Recurrent miscarriage: aetiology, management and prognosis

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Recurrent miscarriage (RM) is a heterogeneous condition. A large number of studies has recently been published, yet many of them have conflicting conclusions. The various aetiological factors, management, prognostic features and outcomes of a subsequent pregnancy in women with RM are reviewed.

Key words: aetiology/prognosis/recurrent miscarriage/treatment

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Introduction

A history of three or more consecutive spontaneous miscarriages occurs in ~0.5–3% of women (Daya, 1993; Tulppala *et al.*, 1993a; Katz and Kuller, 1994). This recurrent loss of pregnancy is often distressing for the patients and frustrating for physicians. In most cases, the cause is not apparent and often requires intensive and expensive clinical and laboratory investigations, despite which there is still a limited understanding of RM.

Definition

Recurrent miscarriage is often defined as three or more consecutive miscarriages. However, some investigators included two or more miscarriages in their series (Quenby and Farquharson, 1993; Stephenson *et al.*, 1998), others did not clarify whether the miscarriages were consecutive (Wilson *et al.*, 1999b). Furthermore some authors only included those with first trimester miscarriages (Clifford *et al.*, 1996) whereas others included losses in the mid-trimester (Stephenson *et al.*, 1998) or did not specify whether or not second trimester miscarriages were included (Kolho *et al.*, 1999). Furthermore, it is essential to specify the types of pregnancy loss, i.e. whether or not the

pregnancy loss occurred after fetal cardiac activity was identified. Bricker and Farquharson considered the pregnancy loss as embryonic loss if it occurred before fetal cardiac activity was identified, and as fetal loss if it occurred after fetal cardiac activity was identified (Bricker and Farquharson, 2002). However, other authors may classify the type of loss differently; for example, it has been proposed that there are three different types of loss: pre-clinical, demise <6 weeks; embryonic loss, demise at ≥6 weeks but ≤10 weeks gestation; fetal loss, demise at ≥10 weeks but <20 weeks gestation (Stephenson *et al.*, 2002). Until a classification is universally accepted, it is important to clearly define the stages at which the loss occurred, which is possible only if serial ultrasound examinations are carried out early on in the pregnancy according to current guidelines (Royal College of Obstetricians and Gynaecologists, 1995). Therefore, when comparing results from different investigators, it is important to understand that there may be different definitions and populations involved.

Aetiology

Identifiable causes of miscarriage

Current medical literature suggests that causes are identified in only ~50% of patients (Plouffe *et al.*, 1992; Tulppala *et al.*, 1993a; Clifford *et al.*, 1994; Katz and Kuller, 1994). The identifiable causes include the following categories.

Parental chromosomal anomalies

Parental chromosomal anomalies occur in ~4% of couples with recurrent miscarriage (RM) as opposed to 0.2% in the normal population. The most common abnormality is a balanced translocation, including reciprocal and Robertsonian transloca-tions resulting in unbalanced translocation in the fetus (Stirrat,

1990; Tulppala *et al*., 1993a; Clifford *et al*., 1994). However, chromosomal anomaly detected using conventional karyotyping techniques represents the 'tip of the iceberg' phenomenon. It is possible that an as yet unidentified micro-deletion or other subtle chromosomal aberrations may contribute to RM. (See also 'Fetal chromosomal anomalies' below.)

A recent study examined X-chromosome inactivation in peripheral blood leukocytes of women with unexplained RM (Uehara *et al*., 2001). X-Chromosome inactivation is a phenomenon which occurs in female mammals, such that one of the two X-chromosomes (one derived maternally and one derived paternally) is randomly inactivated to compensate for the difference in X-linked gene dosage between male and female. The authors found that 7/42 (16.7%) of women with unexplained RM exhibited preferential X-chromosome inactivation compared with 2/36 (5.6%) of control fertile subjects without a history of RM. Further analysis showed that the two-peak pattern appeared to be age-related, with incidence similar in the RM (7.1%) and age-matched control (5.6%) groups. On the other hand, the single-peak pattern, representing completely preferential X-chromosome inactivation, is directly correlated with RM. The underlying causes of preferential X-chromosome inactivation include cryptic X-chromosome aberrations (such as balanced X-autosome translocations and microdeletions of the X chromosome), gene mutation and genomic imprinting.

