ESHRE Campus workshop

NEW TRENDS IN DIAGNOSIS AND MANAGEMENT OF EARLY PREGNANCY FAILURE

Poznan - Poland

15 to 16 December 2006

Contents

Organisation

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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 faillure
                    N. Exalto, Hoofddorp (NL)
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Session 2: Miscarriage

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                    P. Ankum, Amsterdam (NL)
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Session 3: Ectopic Pregnancy

14.00:   Pregnancy of unknown location
                    E. Kirk, London (UK)
14.30:   Ectopic pregnancy
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Session 4: Recurrent miscarriage

16.00:   ESHRE Guideline
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16.30:   Recurrent miscarriage in Poznan
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17.00:   Congenital uterine anomaly
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Session 5: Infection

09.00:   Infection and early pregnancy loss
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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 for 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.

Based on the data of recently published randomized controlled trials (RCTs) and meta-analysis, recommendations are provided for basic investigations of couples presenting with RM and for proposed therapies. The recommendations are summarised in Table II. A large number of therapies, mentioned in the literature require more investigations under strict RCT conditions, whilst others are of no proven benefit or are associated with more harm than good.

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 ß 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 all 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 histopathogical 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 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 $\geq$ 10 weeks of a morphologically normal fetus or one or more premature births at $\leq$ 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.

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

Avoid	Prefer	Ultrasound findings
Egg, Ovum	Oocyte	
Ovum	Embryo	CRL < 10 mm
Embryo	Fetus	CRL ≥ 10 mm and/or fetal heart activity
Embryonic age Postovulatory age Conceptual age Menstrual age	Gestational age based on LMP or CRL	
Abortion Threatened abortion Medical or legal abortion	Miscarriage threatened miscarriage Termination of pregnancy	
Preclinical embryo loss Preclinical abortion Trophoblast in regression Menstrual abortion	Biochemical pregnancy loss or PUL	pregnancy not located or unknown location
Early abortion	Early pregnancy loss	6 - 12 weeks
Anembryonic pregnancy Early embryonic demice Blighted ovum	empty sac	absent or minimal embryonic structure
Embryonic death Missed abortion	fetal loss	CRL + heart activity becoming negative
Late abotion	Late pregnancy loss	≥ 12 weeks
Recurrent abortion Habitual abortion	Recurrent miscarriage (3 early or 2 late losses)	

Table II. Recurrent Miscarriage: recommendations for testing and treatment options.
HSG hysterosalpingography, *BMI* body mass index, *LAC* lupus anticoagulant, *aCL* anticardiolipin antibodies, other abbreviations are mentioned in the text)

Daignosis	Test	Treatment Invalid	Treatment in RCT only	Treatment Beneficial
Maternal age				tender loving care
Number of losses	US data histology karyotype			
Chromosomal abnomalities	Parental karyotype (table III)			
Uterine malformation	(3D) US HSG, hysteroscopy Laparoscopy)		hysteroscopic septum resction	(myomectomy)
Hypothyroidism	thyroid functions			(proper treatment)
Diabetes	HbA1c			(proper control)
PCO syndrome	US, LH, androgens	LH suppression	metformin	
Obesity	BMI index			weight loss
Luteal phase defect		progestagens		
Antiphospholipid Syndrome (APS)	LAC, aCL			low dose aspirin and/or heparin
Inherited Thrombophilia	factor V Leiden Prot C, Prot S prothrombin G20210A			thrombo-prophylaxis in case of high risk
Hyperhomocysteinaemia	homocysteine folic acid		folic acid and/or vitamin B12	
Abnormal Immune response	NK cells in endometrium MBL		glucocorticoids IVIG	
		cellular treatment		
Life style	history	vitamin suppl		diet, reduce alcohol coffee and smoking
Teratology	history			elimination of toxins

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)

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

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

RM_{bs} = a history of ≥2 miscarriages in a brother or sister of either partner, $RM_{parents}$ = 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

Review	Miscarriage n	Abnormal n (%)	Num chrom abn n (% of abn)	Str chrom abn n (% of abn)	Other chrom abn n (% of abn)
Simpson 1987	5318	2696 (**51**)	2584 (96) trisomy 1617 (60) polyploidy 510 (19) monosomy X 457 (17)	80 (3)	32 (1)
Goddijn 2000	4696	2319 (**49**)	2005 (86) trisomy 1216 (52) polyploidy 481 (21) monosomy X 308 (13)	132 (6)	182 (8)

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.

Lomax 2000, Fritz 2001, Schaeffer 2004, Vorsanova 2005, Benkhalifa 2005, Hu 2006

Maternal age
sporadic miscarriage
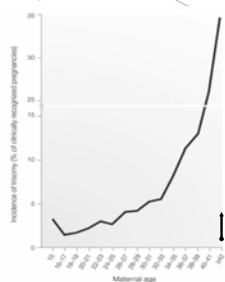
*Hassold Nature Reviews Genetics **2**; 280-291 (2001)*

Mean age of women at birth 1st child

Map 8   Mean age of women at birth of first child around 2003
Carte 8   Âge moyen des femmes à la naissance de leur premier enfant vers 2003

Fetal Karyotype
recurrent miscarriage

	Normal karyotype n (%)	Abnormal karyotype n (%)
Stern 1996	40 (43)	54 (57)
Ogasawara 2000	114 (49)	120 (51)
Carp 2001	89 (71)	36 (29)
Stephenson 2002	225 (54)	195 (46)
Combined n = 873	**468 (54)**	**405 (46)**

Parental chromosome analysis in the Netherlands
recurrent miscarriage

- 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%

Annuals of Postnatal Cytogenetic Analysis in The Netherlands 1992 - 2001

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%

Hook et al. Ann Hum Genet 1989
De Braekeleer en Dao. Hum Reprod 1990

Structural chromosome abnormalities
recurrent miscarriage

Paracentric
inversions
8%

Other
7%

Pericentric
inversions
8%

Robertsonian
translocations
16%

Reciprocal
translocations
61%
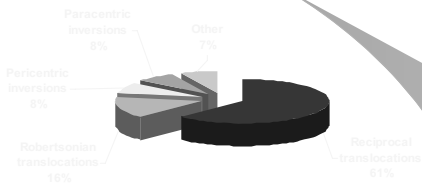
**Identification of risk factors in 279 carrier couples and
428 non-carrier couples**

Franssen BMJ 2005

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

Franssen BMJ 2005

Multivariable logistic regression
recurrent miscarriage

Risk factors	OR	CI	p
Maternal age at *second* miscarriage			.005
< 23 years	**6.2**	1.1 - 34.3	.04
23 - 34 years	**6.1**	1.3 - 27.7	.02
34 – 37 years	**3.3**	0.7 - 16.1	.13
37 – 39 years	**2.3**	0.4 - 12.0	.33
> 39 years	**1.0**	-	-
⩾ 3 miscarriages *(of the couple)*	**1.4**	1.0-2.1	.05
⩾ 2 miscarriages parents	**1.4**	0.9 - 2.2	.10
⩾ 2 miscarriages brother/ sister	**1.9**	1.1-3.2	.02

Franssen BMJ 2005

Risk of carrier status
recurrent miscarriage

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

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

Franssen BMJ 2005

Successful reproductive outcome *(accumulated)*
recurrent miscarriage

	Carriers *n = 247*	Controls *n = 409*
1st pregnancy	148 (60%)	280 (68%)*
2nd pregnancy	173 (70%)	324 (79%)*
3rd pregnancy	194 (79%)	332 (81%)
4th pregnancy	200 (81%)	339 (83%)
Total follow-up	205 (83%)	344 (84%)

* p < 0.05

Franssen BMJ 2006

Adverse reproductive outcome
recurrent miscarriage - carrier couples

	Carriers n = 239		% unbalanced of total no of pregnancies n = 550
	Total	Unbalanced	
No. of terminated pregnancies	6	2*	0.4
No. of children with congenital abnormalities	2	1	0.2
No. of post-partum deceased children	1	1	0.2
No. of stillbirths	3	?	-

* after PND

Franssen BMJ 2006

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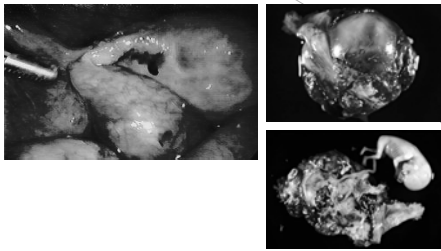
The etiology of 30% of cases remains unknown
Bouyer Am J Epidemiol 2003

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

Honore F+S 1979; Eskes EJOGRB 1984; Coulam Am J Reprod Immunol 1989; Fedele Obstet Gynecol 1989, Saada Contracept Fertil Sex 1997; Nybo Andersen BMJ 2000; De la Roche-brochard HR 2002

Cytogenetic findings
ectopic pregnancy

225			
	140 **(62%)**	146	
	63 **(45%)**	105 **(72%)**	
		7 **(7%)**	

Legend:
- Number of samples
- Successful n (%)
- chr abnormal n (%)

Period '74 - '93
Busch '74/ Poland '76
Elias '81/ Schafer '89
Cohen '93

Period '96-2000
Goddijn '96
Block '98
Coste 2000
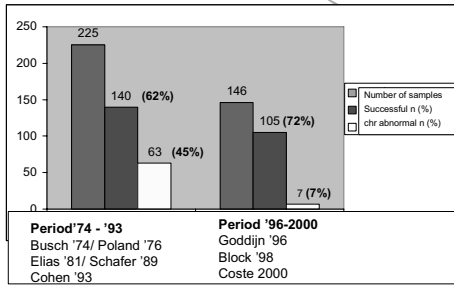
Cytogenetic findings
ectopic pregnancy

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

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Interventions

- Recurrent miscarriage of unknown cause   ➜ **IVF/ PGS**

- Recurrent miscarriage / carrier status of structural chromosome abnormality   ➜ **IVF/ PGD**

Interventions: PGS or PGD
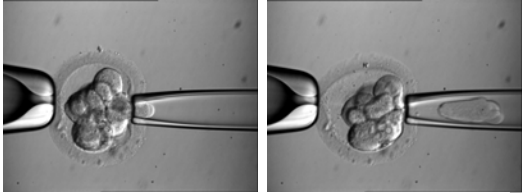
Interventions

	PGS	PGD
Patients	IVF population	Heritable disease
	Advanced maternal age	M Huntington
	Repeated IVF failure	cystic fibrosis
	Recurrent miscarriage eci	.
	Testicular sperm extraction	translocations
Aim	Reduce aneuploidies	Prevention of the birth of a
	Increase no. of pregnancies	seriously handicapped child
Technique	FISH/ CGH	FISH / PCR / CGH
Chromosomes	X, Y, 13,16,18,21,22	Chr specific probes

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[1]
- No RCT's reported comparing IVF-PGS with spontaneous cycles[1]
- High spontaneous chance on ongoing pregnancy[2]

[1]Twisk, Cochrane Review 2006,[2]Brigham 1999

PGD in RM translocation carriers - the evidence

- IVF/PGD is feasible
- > 100 cycles reported[1,2]
- Live birth rate/ ET : 22-23%
- ESHRE-PGD data: low pregnancy rates in translocation carriers but RM subgroup not identified[3]
- No RCT's available

