

---

---

# Clinical Aspects of Miscarriage

---

---

## INTRODUCTION

Miscarriage is the spontaneous (as opposed to induced) loss of a pregnancy before viability, which is considered to be 24 weeks in the UK as this is considered to be the lower limit of viability, although some pregnancies will result in live born infants before that time.

Pregnancies can be lost at a very early stage and present only as a positive pregnancy test and lost before they can be detected by ultrasound; they are often referred to as biochemical pregnancies. At a slightly later stage, ultrasound might demonstrate an apparently empty gestation sac, referred to as early embryonic demise, or later, when a non-viable fetal pole is evident on ultrasound, an early fetal demise. Miscarriage in the first trimester tends to have different aetiologies than those lost in the second trimester, particularly in the later second trimester, although there is overlap between the two trimesters.

Miscarriage is a common complication of pregnancy; 25% of women will experience a miscarriage in their lifetime (Regan, 1997). Of all conceptions, more than 50% will be lost (Kline *et al.*, 1989), many before the woman appreciates that she is pregnant, and between 13.8% (Saraiya *et al.*, 1999) and 22% (French and Bierman, 1962) of recognised pregnancies miscarry.

Most miscarriages are spontaneous — that is, the inevitable loss of an abnormal pregnancy. A small proportion of miscarriages — around 1% — represent the recurrent loss of often normal pregnancies because of a risk factor present in one of the parents.

## SPONTANEOUS MISCARRIAGE

### Introduction

Spontaneous early pregnancy loss is usually secondary to karyotypical or major structural abnormality. One-half of blastocysts and one-quarter of embryos can be seen to be morphologically abnormal to light microscopy and in 25–62% of cases of spontaneous miscarriages, gross chromosomal abnormalities are found (Craven and Ward, 1999). Other factors are also known to have an impact on the risk of spontaneous miscarriage.

### Maternal Age

The risk of spontaneous miscarriage increases with maternal age. The risk in the 20–24 year old age group is 8.9% but in women of 45 years or more is 74.7% (Nybo Anderson *et al.*, 2000). This is largely explained by the increased rate of chromosomally abnormal conceptions with advancing maternal age.

### Subfertility

Hakim *et al.* (1995) found a 70% rate of early pregnancy loss in women with a history of subfertility compared to 21% of women without fertility problems (relative risk 2.6) and suggested that subfertile women have an increased risk of subclinical pregnancy loss, which contributes, at least in part, to their subfertility.

### Psychological Stress

Coste *et al.* (1991) found a threefold increase in the incidence in the rate of spontaneous miscarriage in women who were under psychological stress at the time of conception, whether or not that stress was related to the outcome of the pregnancy.

### Body Mass Index

An increase in spontaneous miscarriage is also seen with increasing body-mass index (BMI). Women with moderate obesity (body/mass index

25–27.9 kg/m<sup>2</sup>) had a significantly greater miscarriage rate than those of normal weight (60% vs 27%,  $p < 0.05$ ) (Hamilton-Fairley *et al.*, 1992). Wang *et al.* (2002) found the rate of spontaneous miscarriage in women receiving treatment for subfertility increased with BMI, with a significance of  $<0.05$  in overweight women,  $<0.01$  in obese women and  $<0.001$  in very obese groups.

## Infection

Acute maternal infection can be associated with early pregnancy loss, with infections such as brucellosis being recognised as precipitating spontaneous miscarriage (Khan *et al.*, 2001) and parvovirus with late miscarriage (Jensen *et al.*, 2000). Parazzini *et al.* (1997) found a history of pelvic inflammatory disease increased the odds ratio fivefold for spontaneous miscarriage.

## Nutritional Factors

Low plasma folate levels are associated with an increased risk of spontaneous miscarriage (George *et al.*, 2002), although hyperhomocysteinaemia (see below), is a genetic disorder, effectively treated by folate supplementation, which is recognised as a predisposing factor for recurrent miscarriage.

## Smoking and Alcohol

The odds ratio of spontaneous miscarriage in women who smoke 20 or more cigarettes each day is 2.0 times that of non-smokers (Mishra *et al.*, 2000). Moderate drinking is also associated with an increased risk of miscarriage; women who drank more than three drinks per week during the first trimester had a higher chance of pregnancy loss (OR 2.3) (Windham *et al.*, 1998), although alcohol consumption prior to pregnancy did not significantly alter the rate of miscarriage.

## Maternal Disease

Spontaneous miscarriage occurs more frequently in women with hypothyroidism (Grossman *et al.*, 1996). Chronic disease increases the risk of

miscarriage, with women with systemic lupus erythematosus (SLE) and no anti-phospholipid antibodies (APA) having 4.7 times the incidence of spontaneous miscarriage that is seen in a normal population (Kiss *et al.*, 2002). Here, there is an overlap with recurrent miscarriage. If a woman's illness is persistent, and not amenable to treatment, her increased risk of miscarriage will be carried into future pregnancies.

## RECURRENT MISCARRIAGE

### Introduction

Recurrent miscarriage is generally considered to be the loss of three or more pregnancies before viability. Although investigation and treatment, particularly if the losses are all in the first trimester, tends to be restricted to women who fit this definition, women with only one miscarriage can be considered as being at higher risk for future problems. Regan *et al.* (1989) studied 630 women in early pregnancy and found that 12% of clinically recognised pregnancies miscarried; the rate was only 5% in the primigravida and 4% in women who had had only successful pregnancies. However, in women whose only previous pregnancy was a miscarriage, the rate was 20%, and this increased to 24% if she had had more than one miscarriage and no successful pregnancies. Knudsen *et al.* (1991) reported the outcome of pregnancy in 300, 500 women. The overall risk of clinical miscarriage in this group was 11%. If a woman had had one previous miscarriage, the risk rose to 16%, two previous miscarriages, 25%, three miscarriages, 45%, and after four miscarriages, the risk of miscarriage was 54%.

There are many factors implicated in recurrent pregnancy loss, which are discussed below, although in half of the cases a cause is not identified.

### Endocrinal Factors

Endocrine deficiency, leading to a poorly implanted and established pregnancy in its early stages, has always been an attractive theory as the cause of recurrent early pregnancy loss. Progesterone and human chorionic gonadotrophin (hCG) have both been proposed as means of supporting the early pregnancy. It is unclear whether reduced gestational hormones are the cause of pregnancy loss, or the result of pregnancy failure.

### *Progesterone*

Progesterone levels reflect the function of the corpus luteum. Levels of progesterone are lower in abnormal pregnancy when compared to normal pregnancy. If low levels of progesterone result from maternal inability to produce functioning corpus lutea, then supplementation with progestogens should improve pregnancy outcome. Contra-wise, supplementation will not help a pregnancy in which progestogen levels are low because the pregnancy is failing. A meta-analysis in 1989 (Goldstein *et al.*, 1989) showed no benefit when progestogens were used to maintain early pregnancies. Concerns have also been raised about potential teratogenic effects, with genital defects in male and female fetuses (Oates-Whithead and Carrier, 2001).