Aldrich *et al*. examined the relationship between HLA-G genotype polymorphisms and unexplained RM (Aldrich *et al*., 2001). HLA-G is a non-classical human leukocyte antigen expressed primarily in fetal tissues at the maternal–fetal interface. It is believed that HLA-G expression plays an important role in the establishment and maintenance of the fetal–placental unit in early pregnancy, possibly via interactions with natural killer (NK) cells (see 'Immunological factors'). The authors found that the presence of HLA-G* 0104 or HLA-G* 0105N allele in either partner was associated with an increased risk for miscarriage. Moreover, the frequency of either *0104 or *0105N carrier was higher in couples with five or more losses compared with couples with two or three previous losses (37 and 26% respectively). As the two alleles are both defined by polymorphisms in the α-2 domain, it is concluded that allelic variation in the α-2 domain of the HLA-G isoforms contributes to RM, perhaps in up to a third of cases.

Uterine pathology

Congenital uterine anomaly. Among the various congenital structural uterine anomalies, the septate uterus is the most common. There is little doubt that the septate uterus is associated with an increased risk of miscarriage due to impairment of implantation (Homer *et al*., 2000). It is now recognized that the septum is rather avascular. Fedele *et al*. used scanning electron microscopy to compare endometrial biopsy specimens obtained from the septum and the lateral uterine wall in the pre-ovulatory phase. They found that the septal endometrium showed defective development, indicative of a reduction in the sensitivity to steroid hormones (Fedele *et al*., 1996). The surgical correction of congenital uterine anomalies has recently been reviewed (Matte *et al*. 2000).

Asherman's syndrome/endometrial fibrosis. Asherman's syndrome is an acquired condition which is due to the presence of post-traumatic intrauterine adhesions, partly or completely obliterating the uterine cavity. Endometrial responsiveness to steroid hormones is reduced in areas affected by intrauterine adhesions or fibrosis. A lesser degree of damage to the endometrium may produce patchy fibrosis without a significant amount of intrauterine adhesion, which is sometimes referred to as partial or incomplete Asherman's syndrome. Successful division of the intrauterine adhesions in cases without extensive fibrosis may restore the responsiveness of the endometrium and lead to regular menstruation. However, the presence of extensive dense fibrosis indicates a poor prognosis.

Uterine fibroid. The situation with respect to uterine fibroid is less clear. Uterine fibroid may also affect implantation and increase the risk of miscarriage. There is convincing observational data from six IVF series to suggest that reproductive outcome is significantly compromised with submucous fibroids (i.e. fibroids distorting the cavity), modestly compromised with intramural fibroids, and possibly compromised with subserosal fibroids (Seoud *et al*., 1992; Farhi *et al*., 1995; Eldar-Geva *et al*., 1998; Ramzy *et al*., 1998; Stovall *et al*., 1998; Bajekal and Li, 2000; Hart *et al*., 2001). It is not known if the endometrium covering a submucous fibroid or close to an intramural fibroid responds suboptimally to steroid hormones. However, it appears from a number of retrospective and cohort studies that there is good evidence that removal of submucous fibroids reduces miscarriage rate, and some evidence that removal of intramural fibroids also reduces miscarriage rate (Li *et al*., 1999; Bajekal and Li, 2000). Nevertheless, the improvement in outcome may be produced independently of the endometrial factor.

Raziel *et al*. examined the prevalence of structural uterine pathology by hysterosalpingogram (HSG) and hysteroscopy in 106 women with RM (Raziel *et al*., 1994). Their findings included: HSG, normal findings 43.6%, uterine septum 17.9% and filling defects and/or uterine wall irregularity 38.7%; hysteroscopy, normal findings 53%, uterine septum 21.7%, intrauterine adhesions 23.6% and endometrial polyp 1% (Raziel *et al*., 1994). We routinely perform HSG in our patients with RM and find a lower prevalence of uterine cavity abnormality (15%) in our own series.