[1]Fridstrom 2001, [2] Kyu Lim 2004, [3]Harper 2006

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

Centrum voor Voortplantingsgeneeskunde

Early Pregnancy Unit AMC

Dr Ankum, Gynaecology
miscarriage

Dr Hajenius, Obstetrics
Ectopic pregnancy

Dr Goddijn, Reproductive Medicine
Recurrent miscarriage

EARLY PREGNANCY UNITS
ESHRE 2006

ESHRE SIGEP
Roy Farquharson UK
Niek Exalto NL
Eric Jauniaux UK
Ole Christiansen DK
Thomas Phillipp Austria

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

1

Standard	Core	Aspirational
Patient Information	Designated Reception Area Universal use of clear, understandable terminology by all staff	Dedicated staff constantly at reception desk to provide greeting, obtain patient details and explain structure and triage function of EPU
Patient Choice of Management	Education of patient relevant to diagnosis and management Open explanation of expectant, medical and surgical options	Dedicated phone line for patient queries and electronic access to protocols from outside unit
Dedicated Quiet Room	Room for breaking bad news away from work area	Single-use room only with soft furnishing and absence of medical equipment
Availability of Service	5 day opening during office hours	7/24 opening and service provision with full staffing and daily scan support
Competence of Scanning	Recognised ultrasound training and RCOG/BMUS preceptor assessment and validation Register of staff competent at scanning	Lead Clinician Presence of RCOG/BMUS trainer in EPU Annual assessment of audited activity
Blood HCG level measurement	Laboratory access to blood HCG measurement and result within 48 hours of sampling	Same day sampling and result with electronic result link to laboratory
Written information leaflets	Visible open access to written information leaflets in EPU	Online external access to PIL
Acknowledgment of Privacy and Dignity	To provide individualised patient support and acknowledge confidentiality	Place one to one care as best practice at all times
Bereavement Counselling	All staff trained in emotional aspects of early pregnancy loss To enable access to counselling and provide immediate support	To provide all emotional and psychological counselling requirements within EPU and supported by dedicated staff and related agencies
Site of EPU	Geographically separate from all maternity	Own EPU entrance/exit

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?

2

Typical EPU presentation – sound familiar?

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

- ?threatened misc

- Day 2
- Reattended with increasing pain
- Tender LIF
- ?left adnexal mass
- Cervical excitation tenderness present
- ?ectopic pregnancy

HCG changes in normal pregnancy
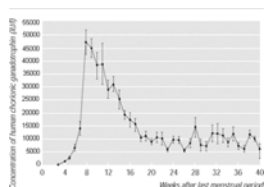
- Mean (SE) serum concentrations of human chorionic gonadotrophin (adapted from Braunstein et al 1976)

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

IUP 'Uncertain viability' → Rescan in 7-10 days

Resolved PUL

Diagnostic algorithm for 'PUL'

Non-viable IUP

Basic diagnostic algorithm for early pregnancy loss

USS – TAS / TVS

'Pregnancy of Known Location' ↔ 'Pregnancy of Unknown Location'

Intrauterine pregnancy ↔ Ectopic pregnancy

Viable IUP

IUP 'Uncertain viability' → Rescan in 7-10 days

Resolved PUL

Diagnostic algorithm for 'PUL'

Non-viable IUP

KEY
USS- Ultrasound scan
TAS- Transabdominal scan
TVS- Transvaginal scan
PUL- Pregnancy of unknown location
IUP- Intrauterine pregnancy

Basic diagnostic algorithm for early pregnancy loss

USS – TAS / TVS

'Pregnancy of Known Location' ↔ 'Pregnancy of Unknown Location'

Intrauterine pregnancy ↔ Ectopic pregnancy

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IUP 'Uncertain viability' → Rescan in 7-10 days

Resolved PUL

Diagnostic algorithm for 'PUL'

Non-viable IUP

KEY
USS- Ultrasound scan
TAS- Transabdominal scan
TVS- Transvaginal scan
PUL- Pregnancy of unknown location
IUP- Intrauterine pregnancy

4

MISCARRIAGE CLINIC
Care Pathway

Miscarriage Nurse clinic History Investigations Bone Density Scan

Consultant Clinic Review with results

Early Pregnancy Loss — Midtrimester loss?

Hysteroscopy and swabs

Oligomenorrhoeic? — HCG until 14/40 and Ultrasound surveillance — Bacterial Vaginosis? Y — Cervical weakness? Y

Thrombophilia — Aspirin +/- Fragmin and Ultrasound surveillance — Intravaginal Clindamycin and oral Erythromycin — Previous failed vaginal suture Y / N

Idiopathic — T.L.C and fortnightly scans — Trans abdominal Cerclage at 9 weeks — vaginal suture at 12 weeks

Pregnancy Support AN Clinic at 14

TVU of Open Cervix at 16 weeks

Transabdominal Cerclage Technique

Knot

Uterine vessels

Double-stranded nylon suture

Uterosacral ligaments

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 bench marking 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

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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.[5-7]

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]

Figure: Natural course of miscarriage with clinical and sonographic findings.
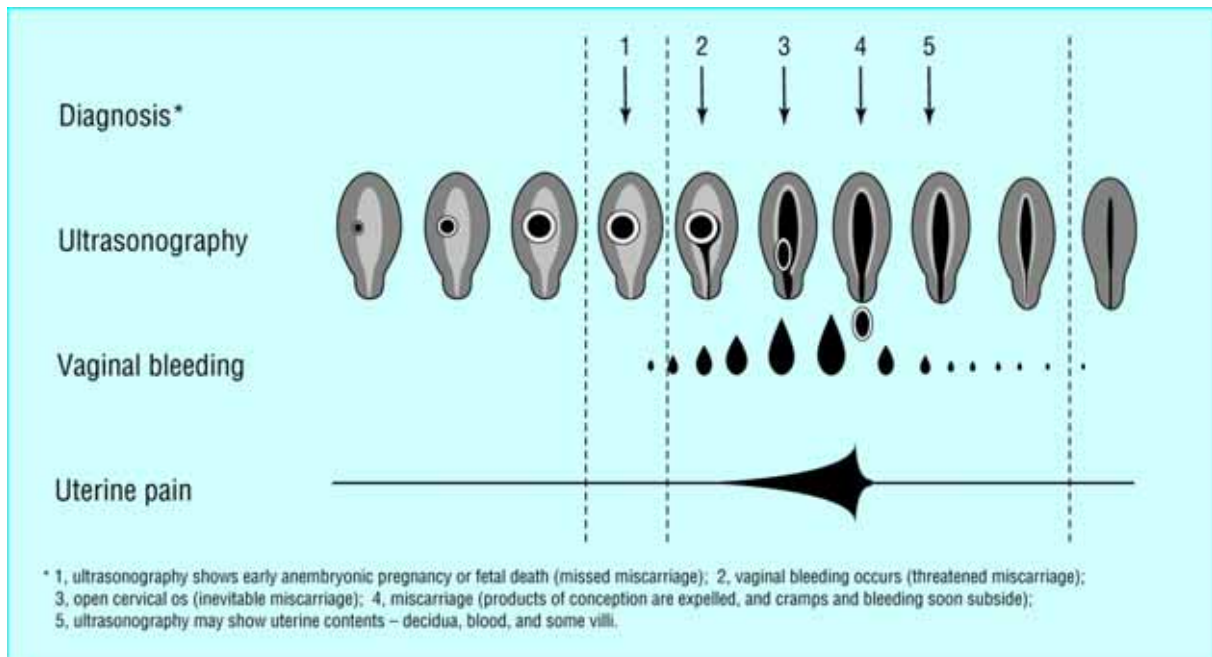
Some bleeding may persist for several weeks, and is followed by a normal period some 4-7 weeks after the actual miscarriage.[9]

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.[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.[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.[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.[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.[15-21] 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.[22,23] 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.[24-26]

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, followed the promising experiences with medical termination of pregnancies by the combined administration of the anti-progestagen *mifepristone* and the prostaglandin *misoprostol*.[27]

The available knowledge from randomised controlled trials comparing various treatment options for miscarriages has been systematically reviewed.[28] 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.[29] In that study, a regimen of daily 600 μg misoprostol administered vaginally upto 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.[30] 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. [31]

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]

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GESTATIONAL TROPHOBLASTIC DISEASE (GTD)
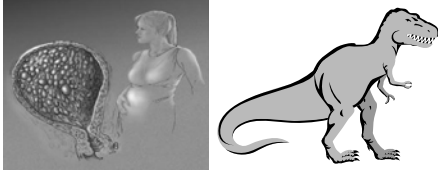
ERIC JAUNIAUX
Royal Free and University College London

GTD

EPIDEMIOLOGY & PATHOPHISIOLOGY

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

⇒EDEMA (HYDROPS).

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

+ *TROPHOBLASTIC HYPERPLASIA*
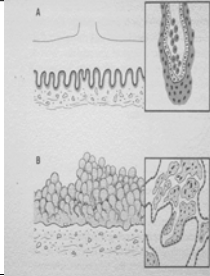
COMPLETE HYDATIFORM MOLE (CHM)

©GENERALIZED TROPHOBLASTIC HYPERPLASIA AND VILLOUS EDEMA=> BUNCH OF GRAPES. NO FETUS.

   (ancient egypt: BC)

ORIGIN OF CHM

23

23
23

Empty egg or with an inactivated genome

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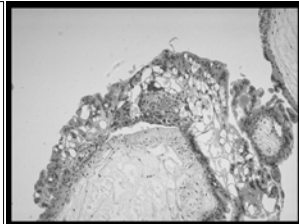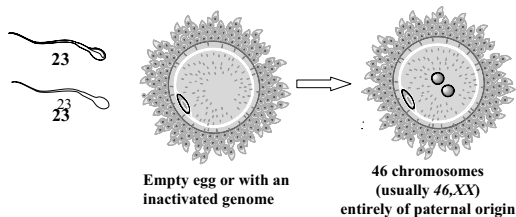
CHM: SEMIOLOGY

- UTERINE ENLARGEMENT (50%)
- VAGINAL BLEEDING (90%)
- HYPEREMESIS (20%)
- ANAEMIA
- HIGH MShCG 10-200MoM
- 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
- MShCG: 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

DIANDRY

23
23
23

69

TRIPLOIDY type I
Partial Mole
(PHM)

DIGINY

23

46

69

TRIPLOIDY type II
non-molar small
placenta
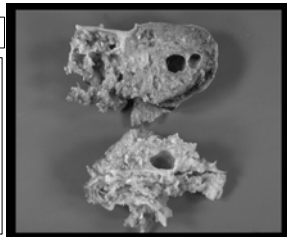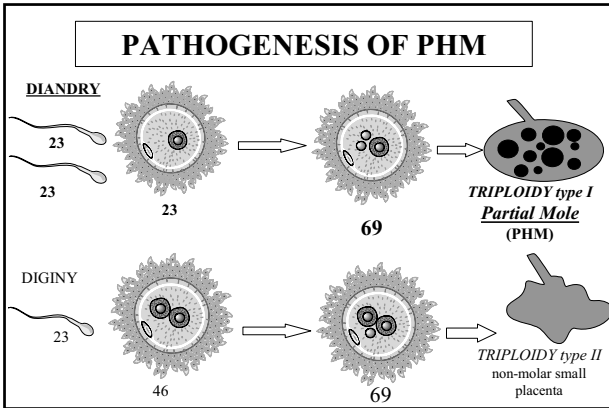
PHM: SEMIOLOGY

- UTERINE ENLARGEMENT (10%)
- VAGINAL BLEEDING (20%)
- HYPEREMESIS (5%)
- MULTICYSTIC OVARIES (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)

10-20%

0.1-3 %

CHM

PHM

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

2% OF CHM PROGRESS TO CHORIOCARCINOMA
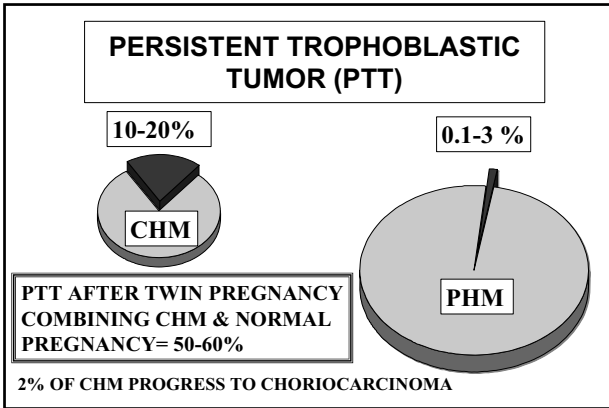
CHORIOCARCINOMA AFTER PHM

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 ???

