hCG > 2000 IU/L or when hCG < 2000 IU/L and hCG ratio < 1.35 Barnhart et al., 2002
  - Protocol 4:
    - Uterine curettage when hCG ratio < 1.5 Stovall et al., 1990
### Results

- 12,572 consecutive women
- 1,003 (8.0%) PULs

<table>
<thead>
<tr>
<th>Protocol</th>
<th>No uterine curettages</th>
<th>No potential TOPs</th>
<th>% potential TOPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – prospective test of model</td>
<td>272</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 – hCG ratio &lt; 1.66</td>
<td>114</td>
<td>14</td>
<td>12.3</td>
</tr>
<tr>
<td>3 – hCG &gt; 2000 IU/L or hCG ratio &lt; 1.35</td>
<td>611</td>
<td>7</td>
<td>1.2</td>
</tr>
<tr>
<td>4 – hCG ratio &lt; 1.50</td>
<td>617</td>
<td>3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

### Conclusions

- Established criteria for the use of uterine curettage in the management of PULs can theoretically result in inadvertent TOPs

- Uterine curettage should therefore not be used in the routine diagnostic workup of women with a PUL

### Use of mathematical models

- Mathematical models have been developed to predict the outcome of PULs

- They do not require any understanding of the behaviour of serum biochemistry in early pregnancy and could possibly lead to more standardised management protocols
The use of a new logistic regression model for predicting the outcome of pregnancies of unknown location


Methods

Positive pregnancy test
Early Pregnancy Unit
Transvaginal ultrasound scan (TVS)

PUL
Serum hCG 0 & 48 hrs

Follow Up

IUP  Failing PUL  EP

Data recorded: hCG & Prog 0 & 48 hrs
Demographic data
Ultrasound features

Univariate & multivariate analysis to identify variables to include in a model

Multi-categorical logistic regression models developed on training set of PULs

One with best performance evaluated using receiver operator characteristic curves (ROC) and tested on a test set of PULs

Cut-off hCG ratios for pregnancy locations identified
Results

3,996 consecutive women attending EPU

381 (9.5%) PULs

Training set 185 PULs

Test set 196 PULs

Significant variables:
- age
- hCG ratio (hCG 48hrs/hCG 0 hrs)
- logarithm of progesterone average
  (progesterone 0 hrs + progesterone 48 hrs / 2)

Mathematical Models

Model M1 hCG ratio (hCG 48 hr/hCG 0 hr)
Model M2 log progesterone average
Model M3 Age
  log progesterone average
  hCG ratio
Current criteria vs New model (Area Under ROC curve)

<table>
<thead>
<tr>
<th></th>
<th>Current</th>
<th>New model (M1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>185</td>
<td>185</td>
</tr>
<tr>
<td>IUP</td>
<td>0.98±0.01</td>
<td>0.96±0.01</td>
</tr>
<tr>
<td>r</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Failing PUL</td>
<td>0.93±0.02</td>
<td>0.99±0.01</td>
</tr>
<tr>
<td>r</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Ectopic</td>
<td>0.61±0.07</td>
<td>0.84±0.04</td>
</tr>
<tr>
<td>r</td>
<td>0.07</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Conclusion

- The model outperforms current single variable hormonal models for the prediction of ectopic pregnancy and allows diagnosis at 48 hours
- Can predict both viability and pregnancy location in PULs

The practical application of a mathematical model to predict the outcome of pregnancies of unknown location (PULs)

3. Use of mathematical models

Model to predict outcome of PULs based on hCG ratio

<table>
<thead>
<tr>
<th>Model</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failing</td>
<td>73.5%</td>
<td>86.2%</td>
<td>98.4%</td>
<td>71.8%</td>
</tr>
<tr>
<td>IUP</td>
<td>86.8%</td>
<td>74.9%</td>
<td>96.6%</td>
<td>69.8%</td>
</tr>
<tr>
<td>EP</td>
<td>82.8%</td>
<td>80.2%</td>
<td>28.2%</td>
<td>97.7%</td>
</tr>
</tbody>
</table>

Subjective Impression

<table>
<thead>
<tr>
<th>Model</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failing</td>
<td>87.0%</td>
<td>91.7%</td>
<td>92.8%</td>
<td>86.3%</td>
</tr>
<tr>
<td>IUP</td>
<td>92.8%</td>
<td>85.9%</td>
<td>94.5%</td>
<td>84.5%</td>
</tr>
<tr>
<td>EP</td>
<td>79.5%</td>
<td>89.9%</td>
<td>54.8%</td>
<td>93.7%</td>
</tr>
</tbody>
</table>

3. Use of mathematical models

Results – 357 PULs

Conclusions

- This model, based on the hCG ratio, can be used to predict the outcomes of PULs.
- It compares favourably with subjective assessment by experienced operators using current strategies to predict the outcome of PULs.
- Can help classify PULs in to those at high and low risk of EP at 48 hours.
4. Time to diagnosis

- Single visit strategy to predict outcome:
  - Single hCG level (discriminatory zone)
  - Single progesterone level
  - Single hCG and progesterone level

- Timing of follow-up visits until final diagnosis known
  - Logistic regression model

---

A prospective evaluation of a single-visit strategy to manage pregnancies of unknown location


---

**Single Visit Protocol**

<table>
<thead>
<tr>
<th>Serum Prog &lt; 10 nmol/L or Serum hCG &lt; 25 IU/L</th>
<th>Serum Prog &gt; 50 nmol/L and Serum hCG &gt; 25 IU/L</th>
<th>Serum Prog 10-50 nmol/L and hCG &gt; 25 IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failing PUL</td>
<td>IUP</td>
<td>EP</td>
</tr>
<tr>
<td>Low Risk PUL</td>
<td>Low Risk PUL</td>
<td>High Risk PUL</td>
</tr>
</tbody>
</table>

The protocol considers a PUL as a low-risk PUL when the progesterone level is < 10 or > 50 nmol/L, or the hCG level is < 25 IU/L, otherwise as high-risk PUL requiring a second visit.
### True diagnosis vs predicted diagnosis

<table>
<thead>
<tr>
<th>Predicted Diagnosis</th>
<th>True Diagnosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-ectopic pregnancy</td>
<td>Ectopic pregnancy</td>
</tr>
<tr>
<td>Non-ectopic PULs</td>
<td>255</td>
<td>10</td>
</tr>
<tr>
<td>Ectopic PULs</td>
<td>48</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>303</td>
<td>15</td>
</tr>
</tbody>
</table>

- Sensitivity: 84.16%
- Specificity: 33.33%
- LR (+): 1.26
- LR (-): 0.48
- Kappa: 0.08

#### Conclusions

- A single hCG / progesterone measurement eliminates almost 85% of non-ectopic pregnancies correctly.

- However 67% of ectopic pregnancies are discharged without adequate follow-up.

- Therefore, a single blood test should not be used as an alternative to the serial hCG measurement (hCG ratio).

---

### A new logistic regression model for predicting ectopic pregnancies based on a single visit

---
Single Visit Model

- 200 PULs, Final Clinical Outcomes Known
  - 0hr Serum hCG and Prog levels
- Logistic Regression Model Developed
  - Using log hCG and log Prog as variables
- Model tested prospectively on 318 PULs

Results

- Predicted probabilities for the different clinical outcomes were calculated given the hCG and progesterone levels:
  1) Failing PUL
  2) IUP
  3) Ectopic Pregnancy

Training Set (n=200)

<table>
<thead>
<tr>
<th>Second Visit</th>
<th>No</th>
<th>Yes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-EP</td>
<td>60</td>
<td>118</td>
<td>178</td>
</tr>
<tr>
<td>EP</td>
<td>0</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

- Sensitivity = 100%
- Specificity = 33.7%
- PPV = 15.71%
- NPV = 100%

- 70% PULs needed a 2nd visit
4. Time to diagnosis

Results

Test Set (n=318)

<table>
<thead>
<tr>
<th>Second Visit</th>
<th>No</th>
<th>Yes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-EP</td>
<td>150</td>
<td>153</td>
<td>303</td>
</tr>
<tr>
<td>EP</td>
<td>4</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>154</td>
<td>164</td>
<td>318</td>
</tr>
</tbody>
</table>

Sensitivity = 73.3%
Specificity = 49.5%
PPV = 6.71%
NPV = 97.4%

51.6% PULs needed a 2nd visit

4. Time to diagnosis

Single Visit - Results

- 4 EPs missed

<table>
<thead>
<tr>
<th>hCG 0 hrs</th>
<th>hCG 48 hrs</th>
<th>hCG ratio</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>372</td>
<td>615</td>
<td>1.65</td>
</tr>
<tr>
<td>2</td>
<td>160</td>
<td>212</td>
<td>1.33</td>
</tr>
<tr>
<td>3</td>
<td>568</td>
<td>517</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>1795</td>
<td>2423</td>
<td>1.35</td>
</tr>
</tbody>
</table>

4. Time to diagnosis

Summary

- Model correctly identifies 73% of EP and brings them back for a 2nd visit
- The number of PULs needing a 2nd visit is reduced to 51%
- Two hCG measurements still superior
Rationalizing the follow-up of pregnancies of unknown location

### Methods

- **0 hrs**
  - TVS

- **48 hrs**
  - Repeat hCG if initial hCG ratio < 0.87

- **Day 7**
  - Repeat TVS
  - Failing PUL
  - IUP
  - EP
  - If PULs - 48 hrs
    - 1 USS - 2 Blood tests
  - If IUP - 2 Blood tests and 2 USS / 7 days - 3 visits
  - If EP - 2 Blood tests and 2 USS / 7 days - 3 visits

**Failing PULs** – 2 blood tests and 1 USS / 48 hrs - 2 visits
**IUP** – 2 blood tests and 2 USS / 7 days – 3 visits
**EP** – 2 blood tests and 2 USS / 7 days – 3 visits

### Results

- **363 PULs**

<table>
<thead>
<tr>
<th></th>
<th>Total number of cases</th>
<th>Number of cases diagnosed within time period</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failing PULs – 48 hrs</td>
<td>229</td>
<td>219</td>
<td>96%</td>
</tr>
<tr>
<td>IUPs – day 7</td>
<td>112</td>
<td>89</td>
<td>80.2%</td>
</tr>
<tr>
<td>EPs – day 7</td>
<td>22</td>
<td>12</td>
<td>54.5%</td>
</tr>
</tbody>
</table>

**Ectopic Pregnancies**
- 96% (21/22) diagnosed within 9 days
- 32% (7/22) returned before and diagnosed by day 5
- 55% (12/22) diagnosed on day 7 (Total 87% by day 7)
- 6% (2/22) still PUL on day 7, then diagnosed on day 9
- 4% (1/22) had diagnostic laparoscopy and ERPC, then after 26 days diagnosed with an interstitial pregnancy
Methods

0 hrs

48 hrs

Day 7

Methods

0 hrs

48 hrs

Day 7

363 PULs
100%

132 PULs
36%

10 PULs
3%

14 PULs
3%

TVS

Repeat hCG if initial hCG ratio > 0.87

TVS

IUP

Confirmed IUP

Repeat hCG if initial hCG ratio > 0.87

Repeat TVS

Failing PUL

Repeat TVS

Ectopic

231 PULs
64%

4. Time to diagnosis

Results

- 88.4% of women given diagnosis of the pregnancy outcome within the time period
- 9% (33) re-presented before the scheduled visit
- 44 (12%) additional serum hCG tests required
- 49 (13%) additional TVS required
- 0.3% (1) underwent surgical intervention
- No adverse outcomes

