European Society of Human Reproduction and Embryology

COURSE 4

Early pregnancy failure

Special Interest Group Early Pregnancy

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Course 4: Special Interest Group "Early Pregnancy" course

"Early pregnancy failure"

PROGRAM

Course co-ordinators: O.B. Christiansen (DK) - E. Jauniaux (UK) - N. Exalto (NL)

Course description: Facts and Fiction about early pregnancy failure. A course for clinicians and scientists about recurrent miscarriage focusing on contradictory results due to methodological pitfalls as well as promising results in the field of immunogenetics and new treatment options.

Session 1: Research methodology and epidemiology of relevance for evaluating studies concerning early pregnancy failure.

09.00 - 09.30: The black box of early pregnancy failure - *N. Macklon (NL)*

09.30 - 09.45: Discussion

09.45 - 10.15: Methodological issues in the evaluation of treatment efficacy in recurrent miscarraige. - *S. Daya (CND)*

10.15 - 10.30: Discussion

10.30 - 11.00: Coffee break

11.00 - 11.40: Methodological pitfalls in recurrent miscarriage research - *O.B. Christiansen (DK)*

11.40 - 12.10: Discussion

12.10 - 13.10: Lunch

Session 2: Immunogenetics of Recurrent Miscarriage

13.10 – 13.35: Cytokines and immune cells in recurrent miscarriage -*S. Laird (UK)*

13.35 – 14.00: Cytokine gene polymorphism in recurrent miscarriage - *G. Unfried (A)*

14.00 – 14.25: The role of HLA-G in recurrent miscarriage - *K. van der Ven (D)*

14.25 – 14.50: Mannan-binding lectin and recurrent miscarriage - *D. Kilpatrick (UK)*

14.50 - 15.00: Discussion

15.00 - 15.30: Coffee break

Session 3: Evidence-based Management of Early Pregnancy Failure

15.30 - 16.00: Evidence-based investigations in early pregnancy failure - *S. Quenby (UK)*

16.00 - 16.30: Evidence-based treatments in early pregnancy failure - *E. Jauniaux (UK)*

16.30 - 17.00: Roundtable discussion between all speakers

The black box of early pregnancy failure

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Learning Objectives

At the end of this lecture, the participant should be able to

1. Discuss the definitions and incidence of early pregnancy loss
2. List the value and limitations of urinary hCG measurements as a marker of early pregnancy loss.
3. Summarize the evidence supporting the embryo as the major cause of early pregnancy loss.
4. Discuss the importance of early pregnancy loss as a determinant of fertility, and of IVF outcome.
5. Understand the limited effects of medical interventions thus far demonstrated in the treatment of early pregnancy loss

Lecture summary

Even when circumstances for conception are deemed optimal, the maximal chance of conceiving a clinically recognized pregnancy in one cycle is around 30%, rising to 40% in young women undergoing timed insemination with sperm from donors of proven fertility (Macklon et al, 2002). Pregnancies may be lost at any time between fertilization and implantation, or up to term. A proportion of these losses are clinically revealed as miscarriages. However, it has become clear that a large number of the conceptions fail before the woman becomes aware that she might have been pregnant. These early pregnancy losses, sometimes termed 'occult' pregnancies, have been defined as pregnancies that terminate so soon after implantation that no clinical suspicion exists as to its having existed. The introduction of sensitive assays for hCG and the possibility provided by in-vitro fertilization (IVF) to observe the events from ovulation to on-going pregnancy has enabled the previously elusive 'black box' of early pregnancy to be investigated (Macklon et al, 2002). With new techniques in cytogenetics, our understanding of the natural limits of human fecundity has grown, with clear implications for where the limits of success may lie for IVF.

The early conceptus produces a wide range of metabolites that may be measured in vitro. Only a limited number of these embryonic factors are secreted in concentrations sufficient to allow detection in maternal serum or urine. The most commonly employed marker of pregnancy, and that on which studies of occult pregnancy have been based, is hCG. In vitro studies have shown that hCG is produced by trophoblastic cells of the unhatched blastocyst and may be detected from 7 days onwards after fertilization. hCG is generally considered to become readily detectable in maternal serum or urine only from the time of implantation onwards. In practical terms this means that it becomes detectable 6.5-9.5 days after the LH surge. The stability of hCG makes it suitable for study since, particularly in early pregnancy, measurements in urine are accurate and consistently represent measurements in the serum. Furthermore, hCG is very stable even in urine which has been repeatedly thawed and frozen. The development of immunoradiometric assays has provided very sensitive and specific means of measuring serum and urinary hCG. In recent years it has become clear that the principal form of hCG produced in normal early pregnancy is hyperglycosylated hCG. However, in pregnancies destined to fail before the onset of menses, little hyperglycosylated hCG is produced,

and regular intact hCG is the principal form expressed in the urine.

Much of our current knowledge of early pregnancy failure is derived from a series of studies carried out by Wilcox, Baird et al in the 1980s on a cohort of 221 women attempting to conceive. Daily urine samples were collected for 6 months, and were analyzed for hCG (Wilcox et al., 1988). Twenty two percent of all pregnancy losses were observed to occur before the woman would have been aware of the pregnancy, being characterized by rise and fall in urinary HCG levels prior to the onset of menses (Figure 1,2). In this group of women, urinary hCG levels rose following implantation, and were observed to fall prior to the onset of menses. This was in contrast to the exponential rise observed in hCG levels when the pregnancy progresses beyond this early phase.

The first appearance of hCG, taken to indicate implantation, varied between 6-12 days after ovulation, with 84% implanting on day 8, 9 or 10. Delayed implantation was clearly associated with occult pregnancy loss, such that no clinical pregnancy was recorded if implantation occurred later than 12 days post-ovulation (Wilcox et al, 1999). Early loss was least likely when implantation occurred by the 9th day. In a more recent study of 518 newly married Chinese women who intended to conceive, conception and pregnancy loss rates were correlated with subsequent delay in achieving a clinical pregnancy (Wang et al 2003). HCG assays of daily urine samples were employed to detect early pregnancy loss. Within the first 12 months, 40% conceived. Of these, 8% ended in clinical spontaneous abortion and 25% in early pregnancy loss. Early pregnancy loss was associated with an increased odds of conception, and shorter time to clinical pregnancy in subsequent cycles. A positive correlation was therefore demonstrated between early pregnancy loss and subsequent fertility. The availability of sensitive hCG assays and pre-implantation genetic screening has made the causes of early pregnancy loss more amenable to study. The pattern of hCG expression may suggest whether the cause lies primarily with the embryo or the endometrium. When the process of implantation, initiated at the normal time, is terminated abruptly, hCG becomes detectable from around day 9 post LH surge but fails to rise exponentially, declining over the next two days. This pattern of hCG secretion may suggest an endometrial factor preventing completion of implantation. However, if implantation is delayed, the rate of rise of hCG is reduced and detection occurs later in the cycle. This pattern of hCG rise may indicate abnormal embryonic development after implantation has occurred (Figure 3).

The likely importance of genetic abnormalities as a cause of early pregnancy loss was made clear by studies of spontaneous abor-tions in which chromosome abnormalities were encountered in more than 50%. Reported proportions of chromosomal abnormal preimplantation embryos ranged between 30% and 70% in embryos at day 2/3 of development (Munné et al., 1997, Verlinsky et al, 2004). The data reported appears to depend on the number of probes applied simultaneously, the type of probes used, embryo morphology, embryo development and the presence of multinucleated blastomeres. Further evidence for the primary role of the embryo as the determinant of early pregnancy loss has come from a study which showed that falls in hCG almost always precede falls in urinary levels of progesterone metabolites in early pregnancy loss prior to the onset of menses (Baird et al, 2003).

Implications for IVF

Despite improvements in laboratory and clinical practice, ongoing pregnancy rates from IVF remain around 20-25% per started cycle. The role of early pregnancy loss in determining clinical outcomes of IVF remains uncertain, as there are few studies of the true rate of early pregnancy loss following IVF. In a recent study comparing early pregnancy loss following IVF with that following oocyte donation treatment, early pregnancy loss was the outcome in 30% of cycles following IVF (Simon

et al., 1999). This compares to 22% observed in spontaneous cycles (Wilcox et al., 1988). While the endometrial milieu is an important factor, the course of events following IVF treatment following the onset of implantation are probably predominantly determined by the quality of the embryo(s). It is becoming clear that morphology does not correlate with the chromosomal status of the embryo (Verlinsky et al, 2004).The incidence of aneuploidy in the normal IVF patient population remains unclear, since most studies thus far performed have focused on subgroups of women at higher risk of aneuploidy, such as older women, or those with recurrent abortion. Pre-implantation genetic screening offers a possible route to improving embryo selection, thus reducing the need for multiple embryo transfer. Moreover, if aneuploidy is the principle cause of early pregnancy loss following IVF, the application of pre-implantation genetic screening could improve success rates from IVF treatment (Figure 4) (Macklon et al, 2002). Finally, until the role of the embryo in determining the outcome of conception is more fully understood, medical therapeutic interventions are unlikely to impact greatly on early pregnancy loss.