### *Human chorionic gonadotrophin*

The hormone hCG is produced by the trophoblast. In normal early pregnancies the levels are seen to double approximately every 48 h. Supplementation of early pregnancy with hCG has been proposed as a treatment for recurrent miscarriage. The same arguments apply to supplementation in early pregnancy with hCG as with progesterone supplementation. A Cochrane review (Scott and Pattison, 2002) found four studies comparing hCG with placebo, but with less than 100 patients in total in each arm. The results suggested that there might be benefit from the use of hCG supplementation in preventing recurrent early pregnancy loss, but the early trials with methodological weaknesses and small numbers might have swung the overall result in favour of hCG. A single study (Quenby and Farquharson, 1994) demonstrated an improved outcome in women with a history of oligomenorrhoea and recurrent miscarriage when hCG was used. It has previously been shown that oligomenorrhoea is a poor prognostic factor in recurrent early pregnancy loss (Quenby and Farquharson, 1993).

### *Luteinising hormone hypersecretion and polycystic ovarian syndrome*

Polycystic ovarian syndrome (PCOS) is associated with both recurrent early pregnancy loss and infertility. It is associated with the clinical features of oligomenorrhoea, acne and hirsutism, secondary to hyperandrogenism, and obesity. Biochemically, it is associated with elevated luteinising hormone (LH),

elevated androgens and insulin resistance/hyperinsulinaemia; elevated plasminogen activator inhibitor-1 (PAI-1) activity has been described by some (Sampson *et al.*, 1996; Atiomo *et al.*, 1998; Glueck *et al.*, 1999). It is characterised on ultrasound scan of the ovary by the presence of 10 or more peripherally placed cysts of between 2 and 10 mm in diameter with a central, echodense stroma. The ultrasound findings can be found in the absence of any biochemical abnormality.

Polycystic ovaries detected by ultrasound were found in 22% of women in a volunteer population and 50% of women presenting for assisted conception (Balen *et al.*, 1993a). In women attending a recurrent miscarriage clinic, an incidence of polycystic ovaries on ultrasound as high as 82% has been reported (Sagle *et al.*, 1988). In assisted conception cycles, the rate of miscarriage was higher in women with polycystic ovaries, being 35.8% compared to 23.6% in the population with normal ovaries (Balen *et al.*, 1993b).

Homburg *et al.* (1988) reported that women with PCOS and elevated LH levels had higher rates of early pregnancy loss. A similar association has been found with PAI-1 (Glueck *et al.*, 1999). It has been proposed that elevated LH results in premature oocyte maturation, resulting in either a failure to achieve fertilisation and subfertility, or fertilisation of an abnormal oocyte and consequently miscarriage (Balen *et al.*, 1993b). Alternatively, elevated PAI-1 activity has been proposed as a mechanism for abnormal ovulation, embryo hatching and implantation (Sampson *et al.*, 1996; Atiomo *et al.*, 1998; Glueck *et al.*, 2001).

There was optimism that if LH hypersecretion was associated with early pregnancy loss, then, suppression of LH with gonadotrophin releasing analogues would improve pregnancy outcome in women with a history of recurrent early pregnancy loss in the absence of infertility. Clifford *et al.* (1996) did not find any improvement in miscarriage rates with the use of buserilin.

Metformin has been used to aid weight loss in women with PCOS and impaired glucose metabolism. This appears to improve fertility in some subjects. It has also been proposed that its continuation into early pregnancy might reduce PCOS related early pregnancy loss, but so far only small trials have been performed. Glueck *et al.* (2001) performed a small prospective trial of metformin in early pregnancy. They found it to be safe, with no adverse effects, and 60% of their trial population (10 women) had normal live births. Previously, they had had a 55% live birth rate when metformin was stopped at confirmation of pregnancy.

## Anatomical Problems

### *Uterine anomalies*

Uterine anomalies have long been thought to contribute to recurrent miscarriage and premature delivery. The problem in considering them as a factor relevant to pregnancy complications is that they are a frequent finding in women with uneventful pregnancies. According to the American Fertility Society, with an arcuate uterus, the fundus is normal, but the uterine cavity is concave. A septate uterus has a septum partially or completely dividing the cavity. For a uterus to be bicornuate, a fundal cleft of at least 1 cm in depth must be present (Anonymous, 1988).

Jurkovic *et al.* (1997) found the incidence of uterine anomalies in women attending for gynaecological ultrasound for a wide variety of reasons, but excluding infertility and recurrent miscarriage, to be 5.4%, with 3.1% having an arcuate uterus and 2.3% having other major abnormalities (subseptate uterus 1.6%, bicornuate uterus 0.4%).

The same group looked at women with a history of recurrent miscarriage or infertility (Woelfer *et al.*, 2001). It was found that a significantly higher proportion of women with a subseptate uterus miscarried in the first trimester, whilst an arcuate uterus was a risk factor for second trimester loss and preterm labour.

Hysteroscopic resection of the septum is said to improve reproductive function. Hickok (2000) reports a pre-resection pregnancy loss in women with a subseptate uterus of 77.4%. This was reduced to 18.2% post-resection. March and Israel (1990), Grimbizis *et al.* (1998) and Porcu *et al.* (2000) also claim improved pregnancy outcomes after hysteroscopic resection of uterine septums. However, these studies are not randomised, and, therefore, it is not possible to say what the pregnancy outcome would have been if these women had not been treated. Seventy-six per cent of women will have a successful pregnancy on the next occasion after only ever having miscarriages (Regan *et al.*, 1989)

### *Cervical factors*

Successful pregnancy and delivery requires the cervix to remain competent until labour at full term. Premature dilatation of the cervix results in mid-trimester miscarriage if it occurs before viability. This might be accompanied by recognisable contractions, and represents preterm labour at its extreme; however, when dilatation of the cervix occurs in the

absence of contractions, or precedes contractions, intrinsic weakness of the cervix is thought to be responsible.

Many factors have been considered to contribute cervical weakness. Congenital associations include association with other uterine anomalies (Golan *et al.*, 1990), Marfan's syndrome (Paternoster *et al.*, 1998), connective tissue disorders such as Ehlers-Danlos syndrome (de Vos *et al.*, 1999), and *in utero* exposure to diethylstilbestrol (Goldstein, 1978). Acquired factors include reasons for surgery to the cervix, such as cone biopsy for the treatment of cervical intraepithelial neoplasia (Moinian and Andersch, 1982), surgical termination of pregnancy (Ratten and Beischer, 1979), previous cervical ectopic pregnancy (Hurley and Beischer, 1989).

Attempts to prevent dilatation of the cervix in the absence of labour are made by placing sutures within the cervix to increase its strength. These can be placed within the portion of the cervix visible vaginally, as in a McDonald's suture (McDonald, 1957). Still using a vaginal approach, the vaginal epithelium can be incised, allowing the underlying tissues, particularly the bladder, to be reflected and the suture to be placed higher, and nearer to the internal os, as in a Shirodkar suture (Shirodkar, 1955; Frieden *et al.*, 1990) and transvaginal cervicoisthmic cerclage (Capsi *et al.*, 1990; Golfier *et al.*, 2001). Alternatively, the suture can be placed at the level of the internal os by an abdominal approach (Anthony and Price, 1986; van Dongen and Nijhuis, 1991). This approach is more invasive, but useful in women in whom there is little vaginal cervix or when other techniques have failed.