Primary endometrial defect. Suboptimal progesterone production and uterine pathology may result in endometrial defects. In some cases, endometrial defect may occur despite normal progesterone levels and in the absence of obvious uterine pathology, in which case it is unexplained. A steroid receptor defect may be an explanation for the observed abnormality. Early investigations into levels of estrogen (ER) and progesterone (PR) receptors in the human endometrium utilized radioactive binding assays and produced conflicting results on the relationship between ER and PR receptors and luteal phase defects (Levy *et al*., 1980; Gravanis *et al*., 1984; McRae *et al*., 1984; Hirama and Ochiai, 1995). Immunostaining for ERα in the luteal phase in endometrium from normal controls shows a steady down-regulation from a mid-cycle peak that is observed in both stroma and glandular epithelium (Lessey *et al*., 1988). However, we have found a wide variation in intensity and pattern of ERα expression and have concluded that

immunocytochemistry is an inadequate method for the investigation of differences in ERα expression between normal and RM women (Li *et al*., 2000). In contrast, immunostaining of luteal phase PR shows a rapid and consistent down-regulation in luminal and glandular epithelium with slower down-regulation in the stroma (Press *et al*., 1988). We observed PR positive gland staining suggestive of delayed down-regulation of PR in about one-third (9/25) of timed endometrial biopsies from RM women, only one of which had been diagnosed with luteal phase defects (Li *et al*., 2000).

The discovery of ERα (Mosselman *et al*., 1996) and PR$_B$ has led to a renewed interest in the complex relationship between the action of steroids and the modifying effects of their different isoforms in the endometrium (Hall and McDonnell, 1999). Moreover, experiments in knock-out mice indicate that the action of progesterone on PR$_A$ and PR$_B$ may be functionally distinct (Mulac-Jericevic *et al*., 2000) and therefore difference in expression pattern might result in different actions in different tissues. Wang *et al*. found no immunostaining for PR$_B$ both in the endometrium of the secretory phase and early pregnancy in normal fertile women (Wang *et al*., 1998); however, dual immunofluorescence has shown persistence of PR$_B$ during the mid-secretory phase implicating a role in gland secretion (Mote *et al*., 1999).

We have also examined the expression of the androgen receptor (AR). Immunostaining for AR was observed mainly in the stroma, did not show any cyclical changes, and no differences were observed between endometrium from normal and RM women (Li *et al*. 2000).

In summary, preliminary results using immunohistochemistry suggest that steroid receptor abnormalities may be present in a small subpopulation of women with unexplained RM. However, further investigations are required, as identification of these differences by steroid receptor immunohistochemistry alone is not sufficiently precise.

Prothrombotic state

Antiphospholipid syndrome. Antiphospholipid syndrome is a well-recognized cause of RM and has been reported in 7–42% of women (Greaves *et al*., 2000). The wide variability is likely to be due to bias in patient selection, inclusion of patients with transient antiphospholipid antibodies and poor standardization of laboratory protocols. A recent study of inter-assay variations showed poor agreement between three laboratories on IgM anticardiolipin antibody testing, and modest agreement on IgG anticardiolipin antibody testing (Roberts *et al*., 2002). More disturbingly, none of the tests was significantly more likely to be positive in women with RM ($n = 36$), compared with 26 controls.

The diagnosis of antiphospholipid syndrome requires fulfilment of at least one of the following clinical criteria (Wilson *et al*., 1999a):

(a) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal abnormalities excluded or:

(b) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus or:

(c) One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe pre-eclampsia or eclampsia or severe placental insufficiency:

In addition, persistent abnormality of one of the following tests when measured at least twice, >6 weeks apart:

(a) Lupus anticoagulant. This can be detected in a number of ways using coagulation-based assays (Greaves, 2002).

(b) Antiphospholipid antibodies. These are IgG or IgM antibodies against cardiolipin and are usually detected using the enzyme-linked immunosorbent assay technique. There are several assay kits on the market and there is significant inter-assay variation in the estimation of these antibodies (Roberts *et al*., 2002). Recently, more specific assays detecting antibodies against the β-2-glycoprotein 1 have been introduced.