Figures

Figure 1

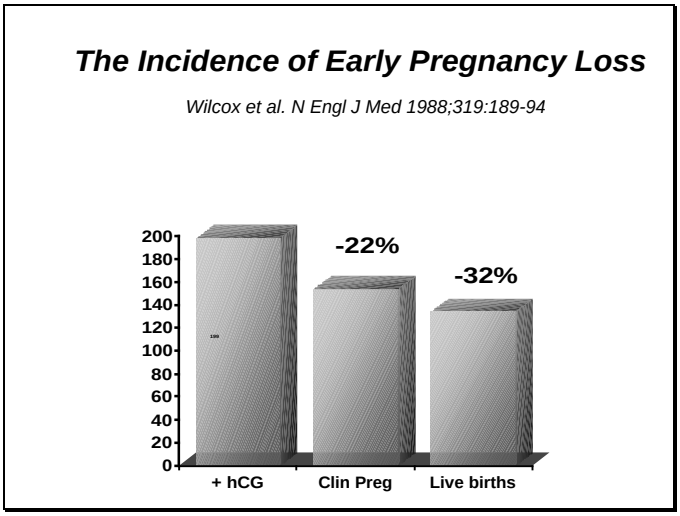


Figure 2

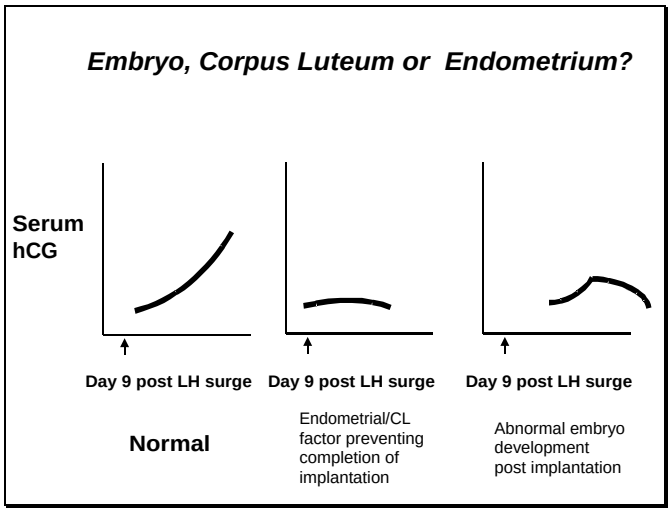
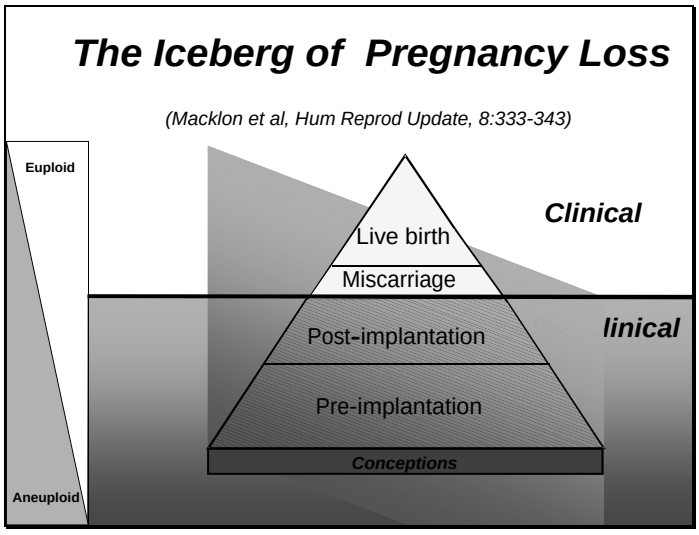


Figure 3



Legends to Figures

Figure 1: The proportion of 707 potentially fertile cycles resulting in conception, early pregnancy loss and clinical miscarriage as demonstrated by Wilcox (1988) are shown.
Figure 2: Three patterns of hCG expression in the urine are depicted, correlating with, from left to right: ongoing pregnancy, early pregnancy loss due to an endometrial or corpus luteum functional deficit, and early pregnancy loss due to an embryo based factor.
Figure 3: The iceberg of pregnancy loss depicts how the majority of pregnancy losses are not 'visible', occurring before the women misses a menstrual period. The relative role of embryo euploidy as a determinant of pregnancy loss is shown to decrease with increasing gestation.

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Methodological issues in the evaluation of treatment efficacy in recurrent miscarriage

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Learning objectives

By the end of the session, the participant will:

1. Have a clear understanding of the definition of recurrent miscariage and its subgroups.
2. Be able to counsel patients on the risk of miscarriage depending on the history and numbers of previous miscarriages.
3. Become familiar with the factors that influence the likelihood of success with treatment and the importance of including them as covariates in trials evaluating the efficacy of treatment for recurrent miscarriage.

Miscarriage is the most common complication of pregnancy, occurring in 10% to 15% of pregnant women. A small number of women has repeated miscarriages. Although accurate prevalence figures are not available, it has been estimated that 2% to 5% of women have three or more miscarriages (1,2). Over the years, increased attention has focused on the evaluation and management of this disorder. These efforts have led to the development of protocols for the diagnostic evaluation of couples with recurrent miscarriage so that a plan of care can be outlined based on the findings. Unfortunately, the paucity of good quality evidence limits the ability to make confident recommendations. There are many issues that have to be addressed in therapeutic trials before inferences can be made that are reliable. Some of these issues will be discussed in this paper.

Definition of miscarriage

The term miscarriage (or abortion) is used to describe a pregnancy that fails to progress resulting in death and expulsion of the embryo or fetus. The generally accepted definition stipulates that the fetus or embryo should weigh 500 g or less (3), a stage that corresponds to a gestational age of up to 20 weeks. Unfortunately, this definition is not used consistently, and pregnancy losses at higher gestational ages are also classified as abortion in some countries. Additionally, the literature is replete with studies on women with pregnancy loss, a term that has been expanded from the original definition of abortion to include pregnancies that have ended in stillbirth and preterm neonatal death. Thus, from a definition perspective, it is important to characterize the population being studied so that comparisons across therapeutic trials can be made more appropriately and reliably. Consensus on this issue is urgently required.

Risk of recurrent miscarriage

There have been several attempts to calculate the likelihood of a pregnancy proceeding to term when preceding pregnancies have ended in miscarriage. The initial estimates have been based on

the assumption that the overall miscarriage rate consists of the sum of two independent rates, one resulting from a random factor and the other from a recurrent factor in miscarriage sequences. From such mathematical calculations, it can be predicted that the chance of a fourth pregnancy going to term in women with three previous miscarriages is considerably less than that of a third pregnancy (1). This observation formed the basis of the definition of 'habitual abortion' (now referred to as recurrent miscarriage) to represent three consecutive miscarriages.

For many years, the mathematical estimates of miscarriage rate were used as control rates against which the efficacy of various therapeutic regimens introduced to prevent miscarriage were assessed. The reliability of these rates was challenged after evidence from a number of clinical studies suggested that the miscarriage rate after three consecutive miscarriages was substantially lower than had been predicted by the earlier models (1). Nevertheless, despite the varied methods of ascertainment, the results of the studies showed remarkable consistency in finding an increasing risk of miscarriage as the number of previous miscarriages increases. The effect of prior losses on subsequent probability of live birth was confirmed using the data from the placebo arm of studies in unexplained recurrent miscarriage and provided a quantitative estimate of the risk (4). It is clear from this evidence that 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. Instead, the approach has been to identify the study sample as a group of women having three or more miscarriages. It is quite likely that by stratifying the sample by number of previous miscarriages that the effect of the experimental intervention will become more easy to demonstrate in those women with higher numbers of previous miscarriage than in those with fewer previous miscarriages because the control event rate is so much lower in the former group (4).

Subgroups of recurrent miscarriage

The pregnancy history in women with recurrent miscarriage may include pregnancies that have ended in live birth. Thus, three different groups can be identified that should be assessed separately because the risk of subsequent miscarriage among these groups varies (1).

a) Primary recurrent miscarriage group
This group consists of women with three or more consecutive miscarriages with no pregnancy progressing beyond 20 weeks' gestation.

b) Secondary recurrent miscarriage group
This group consists of women who have had three or more miscarriages following a pregnancy, having that has gone beyond 20 weeks' gestation, may have ended in live birth, stillbirth or neonatal death.

c) Tertiary recurrent miscarriage group
This is a group that has not been well characterized or studied and consists of women who have had at least three miscarriages that are not consecutive but are interspersed with pregnancies that have progressed beyond 20 weeks' gestation (and may have ended in live birth, stillbirth or neonatal death.)

From these three different groups, it is evident that the study population being evaluated should be clearly specified because the prognosis for a successful outcome will undoubtedly be influenced by

the group being selected. The current approach of lumping all three groups together will not allow the effect of the experimental intervention to be detected easily.

Preconception versus post implantation onset of treatment

There is no standardization in many of the treatment protocols with respect to the onset of treatment. For example, intravenous immunoglobulin has been administered preconceptionally in some studies whereas in other studies treatment is commenced only after confirmation of the pregnancy (5); sometimes treatment is instituted only after fetal cardiac activity has been demonstrated as has been observed in the thrombophilia treatment trials. The likelihood of a successful outcome without treatment once fetal cardiac activity has been demonstrated is relatively high and will result in efficacy studies failing to detect a sizeable treatment effect with the experimental intervention.

Another methodological concern in efficacy trials is the practice of limiting the number of cycles of preconceptional treatment patients may undergo before they are withdrawn from the study, in spite of the fact that they have been randomized into one of the two comparator groups. The overall result becomes biased by excluding the patients who have failed to conceive within a specified (usually short) period of time following randomization. Women with recurrent miscarriage compared to those with sporadic miscarriage have a longer interpregnancy conception interval (i.e. length of time taken for conception to occur in women attempting pregnancy after the miscarriage) (6,7). The pathologic mechanism for this observation is not clear. One possible hypothesis is that fear of miscarriage in a subsequent pregnancy induces significant stress that may adversely influence the hypothalamus and result in subtle ovulatory dysfunction (1). Thus, it is clear that the evaluation of treatment commenced preconceptionally will require many cycles of observation before pregnancy can be achieved. For this reason and for the methodological reasons discussed, women enrolled into such randomized trials should not be withdrawn just because pregnancy has not occurred in the short duration of time.

Female age

Miscarriage – prone women have more pregnancies and have their pregnancies at later age than successful reproducers. Because gravidity is closely linked to female age, it is possible that the increased risk of miscarriage with gravidity, in part, can be ascribed to the effect of maternal age, particularly in view of the fact that chromosomal anomalies are associated with advancing maternal age. It is well established that the risk of miscarriage resulting from trisomic conceptuses increases with maternal age, especially after age 35. Thus, clinical trials of treatment efficacy must take female age into consideration during the design of the trial by using stratification for this covariate.

Effect of male partner

It is well known that some women may have recurrent miscarriages with one male partner and not with another. Therefore, it is important when evaluating treatment efficacy to ensure that the sample is homogeneous from this perspective i.e. consecutive miscarriages with the same partner should be stipulated as an inclusion criterion.

Karyotypic analysis of products of conception

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. The improvement in ultrasonography technology now provides images with better resolution thereby allowing the diagnosis of pregnancy failure to be made much earlier, a process that is assisted with hormone assays. Thus, it is possible to have access to fetal and trophoblast tissue that can be submitted for karyotypic analyses in a non contaminated condition that does not jeopardize the cell culture. Furthermore, improved techniques in cytogenetics have permitted more accurate and reliable assessments of the products of conception. Given these improvements in our diagnostic ability, it is even more important that every effort be taken to study the products of conception in every case of miscarriage in therapeutic trials so that a more valid evaluation can be made on the efficacy of the experimental treatment.

Design issues

The importance of secure randomization, concealment of treatment allocation, adequate sample size, blinding, completeness of follow-up, stratification for important covariates and adequate assessment of outcome are well known to clinical trial methodology and do not require in depth discussion in this paper. It is only by ensuring that a study is valid that one can make confident inferences from the results obtained.