Diagnosis of cervical incompetence is often made on history alone. As this can lead to unnecessary intervention, various methods of evaluating the need for cerclage objectively are described: assessment by hysterosalpingogram (Golan *et al.*, 1990), ultrasound assessment in pregnancy (Guzman *et al.*, 1998; To *et al.*, 2002; Groom *et al.*, 2002) and measurement of cervical resistance outside of pregnancy (Anthony and Price, 1986). Sutures can be inserted before pregnancy, electively in pregnancy, as an emergent procedure, for example, if cervical shortening is seen on ultrasound, or as emergencies after frank dilatation of the cervix has been found. However, it is still unclear whether sutures improve the outcome of pregnancy.

In 1993, the results of a multicentre trial (MRC/RCOG, 1993) found benefit from inserting cervical sutures. The trial randomised women with whom the attending obstetrician was uncertain whether a suture would be of benefit. The rate of premature delivery was reduced in the suture group, but it was estimated that 25 sutures would need to be inserted to improve

outcome in one pregnancy whilst the incidence of puerperal sepsis doubled. Whilst it might be supposed that a greater benefit would be found in a higher risk population, this has not been proved. The cervical incompetence prevention randomised cerclage trial 'CIPRACT Trial' (Althuisius *et al.*, 2000, 2001) did not find a benefit when cerclage was used in an unselected high-risk population, with similar neonatal survival being seen in the cerclage and the observational groups. If cerclage was reserved for women in whom cervical shortening was demonstrated ultrasonographically (cervical length <25 mm), preterm delivery, before 34 weeks, was significantly less frequent and neonatal morbidity was reduced. Rust *et al.* (2000) similarly randomised women when the cervical length was less than 25 mm, but they found no difference in the duration of pregnancy or the perinatal outcome.

## Infection

To be realistically considered as a cause for recurrent pregnancy loss, infection must persist beyond one pregnancy. Bacterial vaginosis is not strictly an infection, rather depletion of the normal, protective lactobacilli by organisms — *Gardnerella vaginalis*, anaerobes and mycoplasmas — that alter the vaginal environment, raising the pH. It is reasonable to expect that factors that predispose to bacterial vaginosis can persist for more than one pregnancy. The association of bacterial vaginosis with preterm labour and rupture of membranes means it is a cause of mid-trimester loss.

Ralph *et al.* (1994) looked at women undergoing *in vitro* fertilisation who were screened for bacterial vaginosis at the time of egg collection. Conception rates were similar in women with normal flora to those with bacterial vaginosis. A significantly greater number of women with bacterial vaginosis miscarried before 13 weeks — 36.1% of women with bacterial vaginosis miscarried, compared to 18.5% with normal flora.

Hay *et al.* (1994) found a prevalence of bacterial vaginosis of 15% in early pregnancy. In their study there was an association of bacterial vaginosis with both preterm delivery (24–37 weeks) and late (16–24 weeks) miscarriage. The presence of bacterial vaginosis, *Mycoplasma hominis* and *Ureaplasma urealyticum* at the first visit in pregnancy (less than 14 weeks gestation) was found to increase the risk of early pregnancy loss.

Oakeshott *et al.* (2002) found the prevalence of bacterial vaginosis in consecutive women presenting with pregnancy before 10 weeks gestation to be 14.5%; it was commoner in the under 25 age group and in Afro-Caribbean or black African women. The overall prevalence of chlamydia

was 2.4%, but again it was higher in the under 25 age group at 8.5%, and 14.4% in teenagers. Women with bacterial vaginosis did not seem to be at increased risk of miscarriage before 16 weeks but the risk of miscarriage between 13 and 15 weeks was increased. Chlamydia infection was not associated with miscarriage, but there was a threefold increase in the prevalence of bacterial vaginosis in the presence of chlamydia. Chlamydia has been studied as a potential cause for miscarriage. However, chlamydia found on urine DNA amplification (Sozio and Ness, 1998), the presence of chlamydia antibody in the blood (Rae *et al.*, 1994; Osser and Persson, 1996; Paukku *et al.*, 1999) or placental tissue (Feist *et al.*, 1999) has not been found to be associated with pregnancy loss.

Ugwumadu (2002) speculated that it is the immunological response to bacterial vaginosis that is responsible for pregnancy loss, rather than a direct effect of the organisms. A normal Th2 response is necessary for normal early pregnancy, with IL-4 and IL-6 inducing trophoblastic release of hCG, which in turn preserves the corpus luteum and resulting progesterone production. If bacterial vaginosis promotes a Th1 response, or suppresses the Th2 response, this might lead to early pregnancy loss. In non-pregnant women endometritis, defined as the presence of plasma cell endometritis, was present in 45% of women with bacterial vaginosis, as opposed to 5% in a control population (Korn *et al.*, 1995). Wennerholm *et al.* (1998) found elevated levels of the Th1 cytokines IL-8 and IL-1 $\alpha$  and Spandorfer *et al.* (2001) found elevated levels of IL-1 $\beta$  and IL-8 in women with bacterial vaginosis.

The impact on pregnancy complications of attempts to eradicate bacterial vaginosis with antibiotic therapy has been assessed. Carey *et al.* (2000) found oral metronidazole had no effect on the rate of premature delivery in women with bacterial vaginosis. Vaginal clindamycin will eradicate bacterial vaginosis from the vagina, but its use has not been shown to be of benefit in preterm labour (Kurkinen-Raty *et al.*, 2000; Rosenstein *et al.*, 2000; Kekki *et al.*, 2001). Ugwumadu *et al.* (2003) looked at the role of oral clindamycin; metronidazole, is effective against anaerobes, but clindamycin has a broader range of activity, including activity against the atypical mycoplasmas. Although vaginal clindamycin may eradicate bacterial vaginosis from the vagina, it may not be effective in treating the associated endometritis, and it is likely that it is the endometritis, rather than the vaginal overgrowth, which is responsible for pregnancy complications. They found that oral clindamycin (300 mg twice daily for 5 days) reduced the rate of late miscarriage and preterm delivery (5.3% vs 15.7%,  $p = 0.0003$ ).

## Recurrent Aneuploidy

Spontaneous miscarriage is often as a result of fetal aneuploidy, as discussed above. It can be seen as reassuring to women with recurrent miscarriage that aneuploidy is found when the products of conception are karyotyped: this is a spontaneous loss, not the further loss of another normal fetus. However, this might not always be the case. There is evidence that some karyotypically normal couples are at risk of recurrent early pregnancy loss because they are at risk of recurrent aneuploidy. Juberg *et al.* (1985) proposed that some couples were at increased risk of non-disjunction, which increased their risk of early pregnancy loss, and also of aneuploidy in ongoing pregnancies, after finding that hypermodal chromosomal spreads significantly more frequently in the lymphocytes of couples with a history of recurrent miscarriage than in control populations. Simon *et al.* (1998) looked at the karyotypes of pre-implantation embryos from subjects undergoing *in vitro* fertilisation. The embryos of couples with a history of recurrent miscarriage had a higher incidence of aneuploidy (58%) than couples with no such history. Pre-implantation genetic diagnosis, and embryo transfer of karyotypically normal embryos, might improve the success rate of infertility treatment in such couples.