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
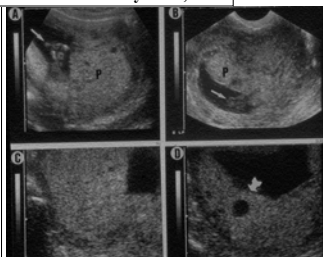
MID-TRIMESTER DIANDRY TRIPLOIDY

PHM placenta, moderate symmetric FGR

MShCG MoM > 5 MoM at 12-20 weeks
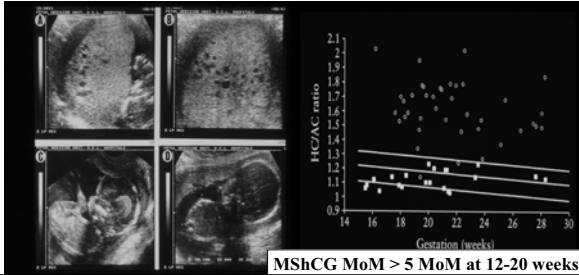
INVASIVE MOLE

Jauniaux, Ultrasound in Obstet Gynecol, 1998

- **RARE COMPLICATION OF CHM.**
- **FOCAL ECHOGENIC HETEROGENEOUS MYOMETRIAL LESION CONTAINING FLUID-FILLED CAVITIES**
- **CONTAINS VILLOUS TISSUE (ACCRETA)**
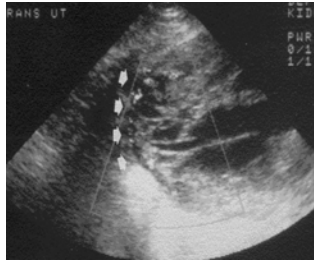
PTT: CDI vs ANGIOGRAPHY

Jauniaux, Ultrasound in Obstet Gynecol, 1998

- TVUS IS AS ACCURATE WITH NO MORBIDITY.
- SERIAL MShCG IS THE GOLD STANDARD FOR DIAGNOSIS AND MONITORING.
- CDI: LACUNAES + NODULES + A-V SHUNTS (PERSIST).
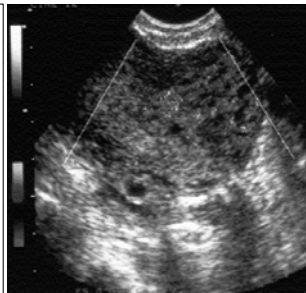
GTD

SCREENING
(First Trimester)

TRIPLOID PHM: 10-14 WKS

Jauniaux et al., AJOG, 1997
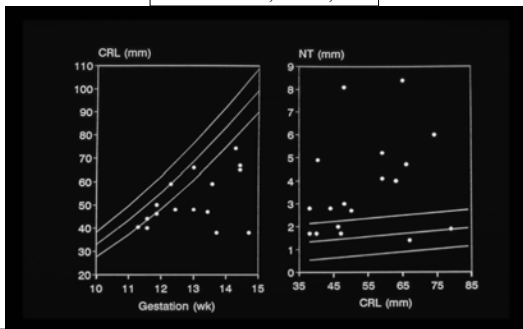
FIRST TRIMESTER US DIAGNOSIS OF CHM (retrospective)

Lazarus et al JUM 1999 (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%.
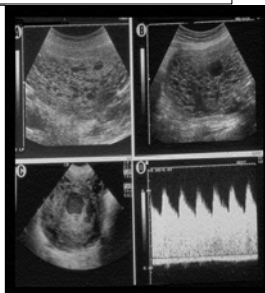
EARLY US DIAGNOSIS OF PHM

- 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.
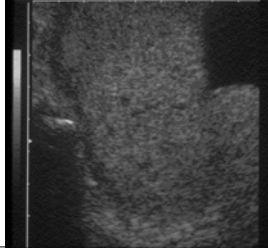
SCREENING FOR GTD IN EPL

- **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
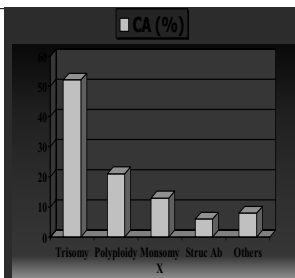
TRIPLOID MISCARRIAGE

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.
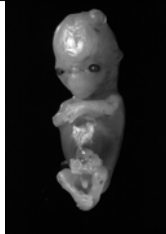
SCREENING FOR GTD IN EPL:
UCLH STUDY 1998-2004 (Johns et al., UOG, April, 2005)

- **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

- <u>VALUES</u> – SCREENING FOR GTD.
  - Investigation of the pathophysiology.
  - Epidemiology.
- <u>PITFALL</u> => 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).

11

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 PUL   EP
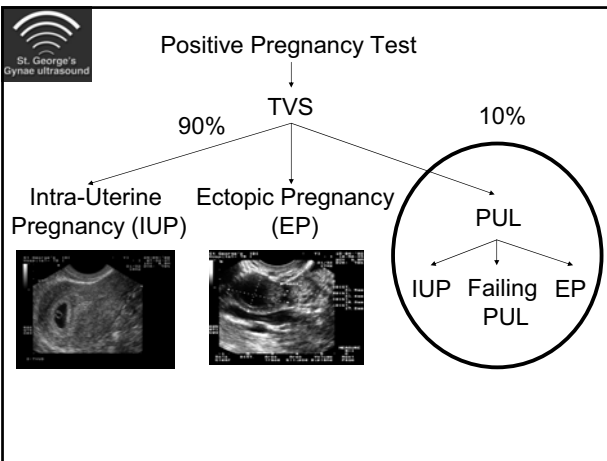
Pregnancies of Unknown Location (PULs)

– Positive pregnancy test
– No pregnancy visualised on scan

Int. Iliac vein   Int. Iliac artery

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

PUL Management

Serum hCG and progesterone

CURRENT CRITERIA
- IUP
-hCG rise > 66% in 48 hrs
- Failing PUL
-Serum progesterone < 20 nmol/L
- Ectopic Pregnancy
-hCG rise < 66% in 48 hours
-Discriminatory zone (hCG 1000 U/L – 2000 U/L)

± TVS ± Curettage ± Laparoscopy

Intrauterine Pregnancy (IUP)

Failing PUL

Ectopic Pregnancy (EP)

PUL Management

Serum hCG and progesterone

60-90%   ± TVS ± Curettage ± Laparoscopy   10-40%

Intrauterine Pregnancy (IUP)

Failing PUL

Ectopic Pregnancy (EP)

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

Hahlin et al 1995, Banerjee et al 1999/2001, Mol et al 2000

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
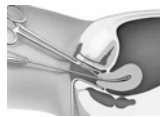
- Serial measurements of hCG and progesterone, TVS and uterine curettage have been combined into various diagnostic algorithms when a pregnancy cannot be seen on TVS

2. Surgical Intervention
Laparoscopy

- 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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2. Surgical Intervention
Laparoscopy

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

Condous et al, Ultrasound Obstet Gynecol 2005; 26:770-5.

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2. Surgical Intervention
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

Prog

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

hCG

PUL Results
hCG > 1500 U/L

Prog

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

hCG

PUL Results
hCG > 2000 U/L

Prog

- 86% of ectopics missed
- 5 laparoscopies to detect one ectopic

hCG

5
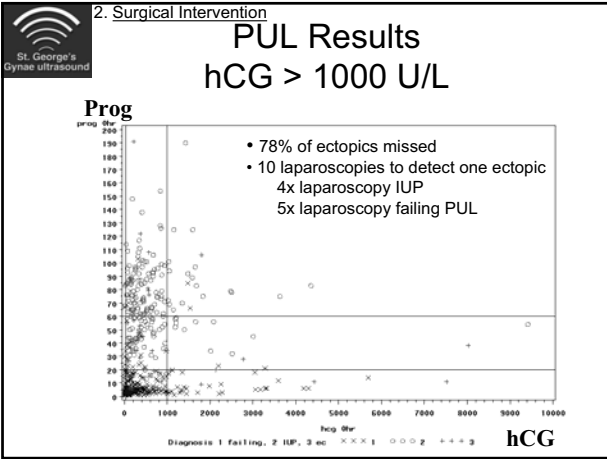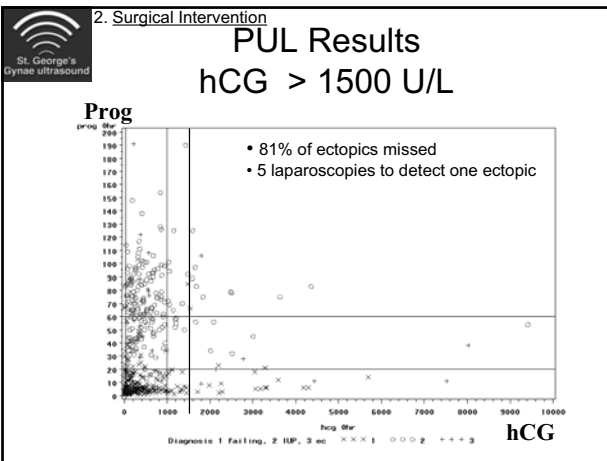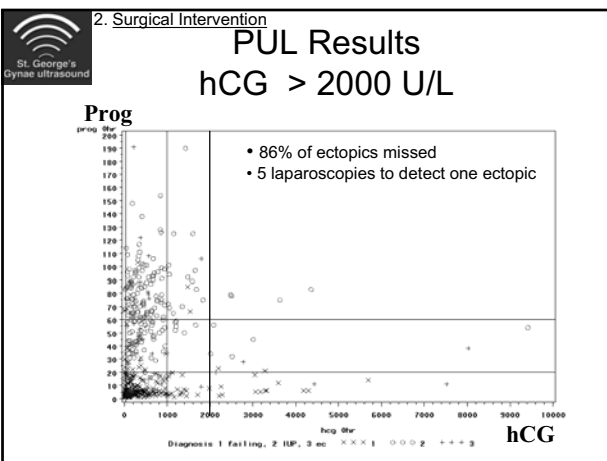
2. Surgical Intervention
Varying discriminatory zones

21.7%	87.3%
15.2%	93.4%
10.9%	95.2%

2. Surgical Intervention
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 asymtomatic woman with a PUL

2. Surgical Intervention
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

2. Surgical Intervention
Curettage

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

Condous et al, Hum Reprod 2006; 21:2706-2710.

2. Surgical Intervention
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

2. Surgical Intervention
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
- 1003 (8.0%) PULs

Protocol	No uterine curettages	No potential TOPs	% potential TOPs
1 — prospective test of model	272	0	0
2 — hCG ratio < 1.66	114	14	12.3
3 — hCG > 2000 IU/L or hCG ratio < 1.35	611	7	1.2
4 — hCG ratio <1.50	617	3	0.5

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

3. 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

Condous et al. Hum Reprod 2004; 19: 1900-10.