Heritable thrombophilia. There are five currently recognized heritable thrombophilic defects. These are deficiencies of antithrombin, protein C or protein S as well as the factor V Leiden and prothrombin 20210A variant. Factor V Leiden can be detected using either a coagulation-based assay (activated protein C resistance) or by direct demonstration of the Arg506Gln change in the factor V gene.

An increased incidence of fetal loss in women with thrombophilia was first reported from a European study (EPCOT) that analysed pregnancy outcome in 571 women with known heritable thrombophilia (Preston *et al*., 1996). Although overall there was a significant association between thrombophilia and miscarriage, this was not found in women with factor V Leiden where the risk of a pregnancy ending in miscarriage was very similar to that found in controls (11.7 versus 12.2%). Whereas deficiencies of antithrombin, protein C or protein S are rare, factor V Leiden is common, affecting 3–4% of the UK population. After this initial observation there have been a number of smaller studies reporting a positive association between factor V Leiden and RM (Grandone *et al*., 1997; Younis *et al*. 2000). More recently, however, Rai *et al*. studied 904 consecutive women with recurrent early and 207 women with late (>12 weeks) miscarriage but failed to find an association with factor V Leiden, although they did observe an association with activated protein C resistance (Rai *et al*., 2001). The reason for the conflicting data is partly due to case selection and to the small size of the studies. In view of the uncertainty about the contribution of heritable thrombophilia to RM, routine testing for these defects is not recommended (Walker *et al*., 2001).

Other haematological abnormalities. A number of other coagulation abnormalities have been reported to be associated with RM, including impaired fibrinolytic activity (Gris *et al*., 1995), factor XII deficiency (Daya, 1994; Ogasawara *et al*., 2001) and reduced activated partial thromboplastin time (Ogasawara *et al*. 1998). The evidence for such associations is, however, at present preliminary and these assays should not be performed outside clinical studies.

Essential thrombocythaemia is known to be associated with an increased risk of fetal loss. In two studies on 106 and 74 pregnancies in women with essential thrombocythaemia, the rate of first trimester miscarriage was 36 and 37% respectively (Griesshammer *et al*., 1996; Wright and Tefferi, 2001). A full

Table I. The prevalence of high LH levels in samples taken in the mid-follicular phase and ultrasonographic evidence of polycystic ovaries, among women with recurrent miscarriage

	Prevalence of polycystic ovaries	Prevalence of high LH (>10 IU/l) levels	LH assay method used	Timing of sample	Blood or urine sample	Control group	LH/FSH ratio
Tulppala *et al*. (1993b)	22/50 (44)[a]	2/50 (4)	Immunometric assay	Mid-follicular phase	Blood	Yes, *n* = 20	⩾2 in 1/50 = 2%
Sagle *et al*. (1988)	46/56 (82)[b]	0/56 (0)	Not specified	Not specified	Blood	Yes, *n* = 11	
Clifford *et al*. (1994)	278/500 (56)	169/294 (57)	Radioimmuno-assay	Mostly mid- or late follicular phase but some in luteal phase	Blood and urine	No	
Carp *et al*. (1995)		56/153 (37)	Radioimmuno-assay	Mid-follicular phase	Blood	No	⩾3 in 22/131 = 14%
Bussen and Steck (1999)	2/42 (5)[c]	1/42 (2–3)[d]	Radioimmuno-assay	Early follicular phase	Blood	Yes, *n* = 42	
Li *et al*. (2000)	8/102 (7.8)	Blood: 7/92 (8) Urine: 0/38 (0)	Immunometric assay	Daily from mid-follicular phase	Blood and urine	Yes, *n* = 22	⩾2 in 3/107 = 2.8% ⩾3 in 2/107 = 1.9%

Values in parentheses are percentages.
[a]Versus control, 4/20 = 20%, *P* = 0.06; [b]versus control, 2/11 = 18%, *P* < 0.001; [c]versus control 1/42 = 2.4% (not significant); [d]versus control 0/42 (not significant), high LH defined as >15 IU/l.

blood count should be an essential investigation in any woman presenting with RM.