Drugan *et al.* (1990) found a 1.6% rate of aneuploidy after amniocentesis or chorionic villus sampling in couples with a history of recurrent early pregnancy loss and normal parental karyotypes, compared to 0.3% in a control group ( $p = 0.02$ ). Ongoing pregnancies conceived by couples with a history of early pregnancy loss may have a greater risk of chromosomal anomaly whilst the parents are less likely to seek pre-natal diagnostic tests because of the fear of pregnancy loss secondary to invasive testing.

Sperm disomy might also have a part to play in recurrent pregnancy loss in some couples. The rate of disomy is higher in sperm samples taken from couples with a history of recurrent early pregnancy compared to controls (Rubio *et al.*, 1999, Egozcue *et al.*, 2000). Aneuploid sperm demonstrate greater motility, with higher rates of sperm aneuploidy being found in Percoll-processed sperm samples than whole specimens (Giorlandino *et al.*, 1998).

## Thrombophilia

Thrombotic events in the placenta have been attributed as contributing to many pregnancy complications. They are also prevalent in the general

population. Bick (2000) found a pro-coagulant defect in 55% of women with a history of three or more miscarriages.

Thrombophilias can be divided into two main groups: the inherited thrombophilias, which are generally gene mutations, and the acquired thrombophilias, principally the anti-phospholipid syndrome.

### *Inherited thrombophilia*

The main inherited thrombophilias are antithrombin III deficiency, deficiencies of protein C and protein S, the factor V Leiden mutation, the prothrombin gene mutation and hyperhomocysteinaemia. The presence of an inherited thrombophilia does not inevitably lead to clinical manifestations, but it does increase the risk. The maternal risk of a thromboembolic episode is increased eightfold in the presence of one of these thrombophilias (Lockwood, 1999). Inherited thrombophilia gives an odds ratio of 3.6 (95% CI 1.4–9.4) of fetal loss after 28 weeks and 1.27 (95% CI 0.94–1.71) before 28 weeks (Preston *et al.*, 1996). This study did not demonstrate an increased risk of fetal loss to the partners of men with thrombophilia, a potential concern, as thrombotic events on the fetal side of the placenta might increase the risk to the pregnancy.

If the pregnancy of a thrombophilic woman is considered to be at risk of thromboembolism, prophylaxis is merited. It is less clear whether this same approach is merited to reduce the risk of pregnancy loss. Aspirin and heparin have been shown to improve the outcome in acquired thrombophilia (see below) and, potentially, a similar approach could be of value in the management of pregnancy in inherited thrombophilias. There is no clear evidence that such a treatment will be of benefit (Girling and de Swiet, 1998).

**Antithrombin III deficiency.** Antithrombin III inactivates thrombin and factors Xa, IXa, XIa and XIIa, limiting the coagulation cascade. Deficiency is inherited in a dominant fashion with more than 80 genetic mutations identified. The 50% lifetime risk of thrombosis (Finazzi *et al.*, 1987) makes antithrombin III deficiency the most thrombogenic of the inherited thrombophilias. It has a prevalence of around 1 in 600 (Tait *et al.*, 1994). The relative risk for miscarriage and stillbirth per pregnancy for women with either antithrombin III, protein C or protein S deficiency is 2.0 (95% CI 1.2–3.3) (Sanson *et al.*, 1996).

**Protein C deficiency.** Protein C inactivates factors Va and VIIIa and its action is enhanced by the presence of protein S. It inhibits coagulation and promotes fibrinolysis. Protein C levels are not altered by pregnancy. It has a prevalence of around 1 in 500 (Tait *et al.*, 1995).

**Protein S deficiency.** Protein S acts as a co-factor for the action of protein C. Levels fall in pregnancy as the amounts of free protein S are reduced. It is estimated that protein S deficiency can be found to be between 0.03% and 0.13% of the population (Dykes *et al.*, 2001).

**Activated protein C resistance — the factor V Leiden mutation.** The prevalence of this mutation varies greatly between racial groups, and it is found more commonly in Europeans with an allele frequency of 4.4%, but rarely in other racial groups (Rees *et al.*, 1995). Carriers of the factor V Leiden mutation appear to have an increased risk of miscarriage with 1.5 times the risk of one miscarriage and 2.5 times the risk of two miscarriages compared to controls (Bare *et al.*, 2000). An increase in the incidence of the factor V Leiden mutation in association with recurrent miscarriage was also found by Younis *et al.* (2000). Increased resistance to activated protein C is found in normal pregnancies (Cumming *et al.*, 1995), and this must be differentiated from the inherited form, which persists, along with its associated increase in risk of thromboembolism, outside pregnancy. Rai *et al.* (2001) found that it was acquired activated protein C resistance in pregnancy, not the inherited condition, that was significantly more common in women with recurrent miscarriage.

**Elevated prothrombin activity — the prothrombin gene mutation.** Carriers of this mutation have higher plasma concentrations of prothrombin and are at increased risk of thrombosis. One study has shown an association with second trimester loss (Kupferminc *et al.*, 2000).

**Thrombomodulin gene mutation.** Thrombomodulin is an endothelial cell receptor for thrombin and accelerates protein C activation. Again, it has been associated with thrombosis, but not yet with an adverse pregnancy outcome.

**Hyperhomocysteinaemia.** Inherited hyperhomocysteinaemia results from a genetic defect affecting the metabolism of methionine to homocysteine. It results in folate deficiency and is associated with recurrent

miscarriage (Ray and Laskin, 1999; Nelen *et al.*, 2000) and neural tube defects (van der Put *et al.*, 1997), as well as an increased risk of atherosclerosis and venous thrombosis. Dietary supplementation with folate, B6 and B12 reduces homocysteine levels and is the basis of treatment (Perry, 1999).

### *Acquired thrombophilia*

**Antiphospholipid syndrome.** APA are antibodies that bind to the negatively charged proteins in the phospholipid component of cell membranes. Normally, the negative charge is not exposed, being on the inner surface of the cell membrane. Production of APA is, therefore, a normal response to any destructive process that exposes the internal surface of the cell membrane and the antibodies are transiently produced in these situations. Production of APA is associated with various autoimmune disorders, of which SLE is the best known, but there exist many other autoimmune disorders. However, APA are not found in all subjects with SLE, with one series finding APA in just 30.4% of subjects with a diagnosis of SLE (Carmona *et al.*, 1999). Their production is also associated with other chronic illnesses, such as Crohn's disease and diabetes mellitus, with many infections, e.g. measles, varicella, pneumococcal pneumonia and Human Immunodeficiency Virus and as response to ingestion of various medications, e.g. the combined oral contraceptive and amoxycillin.

If the antibodies persist beyond the acute event and are associated with either thrombo-embolic disease or recurrent pregnancy loss, a diagnosis of antiphospholipid syndrome (APS) can be made. It is also associated with other medical conditions including thrombocytopenia, migraine and livedo reticularis. Because APA can be a transient finding in a normal individual, the antibodies must be found on at least two occasions more than 6 weeks apart (RCOG, 2001) and the presence of persistent APA in the absence of associated clinical disorders does not constitute a diagnosis of APS. There are various APA, but the two of importance in pregnancy are anticardiolipin and lupus anticoagulant. APA are found in 15% of women presenting with recurrent miscarriage (Rai *et al.*, 1995a)

The antibodies are thought to lead to pregnancy loss by their effect on annexin-V, a phospholipid binding protein present in the syncytiotrophoblast lining the placental villi. Annexin-V is a potent *in vitro* and *in vivo* anticoagulant, and its expression within the syncytiotrophoblast is reduced in the presence of APA, leading to placental thrombosis (Rand *et al.*, 1994).