Conclusion

- The follow-up of PULs can be rationalized by the use of a logistic regression model.
- This is safe and effective although emergency back-up is essential.
PUL Conclusions

- Expectant management – low-risk EP
- There is no role for the routine use of uterine curettage in the management of PULs
- Change in hCG over time, hCG ratio, and not the absolute serum hCG value

PUL Conclusions

- Discriminatory zone not helpful in PUL population where EP diagnosis is TVS based
- Serum progesterone confirms viability of PUL but not location
- Single blood test approach is not appropriate

PUL Conclusions

- Logistic regression is an alternative to current serum markers
- Potential to decrease the number of visits
- Need multi-centre trials to validate or otherwise the use of such models
Use of MTX systematically in a multiple dose regimen with combination with folic acid (as in GTD)

Introduction of single-dose MTX regimen for EP non-surgical treatment

Local treatment with MTX administered into the gestational sac under laparoscopic guidance

Local treatment with MTX administered into the gestational sac under sonographic guidance

MTX=folic acid antagonist
Inactivates dihydrofolate reductase
Depletion of available stores of tetrahydrofolate (essential cofactor in DNA and RNA synthesis during cell multiplication)
Particular vulnerability - rapidly proliferating tissues (trophoblast)
Primary treatment success: no difference
Tubal preservation: no difference
Tubal patency rate: no difference (60-85%)
Future fertility: no difference
Serum hCG clearance time: no difference
Complications/side effects: 61% vs. 12%
Costs: lower in MTX group (if initial hCG<1500 IU/L)

CONCLUSION: no significant differences in major short- and long-term outcome measures

Hajnenius et al. Cochrane Databases of Systematic Reviews, 2000; Issue 1

Notes:
**MTX for treatment of ectopic pregnancy**

**absolute requirements**

- Hemodynamic stability
- No evidence of acute intra-abdominal bleeding
- Reliable commitment to comply with required follow-up
- No contraindications to MTX treatment

---

**MTX for treatment of ectopic pregnancy**

**preferable characteristics**

- Absent or mild symptoms (pain)
- Serum hCG < 10,000 IU/L
- Absent embryonic heart activity
- Ectopic gestational mass < 4 cm in diameter

*MTX is not contraindicated for EPG associated with serum hCG > 10,000 IU/L, fluid > 50 mL or mass > 4 cm BUT THE LIKELIHOOD OF SUCCESS IS SUBSTANTIALLY INCREASED*

---

**MTX for treatment of ectopic pregnancy**

**contraindications**

- Breastfeeding
- Immunodeficiency states
- Alcoholism or evidence of chronic liver disease (elevated transaminases)
- Renal disease (elevated serum creatinine)
- Hematologic abnormalities (severe anemia, leukopenia, trombocytopenia)
- Known sensitivity to MTX
- Active pulmonary disease
- Peptic ulcer disease

*Speroff, Fritz. Clinical Gynecologic Endocrinology and Infertility, 2005*
**NTX for treatment of ectopic pregnancy**

### Intramuscular treatment regimens

<table>
<thead>
<tr>
<th></th>
<th>Single dose</th>
<th>Multidose</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTX (methotrexate)</td>
<td>7 mg/kg</td>
<td></td>
</tr>
<tr>
<td>LEU (leucovorin)</td>
<td>None</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Dosing frequency</td>
<td>One dose, repeat in 7 days if necessary</td>
<td>Alternate daily doses of MTX and LEU until hCG declines by 15%, up to four doses of each</td>
</tr>
<tr>
<td>Monitoring of hCG</td>
<td>Baseline (day 0), day 4, day 7</td>
<td>Baseline (day 0), days 1, 3, 5, 7 until hCG declines 15% from previous value</td>
</tr>
<tr>
<td>Additional doses</td>
<td>2nd dose given if hCG has not declined 15% between days 4 and 7</td>
<td>Give 1st, 3rd, 4th dose if hCG has not declined 15% from the previous value; maximum four doses</td>
</tr>
<tr>
<td>Follow-up after treatment response</td>
<td>Weekly hCG until value &lt;5</td>
<td>Weekly hCG until value &lt;5</td>
</tr>
</tbody>
</table>

**Notes:**

---

**NTX for treatment of ectopic pregnancy**

### Intramuscular NTX dosage

**The Mosteller formula**

\[
BSA (m^2) = \frac{(\text{Height(cm)} \times \text{Weight(kg)})}{3600}
\]

or in inches and pounds:

\[
BSA (m^2) = \frac{(\text{Height(in)} \times \text{Weight(lbs)})}{3131}
\]


**Body surface calculator**

http://www.halls.md/body-surface-area/bsa.htm

**Notes:**

---

**NTX for treatment of ectopic pregnancy**

### Single or multidose?

**Metaanalysis (26 studies, 1327 cases)**

<table>
<thead>
<tr>
<th></th>
<th>SINGLE-DOSE</th>
<th>MULTIDOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failure rate</td>
<td>11.9%</td>
<td>7.3%</td>
</tr>
</tbody>
</table>

**OR=1.71 [CI=1.04-2.32]**


**Notes:**

---

Page 75 of 150
Single-dose MTX should be reserved for women who:
- don’t respond to salpingostomy
- are at low risk for failure of non-surgical management
  - hCG value declining at the time of diagnosis
  - initial hCG value < 1,000 mIU

Barnhardt, Seeber. Contemp OB/GYN 2004

Intramuscular MTX (50mg/m²) administered on days 0 and 4
- 87% of patients were treated successfully
- 3% of tubal ruptures
- Treatment was well tolerated
- 91% of patients satisfied

Barnhart et al. Fertil Steril 2006 Nov 9th

Oral MTX was given at a dose of 60mg/m² in 2 divided doses (2 hrs apart)
- 86% of patients were treated successfully (no impact of initial hCG and ectopic size).
- 86% of patients had increased pain during treatment
- 55% of patients suffered from gastrointestinal side-effects (vomiting, nausea, bloating)
- 32% of patients required more than one treatment cycle

Oral MTX can be used to treat EP successfully but there is little advantage to recommend its use over intramuscular MTX

Lipscomb et al. AJOG 2002;186:1192.
Pretreatment and on day 8

- CBC
- Platelet count
- Renal and liver function tests

Speroff, F. A. Clinical Gynecologic Endocrinology and Infertility, 2005

<table>
<thead>
<tr>
<th>Initial serum β-hCG level (IU/L)</th>
<th>Success Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1,000</td>
<td>98%</td>
</tr>
<tr>
<td>1,000-1,999</td>
<td>93%</td>
</tr>
<tr>
<td>2,000-4,999</td>
<td>92%</td>
</tr>
<tr>
<td>5,000-9,999</td>
<td>87%</td>
</tr>
<tr>
<td>10,000-14,999</td>
<td>82%</td>
</tr>
<tr>
<td>&gt;15,000</td>
<td>68%</td>
</tr>
</tbody>
</table>

Is ectopic gestational sac or mass greater than 3-4 cm a contraindication to MTX treatment?

Common practice: YES  
EBM: NO

No correlation between size and MTX treatment success because:
- EP cannot be distinguished in TV-USG from surrounding blood clot
- Size does not accurately predict viability

Gamzu et al. Hum Reprod 2002;17:2384-2391
Lipscomb et al. AJOG 1998;178:1354-1359
Is the presence of fluid in cul-de-sac a contraindication to MTX treatment?

Common practise: YES  EBM: NO
Free peritoneal fluid can be observed in almost 40% of unruptured EP
Cul-de-sac fluid does not accurately predict the success/failure of MTX treatment


Is the presence of embryonic heart activity a contraindication to MTX treatment?

Common practise: YES  EBM: YES
Treatment failure significantly more often when FRH(+) (good correlation with hCG level)


Persistent trophoblast (inadequately declining serum hCG)
Tubal rupture

Appr. 15% of women initially treated with MTX ultimately require surgery (about half because of tubal rupture)
Tubal rupture does not have independent adverse effect on subsequent fertility

Job-Spira et al. AJOG 1999;180:938.
Classical "15% day 4-7 rule" (>15% decrease in hCG from days 4-7 after single-dose MTX administration)

- sensitivity (PPV) – 93.0%
- specificity (NPV) – 84.2%

New rules
- percentage hCG change days 4-5
- logistic regression models including day 5 hCG and USG findings

CONCLUSION: "15% day 4-7 rule" is a very good indicator of the single-dose MTX treatment success. New rules do not improve ability to predict a successful outcome at an early stage.

Kirk et al. Hum Reprod 2006; Nov 6th

50-80% of women treated with single-dose MTX for EP later achieve intrauterine pregnancy
- 10-25% of women treated with MTX for EP experience a recurrent ectopic pregnancy

Women with EP associated with IUD have better future reproductive prognosis (EP is less likely related to the tubal pathology)

Poorer prognosis concerns:
- older women
- previously infertile women
- those with past history of EP or tubal damage

CONCLUSION: FERTILITY AFTER EP DEPENDS MORE ON ALREADY ESTABLISHED RISK FACTORS THAN ON THE METHOD OF TREATMENT.
Caution: Symptoms of pain commonly emerge or increase over the days following MTX treatment.

Cause is uncertain – most likely it reflects:
- tubal miscarriage (separation pain)
- peritoneal tension resulting from hematoma

This complaint merits re-evaluation but is not an indication for immediate surgery! Most women can be reassured and continue outpatient management (analgesics)


Caution: After MTX therapy, over 50% of EPs followed with serial ultrasonography increase in size.

Most likely it reflects the formation of hematoma

Occasionally the ectopic gestational mass may persist for weeks even after serum hCG have fallen below detection limit

Laparoscopic salpingostomy is the cornerstone of treatment in the majority of women with tubal pregnancy.

Systemic MTX in multiple doses is an alternative treatment option if diagnosis of EP is established noninvasively. This treatment option should be recommended for patients:
- hemodynamically stable
- with unruptured tubal pregnancy
- with no active intra-abdominal bleeding
- presenting with low initial serum hCG level and no fetal cardiac activity in US
- after properly informing them about the risks and benefits of the available treatment options

Hajenius et al. Cochrane Databases of Systematic Reviews, 2000; Issue 1
Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage

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On behalf of ESHRE Special Interest Group for Early Pregnancy (SIGEP).

Recurrent miscarriage (RM; ≥3 consecutive early pregnancy losses) affects around 1% of fertile couples. Parental chromosomal anomalies, maternal thrombophilic disorders and structural uterine anomalies have been directly associated with recurrent miscarriage; however, in the vast majority of cases the pathophysiology remains unknown. We have updated the ESHRE Special Interest Group for Early Pregnancy (SIGEP) protocol for the investigation and medical management of RM. Based on the data of recently published large randomized controlled trials (RCTs) and meta-analyses, we recommend that basic investigations of a couple presenting with recurrent miscarriage should include obstetric and family history, age, BMI and exposure to toxins, full blood count, antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies), parental karyotype, pelvic ultrasound and/or hysterosalpingogram. Other investigations should be limited to particular cases and/or used within research programmes. Tender loving care and health advice are the only interventions that do not require more RCTs. All other proposed therapies, which require more investigations, are of no proven benefit or are associated with more harm than good.