Endocrinological disorders

Hypersecretion of LH. This has been considered to be a marker of miscarriage (Homburg *et al*., 1988; Regan *et al*., 1990). A number of studies examined the prevalence of high LH levels and polycystic ovaries (PCO) among women with RM. The findings are summarized in Table I (Sagle *et al*., 1988; Tulppala *et al*., 1993b; Clifford *et al*., 1994; Carp *et al*., 1995; Bussen *et al*., 1999; Li *et al*., 2000). The prevalence of high LH levels in the follicular phase of women with RM, defined as plasma concentration of ⩾10 UI/l, varied enormously, from 0 to 37%. In addition, Clifford *et al*. (1994) found elevated urinary LH excretion in 57% of women with RM. Such a large variation suggests either that different authors are dealing with very different populations of women with RM, or that different methods were used to measure LH levels.

There are several reasons why it is difficult to compare LH measurements between different studies. First, different assay methods may be used by different investigators. For example, radioimmunoassay based on polyclonal antibodies has been used (Regan *et al*., 1990), whereas a more recent study (Tulppala *et al*., 1993b) employed a fluoro-immunometric assay based on a monoclonal antibody, which is highly sensitive and specific. It is now recognized that radioimmunoassay produces results 30% higher than those of immunometric assays (Balen *et al*., 1993). It is therefore not surprising that Clifford *et al*. (1994) and Carp *et al*. (1995), who employed radioimmunoassay, reported higher prevalence of high LH levels than Tulppala *et al*. (1993b), who employed immunometric assay.

Second, the type of sample (urine or blood) may also affect the result. Whereas most authors measured LH concentration in plasma samples, some measured LH concentration in urine samples. Due to the pulsatile mode of LH secretion, a single blood sample cannot be used reliably to evaluate gonadotrophin pathophysiology. This is because the 95% confidence limits of a single blood sample taken to measure LH range from 50 to 150% of the measured value (Bergendahl *et al*., 1996). Therefore, either repeat measurements of LH on consecutive mornings in the same individual, or repeated samples in the same morning, are recommended to reliably estimate the mean serum LH concentration in any one patient. Some investigators claimed that timed urinary LH levels could be useful because they may reflect LH secretion over a period of time. Watson *et al*. found no difference in serum follicular and luteal phase LH levels between women with RM and controls (Watson *et al*., 1993). However, in the same study population they found significantly higher early morning urine LH levels in the RM patients. Clifford *et al*. measured LH levels in urine samples and reported a significantly higher prevalence (57%) of 'hypersecretion of LH' among women with RM than other investigators. It is therefore possible that LH measurement in urine samples may overestimate the true prevalence of the abnormality and that this may partly explain the lack of observable, beneficial effect of suppression of LH secretion in reducing miscarriage rate in a randomized controlled study (Clifford *et al*., 1994).

Third, even if the measurement is performed on blood samples, the timing of the sample may affect the results as plasma LH levels change in different parts of the cycle. Most investigators obtain samples in the mid-follicular phase, i.e. day 6 or 7 of the cycle (Tulppala *et al*., 1993b; Carp *et al*., 1995; Clifford *et al*., 1996). Samples taken later than the mid-follicular phase, i.e. the

pre-ovulatory phase, are likely to show higher LH levels. Clifford et al. (1996) appeared to have included women with high LH levels in the luteal phase, which is quite different to high LH levels in the follicular phase.

From the above discussions, although the series of Clifford et al. (1994) is the largest study to date ($n = 500$) on the prevalence of high LH levels in women with RM, the prevalence (57%) may represent an over-estimation for two reasons: (i) the use of an older, non-specific assay based on polyclonal antibodies which produces results higher than that of new immunometric assays based on monoclonal antibodies; and (ii) the timing of the sample is not precisely defined: some in mid-follicular phase, some in late follicular phase and some in luteal phase.

Our data (Li et al., 2000), gathered by improved methodology including daily blood samples collected in the mid- to late-follicular phase, and by the use of newer, more precise immunometric assays, suggested that hypersecretion of LH occurs in only 8% of women. Our observation is similar to that of other studies (Sagle et al., 1988; Tulppala et al., 1993b; Bussen et al., 1999); in all these studies the prevalence of high LH levels (⩾10 IU/l) in women with RM appeared low (⩽8%).