This would account for the role of APS in pregnancy loss from the later stages of the first trimester onwards. An alternative explanation is that proposed by Lyden *et al.* (1992) of a non-thrombotic aetiology for pregnancy loss associated with APS in very early pregnancy, with APA directly damaging the trophoblastic layer, leading to defective implantation.

Untreated, there is a high rate of fetal loss in the presence of APS, with 90% of pregnancies in the Rai *et al.* (1995b) series miscarrying. These women had a history of recurrent miscarriage and 94% of their losses were in the first trimester. Lockshin *et al.* (1989) found that the use of steroids does not improve fetal outcome, and might even make the outcome worse. Carmona *et al.* (2001) propose the use of pre-conceptual aspirin to reduce the incidence of fetal loss in APS, with 82% of treated women in their series having a live-born infant as opposed to 25.7% before therapy. Rai *et al.* (2001) found that treatment of APS and three or more miscarriages with aspirin alone had a 42% live birth rate, but when used in combination with heparin there was a significantly higher live birth rate, at 71%. The difference in outcome was accounted for by a reduction in first trimester fetal loss. In this series, aspirin was started when the woman reported a positive pregnancy test.

## Immunological

Adaptations in the immunological response are required in pregnancy. It is known that for a pregnancy to be successful, the embryotoxic Th1 response should be suppressed and the pro-pregnancy Th2 response should become dominant; dominance of the Th1 response in early pregnancy is associated with recurrent early pregnancy loss (Hill *et al.*, 1992; Wilson *et al.*, 1997; Raghupathy *et al.*, 2000; Jenkins *et al.*, 2000). What is not clear is whether the abnormal inflammatory response is an inevitable and innate feature of a subject's response to pregnancy, or whether an abnormal response is initiated by exposure to an immunological event, for example, pregnancy loss, and in some women this persists into future pregnancies.

Women with a history of recurrent miscarriage, as a group, have higher levels of the Th1 cytokines IL-12, IL-18 and IFN $\gamma$  than normal pregnant subjects and do not show the rise in IL-4 seen in normal, pregnant subjects. However, levels of IL-18 in women with a history of recurrent miscarriage are reduced when the pregnancy was successful, and the levels were significantly lower than would be found in the non-pregnant state (Wilson *et al.*,

unpublished). Non-pregnant women with a history of recurrent miscarriage have higher levels of IL-2 receptor, which is a marker of T-cell activation and proliferation (Wilson *et al.*, 2003).

Lipopolysaccharide stimulation of normal peripheral blood polymorphonucleocytes incubated in the plasma of women with a history of recurrent miscarriage results in the release of less IL-10 than controls. The recurrent miscarriage group had heterogeneous aetiologies, but nevertheless, as a group, levels of IL-10 were significantly less. This suggests that the plasma of women with a history of miscarriage contains a factor that attenuates the normal inflammatory response.

Various immunological therapies have been tried. Intravenous immunoglobulin has not been seen to demonstrably increase the chances of successful pregnancy (Stephenson *et al.*, 1998).

## Prognosis

A history of spontaneous miscarriage of a previous pregnancy increases the likelihood of a further miscarriage (Parazzini *et al.*, 1997). Coste *et al.* (1991) found two or more previous fetal losses increased the risk of further miscarriage (OR = 2.3). Although Clifford *et al.* (1996) found suppression of LH did not improve the outcome of pregnancy, they did find that all women in all treatment groups had a good outcome to pregnancy, demonstrating the beneficial effect of support in early pregnancy in women with recurrent losses. This effect has been noted by others, Stray-Pedersen and Stray-Pedersen (1984) found that supportive care alone increased the pregnancy success rate to 86% in women with unexplained miscarriage, compared to 33% ( $p = 0.001$ ) in women who did not receive this support, a finding confirmed by Liddell *et al.* (1991). Any therapy for miscarriage must be seen against the success of supportive therapy alone.

## REFERENCES

- Althuisius SM, Dekker GA, van Geijn HP, Bekedam DJ, Hummel P (2000). Cervical incompetence prevention randomized cerclage trial (CIPRACT): study design and preliminary results. *Am J Obstet Gynecol* 183: 823–829.
- Althuisius SM, Dekker GA, Hummel P, Bekedam DJ, van Geijn HP *et al.* (2001). Final results of the cervical incompetence prevention randomized

- cerclage trial (CIPRACT): therapeutic cerclage with bed rest versus bed rest alone. *Am J Obstet Gynecol* 185: 1106–1112.
- Anonymous (1988). The American Fertility Society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, mullerian anomalies and intrauterine adhesions. *Fertil Steril* 49: 944–955.
- Anthony GS, Price JL (1986). Successful use of transabdominal isthmic cerclage in the management of cervical incompetence. *Euro J Obstet Gynecol Reprod Biol* 22: 379–382.
- Atioma WU, Bates SA, Condon JE, Shaw S, West JH, Prentice AG (1998). The plasminogen activator system in women with polycystic ovary syndrome. *Fertil Steril* 69: 236–241.
- Balen AH, Tan SL, Jacobs SJ (1993a). Hypersecretion of luteinising hormone: a significant cause of infertility and miscarriage. *BJOG* 100: 1082–1089.
- Balen AH, Tan SL, MacDougall J, Jacobs HS (1993b). Miscarriage rates following *in vitro* fertilisation are increased in women with polycystic ovaries and reduced by pituitary desensitisation. *Hum Reprod* 8(6): 959–964.
- Bare SN, Poka R, Balogh I, Ajzner E (2000). Factor V Leiden as a risk factor for miscarriage and reduced fertility. *Aust N Z J Obstet Gynaecol* 40: 118–121.
- Bick RL (2000). Recurrent miscarriage syndrome due to blood coagulation protein/platelet defects: prevalence, treatment and outcome results. *Clin & Appl Thromb/Haemost* 6: 115–125.
- Capsi E, Schneider DF, Mor Z, Langer R, Weinraub Z, Bukovsky I (1990). Cervical internal os cerclage: description of a new technique and comparison with Shirodkar operation. *Am J Perinat* 7: 347–349.
- Carey JC, Klebanoff MA, Jauth JC, Hillier SL, Thom EA *et al.* (2000). Metronidazole to prevent preterm delivery in women with asymptomatic bacterial vaginosis. *NEJM* 342: 534–540.
- Carmona F, Font J, Cervera R, Munoz F, Cararach V, Balasch J (1999). Obstetrical outcome of pregnancy in patients with systemic lupus erythematosus. A study of 60 cases. *Eur J Obstet Gynecol Reprod* 83: 137–142.
- Carmona F, Font J, Azulay M, Creus M, Fabreuges F *et al.* (2001). Risk factors associated with fetal losses untreated antiphospholipid syndrome: a multivariate analysis. *Am J Reprod Immunol* 46: 274–279.
- Clifford K, Rai R, Watson H, Franks S, Regan L (1996). Does suppressing luteinising hormone secretion reduce the miscarriage rate? Results of a randomised controlled trial. *BMJ* 312: 1508–1511.