Summary

The problem of evaluating therapies for recurrent miscarriage is compounded by many issues that are relevant to the design of the study. Apart from the obvious study design issues, it is important to select the sample appropriately by clearly defining the population through strict inclusion criteria. Stratification for relevant covariates (especially number of previous miscarriages) must be undertaken ad hoc. Onset of treatment (preconceptionally or post-implantation) should be supported by biological rationale. Sufficient time should be provided for the intervention to have been adequately tested. Post randomization withdrawals should be avoided. The outcome should be clearly documented and every effort should be made to submit products of conception for karyotypic analysis. Only through such a comprehensive approach can the resulting evidence be used appropriately to direct care of couples with recurrent miscarriages.

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Methodological pitfalls in recurrent miscarriage research

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Learning objectives

1) Give knowledge about methodological pitfalls that threaten the validity of recurrent miscarriage research.
2) Give knowledge about how these methodoloical flaws can affect the results of case-control studies, cohort studies and treatment trials, respectively, in recurrent miscarriage research.
3) Provide the participans with the skills to avoid repeating these flaws in their own recurrent miscarriage research.

Lecture summary

Readers of the extensive literature about recurrent miscarriage (RM), most often defined as three or more consecutive pregnancy losses before gestational week 28, often become confused by the opposing and ever-changing views and results that are being published. Generally, the estimates of the investigated risk factors in RM vary significantly from study to study: e.g. within the same population the frequency of the factor V Leiden mutation among RM patients has ranged from 3.7% to 25%. As for the treatment of RM, the views and published results are just as divergent: in controlled trials, successful outcome after paternal lymphocyte immunisation has been reported to range from 46% to 77% and after low-dose aspirin treatment of antiphospholipid (APL)-positive patients from 42-44% to 72% (1). A major reason for the very diverging results in this area of research is the authors' lack of awareness of the series of epidemiological, methodological and statistical pitfalls threatening the validity of RM research and often rendering comparisons between RM studies difficult. In the following, a review of a series of methodological pitfalls is discussed that are important to be aware of in the design, conductance, reporting and reading of studies on the topic of RM. Some of the pitfalls are exclusively a problem in RM research whereas others display a more widespread distribution. If, in the future, more attention is given to these sources of flaws better studies will hopefully be carried out.

Methodological issues of relevance for RM research

Case-control studies

In RM research, case-control studies retrospectively go back in time and compare women who had already been diagnosed with RM (cases) with women who have not developed RM (controls) regarding the frequency of a potential risk factor. An estimate of a potential risk factor's association with development of RM is given by the odds ratio between cases and controls. Below are given examples of flaws that in RM studies can occur during the sampling of cases and controls, respectively. In addition to case-control studies, these flaws can also invalidate cohort studies and treatment trials.

Incorrect RM diagnosis: With regard to RM, women can be erroneously diagnosed as RM patients due to 1) the women's inconsistent information on previous miscarriages, 2) the increased possibilities to identify biochemical pregnancies and 3) the doctor's lack of adhesion to the strict criteria for RM: a history of at least three miscarriages.

Information Bias: Recall or information bias is a difference in the ability and inclination to remember, interpret or report symptoms or exposures in the past between groups of individuals with or without a particular characteristics (e.g. a disease). Due to their increased attention on pregnancy and miscarriage, women with 1-2 previous confirmed miscarriages may be more prone than other women to interpret delayed menstruations in the past and the future as early miscarriages. More miscarriages may also be reported due to the woman's wish to meet the doctors´ criteria for the RM diagnosis and be offered investigations and treatment. Only 71% of miscarriages reported by non-RM women in a questionnaire could be verified in hospital records (2) and in a retrospective study normal women recalled additionally 30 miscarriages out of a total of 507 miscarriages that were not reported in a prospective study several years before (1). To avoid information bias, documentation for previous miscarriages should be obtained from hospitals' or the practitioners' records.

Biochemical pregnancies: With the availability of very sensitive b-hCG tests of urine or blood a diagnostic problem has raised with regard to RM. Biochemical pregnancies (pregnancies only being documented by a positive hCG test) comprise a considerable proportion of RM patients' pregnancies especially among patients referred to or treated in IVF clinics. A significant proportion of these early failed pregnancies are probably spontaneously resorbed ectopic pregnancies in patients with partial tubal damage. The etiologies of biochemical pregnancies are therefore in most cases different from those of clinical pregnancy losses and inclusion of RM patients with a large proportion of biochemical pregnancies in clinical studies is expected to diminish the risk estimate in case-control studies or the treatment effect in placebo-controlled trials (Table 1). To avoid the impact of biochemical pregnancies on the results of RM studies, documentation for the pregnancies of RM patients being intrauterine (histology or ultrasound) should be searched in hospital records.

Inclusion of women with less than three miscarriages: The diagnosis of RM is quite unique because it is based on the occurrence of a number of incidents (miscarriages). The traditional criteria for RM: three or more consecutive miscarriages are thus arbitrary. In many studies women with only two miscarriages are included. However, there is strong evidence that patients with few miscarriages (two) are different from those with many miscarriages (four or more) with regard to etiological factors (1). Two early miscarriages are most likely caused by de-novo fetal chromosome abnormalities occurring twice by chance. On the other hand, the theoretical risk of experiencing RM as a consequence of consecutive chromosome-abnormal miscarriages declines rapidly with the number of pregnancy losses (3) and in accordance with this, the overwhelming majority of abortuses from patients with four or more miscarriages are found to have normal karyotype (4,5). Including women with only two early miscarriages in a study will increase the etiological heterogeneity of the study group and is expected to "dilute" the risk estimate of the studied risk factor (in case-control and cohort studies) or the treatment effect in controlled trials (Table 1).

Problems relating to sampling and testing of patients and controls

In case-control studies, the quality of the control group is just as important as that of the patient group. Sampling of patients and controls is subject to ascertainment bias, confounding, mismatch with regard to important pregnancy related parameters and inadequacy of the testing procedures.
Ascertainment Bias – Patients: Ascertainment or selection bias happens when patients referred to clinics with special interests are deliberately or unconciously selected because of some clinical or

paraclinical feature reflecting the clinic's interest and expertice. With regard to RM, patients subjected to ascertainment bias are therefore not reflecting the general RM population that should be recognised when interpreting the results of the study. A few examples of ascertainment bias in RM studies can be mentioned. Patients who conceive after artificial reproductive techniques (ART) are often mixed with patients who conceive spontaneously. RM patients investigated in fertility clinics comprise an excess of women with infertility problems treated by ART. The causes of the pregnancy failures in RM patients investigated in ART clinics are expected to be partly different from those among women who conceive spontaneously: some are caused by the ART procedure itself (hormonally induced superovulation, the IVF/ICSI and the embryo transfer procedures), some are caused by factors causing the infertility (e.g. spermatozoal DNA abnormalities, hydrosalpinges, polycystic ovarian syndrome) and finally some are spontaneously resorbed ectopic pregnancies in women with partial tubal damage. Another example of ascertainment bias in RM has been described by Out et al. (6). In this study an increased frequency of APL antibodies was found in RM patients referred to a national centre for APL antibody research. However, when patients with a history of thromboembolic or "lupus-like" symptoms were excluded, the prevalence of APLs in the remaining patients did not differ from controls. The high prevalence of the antibodies in the total patient group was obviously resulting from a preferential referral from specialists of RM patients with non-obstetrical symptoms associated with the presence of APL antibodies.

Ascertainment Bias – Controls: "Normal" women are difficult to sample – therefore healthy individuals consulting the hospital for various reasons are often used as controls – they might come for routine prophylactic screening for cervical dysplasia, routine ultrasonic examination during pregnancy or they may be blood donors. Ascertainment of controls by these methods is often biased or subject to confounding. The controls may differ from the RM patients by social class (women from higher social classes are more likely to attend screening programmes) and may thus differ by potential hazardous exposures (tobacco, alcohol, caffeine, medicine). If a control group is selected for being free of every kind of disease (e.g. blood donors), the frequency of the disease gene will probably be estimated to be lower than in the general population and this can render the group less fitted for comparison with patients. Authors and readers must be aware of the problem when extrapolating a study´s results to other patient groups but ascertainment bias probably remains the main reason for the significant variation often found in study results from different clinics.

Confounding: In case-control studies, a confounding factor is a factor that is both associated with the risk factor and the disorder under study. In the case of RM studies, age is an important and common confounding factor. If e.g. the prevalence of an autoantibody is studied in RM patients and controls, age is a typical confounding factor since increased age is associated with both the risk of the disorder (RM) and the occurrence of autoantibodies in the blood (the risk factor).

Control for confounding should be undertaken by appropriate statistical methods or the controls should be matched to the RM patients by e.g. age, social class and potential hazardous exposures. Mismatch for pregnancy related parameters: Matching of RM patients and controls with regard to gravidity, parity and gestation age of current pregnancy is very important when measuring non-genetic parameters but this criteria is often violated. Often blood samples from pregnant RM patients are compared with samples from women who are non-pregnant or in a different stage of pregnancy. The levels of lymphocytotoxic and blocking alloantibodies, autoantibodies including APL antibodies, cytokines and coagulation factors change significantly between the different trimesters of pregnancy or between pregnant and non-pregnant women (1) and many immunogical parameters change after a term pregnancy (7). To avoid mismatch for parameters influenced by pregnancy between patients and controls, controls should be non-pregnant or pregnant concordant with the patients at the time of the sampling of the test material and controls should be comparable as far as possible with the

patients with regard to previous and current pregnancies. Thus, patients with primary RM should ideally be compared to nulliparous women with repeated legal abortions or, second best, to nulligravida. Patients with secondary RM should ideally be compared to women with at least one term pregnancy. With regard to genetically determined factors (e.g. HLA types, thrombophilia-associated mutations) it is obvious that there is no need for the controls to be pregnant/non-pregnant corresponding to the patients. In these cases controls could be individuals free of the disease being investigated – with regard to RM, multiparous women without miscarriages are optimal.

Testing procedures: In many studies parameters measured in peripheral blood or locally in the uterus are compared between RM patients during the process of miscarrying or just after uterine aspiration and are compared with corresponding parameters in women undergoing a legal abortion (8). Such comparisons are prone to produce invalid results since miscarriage may induce an inflammatory reaction and decline in hormone levels that locally or systemically can affect parameters such as cytokines, lymphocytes, antibodies and coagulation parameters. Abnormal findings after a miscarriage (relative to the findings after a legal abortion) may therefore represent alterations resulting from the process of miscarriage and not being a cause of miscarriage.