Methods

Positive pregnancy test
↓
Early Pregnancy Unit
↓
Transvaginal ultrasound scan (TVS)
↓
PUL
↓
Serum hCG 0 & 48 hrs
↓
Follow Up
↓
IUP   Failing PUL   EP

Methods

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

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3. Use of mathematical models

Results

3996 consecutive women attending EPU

381 (9.5%) PULs

Training set 185 PULs

Test set 196 PULs

2% Persistent PUL

11% EP

54% Failing PUL

33% IUP

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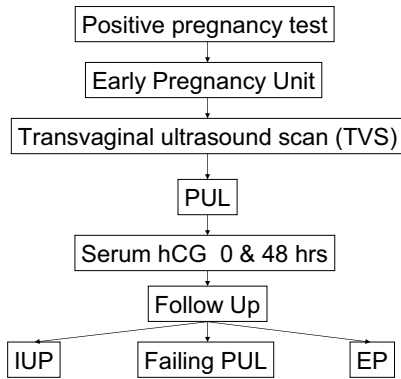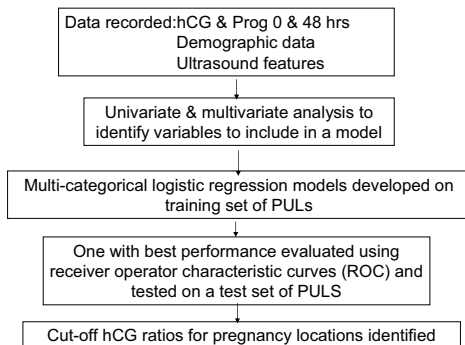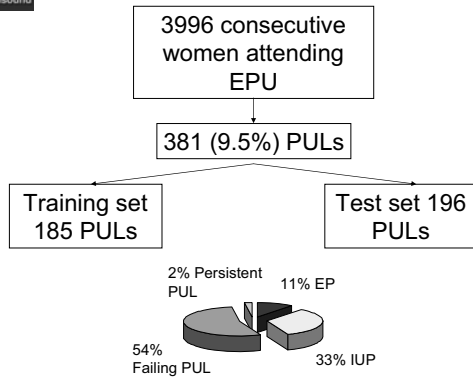
3. Use of mathematical models

Results

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

---

3. Use of mathematical models

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)

0.98±0.01	0.96±0.01	0.98±0.03
0.93±0.02	0.99±0.01	0.97±0.02
0.61±0.07	0.84±0.04	0.89±0.09

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)

Kirk et al, Ultrasound Obstet Gynecol 2006; 27: 311-315.

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3. Use of mathematical models

Model to predict outcome of PULs based on hCG ratio

					pred\true	Fail	IUP	EP	Tot			Senstivity	Specifici	PPV
Prior cost fo each class				Total	Failing	0	1		1	Failing				
1: Failing	2: IUP	3: Ectopic		Accurac	IUP	0	1	0	1	IUP				
Cost(prior)	1.0	1.0	5.00	0.00%	EP	1	0	0	1	EP				
* hcgratio=hcg_0hr/hcg_48hr					Tot	1	2	0	3					

input				Posterior Probability			Probability weighted by cost			Prediced		
ID	hcg_0hr	hcg_48hr	hcgratio*	Fail	IUP	EP	Failing	IUP	EP	Prob for	class
1	100	200	2.0000	0.00	0.93	0.07	0.0033	0.7308	0.2659	0.7308	2
2											
3											
4											

93% probability of an IUP and 7% probability of ectopic pregnancy (EP) based on hCG ratio alone

73% probability of an IUP and 27% probability of EP when probability weighted in order to maximize detection of EP

Overall the model predicts most likely outcome as class 2 – an IUP with a probability of 73%

---

3. Use of mathematical models

Results – 357 PULs

Model

	Sensitivity	Specificity	PPV	NPV
Failing	73.5%	86.2%	98.4%	71.8%
IUP	86.8%	74.9%	96.6%	69.8%
EP	82.8%	80.2%	28.2%	97.7%

Subjective Impression

	Sensitivity	Specificity	PPV	NPV
Failing	87.0%	91.7%	92.8%	86.3%
IUP	92.8%	85.9%	94.5%	84.5%
EP	79.3%	89.9%	54.8%	93.7%

---

3. Use of mathematical models

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

---

4. Time to diagnosis

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

Condous et al; Hum Reprod 2005; 20: 1398-403.

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4. Time to diagnosis

Single Visit Protocol

PUL		
Serum Prog < 10 nmol/L or Serum hCG < 25 IU/L	Serum Prog > 50 nmol/L and Serum hCG > 25 IU/L	Serum Prog 10-50 nmol/L and hCG > 25 IU/L
Failing PUL	IUP	? EP
Low Risk PUL	Low Risk PUL	High Risk PUL
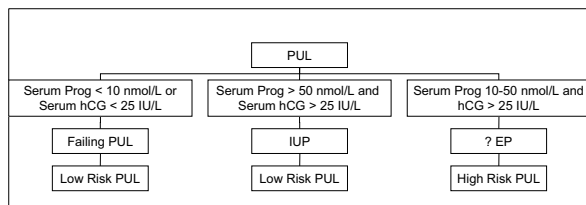
The protocol considers a PUL as a low-risk PUL when the progesterone level is < 10 or > 50 nmol/l, or the hCG level is < 25 IU/l, otherwise as high-risk PUL requiring a second visit.

True diagnosis vs predicted diagnosis
n=318

Predicted Diagnosis	True Diagnosis		Total	
	Non-ectopic pregnancy	Ectopic pregnancy		
Non-ectopic PULs	255	10	265	PPV=96.23%
Ectopic PULs	48	5	53	NPV=9.43%
Total	303	15	318	
	Sensitivity=84.16% LR (+) = 1.26	Specificity=33.33% 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

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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
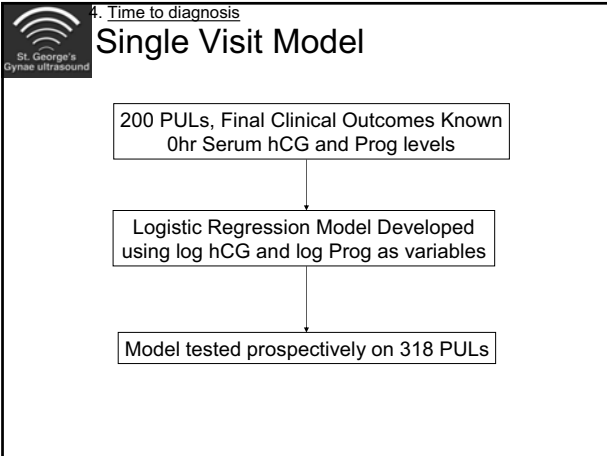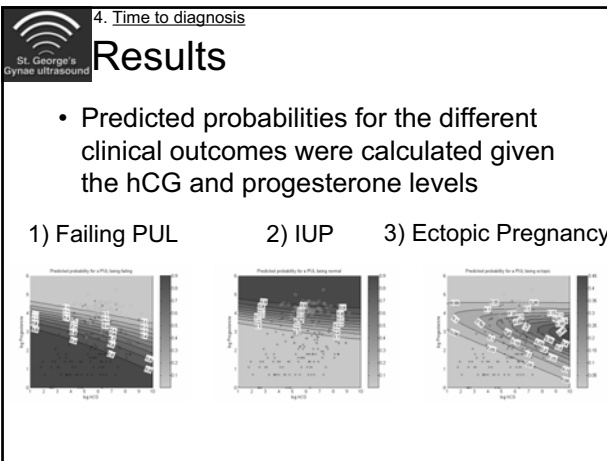
Results

Training Set (n=200)

- Cut-off probability 0.05

	Second Visit		
	No	Yes	
Non-EP	60	118	178
EP	0	22	22
	60	140	200

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

70% PULs needed a 2nd visit

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Results

Test Set (n=318)

	Second Visit		
	No	Yes	
Non-EP	150	153	303
EP	4	11	15
	154	164	318

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

51.6% PULs needed a 2nd visit

Single Visit - Results

- 4 EPs missed

	hCG 0 hrs	hCG 48 hrs	hCG ratio	Management
1	372	615	1.65	Laparoscopic Salpingectomy
2	160	212	1.33	Laparoscopic Salpingectomy
3	568	517	0.91	Laparoscopic Salpingectomy
4	1795	2423	1.35	Laparoscopic Salpingostomy

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

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Rationalizing the follow–up of pregnancies of unknown location

St. George's
Gynae ultrasound

Methods

St. George's
Gynae ultrasound

0 hrs   48 hrs   Confirmed Failing PUL if hCG ratio < 0.87*   Day 7

Failing PUL

Repeat hCG if initial hCG ratio >0.87

TVS

PUL → Repeat hCG → Model Prediction → IUP

Serum hCG

Ectopic

Repeat TVS → Confirmed IUP / Confirmed EP

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

St. George's
Gynae ultrasound

- 363 PULs

	Total number of cases	Number of cases diagnosed within time period	%
Failing PULs – 48 hrs -1 USS -2 Blood tests	229	219	96%
IUPs – day 7 -2 USS - 2 Blood tests	112	89	80.2%
EPs – day 7 -2 USS - 2 Blood tests	22	12	54.5%

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)
- 9% (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
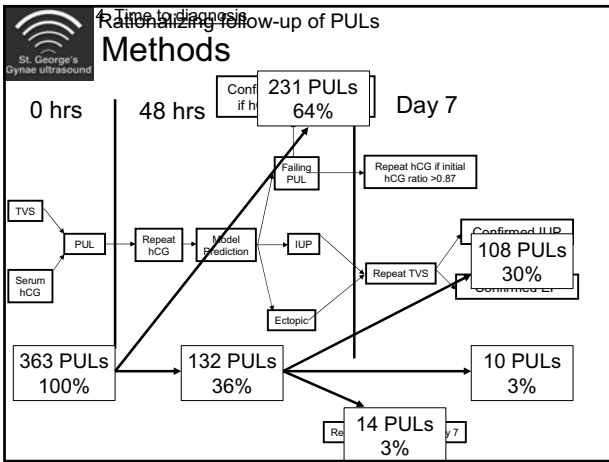
Results

- 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

Methotrexate for ectopic pregnancy

Tomasz Paszkowski
3rd Chair and Department of Gynecology
Medical University of Lublin, Poland

MTX for treatment of ectopic pregnancy
history

- Use of MTX systematically in a multiple dose regimen with combination with folinic acid (as in GTD)

  Ory, Fertil Steril 1986;46:767.-

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

  Stovall et al. Obstet Gynecol 1991;75:754.-

- Local treatment with MTX administred into the gestational sac under laparoscopic guidance

  Pansky et al. AJOG 1989;161:393.-

- Local treatment with MTX administred into the gestational sac under sonographic guidance

  Fernandez et al. Ultrasound Obstet Gynecol 1993;3:357.-

MTX for treatment of ectopic pregnancy
how it acts?