Key words: early pregnancy/evidence-based/management/recurrent miscarriage/treatment

Introduction

Recurrent miscarriage (RM) is traditionally defined as three or more consecutive miscarriages occurring before 20 weeks post-menstruation (Stirrat, 1990; Berry et al., 1995; Bricker and Farquharson, 2002). Around 1% of fertile couples will experience recurrent early pregnancy losses (Berry et al., 1995). The risk of recurrence increases with the maternal age and number of successive losses (Brigham et al., 1999; Andersen et al., 2000). The prognosis is not better for couples with prior live birth (Clifford et al., 1997). Thus, the number of previous miscarriages and maternal age are the most important covariates, and they have to be taken into account when planning therapeutic trials. The ideal trial should have stratification for the number of previous miscarriages and maternal age, with randomization between control and experimental treatments, within each stratum as recently recommended (Christiansen et al., 2005).

Recurrent miscarriage has been directly associated with parental chromosomal anomalies (Franssen et al., 2005), maternal thrombophilic disorders (Rey et al., 2003) and structural uterine anomalies and indirectly with maternal immune dysfunction and endocrine abnormalities (Carrington et al., 2005).

However, as the majority of RM cases following investigation are classified as idiopathic, that is, no identifiable cause in either partner, it is generally accepted that within the idiopathic group there is considerable heterogeneity and it is unlikely that one single pathological mechanism can be attributed to their RM history (Stirrat, 1990). Furthermore, there is considerable debate about the cause and association, as the exact pathophysiological mechanisms of most known etiologies have not been precisely elucidated. Current research is directed at theories on defects in nature’s quality control related to implantation, trophoblast invasion and placentation, as well as factors, which may be embryopathic (Quenby et al., 2002). Most women with recurrent pregnancy loss probably have several risk factors for miscarriage.

Although benefits have been reported for a variety of endocrinologic and immunologic treatment, many therapeutic approaches remain controversial, mainly because of wide variations in patient-selection criteria and treatment protocols. The small sizes of most individual studies, poor stratification bias and matching of cases and controls have limited the translation of results into clinical practice. New randomized controlled trials (RCTs) and meta-analyses have recently been published in the international literature. This has prompted the ESHRE
Special Interest Group for Early Pregnancy (SIGEP) to update its protocol for the investigation and medical management of RM. The surgical treatment of uterine causes of RM and the value of preimplantation genetic screening (PGS) for the embryos of couples presenting with RM, being under the auspices of other SIGs, will not be included in this review.

Evidence-based investigations for couples presenting with RM

Coagulation investigations

Acquired maternal thrombophilia is a well-recognized cause of RM. All women with a history of three or more early pregnancy losses, that is, before 10 weeks, or 1 or more unexplained deaths at ≥10 weeks of a morphologically normal fetus, or 1 or more premature births at ≤34 weeks with severe preeclampsia or placental insufficiency, should be offered a testing for lupus anticoagulant (LAC) and anticardiolipin antibodies (aCL), known collectively as antiphospholipid antibodies (APA), to exclude an antiphospholipid syndrome (APS) (Wilson et al., 1999). More recently, an increased incidence of early and recurrent fetal loss has also been suggested in women with inherited thrombophilia, including Factor V Leiden deficiency, activated protein C resistance, prothrombin G20210A and protein S deficiency (Dawood et al., 2003; Rey et al., 2003); however, other authors have found no association between maternal thrombophilia and pregnancy loss <10 weeks of gestation (Roque et al., 2004). Some studies reported a decreased risk of miscarriage in women with inherited thrombophilia (Carp et al., 2002; van Dunne et al., 2005), whereas one study reported that multiple genetic thrombophilic mutations in either partner seem to increase the risk of miscarriage in a subsequent pregnancy (Jivraj et al., 2006). Larger epidemiological studies are clearly needed to justify testing couples with RM for inherited thrombophilia in routine clinical practice (Robertson et al., 2006). Other coagulation abnormalities, including impaired fibrinolytic activity, factor XII deficiency and reduced activated partial thromboplastin time have also been reported to be associated with RM, but the corresponding epidemiological data are limited (Li et al., 2002), and the assay for these abnormalities should only be performed as part of prospective clinical studies.

Endocrinologic investigations

Early epidemiological data have shown an association between RM and hypothyroidism or diabetes mellitus. Although current evidence indicates that treated hypothyroidism and well-controlled diabetes are not associated with RM (RCOG, 1998), thyroid function tests and HbA1c measurements are accurate and inexpensive and can still be considered as part of the evaluation of RM (Christiansen et al., 2005). Furthermore, a low level of maternal thyroxin and poorly controlled maternal glucose levels in early pregnancy are associated with short- and long-term consequences for fetal development and should therefore be diagnosed and treated before conception.

Obesity is associated with a statistically significant increased risk of first trimester and recurrent miscarriage [odds ratios 1.2 and 3.5, 95% confidence interval (CI) 1.01–1.46 and 1.03–12.01, respectively] (Lashen et al., 2004). Obesity certainly has a wider impact on women’s health, and several studies have shown that the association between polycystic ovary syndrome (PCOS) and RM could be secondary to the association between obesity and miscarriage (Fedorescas et al., 2000; Bellver et al., 2003). Weight loss should be considered as a first option for women who are infertile and overweight (Clark et al., 1998; Morikawa et al., 2004), and there is little doubt that the same concept applies to women with RM. Other endocrinologic disorders, including hypersecretion of LH, high androgen levels, hyperprolactinaemia and luteal phase defects (LPD) have been associated with RM. Current evidence suggest that, as is the case for hypothyroidism, infertility is more likely a problem than pregnancy loss. Further studies are required to examine the relationship between hyperandrogenism and RM (Christiansen et al., 2005).

Immunologic investigations

An excessive maternal immune response against paternal antigens resulting in abnormal immune cells and cytokine production has and is still thought to be one of the causes of RM (Laird et al., 2003). In particular, interest is currently focused on the relationship between RM and Natural Killer (NK) cells. Although much of the evidence is contradictory, these studies suggest differences in the peripheral blood NK-cell levels in women with RM. NK cells are also found in the endometrium and decidua, but the knowledge on their role in human placentalation is limited. There are phenotypic and functional differences between peripheral and uterine NK cells, and tests to measure NK cells in peripheral blood give no useful information on uterine NK cells (Moffett et al., 2004). Furthermore, the percentage of CD56+ NK cells in peripheral blood of healthy individual varies from 5 to 29% and is affected by sex, stress, ethnicity and age. Within this context, testing of peripheral blood NK cells should not be performed routinely in the evaluation of miscarriage in general and RM in particular (Rai et al., 2005; Thum et al., 2005; Wold and Arici, 2005), outside research protocols. Similarly, there is no scientific basis for the introduction of peripheral cytokine level measurements into routine practice. Recent data have shown that a high number of uterine NK cells is found in the endometrium of women with RM and this could be reduced by therapy (Quenby et al., 2005). However, prospective trials are needed to evaluate the possible use of this finding, and currently endometrial sampling should only be offered to women within the context of research programmes.

Mannan-binding lectin (MBL) is a C-type lectin that participates in the innate immune defence by activating complement on the surfaces of micro-organisms. Two large case-controlled studies (Kilpatrick et al., 1995; Kruse et al., 2002) have shown that low levels of MBL are associated with RM and that low MBL is associated with a significantly (20%) higher miscarriage rate in the next pregnancy. However, most women with low MBL levels do not experience RM, suggesting that low MBL as a sole factor probably does not cause RM but may increase the risk of early pregnancy loss when found in conjunction with other immunological disturbances which remain to be investigated.
### Parental cytogenetic investigation

The incidence of structural chromosome abnormalities, usually a balanced translocation, is increased in couples with RM. All the four factors, namely low maternal age at second miscarriage, a history of three or more miscarriages, a history of two or more miscarriages in a brother or sister and a history of two or more miscarriages in the parents of either partner, increase the probability of carrier status (Franssen et al., 2005). A probability of carrier status is obtained when these four factors are combined (Franssen et al., 2005). After one miscarriage, it is generally accepted to refrain from karyotyping. The incidence of carrier status after one miscarriage is 2.2% (Braekeleer de and Dao, 1990). It is thus advised to refer for parental karyotype only when the probability of carrier status is ≥2.2% (see Table I).

### Histopathological and cytogenetic investigations

Whilst it is routine practice to send products of conception for histological examination, mainly to exclude a gestational trophoblastic disorder, the usefulness of histopathological investigation of placental and/or fetal tissue in RM on future pregnancy management for an individual couple remains to be determined (Jauniaux et al., 1996; Jauniaux and Burton, 2005). Overall, the inaccuracy of villous morphology and the limited clinical significance of the finding of an aneuploidy in a sporadic miscarriage have lead some authors to conclude that the histological classification is a valueless clinical exercise (Fox, 1993). New morphologic classifications continue to be proposed (Hakvoort et al., 2006). Overall, these retrospective studies are of academic interest in understanding the pathophysiology of early pregnancy failure, but their practical clinical role has never been demonstrated. In couples with RM, there are a few reports showing an increased incidence of thrombo-inflammatory lesions such as perivillous fibrin deposition, chronic villitis and deciduitis (Doss et al., 1995; Hustin et al., 1996), in particular, when the karyotype is normal (Redline et al., 1999). Although these lesions support the immunologic imbalance concept, the contribution of these histological findings to the management of RM is limited and has not been trialled prospectively.

The risk of live born trisomy following an aneuploidy in a sporadic early pregnancy failure is around 2% (Alberman, 1992). By contrast, chromosomal analyses of the products of conception in couples with RM indicate that a normal conceptus karyotype in a previous pregnancy is a predictor of subsequent miscarriage (Warburton et al., 1987; Ogasawara et al., 2000; Carp et al., 2001; Morikawa et al., 2004). Women <36 years of age with RM have a higher frequency of euploid miscarriage. When stratified for maternal age, there is no difference in the distribution of cytogenetically abnormal miscarriages in couples with RM compared with controls (Stephenson et al., 2002). The possibility that a miscarriage following treatment is the result of aneuploidy must be investigated particularly in efficacy trials. Without this information it is impossible to ascertain whether the pregnancy loss is the result of treatment failure or a de-novo chromosomal anomaly. The magnitude of the size of the treatment effect will be affected without correction for the aneuploidy factor (Christiansen et al., 2005). The cost benefit of performing systematic karyotyping of products of conception after one miscarriage on the overall management of RM needs to be investigated prospectively in large populations.

### Anatomical investigations

The prevalence and impact on reproduction of uterine malformations in the general population have not been clearly established. Traditionally, laparoscopy, hysterosalpingography (HSG) and/or hysteroscopy have been used to diagnose these uterine malformations in women with RM. Ultrasound, and in particular 3D ultrasound, has become an accurate, reproducible, non-invasive, out-patient method for the diagnosis of congenital uterine anomalies (Salim et al., 2003a). Using 3D ultrasound, it has been reported that women with a subseptate uterus have a higher incidence of first trimester loss, whereas women with an arcuate uterus have a greater proportion of second trimester loss and preterm delivery (Woelfer et al., 1990). It is thus advised to refer for parental karyotype only when the probability of carrier status is ≥2.2% (see Table I).