It is advisable to be very careful in stating causality from abnormal findings in samples taken during and just after miscarriage. The best evidence of causality comes when samples taken in good time before miscarriage are compared with samples taken before a legal abortion.

Multiple testing: In many papers an overwhelming series of statistical comparisons are carried out (> 20) and no information from the protocol (if any was made!) is given about what was the prior hypothesis being tested. Defining statistical significance as a P value < 0.05 will result in every 20th statistical comparison being significant by chance. Without a clearly defined prior hypothesis statistical significant post-hoc findings should not be overestimated. To avoid flaws due to multiple testing, the primary and secondary end-points for the study should be clearly stated in the protocol and in the “materials and methods” and only significant findings concerning these end-points should be given full emphasis. New and unexpected significant findings after multiple comparisons should be subjected to the Bonferroni correction (multiplication of the p-value with the number of comparisons undertaken). Significant findings after multiple testing should be confirmed in new and independent studies before full emphasis can be put on the finding.

Cohort studies

With regard to RM research, a cohort study begins by identifying a group of women that possesses a potential risk factor for miscarriage and an otherwise similar group of women without the factor. The groups are observed during a defined time range and the frequency of miscarriage or getting a RM diagnosis is compared between the two groups by calculating the relative risk of miscarriage or RM in women with the risk factor relative to those without the factor. The greatest treat to the validity of a cohort study in RM research is known as differential misclassification

Differential misclassification: Differential misclassification is a biased intensity of monitoring of the exposure to risk factors or outcomes in the different cohorts. Biased intensity of monitoring is a problem in research in human reproduction. Due to the standard procedures in most ART clinics of measuring serum b-hCG 14 days after embryo-transfer more biochemical pregnancies will be registered in cohorts of ART patients compared with women who conceive spontaneously.
An example of the impact of biased intensity of monitoring of pregnancy outcomes in two cohorts of RM patients is shown in Fig. 1. Cohort 1 comprised 89 Swedish RM patients who met the inclusion criteria for a placebo-controlled trial of treatment with intravenous immunoglobulin (IvIg)

and were included in the trial in the next pregnancy when fetal heart action could be demonstrated by ultrasound in gestational week 6-8 (9). Cohort 2 comprised 68 Danish RM patients who agreed to participate in a placebo-controlled trial of IvIg trial when they became pregnant (5), however, unlike in the Swedish trial, the patients were immediately included in the trial as soon as the pregnancy test was positive. In the Swedish study, 50.6% of the patients were classified as not achieving pregnancy and only 6.8% of the pregnant patients had pre-embryonal miscarriages before week 6. In the Danish trial only 14.7% did not report pregnancy in the study period but 22.4% of the 58 pregnancies were classified as pre-embryonal because they happened before fetal echoes could be demonstrated by ultrasound. This very big difference in the frequencies of non-conception and pre-embryonal pregnancies between the two trials reflects the different intensity of monitoring pregnancy outcomes in the two trials. To avoid differential misclassification, rigid and well-defined procedures of monitoring and registration of exposures and outcomes must be carried out.

Treatment trials

With regard to RM, a treatment trial is a study where the pregnancy outcome in women given a specified treatment in the present pregnancy is compared with a control group not receiving this treatment. Sometimes the control group comprises historical controls and sometimes patients are prospectively allocated to non-treatment or another treatment, in the latter case we are talking about a randomized controlled trial.

Historial controls: A typical pitfall in trials of treatment effect is the use of historical controls. In 1938, Malpas stated that the risk of subsequent miscarriage in untreated RM women was 73%. This very high rate of miscarriage was for almost half a century undisputed and any intervention in RM patients followed by a live birth rate of 50-70% (which may reflect the spontaneous outcome) was considered as suggesting that the treatment had had a positive effect. A variant of the use of historical controls is the use of the treated patients as their own controls. In RM research the pregnancy success rate in RM patients before some kind of intervention is often compared with the success rate after the intervention. The spontaneous prognosis for a successful pregnancy is good for the majority of patients with RM and they may have experienced three miscarriages by sheer chance. These patients have an excellent spontaneous prognosis in spite of their poor past reproductive performance - a phenomenon called “regression to the mean” (1). In women included in a trial due to RM, the accumulated past pregnancy success rate will often be < 10%. Comparing this low pre-treatment success rate with an 85% post-treatment success rate (e.g. after cerclage) will not require many patients for obtaining statistical significance, however, this apparent improvement of outcome can completely be attributed to the effect of “the regression to the mean” (1). By using these methods all kind of interventions, also placebo, can be proved to be highly efficient in the treatment of RM (10). In conclusion, historical data of pregnancy outcome cannot be used for comparison in treatment trials neither external data nor data from the treated patients´ previous reproductive histories. Proper evaluation of interventions in RM can only be done in double-blinded placebo-controlled trials.

Premature stop of trial due to results of interim-analyses: Often repeated interim analyses are performed during a placebo-controlled trial and the trial is stopped when a significant result for or against a treatment effect is reached. However, there is a great risk that the trial will be concluded at a point where the difference between the allocation groups for a short moment by random has reached an extreme fluctuation (1). The result at this stopping point is not likely to be typical for the result if the study was conducted to the end.

Inclusion of several pregnancies from the same RM patient: In case-series and controlled treatment trials several pregnancies from the same patients have often been included. The commonly used statistical methods such as the c2-test require that tested variables are independent. Pregnancy

outcome in the same women are not independent variables and thus the common statistical methods cannot be used. Therefore only one pregnancy per patient must be included in treatment trials.

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Legend to figure 1:
Distribution of women who did not become pregnant, had pre-embryonoic pregnancy losses or embryonic pregnancy losses or live births among recurrent miscarriage patients who were included in two placebo-controlled trials of intravenous immunoglobulin.
Cohort 1: 89 patients from the study by Jablonowska et al. (1999) and Cohort 2: 68 patients from the study by Christiansen et al. (2002).

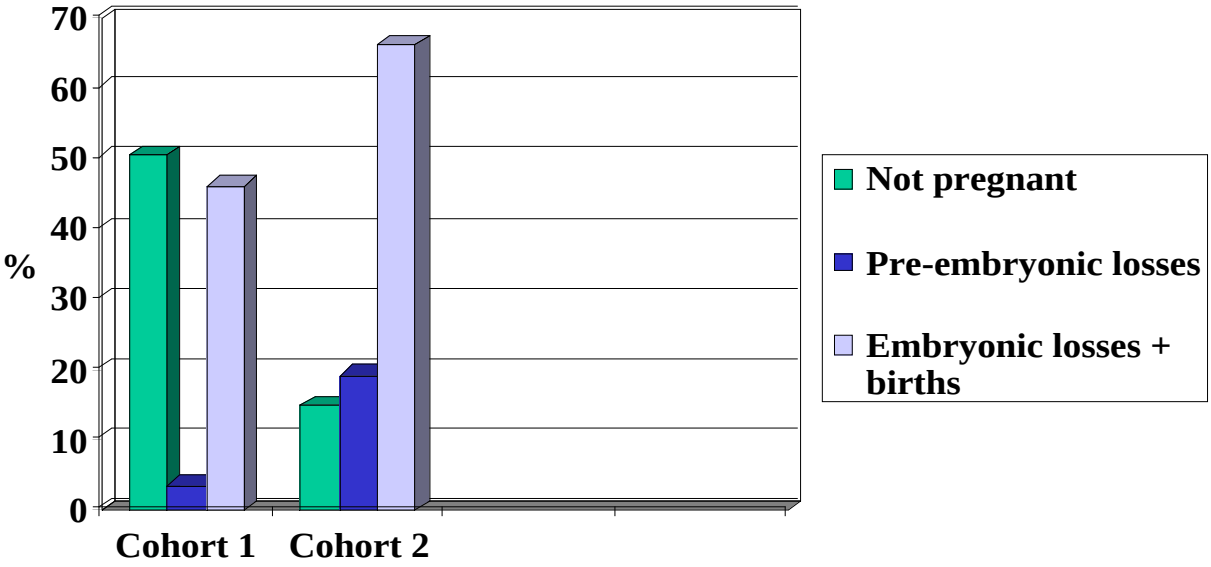

Table 1
Methodological flaws to evaluate in studies of recurrent miscarriage (RM).

Methodological flaw	**Effect on study outcome**
Definition of RM as > 2 miscarriages	Decreases difference in frequency of factor studied in CCS[a] or treatment effect in RCT[b]
Ascertainment bias	Increases prevalence of factor studied
Selection of controls	Increases difference in prevalence of factor studied in CCS or treatment effect in RCT
High age of patients	Increases aneuploid conceptions and thus treatment effect in RCT
Uneven monitoring of two cohorts studied	Increases difference in prevalence of factor
Historical controls	Increase treatment effect in treatment trials
Poor characterization of miscarriage and subgroups of RM	Render comparisons between studies and meta-analyses difficult
Premature termination after interim analysis	Decreases or increases treatment effect in RCT
Inclusion after detection of fetal heart action	Decreases treatment effect in RCT
Inclusion of biochemical pregnancies	Decreases difference in frequency of factor studied in CCS and treatment effect in RCT

a Case-control studies; b Randomized controlled trials

Cytokines and immune cells in recurrent miscarriage

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Learning Objectives

To gain an understanding of the recent literature on the role of selected cytokines and immune cells in recurrent miscarriage

To appreciate the complexity of the immune networks within the placenta and the difficulties of studying immune mechanisms in recurrent miscarriage

To appreciate the importance of compartment and time when studying immune mechanisms of pregnancy loss.

Introduction

The aetiology in approximately 50% of women with recurrent miscarriage is unknown, but it is thought that a proportion of these repeated losses may be due to immune causes. During pregnancy the female reproductive tract is exposed to paternal antigens expressed in the developing fetus. This would normally result in a maternal immune response and destruction of the "foreign" tissue. In successful pregnancies this does not occur. Although the mechanisms that prevent rejection are not clearly understood, it is postulated that one reason for recurrent pregnancy loss may be the breakdown in these protective mechanisms.

Models used to study recurrent miscarriage in women

In women with RM, pregnancy loss usually occurs during the first trimester. Obtaining placental tissue prior to the recognition of pregnancy loss in humans is not possible and therefore the mechanisms of abnormal development which may result in miscarriage are difficult to study. Various alternative approaches have been adopted. These include analysis in 1) peripheral blood of women with RM and fertile women either before or during pregnancy; 2) endometrial tissue from women with RM and fertile women in the peri-implantation period; and 3) placental tissue obtained at the time of miscarriage from women with RM, women with spontaneous, non recurrent miscarriage and women requesting terminations of normal pregnancy. While the study of placental tissue might appear to be the best approach, there are difficulties in determining whether observed differences are due to pro-inflammatory events as a consequence of the miscarriage. Numerous studies have been carried out using animal (particularly mouse) models of recurrent pregnancy loss. However care needs to be taken in extrapolating data directly from rodents to humans as the mechanisms of pregnancy loss may be different.