MTX=folic acid antagonist

⬇

Inactivates dihydrofolate reductrase

⬇

Depletion of available stores of tetrahydrofolate (essential cofactor in DNA and RNA synthesis during cell multiplication)

⬇

Particular vulnerability - rapidly proliferating tissues (trophiblast)

1

MTX for treatment of ectopic pregnancy
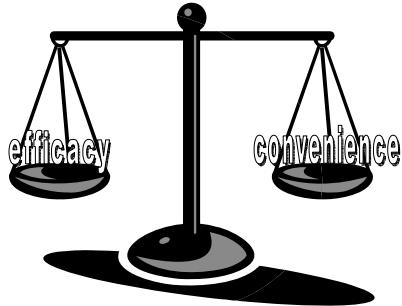
MTX for treatment of ectopic pregnancy
MTX versus laparoscopic salpingostomy

Primary treatment success	no diference
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

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

MTX for treatment of ectopic pregnancy
short-term outcome measures

- **Primary treatment success**
  - hCG clearance time
  - 15% day 4-7 rule
- **Reintervention for**
  - clinical symptoms
  - persistent trophoblast
- **Tubal preservation**
- **Complications/side effects**
- **Costs**

long-term outcome measures

- **Tubal patency**
- **Reproductive performance**
  - Spontaneous intrauterine pregnancies
  - Repeated ectopic pregnancies

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 EPs ASSOCIATED WITH SERUM hCG >10,000 IU/L, FHR(+) OR MASS > 4CM BUT THE LIKEHOOD OF SUCCESS IS SUBSTANTIALLY INCREASED

Speroff, Fritz. Clinical Gynecologic Endocrinology and Infertility. 2005

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, leucopenia, trombocytopenia)
- Known sensitivity to MTX
- Active pulmonary disease
- Peptic ulcer disease

Speroff, Fritz. Clinical Gynecologic Endocrinology and Infertility. 2005

MTX for treatment of ectopic pregnancy
I.M. treatment regimens

	Single dose	Multidose
MTX (methotrexate)	50 mg/m² (BSA calculation)	1 mg/kg
LEU (leucovorin)	None	0.1 mg/kg
Dosing frequency	One dose; repeat in 7 days if necessary	Alternate daily doses of MTX and LEU until hCG declines by 15%; up to four doses of each
Monitoring of hCG	Baseline (day 0), day 4, day 7	Baseline (day 0), days 1, 3, 5, 7 until hCG declines 15% from previous value
Additional doses	2nd dose given if hCG has not declined 15% between days 4 and 7	Give 2nd, 3rd, 4th dose if hCG has not declined 15% from the previous value; maximum four doses
Follow-up after treatment response	Weekly hCG until value <5	Weekly hCG until value <5

Notes:

MTX for treatment of ectopic pregnancy
Intramuscular MTX dosage

The Mosteller formula

$$BSA (m^2) = ([Height(cm) \times Weight(kg)]/ 3600)^{\frac{1}{2}}$$

or in inches and pounds: $BSA (m^2) = ([Height(in) \times Weight(lbs)]/ 3131)^{\frac{1}{2}}$

Mosteller RD. *N Engl J Med* 1987;317:1098

Body surface calculator

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

Notes:

MTX for treatment of ectopic pregnancy
single or multidose ?

Metaanalisis (26 studies, 1327 cases)

	SINGLE-DOSE	MULTIDOSE
Failure rate	11,9%	7,3%

OR=1,71 (CI=1.04-2,82)

Barnhardtet al. Obstet Gynecol 2003;101-778.-

Notes:

MTX for treatment of ectopic pregnancy for whom single-dose MTX ?

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 daclining at the time of diagnosis
  - initial hCG value < 1,000 mIU

Barnhardt, Seeber. Contemp OB/GYN 2004

Notes:

MTX for treatment of ectopic pregnancy "2-dose" regimen

Intramuscular MTX (50mg/m2) administred on days 0 and 4

- 87% of patients were treated succesfully
- 3% of tubal ruptures
- Treatment was well tolerated
- 91% of patients satisfied

Barnhart et al. Fertil Steril 2006 Nov.9th

Notes:

MTX for treatment of ectopic pregnancy intramuscular or oral ?

Oral MTX was given at a dose of 60mg/m^2 in 2 divided doses (2 hrs apart)
- 86% of patients were treated succesfully (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.-

Notes:

MTX for treatment of ectopic pregnancy treatment monitoring

Pretreatment and on day 8

- CBC
- Platelet count
- Renal and liver function tests

Speroff, Fritz. Clinical Gynecologic Endocrinology and Infertility. 2005

MTX for treatment of ectopic pregnancy
Pretreatment prognostic indicators - hCG

initial serum β-hCG level (IU/L)	success rate
<1,000	98%
1,000-1,999	93%
2,000-4,999	92%
5,000-9,999	87%
10,000-14,999	82%
>15,000	68%

MTX for treatment of ectopic pregnancy
pretreatment prognostic indicators - USG-TV

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

Common practise: 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;17:2585.-
Lipscomb et al. AJOG 1998;178:1354.-
Lipscomb et al. NEJM 1999;341:1974.-

MTX for treatment of ectopic pregnancy
pretreatment prognostic indicators - USG-TV

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

Lipscomb et al. NEJM 1999;341:1974.-

Notes:

MTX for treatment of ectopic pregnancy
pretreatment prognostic indicators - USG-TV

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)

Stovall et al. Obstet Gynecol 1991;77:749.-
Lipscomb et al. AJOG 1998;178:1354.-

Notes:

MTX for treatment of ectopic pregnancy
failure

- **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

Sowter et al. Br J Obstet Gynecol 2001;108:192.-
Job-Spira et al. AJOG 1999;180:938.-

Notes:

MTX for treatment of ectopic pregnancy
treatment monitoring/outcome predictors

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

MTX for treatment of ectopic pregnancy
treatment monitoring/outcome predictors

In 85% of women treated with single-dose MTX serum hCG rise between days 1 and 4.

**THIS PHENOMENON IS NORMAL
AND DOES NOT INDICATE FAILED TREATMENT!**

Stovall, Ling. AJOG 1993;168:1759.-

MTX for treatment of ectopic pregnancy
subsequent reproductive performance

- **50-80% of women treated with MTX for EP later achieve intrauterine pregnancy**
  Yao, Tulandi. Fertil Steril 1997;67:421.-
- **10-20% of women treated with MTX for EP experience a recurrent ectopic pregnancy**
  Bernoux et al. Hum Reprod 2000;15:1173.-
- **Women with EP associated with IUD have better future reproductive prognosis (EP is less likely related to the tubal pathology)**
  Bernoux et al. Hum Reprod 2000;15:1173.-
- **Poorer prognosis concerns:**
  - older women
  - previouslu infertile women
  - those with past history of EP or tubal damage
  Ory et al. Fertil Steril 1993;60:231.-

CONCLUSION: FERTILITY AFTER EP DEPENDS MORE ON ALREADY ESTABLISHED RISK FACTORS THAN ON THE METHOD OF TREATMENT

MTX for treatment of ectopic pregnancy adverse effects

- **Prolonged vaginal bleeding**
- **Stomatitis**
- **Conjunctivitis**
- **Elevated hepatic transaminases**
- **Mild abdominal pain of short duration**
- **Nausea, vomiting, diarrhoea**
- **Reversible alopecia**
- **Pneumonitis**
- **Severe bone marrow suppression**
- **Severe hepatotoxicity**

common → rare
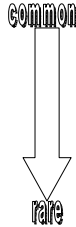
Side effects of MTX are relatively common but usually minor and transient.

Notes:

MTX for treatment of ectopic pregnancy adverse effects

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)

Lipscomb et al. Obstet Gynecol 1999;93:590.-

Notes:

MTX for treatment of ectopic pregnancy adverse effects

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

Gamzu et al. Hum Reprod 2002;17:2585.-

Notes:

MTX for treatment of ectopic pregnancy take-home messages

- **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

Notes:

Notes:

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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 Hb_{A1C} 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 (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; 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 placentation 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 histopathogical 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.*,

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)

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

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$_{bs}$, history of ≥2 miscarriages in a brother or sister of either partner; RM$_{parents}$, 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.

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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 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 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.

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.

Evidence-based medical therapies for couples presenting with RM

Anticoagulants

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 intervention in pregnant women with a history of RM has shown that overall combined unfractionated heparin and aspirin could reduce further pregnancy loss by 54% (Empson *et al.*, 2005). This conclusion was based on only two small non-blinded trials, and one of these lacked adequate allocation concealment. Furthermore, the trial by Farquharson *et al.* (2002), which was not included in the meta-analysis, could detect no effect of heparin/aspirin relative to aspirin alone.

When the data of low molecular weight heparin (LMWH) and unfractionated heparin (UFH) studies are pooled, there is only a 35% reduction in pregnancy loss and premature delivery (Empson *et al.*, 2005). No head-to-head study comparing LMWH and UFH met the inclusion criteria of the systematic review. There is no advantage in high dose over low dose of UFH and aspirin combined with LMWH, and aspirin alone has shown a variable effect in recent studies. The authors of this systematic review also concluded that large RCTs with adequate allocation concealment are needed to further evaluate therapeutic differences between UFH and LMWH (Empson *et al.*, 2005). There is a need for an international collaborative RCT to evaluate the type and duration of thromboprophylaxis in APS, before this treatment is used systematically in routine clinical practice (Table III).

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).

The possible relationship between aspirin in early pregnancy and congenital defects remains controversial. Initial reports such as the Collaborative Perinatal Project which surveyed 14 864 mother–child pairs, exposed to aspirin during the first trimester, found no evidence of a teratogenic effect (Slone *et al.*, 1976). The Food and Drug Administration (FDA) surveillance study involving 1709 newborns exposed to aspirin during the first trimester presented similar conclusions (Briggs, 1998). A more recent meta-analysis has found that the overall risk of congenital malformations in offspring of women exposed to aspirin in early pregnancy is not significantly higher than that in control subjects (Kozer *et al.*, 2002). However, a significant increase in the risk of fetal gastroschisis (odds ratio 2.37, 95% CI 1.44–3.88) was found. A similar risk (odds ratio 2.70, 95% CI 1.20–5.90) was also found in a

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

Basic investigations

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

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

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

Established treatment
   Tender loving care (TLC) and health advices (diet, coffee, smoking and alcohol)

Treatment requiring more RCTS
   Aspirin and/or LMW heparins for women presenting with APS or (multiple) inherited thrombophilias
   Progesterone in women presenting with unexplained early and late RM
   IVIG in women presenting with unexplained secondary RM or late RM
   Folic acid in women presenting with hyperhomocysteinaemia
   Immunization with third-party donor leukocyte

Treatment of no proven benefit
   Immunization with paternal leukocytes or trophoblast membranes
   Multivitamins supplementation

Treatment associated with more harm than benefit
   Daily corticoids during the first half of pregnancy

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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 hypospadia 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 transfusions 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

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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).