### Table I. Probability of carrier status in couples with two or more miscarriages, according to the multivariable logistic regression model (modified from Franssen et al., 2005)

<table>
<thead>
<tr>
<th>Maternal age at second miscarriage</th>
<th>(RM&lt;sub&gt;parents&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</th>
<th>(RM&lt;sub&gt;parents&lt;/sub&gt;)&lt;sup&gt;−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥2 miscarriages</td>
<td>2 miscarriages</td>
</tr>
<tr>
<td>&lt;23 years</td>
<td>10.2</td>
<td>7.3</td>
</tr>
<tr>
<td>(RM&lt;sub&gt;parents&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>5.7</td>
<td>4.0</td>
</tr>
<tr>
<td>(RM&lt;sub&gt;parents&lt;/sub&gt;)&lt;sup&gt;−&lt;/sup&gt;</td>
<td>10.0</td>
<td>7.2</td>
</tr>
<tr>
<td>23–33 years</td>
<td>5.7</td>
<td>4.0</td>
</tr>
<tr>
<td>(RM&lt;sub&gt;parents&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>5.8</td>
<td>4.1</td>
</tr>
<tr>
<td>(RM&lt;sub&gt;parents&lt;/sub&gt;)&lt;sup&gt;−&lt;/sup&gt;</td>
<td>3.2</td>
<td>2.2</td>
</tr>
<tr>
<td>37–38 years</td>
<td>4.0</td>
<td>2.8</td>
</tr>
<tr>
<td>(RM&lt;sub&gt;parents&lt;/sub&gt;)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>(RM&lt;sub&gt;parents&lt;/sub&gt;)&lt;sup&gt;−&lt;/sup&gt;</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>≥39 years</td>
<td>1.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

All values are given in percentage.

Grey area, couples with a probability of carrier status <2.2%. Karyotyping can be withheld in these couples.

RM<sub>parents</sub>, history of ≥2 miscarriages in a brother or sister of either partner; RM<sub>parents</sub>, history of ≥2 miscarriages in parents of either partner; 2 miscarriages, history of ≥2 miscarriages in the couple; ≥3 miscarriages, history of ≥3 miscarriages in the couple.
A large comparative study of the ultrasound morphology of congenital anomalies in women with and without RM has shown no difference in the relative frequency of various anomalies between the two groups of women (Salim et al., 2003b). However, with both arcuate and subseptate uteri, the length of the remaining cavity was shorter, and the size of the fundal distortion was higher in the RM group.

Other investigations
High level of homocysteine (hyperhomocysteinaemia) can be associated with RM (Nelen et al., 2000). Among the genetic causes of this condition, a common one is polymorphism at position 677 in the *methyl tetrahydrofolate reductase* (*MTHFR*) gene, which in the homozygous form leads to a thermodabile enzyme variant (Makris, 2000). Within this context, low plasma folate levels have been associated with an increased risk of first trimester miscarriage (George et al., 2002). Investigation for the above condition remains technically difficult and should not be performed outside a specific clinical context.

Infections with bacteria, viruses or parasites can all interfere with early pregnancy development, but none seems to be a significant cause of RM (Simpson et al., 1996). Toxoplasmosis, Rubella, cytomegalovirus, herpes (TORCH) screen is therefore of limited value in the investigation of RM, outside an acute infectious episode (Li et al., 2002).

A lot of information is available about environmental toxins. The association between miscarriage and ionizing radiation, organic solvents, alcohol, mercury and lead is confirmed, whilst an association to caffeine, hyperthermia and cigarette smoking is suspected (Gardella and Hill, 2000).

The recommended investigations are summarized in Table II.

### Table II. Recommendation for the testing of couple presenting with recurrent miscarriage (≥3 miscarriages)

<table>
<thead>
<tr>
<th>Basic investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obstetric and family history, age, BMI, organic solvents, alcohol, mercury, lead, caffeine, hyperthermia, smoking</td>
</tr>
<tr>
<td>Full blood count (blood sugar level and thyroid function tests)</td>
</tr>
<tr>
<td>Antiphospholipid antibodies (LAC and aLC)</td>
</tr>
<tr>
<td>Parental karyotype (after 2 miscarriages—see Table I)</td>
</tr>
<tr>
<td>Pelvic ultrasound (SIS) and/or hysterosalpingogram and hysteroscopy and laparoscopy in case of inconclusive findings</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Research investigations within the context of a trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feto-placental karyotypes</td>
</tr>
<tr>
<td>Testing of uterine and/or peripheral blood NK cells</td>
</tr>
<tr>
<td>Mannan-binding lectin (MBL) level</td>
</tr>
<tr>
<td>Luteal phase endometrial biopsy</td>
</tr>
<tr>
<td>Homocysteine/folic acid level</td>
</tr>
<tr>
<td>Thrombophilia screening</td>
</tr>
</tbody>
</table>

Evidence-based guidelines for treatment of recurrent miscarriage

Aspirin and/or heparins have become routine treatment for women with APS and inherited thrombophilias and a history of RM, on the basis of limited evidence. A recent meta-analysis of randomized and quasi-randomized RCTs on medical inter-

### Table III. Recommendation for the medical treatment of women with recurrent miscarriage (RM) (≥3 consecutive miscarriages)

<table>
<thead>
<tr>
<th>Established treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tender loving care (TLC) and health advices (diet, coffee, smoking and alcohol)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment requiring more RCTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin and/or LMW heparins for women presenting with APS or (multiple) inherited thrombophilias</td>
</tr>
<tr>
<td>Progestrone in women presenting with unexplained early and late RM</td>
</tr>
<tr>
<td>IVIG in women presenting with unexplained secondary RM or late RM</td>
</tr>
<tr>
<td>Folic acid in women presenting with hyperhomocysteinaemia</td>
</tr>
<tr>
<td>Immunization with third-party donor leukocyte</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment of no proven benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunization with paternal leukocytes or trophoblast membranes</td>
</tr>
<tr>
<td>Multivitamins supplementation</td>
</tr>
<tr>
<td>Treatment associated with more harm than benefit</td>
</tr>
<tr>
<td>Daily corticoids during the first half of pregnancy</td>
</tr>
</tbody>
</table>
retrospective study of maternal use during the first trimester of pregnancy of over-the-counter cough/cold/analgesics including aspirin and other non-steroidal anti-inflammatory drugs (NSAID) (Werler et al., 2002). An association between NSAID use in the first trimester and an increased risk of miscarriage has also been reported, in particular when used around the time of conception (Nielsen et al., 2001; Li et al., 2003). The risk is higher when NSAIDs were used for longer than a week, which indicates a dose–response relationship, though no precise dose information was available (Li et al., 2003). The study by Nielsen et al. (2001) can be criticized on the basis of incomplete ascertainment of both miscarriages and NSAID use during pregnancy and lack of information on important confounders including the reasons for NSAID use. By contrast, the study by Li et al. (2003) identified indications for use of the different drugs, controlled for confounding factors, and evaluated the effect of timing and duration of use. These authors concluded that the association between NSAID use and miscarriage is unlikely to be because of the underlying indications for use of NSAIDs or aspirin.

There is no reported fetal side-effect of heparin use during pregnancy, but osteopenia has been a major concern of long-term heparin therapy, in particular with UFH (Pettila et al., 2002; Hawkins and Evans, 2005). In women with RM, a small decrease of 3.7% of lumbar spine and 0.9% of the neck of femur bone mineral density (BMD) has been reported in one study using both LMWH and UFH (Backos et al., 1999). There was no significant difference in BMD changes, in this uncontrolled study, between the two heparin preparations. A more recent prospective, controlled study has shown that bone loss associated with the use of long-term LMWH for RM and thrombophilia is not significantly different from physiological losses during pregnancy (Carlin et al., 2004). Overall, the decrease in BMD seems to be similar in heparin-treated and untreated pregnant women (Holmberg-Marttila et al., 2000). Fondaparinux sodium, a new indirect activated factor VII inhibitor, does not have a negative effect on BMD and could therefore be a safe and effective alternative to UFH and LMWH (Hawkins and Evans, 2005). Treatment-related thrombocytopenia was not reported in a recent systematic review of 64 studies of LMWH use in pregnancy (Greer and Nelson-Piercy, 2005). Minor local skin reactions are observed in about one-third of RM patients (Deruelle et al., 2005).

Progestational agents

Progesterone has been administered orally, intramuscularly and vaginally for more than five decades in an attempt to prevent miscarriage in early-to-mid pregnancy. Overall, the use of progestational agents during the first and second trimester of pregnancy is not associated with adverse effects in mothers. However, Carmichael et al. (2005) have recently reported that maternal intake of progestins in early pregnancy is associated with an increased risk of hypospadias in the male offspring (odds ratio 3.7, 95% CI 2.3–6.0).

Overall, despite considerable medical use, there is currently insufficient information to allow recommendations regarding optimal dose, route and timing of progesterone supplementation. A recent systematic review found no evidence to support the routine use of progesterone in the first trimester to prevent miscarriage (Oates-Whitehead et al., 2005). The meta-analysis indicated that in a subgroup analysis of three trials involving women with RM, progesterone treatment showed a statistically significant decrease in miscarriage rate compared with placebo or no treatment (odds ratio 0.39, 95% CI 0.17–0.91). The route of administration did not influence the results. All trials were more than 40 years old, and a modern prospective RCT of sufficient power to determine the efficacy of progesterone supplementation in women with RM is needed to confirm these results (Table III).

Immunosuppressant and immunomodulator agents

The use of intravenous immunoglobulin (IVIG), anti-TNFα, glucocorticoids or cellular therapies in order to prevent or reduce an ‘excessive immune response’ and/or abrogate maternal–fetal incompatibility in women with RM remains controversial. IVIG is a pooled blood product, which can be rarely associated with anaphylactic response, fever, flushing, muscle pain, nausea and headache. IVIG preparations may be derived from tens of thousands of donations, and thus there is also the possibility of iatrogenic Creutzfeldt–Jakob disease (CJD), but there is so far no evidence that CJD has been diagnosed following IVIG therapy. Overall, the only downside of IVIG is its high price (Sapir et al., 2005). By contrast, anti-TNFα agents have been reported to be associated with the development of granulomatous disease, lymphoma, systemic lupus erythematosus-like syndromes, congestive cardiac failure and demyelinating diseases (Claudepierre et al., 2005). Multiple courses of glucocorticoids during pregnancy are associated with serious side effects including an increased risk of preterm birth because of premature rupture of membranes and the development of preeclampsia and gestational diabetes (Empson et al., 2002).

In women presenting with RM associated with APA, IVIG shows no reduction in subsequent pregnancy loss (Empson et al., 2005). In these patients, the association of IVIG, heparin and aspirin is associated with an increased risk of pregnancy loss or premature delivery when compared with UFH or LMWH combined with aspirin (relative risk 2.51; 95% CI 1.27–4.95). Similarly, prednisone and aspirin resulted in a significant increase in premature delivery and gestational diabetes in comparison with a placebo, aspirin alone or aspirin combined with heparin (Empson et al., 2005). A small benefit of IVIG in women with RM and APS cannot be excluded on the basis of available studies. Furthermore, IVIG might also be of benefit to women with unexplained RM (Christiansen et al., 2004), but within this context, its use should only occur as part of an RCT. IVIG could be more efficacious in women presenting with secondary RM or repeated second trimester intrauterine fetal deaths (Christiansen and Nielsen, 2005).

The majority of trials using cellular treatment for RM have failed to find any beneficial effect. The most common of these are transusions of paternal leukocytes before conception. So far, meta-analyses have shown no significant benefit of paternal leukocytes, third-party donor leukocytes or trophoblast membranes on pregnancy outcome when compared
with placebo (Scott, 2003; Porter et al., 2005); however, in the three small trials that tested third-party donor leukocytes, the pooled odds ratio for live birth was 1.39 (95% CI 0.68–2.82) in the treatment group compared with the placebo group, emphasizing that this treatment should be further tested in RCTs (Table III).

Other treatments
A small number of non-randomized studies have reported that psychological support, that is, tender loving care (TLC) in early pregnancy, decreases miscarriage rates in women with unexplained RM. Stray-Pedersen and Stray-Pedersen (1984) found that among the RM couples with no abnormal findings, women receiving specific antenatal counselling and psychological support had a subsequent pregnancy success rate of 86%, as compared with a success rate of 33% observed in women who were given no specific antenatal care. Clifford et al. (1997) found that supportive care in early pregnancy conferred a significant beneficial effect on pregnancy outcome with those who attended the early pregnancy clinic having a 26% miscarriage rate in the next pregnancy, compared with 51% for those who did not attend the clinic.