- Coste J, Job-Spira N, Fernandez H (1991). Risk factors for spontaneous abortion: a case-control study in France. *Hum Reprod* 6: 1332–1337.
- Craven CM and Ward K (1999). Embryology and pathology of successful and failed pregnancy. In *Clinical Management of Early Pregnancy*, eds. Prendiville W and Scott JR. Arnold, London.
- Cumming AM, Tait RC, Fildes S, Yong A, Keeney A, Hay CRM (1995). Development of resistance to activated protein C during pregnancy. *Br J Haematol* 90: 725–727.
- de Vos M, Nutinck L, Verellen C, de Paepe A (1999). Preterm rupture of membranes in a patient with the hypermobility type of the Ehlers-Danlos syndrome. A case report. *Fetal Diagn Ther* 14: 244–247.
- Drugan A, Koppitch FC, Williams JC, Johnson MP, Moghissi KS, Evans MI (1990). Prenatal genetic diagnosis following recurrent early pregnancy loss. *Obstet Gynecol* 75: 381–384.
- Dykes AC, Walker ID, McMahon AD, Islam SI (2001). A study of protein S antigen levels in 3788 healthy volunteers: influence of age, sex and hormone use, and estimate for prevalence of deficiency state. *Br J Haematol* 113: 36–41.
- Egozcue S, Blanco J, Vendrell JM, Garcia F, Veiga A *et al.* (2000). Human male fertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. *Hum Reprod Update* 6: 93–105.
- Feist A, Sydler T, Gebbers JJ, Pospischil A, Guscetti F (1999). No association of chlamydia with abortion. *J R Soc Med* 92: 237–238.
- Finazzi G, Caccia R, Barbui T (1987). Different prevalence of thromboembolism in the subtypes of congenital antithrombin III deficiency: a review of 404 cases. *Thromb Haemost* 58: 1094.
- French FE and Bierman JM (1962). Probabilities of fetal mortality. *Public Health Rep* 77: 835–847.
- Frieden FJ, Ordorica SA, Hoskins IA, Young BK (1990). The Shrodkar operation: a reappraisal. *Am J Obstet Gynaecol* 163: 830–833.
- George L, Mills JL, Johansson AL, Nordmark A, Olander B, Granath F, Cnattingius S (2002). Plasma folate levels and risk of spontaneous abortion. *JAMA* 288: 1867–1873.
- Giorlandino C, Calugi G, Iaconianni L, Santoro ML, Lippa A (1998). Spermatozoa with chromosomal abnormalities may result in a higher rate of recurrent abortion. *Fertil Steril* 70: 576–577.
- Girling J, de Swiet M (1998). Inherited thrombophilia and pregnancy. *Curr Opin Obstet Gynecol* 10: 135–144.
- Glueck CJ, Wang P, Fontaine RN, Sieve-Smith L Tracy T, Moore SK (1999). Plasminogen activator activity: an independent risk factor for the high

- miscarriage rate during pregnancy in women with polycystic ovary syndrome. *Metabolism* 48: 1589–1595.
- Glueck CJ, Phillips H, Cameron D, Sieve-Smith L, Wang P (2001). Continuing metformin in women with polycystic ovary syndrome appears to safely reduce mid-trimester spontaneous abortion: a pilot study. *Fertil Steril* 75: 46–52.
- Golan A, Langer R, Wexler S, Segev E, Niv D, David MP (1990). Cervical cerclage — its role in the pregnant anomalous uterus. *Int J Fertil* 35: 164–170.
- Goldstein DP (1978). Incompetent cervix in offspring exposed to diethylstilbestrol *in utero*. *Obstet Gynecol* 52(1): 73S–75S.
- Goldstein P, Berrier J, Rosen S, Sacks HS, Chalmers TC (1989). A meta-analysis of randomised control trials of progestational agents in pregnancy. *BJOG* 96: 265–274.
- Golfier F, Bessai K, Paparel P, Cassingnol A, Vaudoyer F, Raudrant D (2001). Tansvaginal cervicoisthmic cerclage as an alternative to the transabdominal technique. *Euro J Obstet Gynecol Reprod Biol* 100: 16–21.
- Groom KM, Shennan AH, Bennett PR (2002). Ultrasound-indicated cervical cerclage: outcome depends on pre-operative cervical length and presence of visible membranes at time of cerclage. *Am J Obstet Gynecol* 187: 445–449.
- Grossman CM, Morton WE, Nussbaum RH (1996). Hypothyroidism and spontaneous abortions among Hanford, Washington, downwinders. *Arch Environ Health* 51: 175–176.
- Guzman ER, Forster JK, Vintzileos AM, Ananth CV, Walters C, Gipson K (1998). Pregnancy outcomes in women treated with elective versus ultrasound-indicated cervical cerclage. *Ultrasound Obstet Gynecol* 12: 323–327.
- Hakim RB, Gray RH, Zacur H (1995). Infertility and early pregnancy loss. *Am J Obstet Gynecol* 172: 1510–1517.
- Hamilton-Fairley D, Kiddy D, Watson H, Paterson C, Franks S (1992). Association of moderate obesity with poor pregnancy outcome in women with polycystic ovary syndrome treated with low dose gonadotrophin. *BJOG* 99: 128–133.
- Hay PE, Lamont RF, Taylor-Robinson D, Morgan DJ, Ison C, Pearson J (1994). Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. *BMJ* 308: 95–98.
- Hickok LR (2000). Hysteroscopic treatment of the uterine septum: a clinician's experience. *Am J Obstet Gynecol* 182: 414–420.
- Hill JA, Polgar K, Harlow BL, Anderson DJ (1992). Evidence of embryo- and trophoblast toxic cellular immune response(s) in women with recurrent spontaneous abortion. *Am J Obstet Gynecol* 166: 1044–1052.