Polycystic ovaries. In our study of 102 RM women who underwent transvaginal ultrasonography, only eight (7.8%) had the typical features of PCO. The prevalence of PCO in our series is significantly lower than several previous studies (Table I) and also lower than that reported in the normal population (28%) (Kousta et al., 1999). The exact reason for the observed differences is unclear. In a recent study (Rai et al., 2000a), the prevalence of PCO among women with RM was 852/2199 (40.7%). However, the live birth rate was similar in women with PCO (60.9%) and in women with normal ovarian morphology (58.5%). The authors concluded that polycystic ovarian morphology is not predictive of pregnancy loss among ovulatory women with RM who spontaneously conceived.

High androgen levels. Two recent studies have shown that androgen levels in the follicular phase are higher in women who have RM than in normal fertile controls (Okon et al., 1998; Bussen et al., 1999). The observation is independent of the association between polycystic ovarian disease and RM. The high androgen levels were significantly, but negatively, correlated with the concentration of placental protein 14 (glycodelin A), a biochemical marker of endometrial function in uterine flushings (Okon et al., 1998). In-vitro studies have shown that androgens cause an increase in epidermal growth factor receptor concentration (Watson et al., 1998) and reduce the secretory activities of glandular cells as measured by glycodelin A production in a dose-dependent fashion (Tuckerman et al., 2000), which suggest an adverse effect of androgens on endometrial glandular cell function.

Hyperprolactinaemia. Tal et al. reported that prolactin, a stress-related hormone, reduced HCG secretion from the early human placenta in vitro (Tal et al., 1991). Ben-David suggested that hyperprolactinaemia may occur in a transient manner, around the pre-ovulatory phase (Ben-David, 1987). To detect the transient hyperprolactinaemia, several blood samples were necessary: one at the mid-follicular phase, three to four during the expected ovulation period, and one at the mid-luteal phase. A rise of 200% greater than mid-follicular baseline levels at the time of peak follicular maturity indicates transient hyperprolactinaemia, which is associated with unexplained infertility and repeated miscarriages. Hirahara et al. reported the use of bromocryptine in 64 women with two or more miscarriages and hyperprolactinaemia and achieved a successful pregnancy outcome of 85.7%, compared with 52.4% of control subjects (Hirahara et al., 1998). However, the study was open to a number of criticisms including the lack of strict criteria for hyperprolactinaemia and RM (Dlugi, 1998). In our recent study (Li et al., 2000), 3/122 (2.5%) subjects with RM had marginally elevated prolactin levels but none of them had significantly high (⩾1000 IU/l) prolactin. In addition, a subgroup of women ($n = 23$) had daily plasma prolactin measured in the mid-follicular phase to early luteal phase, with no evidence of pre-ovulatory rise of prolactin. Our data suggest that there is no firm evidence for an association between hyperprolactinaemia and RM (Li et al., 2000).

Immunological factors

It is often assumed that immunological mechanisms are involved in successful implantation. The implanting embryo possesses paternal antigens and may therefore be rejected as an 'allograft'. Maternal adaptation of the immunological responses to the implanting embryo is the key to successful establishment of the fetal–placental unit. Miscarriage may therefore be a consequence of implantation failure, secondary to inappropriate humoral or cellular immunological response to the implanting embryo.

Alterations in humoral immune response. Autoantibodies are more common in patients with RM in comparison with control populations. Autoantibodies are found in 18–43% of patients with RM. The antibodies commonly identified include antiphospholipid antibodies (14%) (see 'Prothrombotic state' above) and antinuclear antibodies (7%) (Lin, 1993; Tulppala et al., 1993a); the significance of the latter is uncertain. Although antisperm antibodies are associated with male infertility, there is no association between sperm antibodies and sporadic pregnancy loss (Simpson et al., 1996) or recurrent spontaneous miscarriage (Clarke and Baker, 1993).