Vitamin supplementation has been advocated in the context of an association between poor dietary intake of vitamins and an increased risk of miscarriage. A recent meta-analysis has shown that taking vitamin supplements, alone or in combination with other vitamins, before conception or in early pregnancy does not change the risk of early or late miscarriage (Rumbold et al., 2005). Currently, the data on individual vitamin supplementation in women with RM are insufficient to perform any meaningful analyses (Table III).

References


Submitted on April 5, 2006; accepted on April 7, 2006.
The problem of recurrent miscarriage was given a proper attention only in the past 25 years. The studies conducted during this period were focused on two main aspects: the risk estimation of subsequent miscarriages, the etiologic factor of miscarriages and the treatment methods. The epidemiologic studies have confirmed that the course of the first pregnancy has an influence on the outcome of following pregnancies, and so in a women that has never been pregnant or had only one child the risk of miscarriage is 5% while in those whose pregnancy failed once, even because of miscarriage, the risk of next pregnancy failure is 20%. This risk accumulates with the increase in the number of miscarriages, so in two miscarriages it reaches 28%, in three and more – 43%.

The term “recurrent miscarriage” suggest that the recurrent episodes of early pregnancy loss have a specific cause. Those causes could be grouped into following categories: genetic, anatomical, immunologic, hormonal, infectious and idiopathic. Although the sporadic miscarriages are caused by the same factors, however the causative distribution of these factors is different, e.g. genetic anomalies are frequent among sporadic abortions, while uterine anomalies are more frequent in recurrent miscarriages. However in 30% of cases the causes remain unknown. More so women with repeated pregnancy loses have poorer prognosis regarding the obstetrical outcome than women with sporadic miscarriages.

The aim of this paper was to present the results of our own analysis regarding women with two and more miscarriages.
Material

372 women
with two or more consecutive miscarriages
including 131 women
with three or more consecutive miscarriages

<table>
<thead>
<tr>
<th>Number of miscarriages</th>
<th>Number of women No/%</th>
<th>Maternal age</th>
<th>Gestational age</th>
</tr>
</thead>
<tbody>
<tr>
<td>two</td>
<td>241 (64,7)</td>
<td>33,0</td>
<td>9,0</td>
</tr>
<tr>
<td>three</td>
<td>94 (25,2)</td>
<td>34,0</td>
<td>8,0</td>
</tr>
<tr>
<td>four</td>
<td>25 (6,7)</td>
<td>33,0</td>
<td>8,0</td>
</tr>
<tr>
<td>five and more</td>
<td>12 (3,2)</td>
<td>29,5</td>
<td>8,0</td>
</tr>
</tbody>
</table>

Basic investigation

1. Parental karyotype

2. Pelvic ultrasound and/or hysterosalpingogram and hysteroscopy and laparoscopy in case of inconclusive findings

3. Antiphospholipid antibodies (ACA, aLC)
1. Parental cytogenic investigations
   (routine peripheral blood culture)

   192 couples

   - Carrier couples: n = 16 (8.3%)
   - Non-carrier couples: n = 176 (91.7%)

   - Reciprocal translocations: n = 11
   - Mosaicism: n = 3
   - Inversions: n = 2

   13 (81.25%) carries were women

Number of parental chromosomal abnormalities according to number of miscarriages

<table>
<thead>
<tr>
<th>Number of miscarriages</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of couples</td>
<td>123</td>
<td>55</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Number /% of chromosomal abnormalities</td>
<td>9 (7.3%)</td>
<td>5 (9.1%)</td>
<td>2 (20.0%)</td>
<td>-</td>
</tr>
</tbody>
</table>

Franssen M. et al. BMJ 2005

Low maternal age at second miscarriage, a history of three or more miscarriages, a history of two or more miscarriages in a brother or sister, and a history of two or more miscarriages in the parents of either partner all increase the probability of carrier status.

Selective chromosome analysis could reduce the number of chromosome analysis by 18%
Reproductive outcome in couples with chromosomal abnormalities

16 couples

pregnancy

n =19

live birth
n=8 (42,10%)
miscarriage
n=11 (57,89%)

number of children 10
delivery age ± 34,5 week
birth weight ± 2472 g

Franssen M et al. BMJ 2006
Reproductive outcome after parental chromosome analysis in couples with recurrent miscarriage

<table>
<thead>
<tr>
<th>Reproductive outcome</th>
<th>Carrier couples (n=247)</th>
<th>Non-carrier couples (n=489)</th>
<th>Difference in % (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failure to conceive</td>
<td>8 (3.2)</td>
<td>19 (4.1)</td>
<td>-0.9 (-4.4 to 2.0)</td>
<td>0.56</td>
</tr>
<tr>
<td>One or more miscarriages</td>
<td>120 (48.6)</td>
<td>122 (25.3)</td>
<td>18.8 (11.1 to 26.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>One or more terminated pregnancies</td>
<td>6 (2.4)</td>
<td>8 (2.0)</td>
<td>0.5 (-1.8 to 3.8)</td>
<td>0.60</td>
</tr>
<tr>
<td>One or more aneuploid pregnancies</td>
<td>3 (1.2)</td>
<td>13 (2.7)</td>
<td>-2.5 (-5.1 to 0.1)</td>
<td>0.17</td>
</tr>
<tr>
<td>One or more stillbirths</td>
<td>3 (1.2)</td>
<td>6 (1.3)</td>
<td>-0.1 (-2.3 to 2.2)</td>
<td>0.70</td>
</tr>
<tr>
<td>One or more children who died postpartum</td>
<td>1 (0.4)</td>
<td>4 (1.0)</td>
<td>-0.6 (-2.5 to 1.4)</td>
<td>0.41</td>
</tr>
<tr>
<td>One or more ill or handicapped children</td>
<td>2 (0.8)</td>
<td>11 (2.3)</td>
<td>-5.5 (-8.9 to -2.1)</td>
<td>0.00</td>
</tr>
<tr>
<td>One or more healthy children</td>
<td>205 (83.0)</td>
<td>344 (84.1)</td>
<td>-3.1 (-7.2 to 1.0)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

The prognosis for subsequent live birth is more dependent on

- the number of previous miscarriages
- maternal age
- karyotype of the previous miscarriages
- primary or secondary aborter status
  - rather than on parental karyotype

Carp H et al. Fertil. Steril. 2006
2. Pelvic ultrasound and/or hysterosalpingogram and hysteroscopy and laparoscopy in case of inconclusive findings

Anatomic (congenital and acquired) uterine defects was present in 21.05% women (44/209) with two or more miscarriages

- intrauterine adhesions n=15 (34.09)
- uterus bicornis n=10 (22.72)
- septate uterus n=17 (38.63)
- polypoid lesions n=2 (4.54)

Among 116 women with three or more consecutive miscarriages anatomical abnormalities were found in 28.3%

- intrauterine adhesions n=13 (39.3)
- uterus bicornis n=8 (24.2)
- septate uterus n=12 (36.3)

Anatomic uterine defects – in 15% of women with three or more consecutive spontaneous abortions

Guimaraes Filho HA et al. Arch Gynecol Obstet 2006
Uterine anomalies evaluated through hysteroscopy in 38.3% patients with
26.7% synechies
5.0% polypoid lesions
13.3% shape alterations
Reproductive outcome after hysteroscopic surgical treatment of septate uterus
14 women

- Term live birth: 75%
- Spontaneous abortion: 25%


16 women with a complete septate uterus and pregnancy loss 81%
- Hysteroscopic (n=11) surgical treatment
  - 75% term live births
- Transabdominal (n=5) surgical treatment
  - 100% term live births

Postoperatively 82% term live births
18% spontaneous abortion

3. Antiphospholipid antibodies (ACA and LA)

189 women with two and more miscarriages
- ELISA
  - After first test
    - ACA positive: n=57
      - After second test
        - ACA positive: n=26
          - 15.87% ApL positive patients
            - 3.0% with ≥ 20 GPL or MPL
    - LA positive: n=6
- APTT
Among 65 patients with three and more consecutive miscarriages
18.4% women APL positive
5.0% with ≥ 20 GPL


Among 366 patients with two and more pregnancy loss
21.5% women were APL positive (≥ 20 GPL)
80% patients experienced at least one stillbirth

Research investigations

1. Feto – placental karyotypes
2. Luteal phase endometrial biopsy
3. Trombophilia screening

<table>
<thead>
<tr>
<th>1. Feto – placental karyotypes</th>
<th>first miscarriage</th>
<th>second and third miscarriage</th>
<th>fourth and fifth miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients number</td>
<td>45</td>
<td>41</td>
<td>9</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>22 (48.9%)</td>
<td>30 (73.2%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>Abnormal karyotype</td>
<td>23 (51.1%)</td>
<td>11 (26.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Trisomy 16</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Monosomy</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Triploidy</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>47.XXX</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>49.XXXXX</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
Structural chromosome abnormalities in recurrent miscarriages

- Monosomy 9%
- 47,XXY 9%
- Triploidy 9%
- Trisomy 16 18%
- Trisomy 18 9%
- Trisomy 21 28%
- 49,XXXXY 9%


Theoretical risk of a repeated chromosomal anomaly after
- three losses - 35%
- six losses - 70%


The incidence of embryonic chromosomal aberrations decreases as the number of previous miscarriages increases.


There was no increased risk for trisomy in a second spontaneous abortion after either a previous trisomic abortion or an abortion with another abnormal karyotype.


Data from prenatal diagnosis shows a significant increase in risk for trisomy 21 and other than 21 after previous trisomy 21.
2. Luteal phase endometrial biopsy

was performed in 79 (60.3%) women with three and more miscarriages.

Luteal phase defect was found in 17 (21.5%) patients.

in 6 (35%) plasma progesterone was < 10ng/ml

Li TC, and al. Hum. Reprod. Update 2002

Among 122 patients with RM
33 (27.0%) had delayed endometrial development.