- Homburg R, Armar NA, Eshel A, Adams J, Jacobs HS (1988). Influence of serum luteinising hormone concentrations on ovulation, conception and early pregnancy loss in polycystic ovaries. *BMJ* 297: 1024–1026.
- Hurley VA, Beischer NA (1989). Cervical incompetence in a pregnancy following a cervical ectopic pregnancy. *Aust N Z J Obstet Gynaecol* 29: 358–360.
- Jenkins C, Roberts J, Wilson R, MacLean MA, Shilito J, Walker JJ (2000). Evidence of a Th1 type response associated with recurrent miscarriage. *Fertil Steril* 73(6): 1206–1208.
- Jensen IP, Thorsen P, Jeune B, Moller BR, Vestergaard BF (2000). An epidemic of parvovirus B19 in a population of 3,596 pregnant women: a study of the sociodemographic and medical risk factors. *BJOG* 107: 637–643.
- Juberg RC, Knops J, Mowrey PN (1985). Increased frequency of lymphocytic mitotic non-disjunction in recurrent spontaneous aborters. *J Med Genet* 22(1): 32–35.
- Jurkovic D, Gruboeck K, Tailor A, Nicolaidis KH (1997). Ultrasound screening for congenital uterine anomalies. *BJOG* 104: 1320–1321.
- Kekki M, Kurki T, Pelkonen J, Kurkinen-Raty M, Cacciatore B, Paavonen J (2001). Vaginal clindamycin in preventing preterm birth and periparturient infections in asymptomatic women with bacterial vaginosis: a randomised, controlled trial. *Obstet Gynecol* 97: 643–648.
- Khan MY, Mah MW, Memish ZA (2001). Brucellosis in pregnant women. *Clin Infect Dis* 3: 1172–1177.
- Kiss E, Bhattoa HP, Bettembuk P, Balogh A, Szegedi G (2002). Pregnancy in women with systemic lupus erythematosus. *Eur J Obstet Gynecol Reprod Biol* 101:129–101134.
- Kline J, Stein Z, Susser M (1989). Conception to birth — epidemiology of prenatal development. In *Monographs in Epidemiology and Biostatistics*, Vol. 14. Oxford University Press, Oxford.
- Knudsen UB, Hansen V, Juul S, Secher NJ (1991). Prognosis of a new pregnancy following previous spontaneous abortions. *Eur J Obstet Gynecol Reprod Biol* 39: 31–36.
- Korn AP, Bolan G, Padian N, Ohm-Smith M, Schachter J, Landers DV (1995). Plasma cell endometritis in women with symptomatic bacterial vaginosis. *Obstet Gynecol* 85: 387–390.
- Kupferminc MJ, Peri H, Zwang E, Yaron Y, Wolman I, Eldor A (2000). High prevalence of the prothrombin gene mutation in women with intrauterine growth retardation, abruptio placentae and second trimester loss. *Acta Obstet Gynecol Scand* 79: 963–967.

- Kurkinen-Raty M, Vuopala S, Koskela M, Kekki M, Kurki T *et al.* (2000). A randomised controlled trial of vaginal clindamycin for early pregnancy bacterial vaginosis. *BJOG* 107: 1427–1432.
- Liddell HS, Pattison NS, Zanderigo A (1991). Recurrent miscarriage — outcome after supportive care in early pregnancy. *Aust N Z J Obstet Gynaecol* 31: 320–322.
- Lockshin MD, Druzin ML, Qamar T (1989). Prednisone does not prevent recurrent fetal death in women with antiphospholipid antibody. *Am J Obstet Gynaecol* 160: 439–443.
- Lockwood CJ (1999) Heritable coagulopathies in pregnancy. *Obstet Gynecol Surv* 54: 754–765.
- Lyden TW, Vogt E, Ng AK, Johnson PM, Rote NS (1992). Monoclonal antiphospholipid antibody reactivity against human placental trophoblast. *J Reprod Immunol* 22: 1–14.
- McDonald IA (1957). Suture of the cervix for inevitable miscarriage. *J Obstet Gynaecol Br Emp* 64: 346–350.
- March CM, Israel R (1990). Hysteroscopic management of recurrent abortion caused by septate uterus. *Am J Obstet Gynecol* 162: 598–599.
- Mishra GD, Dobson AJ, Schofield MJ (2000). Cigarette smoking, menstrual symptoms and miscarriage among young women. *Aust N Z J Public Health* 24: 413–420.
- Moinian M, Andersch B (1982). Does cervix conization increase the risk of complications in subsequent pregnancies? *Acta Obstet Gynecol Scan* 61: 101–103.
- MRC/RCOG (1993). Final report of the Medical Research Council/Royal College of Obstetricians and Gynaecologists multicentre randomised trial of cervical cerclage. *BJOG* 100: 516–523.
- Nelen WL, Blom HJ, Steeger EA, den Heijer M, Eskes TK (2000). Hyperhomocysteinemia and recurrent pregnancy loss: a meta-analysis. *Fertil Steril* 74: 1196–1199.
- Nybo Anderson AM, Wohlfart J, Christens P, Olsen J, Melbye M (2000). Maternal age and fetal loss: population based register linkage study. *BMJ* 320: 1708–1712.
- Oakeshott P, Hay P, Hay S, Steinke F, Rink E, Kerry S (2002). Association between bacterial vaginosis or chlamydial infection and miscarriage baffle 16 weeks gestation: a prospective community based cohort. *BMJ* 325: 1334.
- Oates-Whitehead RM, Carrier JAK (2002). Progestogen for preventing miscarriage (Cochrane Review). In *The Cochrane Library*, Issue 4. Update Software, Oxford.

- Osser S, Persson K (1996). Chlamydial antibodies in women who suffer miscarriage. *BJOG* 103: 137–141.
- Parazzini F, Chatenoud L, Tozzi L, Benzi G, Dal Pino D, Fedele L (1997). Determinants of risk of spontaneous abortions in the first trimester of pregnancy. *Epidemiology* 8: 681–683.
- Paternoster DM, Santarossa C, Vettore N, Dalla Pria S, Grella P (1998). Obstetric complications in Marfan's syndrome pregnancy. *Minerva Ginecol* 50: 441–443.
- Paukku M, Tulppala M, Puolakkainen M, Anttila T, Paavonen J (1999). Lack of association between serum antibodies to *Chlamydia trachomatis* and a history of recurrent pregnancy loss. *Fertil Steril* 73: 656–657.
- Perry DJ (1999). Hyperhomocysteinaemia. *Bailliere's Best Pract Res Clin Haematol* 12: 451–477.
- Porcu G, Carvello L, D'Ercole C, Cohen D, Roger V, de Motgolfier R, Blanc B (2000). Hysteroscopic metroplasty for separate uterus and repetitive abortions: reproductive outcome. *Eur J Obs Gyn Rep Biol* 88: 81–84.
- Preston FE, Rosendaal FR, Walker ID, Briët E, Berntorp E *et al.* (1996). Increased fetal loss in women with heritable thrombophilia. *Lancet* 348: 913–916.
- Quenby SM, Farquharson RG (1993). Predicting recurring miscarriage: what is important? *Obstet Gynecol* 82: 132–138.
- Quenby SM, Farquharson RG (1994). Human chorionic gonadotrophin supplementation in recurring pregnancy loss: a controlled trial. *Fertil Steril* 62: 708–710.
- Rae R, Smith IW, Liston WA, Kilpatrick DC (1994). Chlamydial serologic studies and recurrent spontaneous abortion *Am J Obstet Gynecol* 170: 782–785.
- Raghupathy R, Makhseed M, Azlieh F, Omu A, Gupta M, Farhat R (2000). Cytokine production by maternal lymphocytes during normal human pregnancy and in recurrent spontaneous abortion. *Hum Reprod* 15: 713–718.
- Rai RS, Regan L, Clifford K, Pickering W, Dave M, Mackie I, McNally T, Cohen H (1995a). Antiphospholipid antibodies and beta 2-glycoprotein-I in 500 women with recurrent miscarriage: results of a comprehensive screening approach. *Hum Reprod* 10: 2001–2005.
- Rai RS, Clifford K, Cohen H, Regan L (1995b). High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies. *Hum Reprod* 10: 3301–3304.