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Recurrent miscarriage in Poznań

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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 misarriages

Material

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

Basic investigation

1. Parental karyotype

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

3. Antiphospolipid antibodies (ACA, aLC)

1. *Parental cytogenic investigations*
(routine peripheral blood culture)

192 couples

carrier couples   **non-carrier couples**
n = 16 (8,3%)   **n = 176 (91,7%)**

reciprocal translocations   n=11
mosaicism n=3
                            13 (81,25%) carries were women
inversions   n=2
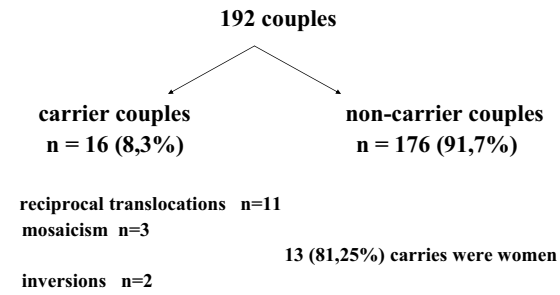
**Number of parental chromosomal abnormalities
according to number of miscarriages**

Number of miscarriages	2	3	4	5
Number of couples	123	55	10	4
Number /% of chromosomal abnormalities	*9 (7,3%)*	*5 (9,1%)*	*2 (20,0%)*	-

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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

Selectvie chromosome analysis could reduce the number of chromosome analysis by 18%

2

Reproductive outcome
in couples with chromosomal abnormalities

16 couples

pregnancy
n =19

live birth
n=8 (42,10%)

miscarriage
n=11 (57,89%)
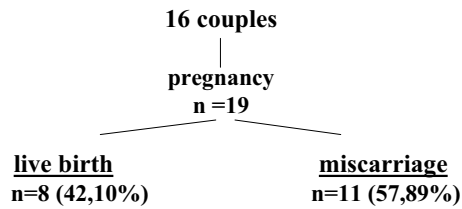
number of children 10
delivery age ± 34,5 week
birth weight ± 2472 g

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

Reproductive outcome	Carrier couples (n=247)	Non-carrier couples (n=409)	Difference in % (95% CI)	P value
Failure to conceive	8 (3.2)	19 (4.6)	-1.4 (-4.4 to 2.0)	0.38
One or more miscarriages	**120 (48.6)**	122 (29.8)	18.8 (11.1 to 26.3)	<0.01
One or more terminated pregnancies	6 (2.4)	8 (2.0)	0.5 (-1.8 to 3.4)	0.69
One or more ectopic pregnancies	3 (1.2)	13 (3.2)	-2.0 (-2.1 to 2.2)	0.11
One or more stillbirths	3 (1.2)	6 (1.5)	-0.3 (-2.1 to 2.2)	0.79
One or more children who died postpartum	1 (0.4)	4 (1.0)†	-1.6 (-2.1 to 1.4)	0.41
One or more ill or handicapped children	2 (0.8)	11 (2.7)‡	-1.9 (-4.0 to 0.5)	0.09
One or more healthy children	**205 (83.0)**	344 (84.1)	-1.1 (-7.2 to 4.6)	0.17

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)**
- **polypoids 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)**

Devi Wold AS, Pham N, Arici A. Semin. Reprod. Med. 2006

Anatomic uterine defects – in 15% of women with

three or more consecutive

sponteneous 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   **spontaneous abortion**
75%   **25%**

Patton PE et al. Am. J. Obstet. Gynecol 2004

16   women with a complete septate uterus
and pregnancy loss 81%

hysteroscopic (n=11)   **transabdominal (n=5)**
surgical treatment   **surgical treatment**

75%   **100%**
term live births   **term live births**

Postoperatively 82% term live births
18% spontaneous abortion

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3. *Antiphospholipid antibodies (ACA and LA)*

189   women with two and more miscarriages
ELISA   **APTT**
after first test

ACA positive   **LA positive**
n=57   **n=6**

after second test

ACA positive   **LA positive**
n=26   **n=4**

15,87% ApL positive patients
3,0%   with ≥ 20 GPL or MPL

Among **65** patients with tree and more consecutive miscarriages

18,4% women ApL positive

5,0% with ≥ **20** GPL

Oshiro BT et al. Obstet.Gynecol 1996

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**

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1. Feto – placental karyotypes

	first miscarriage	second and third miscarriage	fourth and fifth miscarriage
Patients number	45	41	9
Normal karyotype	22 (48,8%)	30 (73.2%)	9 (100%)
Abnormal karyotype	**23 (51,1%)**	**11 (26,8%)**	**0**
Trisomy 21	3	4	0
Trisomy 16	6	2	0
Trisomy 18	0	1	0
Monosomy	5	1	0
Triploidy	7	1	0
47,XXX	0	1	0
49,XXXXX	1	1	0

**Structural chromosome abnormalities
in recurrent miscarriages**
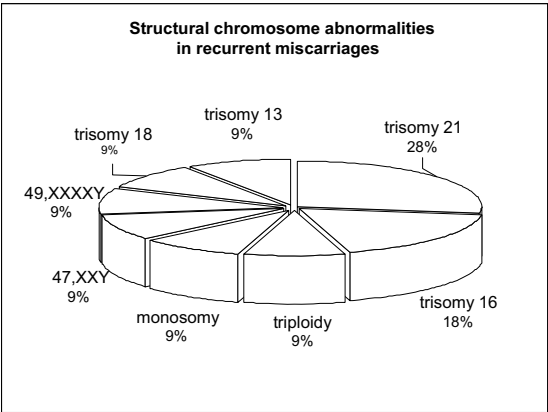
Coulam CB et al. Am. J. Reprod. Immunol. 1996

Theoretical risk of a repeated chromosomal anomaly
     after

  three losses - **35%**

  six losses   - **70%**

Ogsawara M et al. Fertil. Steril.2000

The incidence of embryonic chromosomal aberrations
        decreases as

 the number of previous miscarriages
        increases

Warburton D et al. Am. J. Hum. Genet. 1987

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**

Warburton D et al. Am. J. Hum. Genet. 2004

**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 (nmol/l)	Endometrial dating	
	Normal	Retarded
≥ 30	55	16
< 30	7	8

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

	Group	Number	MUC-1 U/ml	SD	P	
1.	Two and more miscarriages	37	26,26	11,56	0,023 1. vs 2.	0,027 1.vs 3.
2.	Infertility	46	21,44	7,33	0,0001 2. vs 3.	
3.	Control	19	33,78	12,06		

J.Skrzypczak et al. Pol. J. Gynecol. Investing 2006

Concentration of glycodelin (ng/ml) in uterine fluid								
Lp		number	min	max	mediane	25 perc	75 perc	P
1	Two or more miscarriages	42	0	102	45,75	16	55,25	0,026 1. vs 2.
2	Infertility	52	0	75,39	23,0	5,73	46,66	0,052 1. vs 3.
3	Control	21	9,1	97.47	48,91	39,7	69,46	0,0001 2. vs 3.

J. Skrzypczak et al. Ginekol. Pol 2005

Concentration of MMP9, TIMP, uPA and uPAR in uterine fluid									
Group	number	MMP9	*p*	TIMP1 ng/ml	*p*	uPA pg/ml	*p*	uPAR ng/ml	*P*
Idiopatic infertility	16	13.46	0,0007	12.98	0.039	210.60	0.013	4.87	NS
Recurrent miscarriage	13	18.28	0,034	13.31	0.055	312.92	0.038	3.59	0.026
Control	14	36.24	NA	27.37	NA	790.80	NA	7.197	NA

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

3. Trombophilia sreening

Kovalsky G et al. Intern. Med. 2004

A meta-analysis
The combined ORs for association between

RPL and factor V Leiden   2.0 (95%CI, 1,5-2,7)
RPL and G20210 A   2.0 (95%CI, 1,0-4,0)

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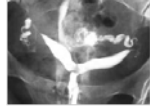
1st Department of Gynecology
University School of Medicine, Lublin, Poland

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embryology

| 6 weeks of pregnancy | 2 paramesonephric (mullerian) ducts | 2 mesonephric (wolffian) ducts |

XX phenotype
abscence of TDF
(Testis-Determining Factor)
↓
abscence of
antimullerian hormone

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

embryology

UTERINE MALFORMATIONS
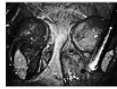
The result of:

1. Failure of 1 or 2 mullerian ducts to develop
(agenesis, 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
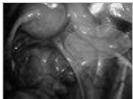
The overall incidence of mullerian defects- 5%

- 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

epidemiology

The presence of mullerian duct anomalies:

- not associated with increased mortality compared to
        the general population
- increased morbidity in patients with hematosalpinx
        hematocolpos retrograde manstruations,
        endometriosis
- high association with renal anomalies
        (unilateral renal agenesis mainly)
- no racial predilection
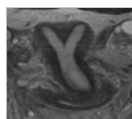
DIAGNOSIS

- Ultrasonography
- 3D Ultrasonography
- Hysterosalpingography
- Magnetic resonance imaging (MRI)
- Hysteroscopy
- Hysteroscopy/ laparoscopy
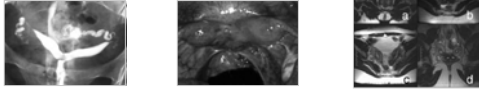
Classification of mullerian ducts anomalies
1988 American Fertility Society
(now American Society of Reproductive Medicine)

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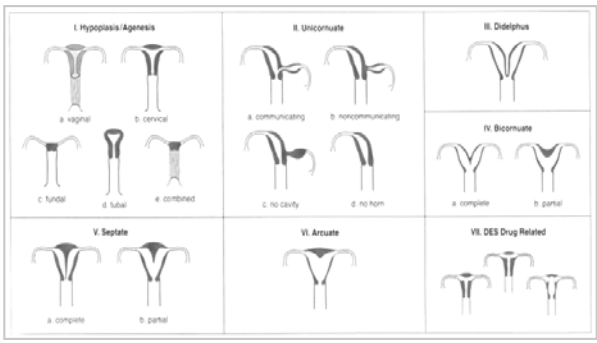
classification

Class I (agenesis/hypoplasia)

Uterine/cervical agenesis or hypoplasia

The most common is the Mayer-Rokitansky-Kuster-Hauser Syndrome (abscence of uterus and upper vagina)

Primary amenorrhoae

No reproductive potential aside from medical intervention

classification

Class II (unicornuate uterus)

Complete or almost complete, arrest of development of 1 mullerian 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

---

classification

Class III (didelphus uterus)

Complete nonfusion of both mullerian ducts

The individual horns are fully developed

Two cervices are inevitably present

Patients have been known to carry pregnancy to full term

---

classification

Class IV (bicornuate uterus)

partial nonfusion of both mullerian ducts

Some degree of fusion between the two horns

Horns are not fully developed

classification

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

---

classification

Class VI (arcuate uterus)

Single uterine cavity with a conflex or flat uterine fundus

Small fundal cleft or impression ≥ 1.5cm

Not significantly associated with the increased risk of pregnancy loss

---

classification

Class VII (dietylstilbestrol-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)

Rock and Adam AFS modified classification

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.