Plasma progesterone Endometrial dating
(nmol/l) Normal Retarded

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Retarded</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 30</td>
<td>55</td>
<td>16</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Concentration of MUC-1 in uterine fluid in women with impaired fertility

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>MUC-1 U/ml</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Two and more miscarriages</td>
<td>37</td>
<td>26.26</td>
<td>11.56</td>
<td>0.023 1. vs 2. 0.027 1. vs 3.</td>
</tr>
<tr>
<td>2. Infertility</td>
<td>46</td>
<td>21.44</td>
<td>7.33</td>
<td>0.0001 2. vs 3.</td>
</tr>
<tr>
<td>3. Control</td>
<td>19</td>
<td>33.78</td>
<td>12.06</td>
<td></td>
</tr>
</tbody>
</table>

### Concentration of Glycodelin (ng/ml) in Uterine Fluid

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>25 perc</th>
<th>75 perc</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two or more miscarriages</td>
<td>42</td>
<td>0</td>
<td>162</td>
<td>45.75</td>
<td>16</td>
<td>55.25</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1. vs 2.</td>
</tr>
<tr>
<td>Infertility</td>
<td>52</td>
<td>0</td>
<td>75.39</td>
<td>23.0</td>
<td>5.73</td>
<td>46.66</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1. vs 3.</td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>9.1</td>
<td>97.47</td>
<td>48.91</td>
<td>39.7</td>
<td>69.46</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. vs 3.</td>
</tr>
</tbody>
</table>

*J. Skrzypczak et al. Ginekol. Pol 2005*

### Concentration of MMP9, TIMP, uPA and uPAR in Uterine Fluid

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>MMP9 ng/ml</th>
<th>TIMP1 ng/ml</th>
<th>uPA pg/ml</th>
<th>uPAR ng/ml</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic infertility</td>
<td>16</td>
<td>13.46</td>
<td>32.98</td>
<td>0.013</td>
<td>0.035</td>
<td>4.87</td>
</tr>
<tr>
<td>Recurrent miscarriages</td>
<td>13</td>
<td>18.28</td>
<td>13.31</td>
<td>0.015</td>
<td>0.038</td>
<td>3.59</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>36.24</td>
<td>NA</td>
<td>27.37</td>
<td>790.80</td>
<td>7.197</td>
</tr>
</tbody>
</table>

*J. Skrzypczak et al. Am. J. Reprod. Immun. in press*

### 3. Trombophilia screening

*Kovalsky G et al. Intern. Med. 2004*

A meta-analysis

The combined ORs for association between

<table>
<thead>
<tr>
<th>Condition</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPL and factor V Leiden</td>
<td>2.0 (95%CI, 1.5-2.7)</td>
</tr>
<tr>
<td>RPL and G20210 A</td>
<td>2.0 (95%CI, 1.0-4.0)</td>
</tr>
</tbody>
</table>
In our material
55 women with three and more recurrent miscarriages

- *factor V Leiden mutation* – 3 patients (5.45%) in mean age 30 years old, they had total of 11 miscarriages

- *prothrombin G20210A mutation* – 1 patient (1.81%) 21 years old, with two miscarriages

- *activated protein C resistance* – in two (2.63%) patients associated with factor V Leiden mutation
CONGENITAL UTERINE ANOMALY AND RECURRENT MISCARRIAGE

Jan Kotarski

1st Department of Gynecology
University School of Medicine, Lublin, Poland

6 weeks of pregnancy
XX phenotype
absence of TDF (Testis-Determining Factor)
absence of antimullerian hormone

9 weeks of pregnancy
2 paramesonephric (mullerian) ducts
2 mesonephric (wolffian) ducts
Degeneration and formation a matrix for paramesonephric ducts

9 weeks of pregnancy
Fallopian tubes
Uterus
Four-fifths of the vagina

Embryology

9 weeks of pregnancy
Regression of uterine septum as a result of bcl-2 mediated apoptosis

12 weeks of pregnancy
Normally developed uterine configuration
UTERINE MALFORMATIONS

The result of:

1. Failure of 1 or 2 mullerian ducts to develop (agensis, uniconuate uterus without rudimentary horn)
2. Failure of the ducts to canalize (uniconuate uterus with rudimentary horn)
3. Failure of, or abnormal fusion of, the ducts (uterus didelphys, bicornuate uterus)
4. Failure of reabsorption of the midline uterine septum (septate uterus, arcuate uterus)

Epidemiology

The overall incidence of mullerian defects - 5%
- 2-3% in fertile women
- 3% in infertile women
- 5-10% in women with recurrent miscarriages
- 25% in women with late miscarriages and preterm deliveries

The presence of mullerian duct anomalies:
- not associated with increased mortality compared to the general population
- increased morbidity in patients with hematosalpinx hematocolpos retrograde menstruations, endometriosis
- high association with renal anomalies (unilateral renal agenesis mainly)
- no racial predilection
DIAGNOSIS
- Ultrasonography
- 3D Ultrasonography
- Hysterosalpingography
- Magnetic resonance imaging (MRI)
- Hysteroscopy
- Hysteroscopy/ laparoscopy

Classification of mullerian ducts anomalies
1988 American Fertility Society
(now American Society of Reproductive Medicine)

Class I (agenesis/hypoplasia)
Uterine/cervical agenesis or hypoplasia
The most common is the Mayer-Rokitansky-Kuster-Hauser Syndrome (absence of uterus and upper vagina)
Primary amenorrhoeae
No reproductive potential aside from medical intervention
Class II (unicornuate uterus)

Complete or almost complete, arrest of development of 1 müllerian duct
If the arrest is incomplete (90%) a rudimentary horn is present
If the contralateral healthy horn is fully developed, a full pregnancy is believed to be possible

Class III (didelphus uterus)

Complete nonfusion of both müllerian ducts
The individual horns are fully developed
Two cervices are inevitably present
Patients have been known to carry pregnancy to full term

Class IV (bicornuate uterus)

Partial nonfusion of both müllerian ducts
Some degree of fusion between the two horns
Horns are not fully developed
Class V (septate uterus)

- Failure of resorption of the septum between two horns
- The septum can be partial or complete
- The highest incidence of reproductive complications

Class VI (arcuate uterus)

- Single uterine cavity with a confluent or flat uterine fundus
- Small fundal cleft or impression ≥ 1.5 cm
- Not significantly associated with the increased risk of pregnancy loss

Class VII (diethylstilbestrol-related anomaly)

- DES prescribed to prevent miscarriages (1945-1971)
- Uterine hypoplasia
- T-shaped uterine cavity
- Abnormal transverse ridges
- Stenosis of the cervix
- Adenosis of vagina
- Vaginal clear cell carcinoma
Rock and Adam AFS modified classification

Class 1
identical to AFS class I (agenesis/hypoplasia)

Class 2
All anomalies in which incomplete fusion occurs between the descending mullerian and the ascending urogenital sinus. This class also include obstructive or non-obstructive transverse vaginal septa, cervical agenesis, and dysgenesis with or without obstruction.

Class 3
Disorders of lateral fusion such as didelphys, unicorunate, bicornuate, and septate uteri (AFS classes II, III, IV, V)
A. Symmetric unobstructed form (5 types): septate, bicornuate, didelphys, DES, unicorunate uterus with or without rudimentary horn
B. Asymmetric obstructed form (3 types): unicorunate with noncommunicating obstructed horn, unilaterally obstructed double uterus, unilateral vaginal obstruction
Mullerian duct anomaly (1392 cases):
- Septate uterus 34.9%
- Bicornuate uterus 26%
- Arcuate uterus 18.3%
- Unicornuate uterus 9.6%
- Uterus didelphys 8.2%
- Mullerian agenesis/hypoplasia 2.9%

Grimbizis GF et al. Hum Reprod Update 2001;

Recurrent abortion:
- Loss of three or more consecutive pregnancies
- 0.5-1% pregnant women

The pattern of pregnancy loss in women with recurrent miscarriage

25-40% unexplained
30-65% immunologic factor
10-30% structural uterine anomaly
5-17% endocrine/endometrial defects
5-7% chromosomal anomaly
2-4% infections
Possible mechanisms leading to pregnancy losses in women with uterine anomalies

Endometrium alterations
- reduction and irregular distribution of glandular ostia
- incomplete ciliogenesis
- reduced ciliated cell ratio
- reduced vascular supply
- decreased sensitivity to hormones

Inadequate placentation
Space constraints
ER and PR deficiency
Cervical incompetence

Pregnancy outcome associated with reproductive tract anomalies

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Term pregnancy outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transverse vaginal septum</td>
<td>50%</td>
</tr>
<tr>
<td>Absence of cervix</td>
<td>rare pregnancy</td>
</tr>
<tr>
<td>Rudimentary horn</td>
<td>64-85% (normal horn)</td>
</tr>
<tr>
<td>Unicollis</td>
<td>69%</td>
</tr>
<tr>
<td>Septate uterus</td>
<td>15% (78% after repair)</td>
</tr>
<tr>
<td>Bicornuate uterus</td>
<td>10% (84% after repair)</td>
</tr>
<tr>
<td>Didelphic uterus</td>
<td>57%</td>
</tr>
</tbody>
</table>

ACOG Technical Bulletin „Pregnancy loss“

Pregnancy outcome in patients with untreated unicornuate uterus (n 151)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopics</td>
<td>1.2%</td>
</tr>
<tr>
<td>Abortions</td>
<td>36.5%</td>
</tr>
<tr>
<td>Preterm deliveries</td>
<td>16.2%</td>
</tr>
<tr>
<td>Term deliveries</td>
<td>44.6%</td>
</tr>
<tr>
<td>Live births</td>
<td>54.2%</td>
</tr>
</tbody>
</table>

Grimbizis GF et al. Hum Reprod Update 2001;7
Pregnancy outcome in patients with untreated didelphys uterus (n 114)

Ectopics 1.3%
Abortions 32.2%
Preterm deliveries 28.3%
Term deliveries 36.2%
Live births 55.9%

Grimbizis GF et al. Hum Reprod Update 2001;7

Pregnancy outcome in patients with untreated bicornuate uterus (n 261)

Ectopics 0.3%
Abortions 36%
Preterm deliveries 23%
Term deliveries 40.6%
Live births 55.2%

Grimbizis GF et al. Hum Reprod Update 2001;7

Pregnancy outcome in patients with untreated septate uterus (n 198)

Ectopics 0.6%
Abortions 44.3%
Preterm deliveries 22.4%
Term deliveries 83.1%
Live births 50.1%

Grimbizis GF et al. Hum Reprod Update 2001;7
Pregnancy outcome in patients with untreated arcuate uterus (n 102)

- Ectopics: 2.9%
- Abortions: 25.7%
- Preterm deliveries: 7.5%
- Term deliveries: 62.7%
- Live births: 66%

Grimbizis GF et al. Hum Reprod Update 2001;7

Reproductive outcome in uterine anomalies

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>N</th>
<th>Preterm deliveries</th>
<th>Ectopic deliveries</th>
<th>Live deliveries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unicorunate uterus</td>
<td>393</td>
<td>43.3%</td>
<td>4.3%</td>
<td>54.2%</td>
</tr>
<tr>
<td>Didelphic uterus</td>
<td>86</td>
<td>29.4%</td>
<td>2.3%</td>
<td>58.6%</td>
</tr>
<tr>
<td>Bicornuate uterus</td>
<td>56</td>
<td>25%</td>
<td>0</td>
<td>62.5%</td>
</tr>
<tr>
<td>Uterine septum</td>
<td>1459</td>
<td>10%</td>
<td>1.9%</td>
<td>58.1%</td>
</tr>
<tr>
<td>Arcuate uterus</td>
<td>283</td>
<td>5.1%</td>
<td>3.5%</td>
<td>66.2%</td>
</tr>
</tbody>
</table>

Lin PC J Women's Health 2004;13

Reproductive outcome of patients with uterine anomalies in their first and in all their pregnancies

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>1st (n 40)</th>
<th>all (n 66)</th>
<th>1st (n 66)</th>
<th>all (n 176)</th>
<th>1st (n 176)</th>
<th>all (n 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopics</td>
<td>7%</td>
<td>5%</td>
<td>0</td>
<td>0</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>Abortions</td>
<td>33%</td>
<td>39%</td>
<td>21%</td>
<td>23%</td>
<td>38%</td>
<td>46%</td>
</tr>
<tr>
<td>Preterm deliveries</td>
<td>13%</td>
<td>8%</td>
<td>8%</td>
<td>23%</td>
<td>31%</td>
<td>22%</td>
</tr>
<tr>
<td>Term deliveries</td>
<td>43%</td>
<td>48%</td>
<td>7%</td>
<td>54%</td>
<td>31%</td>
<td>45%</td>
</tr>
</tbody>
</table>

Grimbizis GF et al. Hum Reprod Update 2001;7
Uterine reconstructive surgery

- Abdominal metroplasty
- Jones wedge metroplasty
- Strassman procedure
- Tompkins’ fundal bivalve metoplasty

Hysteroscopic septum resection

Steps in the operative pair of septate uterus by excision of a wedge (1)

RB Hunt
Atlas of female infertility surgery
Steps in the operative pair of septate uterus by excision of a wedge (2)

Lysis of complete septum

... Patients treated with abdominal metroplasty are subject to the risks of prolonged anesthesia time, significant blood loss, postoperative infection, and intraperitoneal adhesions... these patients traditionally undergo cesarean delivery...