Cytokines and recurrent miscarriage

Th1 and Th2 cytokines and recurrent miscarriage

Cytokines can be divided into two groups depending on their major function. Th1 cytokines, include IFN_, TNF_ and IL2 and control cell-mediated immune responses. Th2 cytokines, include IL4, IL6 and IL10 and control antibody mediated responses. Studies in rodents have suggested that successful pregnancy outcome is associated with a predominant Th2 cytokine profile and that Th1 cytokines are detrimental to pregnancy outcome. Matings between CBA/J female and DBA/2 male mice result in a high number of abortions. Placental and peripheral blood lymphocyte production of Th1 cytokines is higher in these mice than in matings between CBA/J and BALC/C mice, which result in normal pregnancy outcomes. Injection of TNFa, IFNg or IL2 into non-abortion prone mice results in increased abortion rates, while injection of IL10 (a Th2 cytokine) into abortion prone mice decreases abortion rates (1).

An increase in the Th2/Th1 cytokine ratio during pregnancy in humans is suggested by the fact that diseases driven by a Th1 cytokine response, such as rheumatoid arthritis, are ameliorated during pregnancy, while diseases driven by a Th2 cytokine response, such as systemic lupus erythematosus, are increased. Increased production of IL10 and IL4 and decreased production of IFN_ and TNF_ by stimulated peripheral blood mononuclear cells (PBMCs) from pregnant women compared to non-pregnant women has also been shown (2).

The evidence supporting a role for Th2 cytokines in preventing pregnancy loss in humans is more controversial. Studies comparing peripheral blood Th1 and Th2 levels are summarised in Table 1 and show conflicting results which are probably due to the timing of the blood sample and whether the sample was taken at the time of miscarriage.

Cytokines in the maternal blood will have less access to trophoblast cells than cytokines produced by placental cells. However, there are few reports on placental cell production of cytokines in women with RM, probably because of difficulties in obtaining tissue. One study has shown significantly lower production of IL4 and IL10 by stimulated T-cell clones isolated from decidua from RM women during miscarriage compared to stimulated decidual T-cell clones from women undergoing terminations. However, these cells underwent considerable in vitro manipulations (cloning and artificial stimulation) before cytokine measurement (7).

A study comparing cytokine mRNA expression in the intact endometrium of fertile and RM women during the peri-implantation period showed that fewer women with RM had detectable levels of IL6 in their endometrium, but more had detectable levels of TNFb, IFNg, IL2 and IL12, compared to fertile women (8). Other studies have shown a decrease in the number of IFN_ and TNF_ producing CD4+ cells in the endometrium of women with RM compared to controls (5). Cytokines are also produced by endometrial epithelial and stromal cells which will be included in the earlier study; this might account for the differences in the results of the two studies.

Proinflammatory cytokines

IL11, LIF, IL18 and IL12 are proinflammatory cytokines and may play a role in successful pregnancy outcome.

LIF and IL11

Implantation does not occur in LIF knock-out mice although transfer of LIF negative blastocysts to wild-type mice results in normal implantation and pregnancy outcome. Decreased expression of LIF in the endometrium of non-pregnant RM women has been shown (10), while isolated stimulated decidual T-cell clones obtained during miscarriage from RM women produced less LIF than stimulated decidual T-cell clones from women undergoing terminations (7). In IL11 receptor _ chain (IL11R_) knock-out mice blastocyst implantation occurs, but only small decidua form and this results in pregnancy loss. A decreased expression of epithelial cell IL11, but not IL11R_ in the endometrium of RM women compared to controls has also been shown (11).

IL18 and IL12

IL18 and IL12 promote a Th1 response mainly through induction of IFN_. High levels were therefore expected to be associated with pregnancy loss. However the placenta of mice from abortion prone matings (CBA x DBA/2) produce less IL18 than those from non-abortion prone matings (CBA x BALB/c). Studies in women have suggested that abnormal endometrial expression of IL18 is associated with infertility (12) and that non-pregnant IL18 plasma levels are lower in women with RM compared to fertile women (13). IL12 is produced by the murine fetal placental unit and co-injection of IL12 and IL18 into mice induces abortion Abnormal endometrial IL12 production is also associated with infertility (12). Higher plasma levels of IL12 have been shown in non-pregnant RM women compared to controls (5), but another study has shown no differences in plasma IL12 levels in women undergoing terminations and spontaneous miscarriage (14).

Limitations of cytokine studies

Many studies have concentrated on production of cytokines by immune cells particularly T-cells. However cytokines are also produced by endometrial epithelial and stromal cells and by decidual and cytotrophoblast cells of the placenta. In addition cytokines are designed to act locally. Therefore measurements of amounts present in the feto-placental unit post-implantation are of greater significance than measurements in peripheral blood, or measurements prior to implantation.

Immune cell populations in women with recurrent miscarriage

The human endometrium and decidua contain a unique population of lymphocytes which consist mainly of uNK cells, macrophages and T-cells. In contrast to those in peripheral blood the majority of uNK cells express large amounts of CD56, but are CD16 negative. There is also a small population of CD16+ cells which express small amounts of CD56 (CD56dimCD16+ cells). While the number of macrophages and T cells in the endometrium and decidua remain fairly constant the numbers of CD56+ cells increase in the secretory phase of the menstrual cycle and during the first trimester of pregnancy (15).

CD56+ Cells

Because of their name, the fact that they show cytolytic activity and that there are high numbers present in the placenta of abortion prone mice, it is often assumed that there will be high numbers of CD56+ cells in women with RM and that they are the cause of miscarriage. However, the evidence for a relationship between CD56+ cells and RM is not conclusive (16).

Several studies have shown increased numbers of CD56+ NK cells in the peripheral blood of women with RM either prior to, or during pregnancy compared to non-pregnant or pregnant controls and that numbers of peripheral blood CD56+ cells can predict pregnancy outcome in women with RM

(10). Other studies have shown that high numbers of CD56+ NK cells are only seen in pregnant RM women with chromosomally normal fetuses suggesting that the presence of high CD56+ numbers are a cause rather than effect of miscarriage (17).

Although NK cells in the blood will have contact with the syncytiotrophoblast cells they will have less contact with the developing fetus than decidual uNK cells. In contrast to the increased numbers of CD56+ cells in peripheral blood a decreased number of decidual CD56+ NK cells are reported in miscarried placental tissue from RM women compared to tissue from spontaneous miscarriages and women requesting terminations (18).

The non-pregnant endometrium of women with RM is reported to contain higher numbers of CD56+ cells than control women and lower numbers are reported in women with RM who subsequently had a live birth compared to those who miscarried (19). In contrast other studies have shown similar numbers of CD56+ cells in the endometrium of women with RM and control subjects, although in one study, women with RM had increased numbers of endometrial CD56dim CD16+ cells compared to controls (20,9).

These results suggest that alterations in numbers of CD56+ cells in may be present in women with RM. However, whether these are increased or decreased depends on the compartment which is sampled. CD56+ cells comprise <10% of peripheral blood leucocytes and therefore these changes may not be significant to total peripheral blood cell activity. The decreased numbers of CD56+ cells in the decidua and possible increased numbers in the endometrium is difficult to explain, but may be due to the presence of two different populations of CD56+ cells (CD16+ or CD16-). More studies are required to determine the role of decidual and endometrial NK cells in recurrent pregnancy loss and to define normal numbers of these cells.

CD3+ T Cells

Several studies have shown no differences in the numbers of CD3+ T cells in either the peripheral blood or the endometrium prior to pregnancy or in the decidua in early pregnancy in control and RM women. T-cells can be classified according to protein components of their CD3 receptor. The majority of T cells express the ab receptor but some express gd. Large numbers of gd T cells are found in the decidua of abortion prone mice. The ratio of specific sub-populations of peripheral blood gd T cells (Vg1,Vd1 to Vg9Vd2) is reported to be different in pregnant women with RM compared to controls (10). Endometrial CD4+ and CD8+ T cell subsets have also been investigated and studies have shown a shift towards a higher CD4+/CD8+ ratio in endometrial biopsies from women with RM (19). Thus although there appears to be no differences in the total T cell numbers in women with RM there may be differences in sub-populations of T cells.

Macrophages

No significant difference in the number of macrophages in first trimester decidua from women with RM compared to decidua from spontaneous abortions and controls have been found (18). However an increase in the number of macrophages in the non-pregnant endometrium of women with RM compared to controls has been reported (19).

Cell activation markers

Differences in the absolute numbers of leukocytes may not reflect differences in the activity and function of the cells. The activation status of both T cells and CD56+ cells has been investigated by

measurement of expression of CD25 (IL2 Ra) and CD69 activation markers. An increased number of CD25+ cells have been shown in the first trimester decidua of women with RM with chromosomally normal fetuses compared to decidua from elective terminations and women with RM with chromosomally abnormal fetuses (18). Increased expression of CD69 on peripheral blood CD56+ cells of women with unexplained RM compared to controls has also been reported (21). Further work is required on the activity of these cells in women with RM, particularly the investigation of functional parameters such as cytolysis and secretory activity.

Conclusions

The immune cell interactions which occur during implantation and which may be abnormal in women with unexplained RM are still not clearly understood. One of the problems in this field is the amount of contradictory findings. Some of this variation occurs due to difficulties in obtaining the optimum tissue. Others may be due to differences in study design. Factors which might account for differences are the compartment (peripheral blood, endometrium or decidua) which is sampled. The measurement of factors such as cytokines (which are known to act locally) in peripheral blood may have little significance as this compartment has less contact with the trophoblast than the endometrium, or decidua. In addition the peripheral blood cell population is considerably different to that in the endometrium and decidua. The timing of sampling is also important, both with respect to the point in the menstrual cycle and pregnancy and whether it is at the time or just after miscarriage, as both of these factors will affect the expression of cytokines and cell number and activity.