- Rai RS, Shlebak A, Cohen H, Backos M, Holmes Z, *et al.* (2001). Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage. *Hum Reprod* 16: 961–965.
- Ralph SG, Rutherford AJ, Wilson JD (1994). Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. *BMJ* 319: 220–223.
- Rand JH, Wu XX, Guller S, Gil J, Guha A, Scher J, Lockwood CJ (1994). Fetus–placenta–newborn: reduction of annexin-V (placental anticoagulant protein-1) on placental villi of women with antiphospholipid antibodies and recurrent spontaneous abortion. *Am J Obstet Gynecol* 17: 1566–1572.
- Ratten GJ, Beischer NA (1979). The effect of termination of pregnancy on maturity of subsequent pregnancy. *Med J Aust* 1: 479–480.
- Ray JG, Laskin CA (1999). Folic acid and homocysteine metabolic defect and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systematic review. *Placenta* 20: 519–529.
- RCOG (2001). Guideline No. 25: Early pregnancy loss—management. Clinical Green Top Guidelines.
- Rees DC, Cox M, Clegg JB (1995). World distribution of factor V Leiden. *Lancet* 346: 1133–1134.
- Regan L (1997). Sporadic and recurrent miscarriage. In *Problems in Early Pregnancy* eds. Grudzinskas JG and O'Brien PMS. RCOG Press, London.
- Regan L, Braude PR, Trembath PL (1989). Influence of past reproductive performance on risk of spontaneous abortion. *BMJ* 299: 541–545.
- Rosenstein IJ, Morgan DJ, Lamont RF, Sheehan M, Dore CJ *et al.* (2000). Effect of intravaginal clindamycin cream on pregnancy outcome and on abnormal vaginal microbial flora of pregnant women. *Infect Dis Obstet Gynecol* 8: 158–165.
- Rubio C, Simon C, Blanco J, Vidal F, Minguez Y *et al.* (1999). Implications of sperm chromosome abnormalities in recurrent miscarriage. *J Assist Reprod Genet* 16: 253–258.
- Rust OA, Atlas RO, Jones KJ, Benham BN, Balducci J (2000). A randomised trial of cerclage versus no cerclage among patients with ultrasonographically detected second-trimester preterm dilatation of the internal os. *Am J Obstet Gynecol* 183: 830–835.
- Sagle M, Bishop K, Ridley N, Alexander FM, Michel M, Bonney RC, Beard RW, Franks S (1988). Recurrent early miscarriage and polycystic ovaries. *BMJ* 297: 1027–1028.

- Sampson M, Kong C, Patel A, Unwin R, Jacobs HS (1996). Ambulatory blood pressure profiles and plasminogen activator inhibitor (PAI-1) in lean women with and without the polycystic ovary syndrome. *Clin Endocrinol* 45: 623–629.
- Sanson BJ, Friederich PW, Simioni P, Zanardi S, Hilsman MV *et al.* (1996). The risk of abortion and stillbirth in antithrombin-, protein C- and protein S-deficient women. *Thromb Haemos* 75: 387–388.
- Saraiya M, Berg C, Shulman H, Green CA, Atrash HK (1999). Estimated of the annual number of clinically recognised pregnancies in the United States, 1981–1991. *Am J Epidemiol* 149: 1025–1029.
- Scott JR, Pattison N (2002). Human chorionic gonadotrophin for recurrent miscarriage (Cochrane Review). In *Cochrane Library*, Issue 4. Update Software, Oxford.
- Shirodkar VN (1955). A new method of operative treatment for habitual abortions in the second trimester of pregnancy. *Antiseptic* 52: 299–300.
- Simon C, Rubio C, Vidal F, Gimenez C, Moreno C, Parrilla JJ, Pellicer A (1998). Increased chromosome abnormalities in human preimplantation embryos after *in vitro* fertilization in patients with recurrent miscarriage. *Reprod, Fertil Dev* 10(1): 87–92.
- Sozio J, Ness RB (1998). Chlamydial lower genital tract infection and spontaneous abortion. *Infect Dis Obstet Gynecol* 6: 8–12.
- Spandorfer SD, Neuer A, Giraldo PC, Rosenwaks Z, Witkin SS (2001). Relationship of abnormal vaginal flora, proinflammatory cytokines and idiopathic infertility in women undergoing IVF. *J Reprod Med* 46: 806–810.
- Stephenson MD, Dreher K, Houlihab E, Wu V (1998). Prevention of unexplained recurrent spontaneous abortion using intravenous immunoglobulin: a prospective, randomised, double-blinded, placebo-controlled trial. *Am J Reprod Immunol* 39: 82–88.
- Stray-Pederson B, Stray-Pederson S (1984). Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. *Am J Obstet Gynecol* 148: 140–146.
- Tait RC, Walker ID, Perry DJ, Islam SI, McCall F (1994). Prevalence of antithrombin deficiency in the healthy population. *Br J Haematol* 87: 106–112.
- Tait RC, Walker ID, Reitsma PH, Islam SI, McCall F, Poort SR, Conkie JA, Bertina RM (1995). Prevalence of protein C deficiency in the healthy population. *Thromb Haemost* 73(1): 87–93.

- To MS, Palaniappan V, Skentou C, Gibb D, Nicolaidis KH (2002). Elective cerclage vs. ultrasound-indicated cerclage in high-risk pregnancies. *Ultrasound Obstet Gynaecol* 19: 475–477.
- Ugwumadu A (2002). Bacterial vaginosis in pregnancy. *Curr Opin Obstet Gynecol* 14(2): 115–118.
- Ugwumadu A, Manyonda I, Reid F, Hay P (2003). Effect of early clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial. *Lancet* 361: 983–988.
- van der Put NMJ, Eskes TK, Blom HJ (1997). Is the common 667C-T mutation in methylene tetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. *Q J Med* 90: 111–115.
- van Dongen PW, Nijhuis JG (1991). Transabdominal cerclage. *Eur J Obstet Gynecol Reprod Biol* 412: 97–104.
- Wang JX, Davies MJ, Norman RJ (2002). Obesity increases the risk of spontaneous abortion during infertility treatment. *Obes Res* 10: 551–554.
- Wennerholm UB, Holm B, Mattsby-Baltzer I, Nielson T, Platz-Christensen JJ *et al.* (1998). Interleukin-1 alpha, interleukin-6 and interleukin-8 in cervico/vaginal secretion for screening preterm birth in twin gestation. *Acta Obstet Gynecol Scan* 775: 508–514.
- Wilson R, McInnes I, Leung B, McKilltop JH, Walker JJ (1997). Altered interleukin 12 and nitric oxide levels in recurrent miscarriage. *Eur J Obstet Gynecol Reprod Biol* 75: 211–214.
- Wilson R, Moore J, Jenkins C, Miller H, MacLean MA, McInnes IB, Walker JJ (2003). Abnormal IL-2 receptor levels in non-pregnant women with a history of recurrent miscarriage. *Hum Reprod* 18: 1529–1530.
- Windham GC, Von Behren J, Fenster L, Schaefer C, Swan SH (1997). Moderate maternal alcohol consumption and risk of spontaneous miscarriage. *Epidemiology* 8: 509–514.
- Woelfer B, Salim R, Banerjee S, Elson J, Regan L, Jurkovic D (2001). Reproductive outcomes in women with congenital uterine anomalies detected by three-dimensional ultrasound. *Obstet Gynecol* 98: 1099–1103.
- Younis JS, Brenner B, Ohel G, Tal J, Lanir N, Ben-Ami M (2000). Activated protein C resistance and factor V Leiden mutation can be associated with first- as well as second-trimester recurrent pregnancy loss. *Am J Reprod Immun* 43: 31–35.