... Hysteroscopic management of the uterine septum significantly reduces surgical complications and has a comparable success rate...

ACOG Technical Bulletin „Hysteroscopy“
... hysteroscopic metroplasty is now considered for the first therapeutic option for septate uterus ...

Pabuccu and Gomel Fertil Steril 2004;81

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Crude pregnancy rate after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fayez</td>
<td>7</td>
<td>71</td>
</tr>
<tr>
<td>Perino et al.</td>
<td>8</td>
<td>63</td>
</tr>
<tr>
<td>Daly et al.</td>
<td>15</td>
<td>47</td>
</tr>
<tr>
<td>Querleu et al.</td>
<td>9</td>
<td>67</td>
</tr>
<tr>
<td>Marabini et al.</td>
<td>14</td>
<td>44</td>
</tr>
<tr>
<td>Pabuccu et al.</td>
<td>10</td>
<td>63</td>
</tr>
<tr>
<td>Colacurci et al.</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>84</strong></td>
<td><strong>48</strong></td>
</tr>
</tbody>
</table>

Pregnancy outcome in patients with septate uterus before and after hysteroscopic metroplasty (n 466)

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopics</td>
<td>0.3%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Abortions</td>
<td>86.4%</td>
<td>16.4%</td>
</tr>
<tr>
<td>Preterm deliveries</td>
<td>9.8%</td>
<td>6.8%</td>
</tr>
<tr>
<td>Term deliveries</td>
<td>3.3%</td>
<td>76.2%</td>
</tr>
<tr>
<td>Life births</td>
<td>6.1%</td>
<td>83.2%</td>
</tr>
</tbody>
</table>

Grimbizis GF et al. Hum Reprod Update 2001;7

Comparison of reproductive outcome before and after hysteroscopic metroplasty

<table>
<thead>
<tr>
<th></th>
<th>Primary infertility</th>
<th>91.8%</th>
<th>96.1%</th>
<th>94.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spontaneous abortion</td>
<td>109</td>
<td>3.8%</td>
<td>5.1%</td>
</tr>
<tr>
<td></td>
<td>Habitual abortion</td>
<td>59</td>
<td>1.1%</td>
<td>2.4%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>361</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abortions</td>
<td>Before metroplasty</td>
<td>-</td>
<td>10.4%</td>
<td>16.1%</td>
</tr>
<tr>
<td></td>
<td>After metroplasty</td>
<td>33.3%</td>
<td>10.2%</td>
<td>16.1%</td>
</tr>
<tr>
<td>Preterm deliveries</td>
<td>Before metroplasty</td>
<td>-</td>
<td>7.1%</td>
<td>5.1%</td>
</tr>
<tr>
<td></td>
<td>After metroplasty</td>
<td>15.5%</td>
<td>20.4%</td>
<td>18.8%</td>
</tr>
<tr>
<td>Term deliveries</td>
<td>Before metroplasty</td>
<td>-</td>
<td>1.1%</td>
<td>0.4%</td>
</tr>
<tr>
<td></td>
<td>After metroplasty</td>
<td>51.1%</td>
<td>69.3%</td>
<td>65%</td>
</tr>
<tr>
<td>Live babies</td>
<td>Before metroplasty</td>
<td>-</td>
<td>4.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td></td>
<td>After metroplasty</td>
<td>57.7%</td>
<td>79.5%</td>
<td>75%</td>
</tr>
</tbody>
</table>

Saygılı-Yıldız E et al. Arch Gynecol Obstet 2003;269
The rate of rehysterectomy according to the type of septate uterus

<table>
<thead>
<tr>
<th>Type of Septate Uterus</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtotal septate</td>
<td>7%</td>
</tr>
<tr>
<td>Total septate</td>
<td>25%</td>
</tr>
<tr>
<td>Total septate with double cervix</td>
<td>27.5%</td>
</tr>
<tr>
<td>Total</td>
<td>13.6%</td>
</tr>
</tbody>
</table>

Saygili-Yildiz E et al. Arch Gynecol Obstet 2003;268

Preoperative preparation of the endometrium

- **aGnRH**: reduction in the thickness, glandularity, and vascularity
- **Progestins**: vascular telangiectasia, tissue edema without causing atrophy
- **Danazol**: reduced vascularity

Indications: reduce bleeding and improve vision

- **septa completa**

Scissors, electrosurgery, or laser?

- **Scissors**: no risk of thermal vascular damage, longer operation time, inability to achieve simultaneous hemostasis
- **Electrosurgery**: inexpensive, simple method, easily achieved hemostasis
- **Laser**: short operation time, minimal blood loss, good hemostasis, safety
The role of laparoscopy

- To differentiate between a septate and a bicornuate uterus
- To monitor hysteroscopic surgery
- To complete the diagnostic workout in patients with infertility
- To aspirate the fluid that has leaked into the peritoneal cavity

Postoperative management

IUD  reduction of adhesions and promotion of epithelialization
inflammation/ bleeding/ infections

Antibiotics  the role of routine administration not established

Estrogens  promotion of epithelialization
the role not established

Follow up
1-2 months after surgery
(USG/HSG/hysteroscopy)

Reproductive outcome before and after metroplasty in women with bicornuate uterus

<table>
<thead>
<tr>
<th></th>
<th>before</th>
<th>after</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birth rate</td>
<td>0</td>
<td>80%</td>
</tr>
<tr>
<td>Abortion rate</td>
<td>64%</td>
<td>20%</td>
</tr>
</tbody>
</table>

Maneschi et al. 1993

<table>
<thead>
<tr>
<th></th>
<th>before</th>
<th>after</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birth rate</td>
<td>21%</td>
<td>82%</td>
</tr>
<tr>
<td>Abortion rate</td>
<td>80%</td>
<td>20%</td>
</tr>
</tbody>
</table>

Capraro et al. 1968

Surgical reconstruction can be considered for patients with recurrent miscarriages and no other obvious etiologies...

*Lin PC J Women’s Health 2004;13*
Infection and early pregnancy loss

Tomasz Niemiec
Associate Professor in Obstetrics and Gynecology
Research Institute of Mother and Child
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tomaszniemiec@hotmail.com

The etiology of early pregnancy loss is varied and often controversial. More than 1 etiologic factor is often present. Although infection has been reported as a cause of pregnancy loss, few studies have been conducted, and results are inconsistent. Numerous organisms have been implicated in sporadic causes of miscarriage, but common microbial causes have not been confirmed. In fact, infection is viewed as a rare cause of recurrent miscarriage.

Organisms implicated with spontaneous abortion include the following:

- **Bacteria**
  - *Listeria monocytogenes*
  - *Chlamydia trachomatis*
  - *Ureaplasma urealyticum*
  - *Mycoplasma hominis*
  - Bacteria causing vaginosis

- **Viruses**
  - Cytomegalovirus (CMV)
  - Rubella
  - Herpes simplex virus (HSV)
  - HIV
  - Human papillomavirus (HPV)
  - Parvovirus

- **Parasites**
  - *Toxoplasma gondii*
  - Plasmodium falciparum

- **Spirochetes** - *Treponema pallidum*

Different theories have been postulated to explain exactly how an infectious agent leads to miscarriage. These include the following:

- Toxic metabolic byproducts, endotoxin, exotoxin, or cytokines may have a direct effect on the uterus or the fetoplacental unit.
- Fetal infection may cause fetal death or severe malformation incompatible with fetal viability.
- Placental infection may result in placental insufficiency, with subsequent fetal death.
- Chronic infection of the endometrium from ascending spread of organisms (eg, *M hominis*, *Chlamydia* organisms, *U urealyticum*, HSV) from the lower genital tract may interfere with implantation.
- Amnionitis in the first trimester may play a role similar to chorioamnionitis in the third trimester, resulting in preterm labor (related to various common gram-positive and gram-negative bacteria, *L monocytogenes*).
- Induction of a genetically and anatomically altered embryo or fetus may occur because of viral infection (eg, rubella, parvovirus B19, CMV, chronic CMV, coxsackievirus B, varicella-zoster, HSV, syphilis, Lyme disease, HPV) during early gestation.

A recent review failed to show sufficient evidence for the notion that any type of infection can be identified as a causal factor for recurrent miscarriage. Most patients with a history of recurrent miscarriage do not benefit from an extensive infection workup. Exposure to a microbe that can establish chronic infection that can spread to the placenta in a patient who is immunocompromised is probably the most obvious risk situation in recurrent abortions.

Specific pathogens include *Neisseria gonorrhoeae*, which is associated with premature rupture of membranes and chorioamnionitis, and *C trachomatis*. Previous chlamydial infection is not associated with fetal loss in women with recurrent abortion. Serologic studies have suggested an association between *C trachomatis* and recurrent abortion, and routine *C trachomatis* screening has been recommended for all patients undergoing an infertility workup. However, microbiologic testing for endocervical chlamydial infection during pregnancy has failed to confirm the association with recurrent abortion. Bacterial vaginosis is associated with preterm labor, intrauterine growth retardation, chorioamnionitis, and late miscarriage. However, no studies have been conducted to investigate its role in women with recurrent miscarriages. Most women are screened at their first prenatal visit and more frequently than this if they have a history of late miscarriages or preterm delivery. Regarding genital mycoplasma *M hominis* and *Ureaplasma* species are isolated from the vagina in as many as 70% of pregnant women. Although these organisms are most frequently found in women with recurrent miscarriages, their elimination has not improved subsequent pregnancy outcome. Therefore, screening for *Mycoplasma* and *Ureaplasma* species is not recommended for the typical patient with a history of recurrent miscarriage. *L monocytogenes* typically produces asymptomatic colonization of the maternal lower genital tract, though symptomatic maternal listeriosis characterized by bacteremia and influenza-like symptoms may occur. Symptomatic listerial infection is typically described as a complication of the third trimester, resulting from ingestion of unpasteurized milk or cheese. No evidence suggests that *Listeria* organisms play a role in
patients with a history of recurrent pregnancy loss. Screening for *Listeria* during pregnancy or in routine cases of recurrent miscarriage is not recommended. *T. pallidum* is known to cause stillbirth and abortion in the second trimester. The timing of death is probably associated with the maturation of the fetal immune system at the 20th week of gestation. However, syphilis is unlikely to substantially contribute to the general problem of recurrent miscarriage. CMV is associated with random miscarriage but not recurrent miscarriage. Primary HSV infection has been associated with spontaneous abortion, and chronic HSV infection is a possible cause of recurrent abortion (especially in a patient who is immunocompromised). The incidence of recurrent abortion secondary to chronic HSV infection is extremely low in the general population and does not warrant routine screening in patients with recurrent pregnancy loss. Malaria due to *P. falciparum* during pregnancy is associated with spontaneous abortion, stillbirth, low birth weight, and prematurity. Screening is only important in those women who live where the disease is endemic or in symptomatic patients who have traveled to endemic countries. Primary toxoplasmosis can lead to miscarriage and stillbirth. However, if the infection develops during the first trimester, the risk is less than 5%. Human papilloma virus (HPV) infection is one of the most frequently observed sexually-transmitted diseases. In pregnant women, as well as accelerating the evolution of dysplasia to cervical cancer, the infection may be transmitted to the fetus during gestation or at the time of birth. HPVs are elevated in spontaneously aborted products of conception. Furthermore, these results suggest the possibility that HPVs may be etiologic agents of at least some spontaneous abortions. HPV-infected trophoblasts may have altered characteristics, which may lead to a compromised gestation. Low-risk cervical HPV infection might be associated with a slightly higher risk of abnormal fetal karyotype. Studies have failed to show an increase in miscarriage rates for asymptomatic patients with HIV infection.