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Table 1. Studies on peripheral blood levels of Th1 and Th2 cytokines in RM and control women

Reference	Findings	Time of sampling	Comments
Raghupathy et al. 2001 (3)	Decreased Th2 and increased Th1 cytokine production by stimulated PBMCs from RM women	First trimester miscarriage and delivery (controls) First trimester miscarriage and first trimester on-going pregnancy	Different times and presence of miscarriage may affect cytokine production
Hill 1995 (4)	Production of TNF_ and IFN_ by stimulated PBMCs from RM women. Production of IL6 by stimulated PBMCs from control women.	Prior to pregnancy	Only seen in women with unexplained RM with chromosomally normal fetuses.
Wilson et al. 2004 (5) Dahar et al 2004 (6)	Increased plasma IFN_ or increased PBMC IFN_ production in women with RM	Prior to pregnancy	
Bates et al. 2002 (2)	Increased IL4 and IL10 and decreased IFN_ and TNF_ production from PBMCs from RM women	Early pregnancy before miscarriage and gestationally matched controls	Opposite findings to others, but only study to investigate pregnant women prior to miscarriage.

Cytokine gene polymorphism in current miscarriage

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NOTES

The role of HLA-G in recurrent miscarriage

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Learning objectives

-To gain knowledge about the structure and genes of the Human Major Histocompatibility Complex (MHC)
-To understand the specific antigenic composition of the maternal-fetal interface of the placenta
-To learn about the specific characteristics of the non-classical MHC gene HLA-G and its role in maternal-fetal immune tolerance
-To be able to judge on the potential relevance of HLA-G in recurrent pregnancy loss on the basis of potential mechanisms of HLA-G action in early pregnancy

Lecture Summary

Structure and genes of the human MHC

The human Major Histocompatibility Complex (MHC) is a group of approximately 200 genes located on the short arm of chromosome 6 which can be subdivided into three different regions, MHC-class I, II and III. The highly polymorphic Human Leukocyte Antigens (HLA) which are essential for immune recognition, development of immune competence and interaction with lymphocytes are encoded by approximately 40 genes of the MHC class I and II regions. In contrast, the MHC class III region contains non-HLA genes which are partially involved in immune interactions. HLA-class I genes are expressed on virtually all cells of the human body, whereas HLA- class II molecules are mainly expressed on immune cells.

The MHC class I region contains three highly polymorphic classical HLA-gene loci , HLA-A, -B and –C and three additional gene-loci with as yet unknown functions, HLA-E, -F and -G . Those loci show high homology with classical HLA-loci as far as nucleotide sequence and gene structure are concerned but differ in their degree of nucleotide and amino acid polymorphism and expression profile and are thus called non-classical HLA-genes.

The non-classical MHC-gene HLA-G

HLA-G is a nonclassical HLA class Igene. Although structurally similar with classical HLA-class I gene products, it exhibits only a low degree of polymorphism (Kirszenbaum et al, 1999). However, the HLA-G transcript is alternatively spliced into at least seven membrane-bound and soluble isoforms, which guarantees a certain degree of variability on the mRNA and protein level. HLA-G was found to be the major HLA-antigen on extravillous cytotrophoblasts at the maternal-fetal interface (Ellis et al, 1990; Kovats et al., 1990). Although transcripts could be identified in several

fetal and adult tissues (Carosella et al., 1996), HLA-G protein expression is restricted to extra-embryonic tissues and membranes (McMaster et al., 1995; Loke et al., 1997). The HLA-G molecule seems to maintain a broad panel of functions like antigen presentation (Lee et al, 1998), interaction with and inhibition of natural killer cells (King et al., 1998, Colonna et al, 1999, refs), regulation of chorionic angiogenesis (Le Bouteillier, 1999) and other immune interactions (Bainbridge et al, 2000; Kapasi et al, 2000, Moreau et al, 1999).

The limited expression and functional specification triggered the speculation that HLA-G has a key role in maintenance of pregnancy but may also be involved in the pathogenesis of pregnancy-associated disorders like preeclampsia or recurrent spontaneous abortions.

Antigenic composition of the maternal fetal interface

The HLA-antigen expression profile of the placenta differs significantly from other body tissues. The highly polymorphic classical HLA-class I and II antigens are not expressed at the maternal-fetal interface with the exception of HLA-C, which can only be detected in low amounts. Extravillous cytotrophoblasts, the cells which are in direct contact with maternal decidua and blood vessels, express the nonclassical class I antigens HLA-G and HLA-E (Ellis et al, 1990; Kovats et al., 1990, King et al, 2000). However, HLA-G is the dominant molecule at the fetal-maternal interface. Although the exact functions of these molecules in the placenta have not been fully elucidated, they seem to participate in an intrinsic network that guarantees the survival of the fetal allograft.

Studies on HLA-G and recurrent spontaneous abortions (RSA)

Research on the potential role of HLA-G on the etiology of recurrent spontaneous abortions has been pursued under different pathophysiological concepts.

The HLA-system as major determinant of tissue acceptance or – rejection in transplantation medicine has been implicated in research of recurrent miscarriage under the concept of the fetus as a semiallograft.

For classical HLA-antigens, it was initially suggested that maternal recognition of paternally derived HLA is required or may be beneficial in normal pregnancy (Beer and Billingham, 1974). As a result, early studies on classical HLA in recurrent spontaneous abortions were based on the hypothesis that increased HLA similarity between partners would lead to inadequate maternal protective immune responses and fetal loss. This hypothesis was also applied to initial studies of the dominant trophoblast antigen HLA-G and the co-expressed HLA-E and –C and recurrent abortion. However, studies on polymorphisms of HLA-C and -E or sharing of HLA-C or –E alleles in patients with recurrent pregnancy loss could not provide evidence for a key role of those antigens in the pathogenesis of recurrent abortion (Christiansen et al., 1997; Steffensen et al., 1998; Kanai et al., 2001; Imai et al., 2001; Pfeiffer et al., 2001).

In contrast to classical HLA-class I loci, only few alleles including the null-allele G*0105N, could be identified for HLA-G (Suarez et al., 1997). Several studies have investigated the potential role of HLA-G in the pathogenesis of habitual abortion based on the concept of immunological effects of the different HLA-G alleles in analogy to classical HLA. Earlier studies which were based on RFLP typing and thus did not discriminate between the different HLA-G alleles, did not find HLA-G to be involved in recurrent pregnancy loss (Steffensen et al., 1998; Karhukorpi et al., 1998;Yamashita et al., 1999). More recent studies based on molecular determination of HLA-G alleles and HLA-G nucleotide polymorphisms suggest that women with recurrent spontaneous

abortions who carry specific HLA-G alleles may have lower chances for a successful pregnancy (Pfeiffer et al., 2001; Aldrich et al., 2001). Those results have not been confirmed in a third study in a Danish population (Hviid et al., 2002) and the pathophysiological basis of those allele-associations has yet to be clarified.

Clinical data on HLA-G*0105N homozygotes provided the important information that the major membrane-bound isoform of the HLA-G protein, HLA-G1, is not essential for fetal survival (Ober et al., 1998; Castro et al., 2000). Nevertheless, serum levels of soluble HLA-G seem to be a predictor of pregnancy success and decreased HLA-G expression in trophoblast has been described in spontaneous abortions (Athanassakis et al., 1999; Pfeiffer et al., 2000; Emmer et al., 2002). Furthermore, embryos expressing or secreting HLA-G seem to have a better chance to implant (Jurisicova et al., 1996; Fuzzi et al., 2002, Sher et al 2005). Those data provide some evidence that HLA-G or some of its soluble isoforms, although not essential for fetal survival, may still influence pregnancy development.

More recent investigations focussed on potential consequences of HLA-G polymorphisms for molecule function and the amount of available HLA-G protein. For example, HLA-G polymorphisms in the coding and non-coding region are associated to differences in the expression levels of HLA-G mRNA and the pattern of alternatively spliced HLA-G mRNA isoforms (Rebmann et al., 2001; Ober et al., 2003; Hviid et al., 2004), which in turn determine the absolute levels of functional HLA-G protein. Indeed, polymorphic variants in the upstream regulatory region and the 3'untranslated region of the HLA-G gene were found to be associated with an increased risk for miscarriage (Ober et al., 2003; Hviid et al., 2004). Thus, although the major isoform of HLA-G, HLA-G1 is not essential for fetal survival , the absolute amount of functional HLA-G protein or the relative levels of its functional isoforms may partially determine the likelihood of a successful pregnancy.

Conclusions

HLA-G is the dominant HLA-antigen at the maternal-fetal interface and has thus been investigated thoroughly with regard to its role in the etiology of recurrent pregnancy failure. Whereas initial studies aimed at potential immunological effects of HLA-G alleles or HLA-G sharing between partners, more recent studies focussed on consequences of HLA-polymorphisms for molecule function and /or the amount of HLA-G mRNA and –protein. Although it has been demonstrated that the major membrane-bound isoform of HLA-G is not essential for fetal survival, current data suggest that the functional level of HLA-G protein or the expression pattern of its isoforms may still determine pregnancy outcome and especially the risk of recurrent miscarriage.

Mannan-binding lectin and recurrent miscarriage.

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Learning objectives

_ To understand the biochemistry and genetics of mannan-binding lectin (MBL) in outline
_ To evaluate the putative association between MBL and recurrent miscarriage
_ To appreciate the limitations of disease associations studies based on statistical analyses

Lecture summary

Recurrent miscarriage (RM) is a complex and heterogeneous disorder. There is some evidence for a genetic component to RM, but recurrent embryonic/fetal death clearly does not follow a simple pattern of inheritance; indeed, it is likely to be a multifunctional condition to which several unrelated genetic components may be relevant [1]. These include the HLA system which has received much attention. Another relevant immunogenetic factor is mannan-binding lectin (MBL), also known as mannose-binding lectin [2-5].

MBL is a collectin, a member of a subfamily of animal lectins [6], 4-domain proteins possessing both a collagen-like region and a C-type carbohydrate recognition domain. Along with surfactant proteins A and D, MBL is one of three similar collectins encoded on human chromosome 10, and (with L-ficolin) is one of two major soluble pattern recognition proteins in the circulation. MBL polypeptides (Mr = 25K) combine to form a basic triplet subunit through the formation of a collagen-like triple helix and covalent attachment near the N-terminus. This basic subunit has a genetically-determined capacity to form oligomers, from dimers up to hexamers. It is only the higher oligomers (trimers upwards) that has the capacity to activate complement through the lectin pathway of complement activation and to bind carbohydrate structures with high avidity. Usually, when MBL is measured in serum by enzyme-linked immunosorbent assay (ELISA), it is mainly the higher oligomers that are detected. On that basis, apparent MBL concentrations in healthy individuals vary approximately 2000-fold. Although many individuals have substantial MBL protein circulating in the form of monomeric subunits, and these may possess some biological activity, those described as MBL deficient (about 10% of the healthy population) or MBL insufficient (about 40% of the population) are actually those individuals lacking functional higher oligomers of MBL. MBL deficiency/insufficiency was initially associated with an increased susceptibility to infections in childhood, but there are now numerous clinical studies providing circumstantial evidence that MBL can act as a disease modifier in a wide variety of clinical settings [2-6].