Rock and Adam AFS modified classification

Class 3
    Disorders of lateral fusion such as didelphys,
    unicornuate, bicornuate, and septate uteri (AFS classes
    II, III, IV, V)
A. Symmetric unobstructed form (5 types): septate,
    bicornuate, didelphys, DES, unicornuate uterus with or
    without rudimentary horn
B. Asymmetric obstructed form (3 types): unicornuate with
    noncommunicating obstructed horn, unilaterally
    obstructed double uterus, unilateral vaginal obstruction
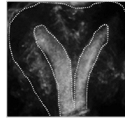
Mullerian duct anomaly (1392 cases):

- 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;7

Recurrent abortion:

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

The pattern of pregnancy loss in women with reccurent 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
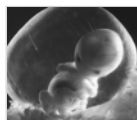
**Possible mechanisms leading to pregnancy losses
in women with uterine anomalies**

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
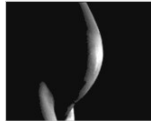
**Pregnancy outcome associated with
reproductive tract anomalies**
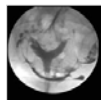
Anomaly	Term pregnancy outcome
Transverse vaginal septum	50%
Abscence of cervix	rare pregnancy
Rudimentary horn	64-85% (normal horn)
Unicollis	69%
Septate uterus	15% (78% after repair)
Bicornuate uterus	10% (84% after repair)
Didelphic uterus	57%

ACOG Technical Bulletin „Pregnancy loss"

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

Ectopics	1.2%
Abortions	36.5%
Preterm deliveries	16.2%
Term deliveries	44.6%
Live births	54.2%
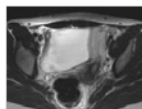
Grimbizis GF et al. Hum Reprod Update 2001;7

**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%
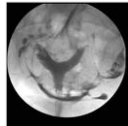
Grimbizis GF et al. Hum Reprod Update 2001;7

**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%
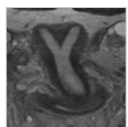
Grimbizis GF et al. Hum Reprod Update 2001;7

**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**
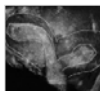
	N	Preterm deliveries	Ectopic	Live deliveries
Unicornuate uterus	393	43.3%	4.3%	54.2%
Didelphic uterus	86	24.4%	2.3%	58.6%
Bicornuate uterus	56	25%	0	62.5%
Uterine septum	1459	10%	1.9%	58.1%
Arcuate uterus	283	5.1%	3.5%	66.2%

Lin PC J Women's Health 2004;13

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

	Arcuate (n 40)		Septate (n 31)		Bicornuate (n 66)		Malformed (n 176)		Normal (n 28)	
	1st	all	1st	all	1st	all	1st	all	1st	all
Ectopies	7%	5%	0	0	0	1%	1%	2%	0%	0%
Abortions	33%	39%	21%	23%	38%	46%	31%	36%	12%	8%
Preterm deliveries	13%	8%	8%	23%	31%	22%	24%	8%	8%	6%
Term deliveries	43%	48%	71%	54%	31%	31%	45%	44%	81%	85%

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
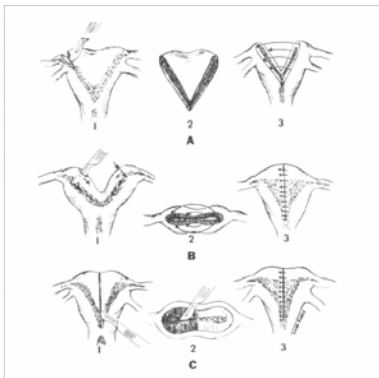
Jones wedge metroplasty

Strassman procedure

Tompkins' fundal bivalve metoplasty
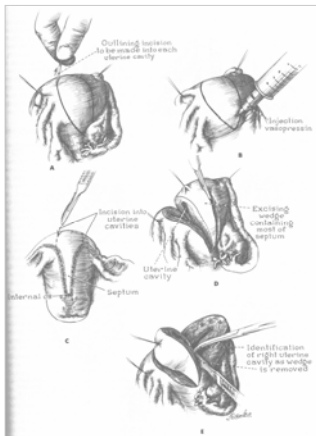
Steps in the operative pair of septate uterus by excision of a wedge (1)

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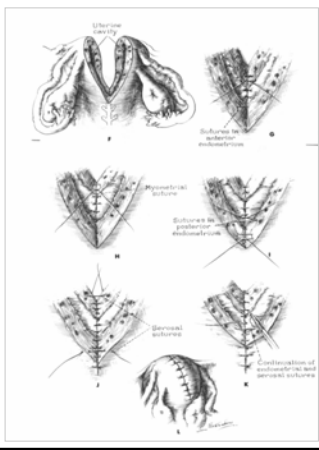
Steps in the operative pair of septate uterus by excision of a wedge (2)

RB Hunt
Atlas of female infertility surgery
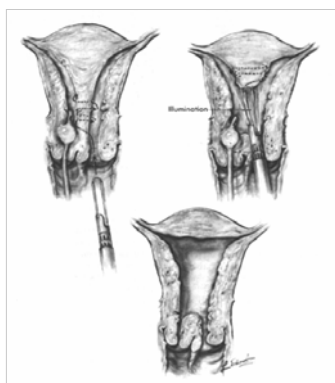
Lysis of complete septum

RB Hunt
Atlas of female infertility surgery

septum resection

… 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 succes rate ...

ACOG Technical Bulletin „Hysteroscopy"

	septum resection	
	n	Crude pregnancy rate after treatmnet
Fayez	7	71
Perino et al.	8	63
Daly et al.	15	47
Querleu et al.	9	67
Marabini et al.	14	44
Pabuccu et al.	10	63
Colacurci et al.	21	29
total	84	48

... hysteroscopic metroplasty is now considered for
the first therapeutic option for septate uterus ...

Pabuccu and Gomel Fertil Steril 2004;81

---

septum resection

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

	before	after
Ectopics	0.3%	0.3%
Abortions	86.4%	16.4%
Preterm deliveries	9.8%	6.8%
Term deliveries	3.3%	76.2%
Life births	6.1%	83.2%

Grimbizis GF et al. Hum Reprod Update 2001;7

Comparison of reproductive outcome before *septum resection* and after hysteroscopic metroplasty

	Primary infertility n 193	Spontaneous abortion n 109	Habitual abortion n 59	Total n 361
Before metroplasty				
Abortions	-	91.8%	96.1%	94.3%
Preterm deliveries	-	7.1%	3.8%	5.1%
Term deliveries	-	1.1%	-	0.4%
Live babies	-	4.3%	1.1%	2.4%
After metroplasty				
Abortions	33.3%	10.4%	10.2%	16.1%
Preterm deliveries	15.5%	19.7%	20.4%	18.8%
Term deliveries	51.1%	69.7%	69.3%	65%
Live babies	57.7%	81.3%	79.5%	75%

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

septum resection

The rate of rehysteroscopy according to the type of septate uterus

	%
Subtotal septate	7%
Total septate	25%
Total septate with double cervix	27.5%
Total	13.6%

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

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septum resection

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

---

septum resection

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

septum resection

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

---

septum resection

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)

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bicornuate uterus
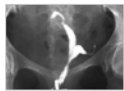
Reproductive outcome before and after metroplasty
in women wirh bicornuate uterus

		before	after
Maneschi et al. 1993	Live birth rate	0	80%
	Abortion rate	64%	20%
Capraro et al. 1968	Live birth rate	21%	82%

Surgical reconstruction can be considered for patients with
recurent miscarriages and no other obvious etiologies ...
Lin PC J Women's Health 2004;13

16

Infection and early pregnancy loss

Tomasz Niemiec
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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

University of Liverpool
School of Reproductive and Developmental Medicine

What is recurrent miscarriage?

- 3 consecutive miscarriages
- Very distressing because cycle of
- Occurs over 1-2 years
  - Increasingly desperate for baby

Try again

Happy pregnant

Few week later
Pain, bleeding loss

Recover

Liverpool School of Reproductive and Developmental Medicine
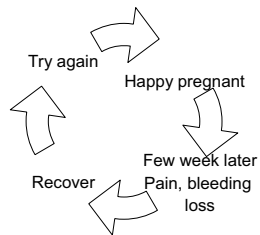
How common?

- 3% couples trying for a baby
- 2 million births in UK per year
- 60,000 couple in UK per year

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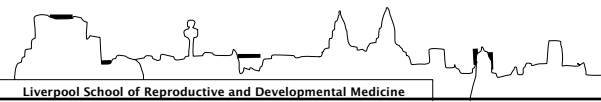
Historical perspective

- 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%
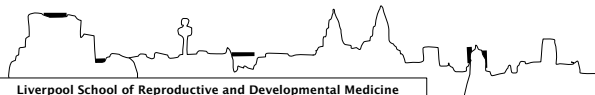
Liverpool School of Reproductive and Developmental Medicine

Medawar

- "How does the pregnant mother continue to nourish within itself for many weeks or months a fetus that is an antigentically foreign body?"
- 1953
- 50 years later no effective treatment
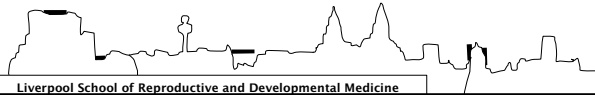
Liverpool School of Reproductive and Developmental Medicine

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?**
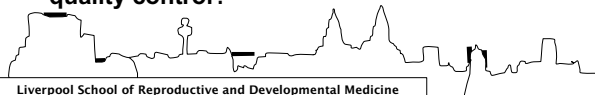
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The decidua

- 90% karyotypically abnormal pregnancies miscarry in the first trimester
- 93% karyotypically normal pregnancies continue
  - McFadyen, 1989
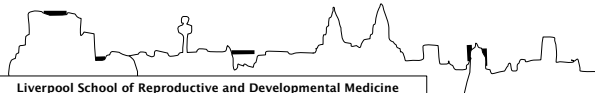
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Balanced translocations

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

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Balanced translocations

- unbalanced translocations
  - 38% of miscarriages
  - 0% of ongoing pregnancies
    - Stephenson and Sierra 2006
- 0% of on going pregnancies unbalanced translocations n= 41
  - No miscarriages not ≈ incidence of translocation
    - Godijin 2004
- 1 infant unbalanced translocation n=95
    - Sugiura-Ogasawara 2004

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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)

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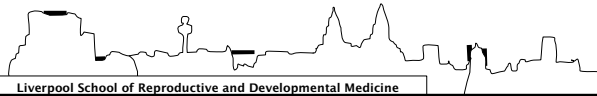
How does selection occur

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

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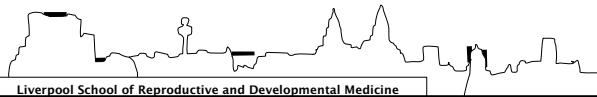
Aneuploidy miscarriages

- RM women miscarried aneuploidy fetus found normal trophoblast invasion and plugging of spiral arteries
  - Sebire et al 2002
- Therefore not fetal problem
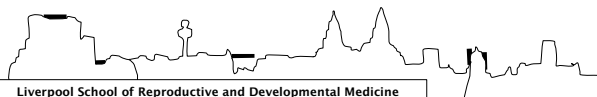
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Maternal endometrium
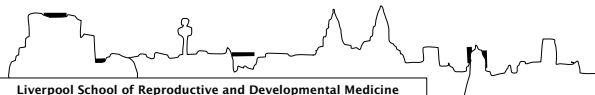
MUC-1

- 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*

5

Endometrium different in RM normal v abnormal?
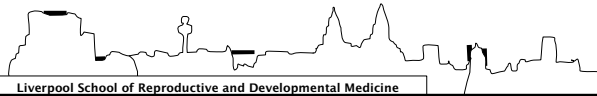
Karyotypical abnormality

- 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
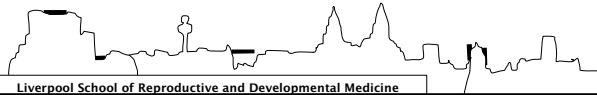
	Definition	Gestation in weeks	Ultrasound findings
Empty sac	Trophoblast development without the development of an embryo	<12	Empty gestation sac diameter >20mm or <20 mm no change 7 days later
Embryonic loss	An early embryo loss before fetal heart activity	<8	Embryo >5mm size, no FH, CRL <5mm with no change 7 days later
Fetal Loss	Death of a fetus in the first trimester	8-12	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 karyptypically abnormal
    - Marikito et al., 2004
- In sporadic fetal losses
  - 86% abnormal
    - Phillip et al., 2003
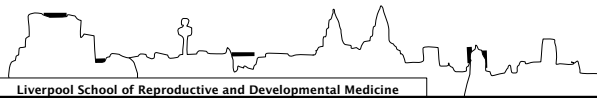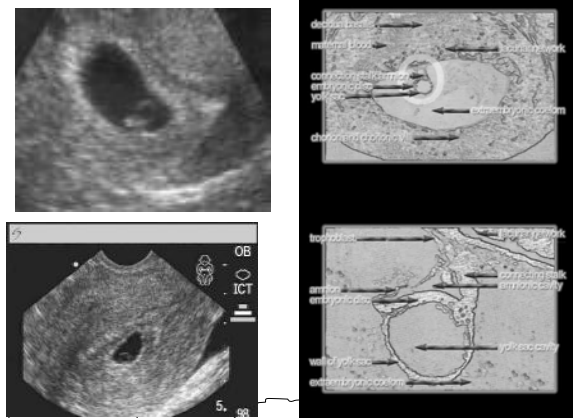
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Luminal epithelium

b: α4

c: β1

d: αvβ3

P=0.0017

P=0.0001

P=0.0004

P=0.0034

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Glandular epithelium

a: α1

b: α4

c: β1

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NATURE MEDICINE 2006

Killers become builders during pregnancy

Philippe Le Bouteiller & Julie Tabiasco

Circulating natural killer cells might be best known for their ability to disable and maim target cells. But in the pregnant uterus of humans these cells seem to have a positive effect, regulating placental development and angiogenesis (pages 1065–1074).