Although an effective instrument in early pregnancy loss prevention is still elusive, the studies conducted so far improved our understanding of infection-mediated spontaneous abortion in human parturition.
Recurrent Miscarriage: Failure of nature’s selection

Siobhan Quenby
University of Liverpool
Liverpool Women’s Hospital

What is recurrent miscarriage?
• 3 consecutive miscarriages
• Very distressing because cycle of
  • Occurs over 1-2 years
    • Increasingly desperate for baby

How common?
• 3% couples trying for a baby
• 2 million births in UK per year
• 60,000 couple in UK per year
Historical perspective

- Definition RM: 3 consecutive pregnancy losses before the 24th weeks
- Sporadic miscarriage rate is 15%
- RM rate $0.15^3 = 0.3$-$0.4\%$.
- The actual prevalence of RM is 1-3%

Medawar

- “How does the pregnant mother continue to nourish within itself for many weeks or months a fetus that is an antigenically foreign body?”
- 1953
- 50 years later no effective treatment

New paradigm

- The selection failure hypothesis
  - *Recurrent miscarriage is the result of failure of the prevention of "poor quality" embryos implanting, allowing embryos that are destined to fail to implant and present clinically as recurrent miscarriage.*
- *Recurrent miscarriage: a defect in nature’s quality control?*
The decidua

• 90% karyotypically abnormal pregnancies miscarry in the first trimester
• 93% karyotypically normal pregnancies continue
  – McFadyen, 1989

Balanced translocations

• High miscarriage rate
  – 60-70%
  • Sugiura-Ogasawara 2004
  – 29%
  • Stephenson and Sierra 2006
• Some miscarriage in these women aneuploidy (30%)
  • Stephenson and Sierra 2006

Balanced translocations

• unbalanced translocations
  – 38% of miscarriages
  – 0% of ongoing pregnancies
  • Stephenson and Sierra 2006
• 0% of ongoing pregnancies unbalanced translocations n= 41
  – No miscarriages not = incidence of translocation
  • Godijn 2004
• 1 infant unbalanced translocation n=95
  • Sugiura-Ogasawara 2004
Fetal genome

- Mutation carriage in either partner equally important in predicting miscarriage
  - Jivrai et al., 2006
- Hutterite population
  - No increase in loss in MTHFR, FVL
  - Children of carriers deficit of FVL therefore more losses of fetus with FVL
    - Seirra and Stephenson (SGI 2006)

How does selection occur

- abnormal trophoblast does not invade?
- Maternal endometrium can select?

Aneuploidy miscarriages

- RM women miscarried aneuploidy fetus found normal trophoblast invasion and plugging of spiral arteries
  - Sebire et al 2002
- Therefore not fetal problem
Maternal endometrium

MUC-1

- MUC-1 surface mucin
- Barrier to implantation
- Expressed in the implantation window
- Loss MUC1 implantation site after embryo-epithelial interaction
- Less in women with RM immunohistochemistry and flushings – Aplin and co-workers

- Epithelial abnormality with impairment of barrier function could lead to a situation in which “poor quality” embryos that are otherwise destined to fail, implant and present later as miscarriages
Endometrium different in RM normal v abnormal?

Karyotypical abnormality
- High (29-57%) in RM population
  - Stern et al., 1996,
  - Ogasawara et al., 2000,
  - Carp et al., 2001,
  - Stephenson et al., 2002
- Same rate recurrent and spontaneous miscarriage

<table>
<thead>
<tr>
<th>Definition</th>
<th>Gestation in weeks</th>
<th>Ultrasound findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty sac</td>
<td>&lt;12</td>
<td>Empty gestation sac diameter &gt;20mm or &lt;20 mm no change 7 days later</td>
</tr>
<tr>
<td>Embryonic loss</td>
<td>&lt;8</td>
<td>Embryo &gt;5mm size, no FH, CRL &lt;5mm with no change 7 days later</td>
</tr>
<tr>
<td>Fetal Loss</td>
<td>8-12</td>
<td>Fetus of 8-12 weeks size with no FH</td>
</tr>
</tbody>
</table>

Fetus of 8-12 weeks size with no FH
Death of a fetus in the first trimester
Embryo >5mm size, no FH, CRL <5mm with no change 7 days later
Fetus of 8-12 weeks size with no FH
Empty gestation sac v Fetal

- In RM
  - Empty gestation sac loss > karyotypically normal than fetal
  - 80% fetal losses karyotypically abnormal
    - Mankito et al., 2004
- In sporadic fetal losses
  - 86% abnormal
    - Philip et al., 2003
Glandular epithelium

P=0.003

P=0.007

P=0.001

uNK cells in endometrium

Control

RM patient

 killers become builders during pregnancy

NATURE MEDICINE 2006

 Circulating natural killer cells might be harnessed for their ability to disable and eviscerate microorganisms, such as in the prevention of human disease. These cells are host to a particular effect, regulating perinatal development and pregnancy (pp. 1999-2010).
uNK cells and RM

- 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
- More numerous in chromosomally abnormal than normal miscarriages
  - Yamamoto et al, 1999, Quack et al., 2001

Quantification of vascular arteriolar smooth muscle differentiation

- Always see epithelium
- Same area slide
- 2 blinded observers

- Count complete and partials
- Calculate
  - Completes
  - Partial + completes X 100
VSMC differentiation - αSMA and SMM
uterine artery PI (n=28)

% uNK cells

D

SMA (P = 0.02)

SMM (P = 0.01)

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Vascular smooth muscle differentiation
% uNK cells
(N = 28)

% uNK cells

SMA and SMM

uterine artery PI (n=28)

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% uNK cell per uterine flow N=46

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cystokines

• Failure of the Th1 to Th2 shift to occur in pregnancy is cause of miscarriage
  – (Hill et al., 1995)
• Therefore, a failure of this shift to occur should precede miscarriage
• Other workers investigated after miscarriage occurred
• Our study prospective

Cytokine production by cultured PBMC

<table>
<thead>
<tr>
<th>RPL</th>
<th>baby</th>
<th>misc</th>
<th>pregnant</th>
<th>non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=33</td>
<td>n=13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>TNF-α</td>
<td>↓</td>
<td>↓↓</td>
<td>↓</td>
<td>—</td>
</tr>
</tbody>
</table>

Was the pregnancy normal?

• Ultrasound
• Karyotype
• Morphology
• Embryoscopy
Conclusions

- Classify early pregnancy failure
- Abnormal
  - Parental karyotype if recurrent loss
- Normal
  - APS
  - Thrombophilia screen
  - Aspirin and LMWH
- All need supportive care

Acknowledgements
Leukemia Inhibitory Factor (LIF) and implantation

Mateusz Mikołajczyk
Division of Reproduction
Department of Gynecology and Obstetrics
Poznan, Poland
Markers of successful implantation?

- Endometrial assessment
  - Histology (Noyes&Hertig)
  - Scanning microscope

- Ultrasonographic assessment of endometrium


Global gene profiling in human endometrium during the window of implantation.

Endocrinology 2002;145:2119-38

LIF-Leukemia Inhibitory Factor

- Gearing 1987

- Pleiotropic glycoprotein with many functions in the organism
LIF- role in reproduction

- Animal studies
  - Bhatt 1991
  - Stewart 1992
    - gene knock-out

LIF- human reproduction

- Kojima – LIF in endometrium
- Delage – infertile women
- Laird, Ledee-Bataille – uterine flushing

Aim of the study

- assess the concentration of LIF in uterine cavity and serum
- correlation of uterine flushing results with mRNA expression for LIF in the endometrium
- assessment of possible relationship between LIF level in uterine cavity and histological endometrial dating
- test a new way to flush the uterus
Material

A total of 230 infertile patients were qualified for the study in Division of Reproduction between 2001 and 2003

Material

Endometriosis grade I and II – 14
Luteal phase deficiency – 13
Idiopathic infertility - 27

Diagnostic process

- Hormonal studies (I phase of the cycle – LH, FSH; II phase of the cycle – progesterone, PRL)
- Detailed ultrasound with ovulation tracking (infertile women – additional semen analysis and penetration test)
- HSG
- Histological evaluation of the endometrium
Method

- Blood sample
- Endometrial biopsy
- Aspiration of fluid from the uterine cavity

Method

- Uterine flushing

Method

- Evaluation of LIF in uterine flushing and serum – ELISA (Bender MedSystems)
- Histological grading of endometrium according to Noyes and Hertig criteria
- LIF mRNA expression in endometrium (Qiagen)
Patient’s characteristic

- Mean age
  - Primary infertility – 28 years (20-41)
  - Control – 24 years (24-35)

- Mean duration of infertility: 5.6 years (3-14)

Results – LIF in uterine flushing

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Median</th>
<th>Range</th>
<th>25-75% confidence interval</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertile women</td>
<td>54</td>
<td>13.64</td>
<td>0-320</td>
<td>2.6-27.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Idiopathic infertility</td>
<td>27</td>
<td>4.21</td>
<td>0-470.87</td>
<td>0.25-14.63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Luteal phase defect</td>
<td>13</td>
<td>11.33</td>
<td>0-186.8</td>
<td>4.69-49.49</td>
<td>NS</td>
</tr>
<tr>
<td>Infertile women with endometriosis</td>
<td>14</td>
<td>25.53</td>
<td>0-379.14</td>
<td>12.63-43.32</td>
<td>NS</td>
</tr>
<tr>
<td>Control group</td>
<td>21</td>
<td>38.46</td>
<td>0-324.6</td>
<td>13.95-60.47</td>
<td>-</td>
</tr>
</tbody>
</table>

LIF – a new diagnostic test?

cut-off value <8.63 pg/ml; sensitivity - 70.4 %; specificity - 95.2 %;
AUC=0.833
Expression of LIF mRNA

n=52

<table>
<thead>
<tr>
<th>number</th>
<th>mRNA LIF/GAPDH expression</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (25-75% confidence interval)</td>
<td></td>
</tr>
<tr>
<td>Infertile patients</td>
<td>54</td>
<td>0.914486</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.753495-1.157888)</td>
</tr>
<tr>
<td>Control group</td>
<td>10</td>
<td>1.426640</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.094208-1.667347)</td>
</tr>
</tbody>
</table>

Correlation between LIF mRNA and LIF in uterine flushing

Future directions

LIF – a predictive factor?
Questionnaire

- obstetric outcome
- use of additional treatment (ovulation induction, IUI etc.)

- out of 54 initial patients with infertility 26 qualified for workup (48%)
- 9/26 (34.6%) did not achieve pregnancy
- the remaining 17/26 patients (65.4%) did get pregnant and a majority of them carried the pregnancy to term. Only one patient has had an ectopic pregnancy

LIF and pregnancy

cut-off value > 2.31 pg/ml; sensitivity – 95.7%; specificity – 81.8%;
AUC - 0.875
Conclusions

- Evaluation of LIF in uterine flushing is more informative than the level of LIF in the serum
- LIF level in uterine cavity is independent of endometrial development
- Lower LIF levels than initially expected are necessary for successful implantation

Special thanks

Division of Reproduction