The genetic basis of MBL deficiency or insufficiency is largely dependent on dimorphisms in the first exon of the 4-exon structural gene. The normal or wild-type allele (denoted A) at codon 54 encodes glycine, but a point mutation (B) results in the substitution of an aspartic acid residue. This is the commonest cause of low MBL concentrations in Europeans. However, similar point mutations can occur at codon 52 (D mutation; arg_cys) and codon 57 (C mutation; gly_glu). Individuals who are homozygous for structural gene mutations have very little functional MBL; heterozygotes have

approximately 10% of the functional MBL found in wild-type individuals.

Point mutations also occur in the promoter region. Their influence is more modest, but one of them (the X variant) has a significant effect on circulating MBL: X variant homozygotes have MBL concentrations comparable to structural gene heterozygotes. There is also a genetic dimorphism (P/Q) in an untranslated region of the structural gene, but it has a modest influence on concentration. There are strong linkage disequilibria between promoter, P/Q and exon-1 variants. Consequently, only nine haplotypes have been described so far. Of those the commonest are HYPA, LYQA (both high MBL producers), LXPA (a medium MBL producer) and LYPB (a low MBL producer). Of course, each individual inherits a genotype consisting of two haplotypes. For example, the commonest genotype, HYPA/HYPA is associated with normal (high) circulating MBL, while HYPD/LYPB is associated with barely detectable serum MBL. It must be stressed, however, that while a strong and predictable correlation between genotype and serum MBL exists at the population level, one cannot predict an individual's MBL concentration with confidence from his genotype. Indeed, some surprising discrepancies have been found in some subjects. Some disease association studies relate serum MBL to clinical features; others infer MBL insufficiency from genotyping or the presence of allelic variants; and some studies use both DNA and protein-based data.

An association between low serum MBL concentrations and RM was was first reported in Scottish couples in 1995 [7]. The relationship was equally strong for male and female partners, and it was suggested that a deficiency of MBL within the feto-placental unit could be important. That was supported by detection of MBL by immunohistochemistry in various cells within first trimester placental sections. It was conjectured that fetal inheritance of mutant MBL alleles would result in an increased susceptibilty to intrauterine infections, possibly resulting in a shift in the Th-1:Th-2 type cytokine balance.

This association was subsequently investigated in a Danish cohort [8]. The association was confirmed for female partners, and indeed a significant correlation between the frequency of MBL deficiency in RM women and the number of their previous miscarriages was found. No corresponding relationships were found with the male partners, but samples were available from relatively few men.

Studies on both Scottish and Danish patients were subsequently extended. Amongst Scottish subjects, MBL deficiency in male and female partners was confirmed as a risk factor, and a cut-off level of 100 ng/ml was established as the most appropriate. These data were also used to predict that genotyping would be a much less sensitive means of identifying patients at risk. An extended Danish study found no evidence for male involvement in disease susceptibility, but confirmed the association with female partners and the appropriateness of a 100 ng/ml cut-off level. Moreover, and most importantly, women with [MBL] < 100 ng/ml who went on to have further pregnancies had a significantly lower live birth rate than women with [MBL] > 100 ng/ml and, if successful, produced babies with lower birth weights.

The extended Danish study also analysed MBL genotypes in relation to pregnancy outcome.There was a significantly higher frequency of low MBL-conferring genotypes in women with a history of ≥4 miscarriages, but the frequency in the group as a whole did not reach statistical significance. The prediction that any relationship with genotyping would be statistically weaker was thus realised. These findings were therefore consistent with another, smaller study of RM patients in London which found no association between MBL genotype and RM [11].

All the above studies were hospital-based and relatively small. In contrast, Dahl et al [12] have

carried out a large population-based study of morbidity and mortality from infectious and other disorders in the adult Danish population. They found no statistically significant difference between genetically-defined MBL-insufficient individuals and controls in relation to infectious diseases or mortality and concluded that MBL deficiency is of little clinical importance in the general population. Nevertheless, the strongest association of homozygosity for mutant MBL alleles with morbidity leading to hospitalization was with miscarriage (relative risk 1.7; p=0.1). That compares with a relative risk of 1.1 (p=0.47) for pneumonia, an infectious disease generally believed to be associated with MBL deficiency.

In conclusion, the present evidence indicates that there is a genuine association between very low serum MBL in women and spontaneous abortion and that [MBL]
< 100 ng/ml has predictive value. Furthermore, the primary association is with plasma protein rather than at the DNA level and therefore is unlikely to arise from linkage to a separate abortion gene on chromosome 10. Any association between RM and paternal serum MBL, however, is uncertain. MBL deficiency is likely to be one of several factors making a modest contribution to miscarriage susceptibility, but direct proof requires the application of MBL replacement therapy in this condition.

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Note

Reference 1 provides a comprehensive introduction to factors that could be relevant to pregnancy outcome, including immunogenetic factors. References 2-5 are general reviews detailing the biochemistry, genetics, clinical significance and therapeutic potential of MBL. Reference 6 is a monograph on animal lectins which would help the reader to place MBL and the collectins in a broader context. References 7-12 comprise the original literature on which the lecture is based.

Evidence-based investigations in early pregnancy failure

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Learning Objectives

The learning objectives of this lecture are;

To accurately classification early pregnancy failure using;
- gestation,
- ultrasound findings,
- karyotype results
- morphological appearance of fetus

To categorize the pregnancy loss into that of embryonic and that of maternal aetiology.

To use investigations appropriate to the type of pregnancy loss.

Introduction

With the frequent use of high resolution ultrasonography and widespread access to sophisticated genetics laboratories there is a need to impart detailed information regarding the type and cause of pregnancy loss to patients. The clinician needs to use all information available to them in order to accurately inform the patient of the cause of the pregnancy demise and target investigations appropriately.

Classification of early pregnancy failure

Defining terms

The term miscarriage has replaced "abortion" to describe spontaneous pregnancy loss following pressure from patient groups that say that for the patient the term "abortion" is confuses therapeutic termination of pregnancy and spontaneous pregnancy loss. Miscarriage is not a precise diagnosis at it can mean a range of pregnancy loses from a raised human chorionic gonadotrophin (HCG) level in the absence of ultrasound confirmation of pregnancy, the demise of an empty sac to extreme preterm labour at 23 weeks gestation. A suggested classification is delineated in Table 1(Dawood et al., 2004).

Was the loss of a normal or abnormal pregnancy?

Ultrasound

High resolution ultrasonography is an extensively used tool to detect early pregnancy demise. However, information from an ultrasound scans is underutilised as it should also be used to categorize the type of demise (Table1) and to detect the following fetal structural anomalies.

– Nuchal translucency/cystic hygroma which are associated with
• Down's and Turner's syndromes and Cardiac defects
– Body stalk defect
– large body wall defects
– Dwarfism (lethal)
– anencephaly
– encephalocele
– holoprosencephelopathy associated with
• Edward's and Patau's syndromes

Karyotype

It used to be assumed that couples having three or more miscarriages had parental factors contributing to their losses. However, a series of papers have reported an incidence of 29-57% karyotypical abnormality rate in the pregnancies miscarried from women suffering three or more losses (Table 2). Therefore, karyotypical abnormality in the miscarried pregnancy should be excluded before further causes are investigated.

The conventional technique of culture and karyotyping of miscarried tissue has many difficulties. Trophoblasts do not survive in culture long enough to allow conventional karyotyping with G banding; this means that the cyogenetists, in fact, culture the less common fetal mesenchymal stromal cells and leucocytes. Difficulties in getting miscarried fetal cells to grow means that, conventional karyotyping is limited by external contamination, culture failure and selective growth of maternal cells. A series of options are available to improve the diagnostic accuracy for karyotypical abnormalities;

- Comparative genomic hybridisation (CGH), a technique that detects chromosomal imbalances without the need for culture, has been demonstrated to improve detection rates.
- Fluorescence in-situ hybridization (FISH) has also been used effectively to detect aneuploidy in miscarried tissue.

Morphology

Karyotyping does not detect pregnancies complicated by structural abnormalities in the presence of morphologically normal chromosomes. The newly introduced medical therapeutic abortion methodology has allowed detailed examination of undamaged first trimester pregnancies. Severe structural abnormalities likely to be incompatible with survival into the second trimester were found in 34% of specimens examined (Blanch et al 1998).

Embryoscopy

Philipp and co-workers (2003) have suggested embryoscopy using a standard hysterocope, immediately prior to evacuation of the pregnancy under general anaesthetic. They found a similar pattern of disorders in first trimester fetal deaths to those reported by Blanch and co-workers (1998) from medical therapeutic abortions (Table 3). Importantly Philipp and co-workers (2003) found that 66% of karyotypically normal pregnancies had serious structural abnormalities.

It is important to attempt to diagnose fetal abnormality using ultrasound, embryoscopy, examination of products of conception and karyotyping in order to give women a reason for their pregnancy loss

If the loss was of an abnormal pregnancy will the abnormality recur?

Parental peripheral blood karyotypes

Abnormalities in parental peripheral blood karyotypes are reported in 3 to 5 % of couples suffering recurrent miscarriage (RM) and testing of both partners is therefore advocated. The commonest abnormalities appear to be balanced reciprocal and Robertsonian translocations if present these may lead to recurrence of the genetic cause of the pregnancy loss. (Dawood et al., 2004).

Maternal oocyte abnormalities

A study of microsatellite markers in miscarried tissue found that most karyotypical abnormalities were of maternal oocyte origin and were age related (Stephenson et al., 2002).

Skewed X chromosome inactivation and Telomer abnormality have been postulated as genetic causes of recurrent fetal abnormality in recurrent miscarriage but detailed studies have not proven these to be contributing factors.

Endometrial adhesiveness

We have postulated that some women with recurrent miscarriage have excessively adhesive endometrium so that those embryos of poor quality implant, allowing embryos that are destined to fail to implant and present clinically as recurrent miscarriage (Quenby et al., 2002).

If the loss was of a normal pregnancy is there an underlying maternal cause?

Hormonal

Although polycystic ovaries (PCOS) have been reported as having a higher prevalence in RM women compared to the general population. There is a strand of evidence supporting a causal relationship between hyperprolactinaemia and RM. The entity of oligomenorrhea is over represented in the RM population (10 to 15%), in contrast to 1% in the general population. Women with oligomenorrhea have been shown to have lower luteal phase oestradiol levels, which may alter endometrial receptivity with subsequent compromised implantation. The presence of oligomenorrhea has also been shown to have a higher chance of a normal karyotype miscarriage.