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uNK cells in endometrium

Control

RM patient

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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

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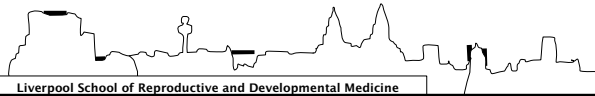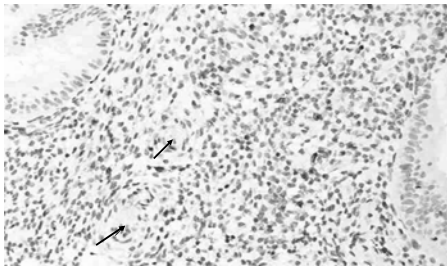
•uNK cells accumulate in perivascular areas of the endometrial stroma

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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

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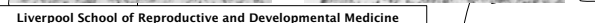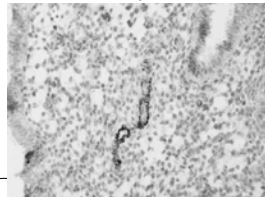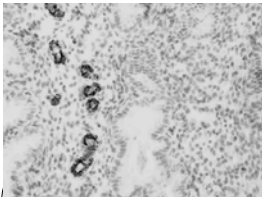
VSMC differentiation - αSMA and SMM
uterine artery PI (n=28)

αSMA (P = 0.02)
SMM (P = 0.01)
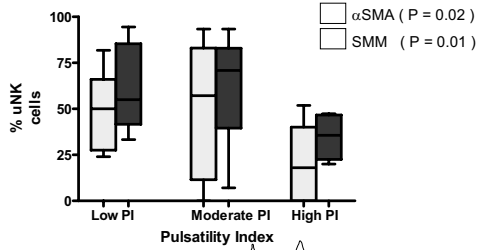
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Vascular smooth muscle differentiation
%uNK cells
(N = 28)
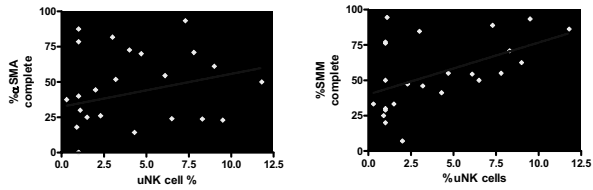
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% uNK cell per uterine flow N=46

P=0.0004
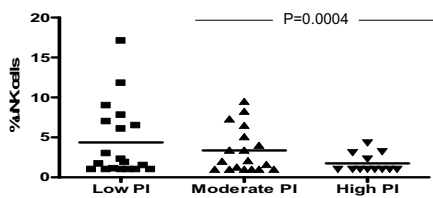
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cytokines

- 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

	RPL		pregnant	non-pregnant
	baby n=33	misc n=13		
IL-10	↑↑	↑↑	↑	—
IFN-γ	↓↓	↓	↓	—
TNF-α	↓	↓↓	↓	—

Was the pregnancy normal?

- Ultrasound
- Karyotype
- Morphology
- Embryoscopy

11

Conclusions

- Classify early pregnancy failure
- Abnormal
  - Parental karyotype if recurrent loss
- Normal
  - APS
  - Thrombophilia screen
  - Aspirin and LMWH
- All need supportive care
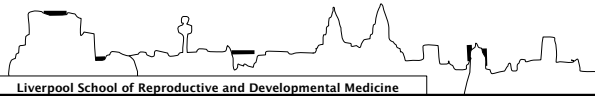
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Acknowledgements
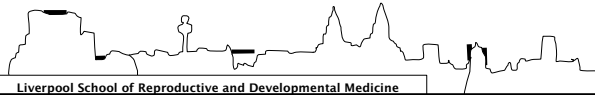
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Leukemia Inhibitory Factor (LIF) and implantation

Mateusz Mikołajczyk

Division of Reproduction
Department of Gynecology and Obstetrics
Poznan, Poland

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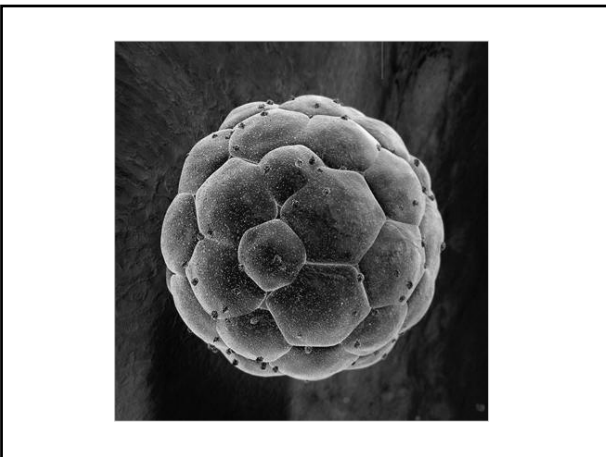
Markers of succesful implantation ?

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

- Ultrasonographic assessment of endometrium
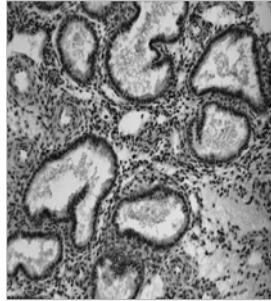
Markers of succesful implantation ?

Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A, et al.

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 function 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

3

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

	number	median	range	25-75% confidence interval	p*
Infertile women (the whole group)	54	13,64	0-320	2,6-27,33	<0,05
Idiopathic infertility	27	4,21	0-470,87	0,25-14,63	<0,01
Luteal phase defect	13	11,33	0-186,8	4,69-49,49	NS
Infertile women with endometriosis	14	25,53	0-379,14	12,63-43-32	NS
Control group	21	38,46	0-324,6	13,95-60,47	-

LIF – a new diagnostic test ?

LIF in uterine fluid (pg/ml)
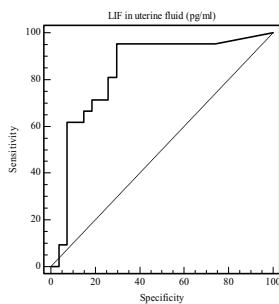
cut-off value <8,63 pg/ml; sensitivity - 70,4 %; specificity - 95,2 %; AUC-0,833

LIF

? =

? = ↑ LIF

LIF mRNA expression
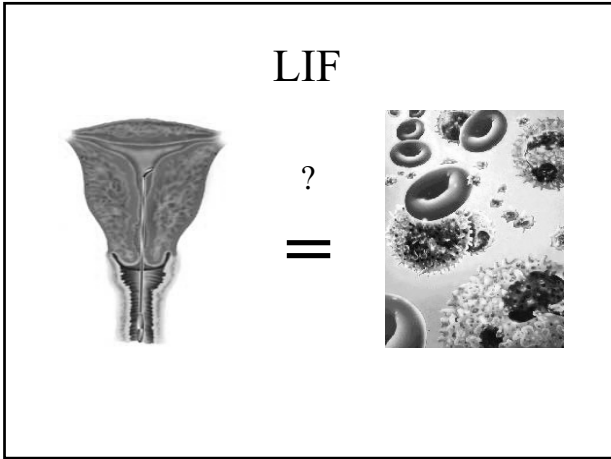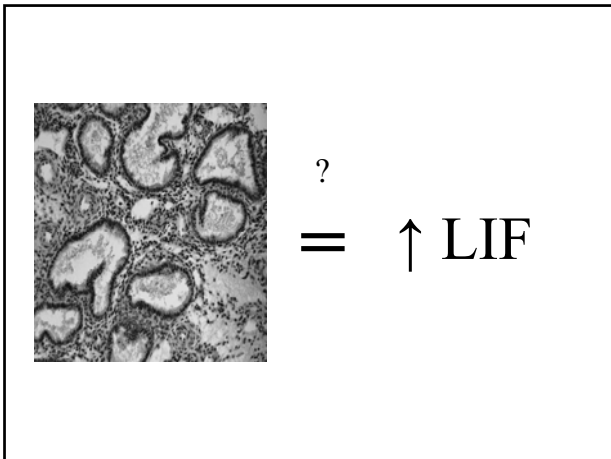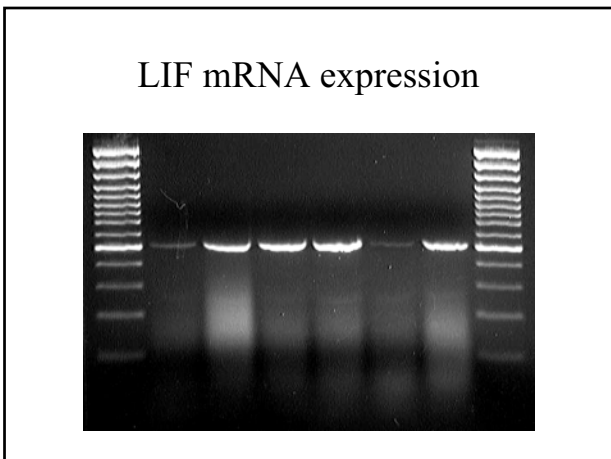
Expression of LIF mRNA
n=52

	number	mRNA LIF/GAPDH expression median (25-75% confidence interval)	p*
Infertile patients	54	0,914486 (0,753495-1,157888)	<0,01
Control group	10	1,426640 (1,094208-1,667347)	-

Correlation between LIF mRNA and LIF in uterine flushing
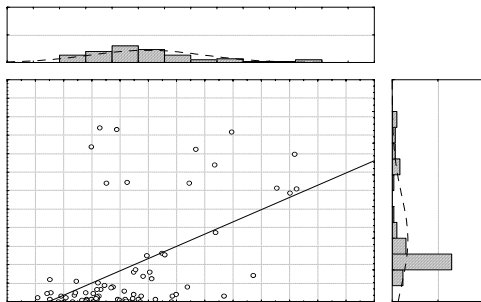
Future directions

LIF – a predictive factor ?

Questionnaire

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

Questionnaire

- out of 54 initial patients with infetility 26 qualified for workoup (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
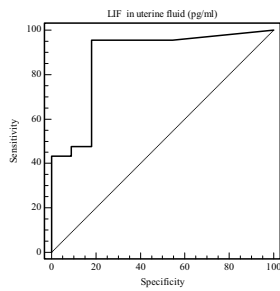

cut-off value > 2,31 pg/ml; sensitivity – 95,7%; specificity – 81,8%; AUC - 0,875

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