Antiphospholipid syndrome (APS)

The antiphospholipid syndrome (APS) remains entrenched as one of the most important causes of fetal loss. It is characterised by a clinical history of recurrent miscarriage, thrombosis or thrombocytopenia, accompanied by persistently positive tests for antiphospholipid antibodies. Tests for antiphospholipid antibodies are usually a medium to high titre of antibody to cardiolipin (ACA) and/or abnormalities of coagulation based assays consistent with the presence of a lupus anticoagulant and should be positive on two occasions at least six weeks apart.

Clinical obstetric criteria to diagnose APS are:
- Three or more consecutive spontaneous losses before the tenth week of gestation
- One or more premature births before 34 weeks for severe pre-eclampsia or impaired fetal growth
- One or more unexplained intrauterine deaths beyond 10 weeks' gestation

Other Thrombophilias

Thrombophilic disorders have generated considerable interest in the field of RM. Thrombophilia is heralded by a predisposition to thrombosis due to a procoagulant state. Several blood clotting disorders are grouped under the umbrella term of thrombophilia. Amongst these, are Activated Protein C Resistance (APCR), protein S deficiency, protein C deficiency, prothrombin mutation, antithrombin III deficiency and hyperhomocysteinaemia (methylenetetrahydrofolate reductase mutation ,C677T MTHFR).

A meta-analysis of 31 papers investigating the relationship between fetal loss and thrombophilias concluded that "the magnitude of the association between thrombophilia and fetal loss varies, according to type of fetal loss and type of thrombophilia" (Rey et al., 2003). Factor V Leiden was associated with all types of pregnancy loss, activated protein C resistance was associated with early recurrent fetal loss, the prothrombin G20210A mutation and protein S deficiency were associated with both recurrent and late non-recurrent fetal loss. However, Methylenetetrahydrofolate mutation, protein C, and antithrombin deficiencies were not significantly associated with fetal loss.

Second trimester Miscarriages

Second trimester Miscarriages refer to those that occur between 12 and 24 weeks of gestation and constitute approximately 5 % of all pregnancy losses. The presence of dual pathology occurs in 10-15 % of mid-trimester losses.

Late fetal losses

Antiphospholipid syndrome is one of the most important cause of late fetal losses but testing for other thrombophilias is also warranted.

Spontaneous mid-trimester losses

Anatomical

The incidence of uterine anomaly is less than 5% in RM women. Although traditionally associated with mid-trimester losses, uterine abnormalities may also be implicated in first trimester losses. The use of non-invasive 3-dimensional ultrasound scan is gaining vogue as it provides both diagnosis and classification of uterine malformation rendering hysteroscopy unnecessary.

Cervical weakness accounts for approximately 10% of mid-trimester losses. It may follow previous damage caused by cone biopsy, operative dilatation of the cervix, or rarely due to a congenital defect and presents classically as painless dilatation of the cervix, which may then lead to spontaneous rupture of membranes. Following this specific history, a pre-conceptual confirmatory hysteroscopy is recommended to assess cervical resistance and length. Alternatively, serial transvaginal ultrasonography has been used to assess cervical length in pregnancy and effectively predict second trimester miscarriage and preterm labour.

Bacterial Vaginosis

Bacterial vaginosis (BV) is a vaginal infection caused by an imbalance in the polymicrobial vaginal flora. It is not fully understood as to what causes this increase in commensal organisms. BV is more commonly associated with mid-trimester losses than first trimester losses. In women found to have BV in early pregnancy, a five-fold increase in mid-trimester losses has been demonstrated. There is also a substantial body of evidence implicating BV with an increased risk of pre-term delivery and associated poor perinatal outcome.

It should be borne in mind that as is the case with first trimester losses, the aetiology of mid-trimester losses is heterogeneous. The presence of dual pathology, such as cervical weakness and bacterial vaginosis or antiphospholipid syndrome, may occur in around 10% of mid-trimester losses.

Conclusion

It is important that all the information that can be possibly obtained is used to assess whether the loss was of a normal or abnormal pregnancy and the gestation at which it occurred. Once this process has occurred then investigations should be targeted at assessing the risk of recurrence of the loss.

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Table 1

Terminology of miscarriage

	Definition	**Gestation in weeks**	**Ultrasound findings**
First trimester Miscarriage			
Biochemical loss	Rise in HCG no other evidence of pregnancy	<6	No evidence of pregnancy
Empty sac	Trophoblast development without the development of an embryo	<12	empty gestation sac (diameter >20mm, no embryonic pole or yolk sac) or if diameter <20mm with no change on rescan 7 days later
Embryonic loss	An early embryo loss before fetal heart activity	<8	an embryo >5mm size but up to eight weeks size, with no cardiac activity. Or crown rump length <5mm with no change on rescan 7 days later
Fetal Loss	Death of a fetus in the first trimester	8-12	Fetus of 8-12 weeks size with no fetal heart activity
Second trimester Miscarriage			
Late fetal loss	Death of a fetus in the second trimester	12-24	Fetus of 12-24 weeks size with no fetal heart activity
Spontaneous second trimester loss	Pregnancy loss associated with SROM or cervical dilation	12-24	Fetus of 12-24 weeks size with fetal heart activity

Table 2
Cytogenetic Analysis of Pregnancy Loss in Recurrent Miscarriage
Trisomy Frequency in Descending Order

Philipp et al, Hum Rep,2003 (n=221) Culture 70%+CGH	Stephenson et al, Hum Rep, 2002 (n=420) Culture 82%+CGH	Rubio et al, Hum Rep, 2003 (n=71) PGD+FISH	Sullivan et al O&G, 2004 (n=122) Culture 85%
15	15	16	16
16	16	21	15
21	22	13	NA
22	21	22	NA
14	14	18	NA

Table 3

Structural abnormalities in early pregnancy reported from two different studies

	Blanch et al., 1998 BMJ *N=121*	*Philipp et al., 2003 Hum Reprod.* *N=233*	*Specimens with Abnormal Karyotype*
Reason for pregnancy loss	Medical therapeutic abortion	Fetal loss	75%
Method of examination	Binocular microscope	Embryoscopy	Culture and G banding
Normal Embryos	40%	14%	48%
Anembryonic pregnancies	27%	Excluded	-
Ruptured sac	12%	Excluded	-
Growth disorganised	12%	31%	69%
Combined defects	6%	51%	86%
Isolated defects	3%	4%	60%

Evidence-based treatments in recurrent miscarriage

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Learning objectives

- List the drugs that are used in early pregnancy in our department.
- Define the potential role of the different drugs used in your practice.
- Name the drugs that have been tested by RCT for the treatment of recurrent miscarriage and describe their effect.
- Compare the advantages and side-effects of drugs used routinely in early pregnancy.

Lecture summary

Implantation in human is a complex, closely regulated, highly selective and relatively poorly understood process. Humans have the highest rate of miscarriage in mammals and various pharmacological manipulations have been used to minimise pregnancy losses in both spontaneous pregnancies and pregnancies resulting from assisted reproduction technology (ART). The widespread application of protocols using numerous drugs in women presenting with recurrent miscarriages has led to an increasing number of pregnancies exposed to these drugs. The vast majority of these protocols have been based on data from a few observational and often retrospective clinical studies. This lecture presents a critical evaluation of the recent literature on drug interventions in early pregnancy with particular focus on their use in recurrent miscarriages.

Following the discovery in the forties that women with miscarriages had lower urinary oestrogen levels, Diethylstilboestrol (DES) started to be prescribed routinely for pregnant women at risk of early and late pregnancy loss (1). The use of DES for the prevention of miscarriage was further promoted by the uncontrolled trial of Smith (2). Reports that DES increases the risk of cancer in laboratory animals were published as early as the end of the thirties (1). In 1950s, Dieckmann et al published a placebo-controlled trial showing the DES was ineffective in the prevention of miscarriage and other pregnancy complications (3). However, it is only in 1971, when Herbst and colleagues (4) published their first case-control study showing that maternal use of DES could result in cancer and reproductive abnormalities 20 years after exposure, that the red flag was raised. In the mean time DES had been marketed under more than 200 brand names and it is estimated that around 5 million women were prescribed DES in the US alone before it was banned for use by the Food and Drug Administration (1). In some countries, like France, DES continued to be prescribed to women until 1977 and it was still prescribed during pregnancy in Mexico, Poland and Uganda in the early 1990s. By contrast with DES, teratogenic effects of thalidomide became quickly apparent, and it was subsequently withdrawn from the market in 1961. Phocomelia is a very common birth defect seen with thalidomide use. Additionally, thalidomide can also affect the development of the fetal eye, ear, heart, genitals, kidneys, digestive tract, and nervous system.

In both cases, the pharmaceutical industry ignored the lack of efficacy of their products and failed to assess adverse health effects but promoted its effectiveness and safety to doctors and consumers. These tragic lessons forced the medical scientific community to abandon the concept that the fetal environment is a safe place well protected by the placental barrier. The entire field of teratology was established following these episodes and the toxicity evaluation of drugs for use during pregnancy is now much more comprehensive and thorough. Nonetheless, many drugs that were first marketed prior to current more stringent regulations have not been evaluated with respect to efficacy. Our recent exhaustive review of the literature (Table 1) indicates that there are still numerous issues about the safety of most drugs for both the women and their fetus. In many cases, the benefits are theoretical and the possible long-term side-effects are untested. There is an urgent need for more epidemiological studies and randomised controlled trials to explore the use, efficacy and side effects of both old and new drugs in early pregnancy.

Table 1: Drug interventions in early pregnancy.

Recurrent Miscarriage: Anti phospholipids Antibody Syndrome	
Treatments which are likely to work	Low dose aspirin and heparin
Treatments requiring further study	Aspirin Intravenous immunoglobulin
Treatments that do more harm than good	Steroids
Infertility: Multiple Failed IVF Treatment: Anti phospholipids Antibody Seropositivity	
Treatments requiring further study	Low dose aspirin and heparin.
Treatments that do more harm than good	Steroids
Infertility: Poor Pelvic Perfusion	
Treatments requiring further study	Low dose aspirin Nitric Oxide
Infertility: Pituitary Down-regulation	
Treatments which are likely to work	Progesterone hCG
Infertility and Recurrent Miscarriage: Hyper-prolactinaemia	
Treatments requiring further study	Dopaminergic agents
Infertility and Recurrent Miscarriage: PCOS	
Treatments requiring further study	Suppression of high LH Metformin
Infertility and Recurrent Miscarriage: Oxidative Stress	
Treatments requiring further study	Vitamin E Vitamin C

References & further reading

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