The association between thyroid antibodies and RM has been examined in a number of studies. Pratt et al. found that 31% of women with RM had positive test results for one or both thyroid antibodies (peroxidase and thyroglobulin), compared with 19% in the control group, although the difference did not reach statistical significance (Pratt et al., 1993a,b). Bussen and Steck found that the prevalence of thyroid autoantibodies, including thyroglobulin or peroxides antibodies are significantly increased in women with RM (8/22 = 36%) compared with a group of nulligravidae (2/22 = 9%) and multigravidae (1/22 = 5%) control subjects (Bussen and Steck, 1995). The association between RM and thyroid antibodies may be a result of: (i) a direct effect of these autoantibodies on fetal tissue; or (ii) the thyroid antibodies representing an underlying more generalized defect in auto-immunity.

The prognostic value of thyroid antibody. Among women with RM known to be positive for thyroid peroxidase antibody, those who miscarried again in a subsequent pregnancy had a significantly higher antibody titre than those who had a live birth

(Wilson *et al*., 1999b), which suggests that the antibody titre is of prognostic value. Moreover, in those pregnancies which continued to term, the titre and avidity declined as pregnancy progressed. However, a more recent study suggested that the presence of thyroid autoantibodies does not affect the future pregnancy outcome of women with a history of RM (Rushworth *et al*., 2000). The prognostic value of thyroid autoantibodies therefore remains uncertain.

Eblen *et al*. investigated the reactivity of maternal antibodies with endometrium-derived antigens by Western immunoblot techniques (Eblen *et al*., 2000). The serum from women with RM had a significantly higher rate of recognition of antigens (21 and 28 kDa) derived from endometrial tumours than control multiparous women. In addition, women with RM had significantly lower levels of asymmetric (blocking) IgG compared with controls. They concluded that women with RM had an altered immunological recognition of endometrial antigens.

Wilson *et al*. reported that normal pregnancy was associated with a significant increase in total IgG production and an increase in IgG subclasses 1, 2 and 3 but not subclass 4 (Wilson *et al*., 2001). However, women with a history of RM and whose pregnancy again ended in miscarriage had attenuated increased production of the total IgG and the subclasses 1, 2 and 3, as well as reduced production of IgG subclass 4. The increase in IgG production is mediated via Th-2 type cytokines (IL-10 and IL-4) and inhibited by TH-1 type cytokine interferon γ.

Alterations in cellular immune response. Several recent studies have reported significant changes in the circulating immunocompetent cells of women with RM. Ntrivalas *et al*. showed that peripheral blood NK cells of women with RM have a higher proportion of activated NK cells *in vivo* than control subjects (Ntrivalas *et al*., 2001). Emmer *et al*. examined the peripheral NK cell cytotoxicity and NK and T cell numbers and found that women with RM whose subsequent pregnancy progressed to term delivery had a lower NK cell number than women whose subsequent pregnancies miscarried again (Emmer *et al*., 2000). Kotlan *et al*. found significantly higher antipaternal cytotoxic T-lymphocyte precursor frequencies in women with unexplained RM (Kotlan *et al*., 2001). Makhseed *et al*. examined the cytokine profile secreted by peripheral blood mononuclear cells obtained at the time of delivery or miscarriage in response to stimulation by phytohaemagglutinin and found that cells from women with RM who miscarried had an increased Th1:Th2 cytokine ratio compared with cells from women with RM who had a live birth or control subjects (Makhseed *et al*., 2001).

The alterations in cellular immunology have also been examined locally, in the endometrium and decidua by a number of investigators. The immunological aspects of human implantation and the interaction of decidual NK cell and trophoblast have recently been reviewed (Loke and King, 2000a,b). Around the time of implantation, ~20% of endometrial stroma cells are leukocytes, of which the majority are large granular lymphocytes (LGL) (Bulmer *et al*., 1987). The LGL are similar to peripheral NK cells, but express a different set of surface markers: CD56$^+$, CD16$^-$ and CD3$^-$. Quenby *et al*. examined the leukocyte population in mid-luteal phase of the endometrium of 22 women suffering from unexplained recurrent (≥3) miscarriage and compared the results with nine control subjects (Quenby *et al*.,

1999). All the biopsies were timed from the last menstrual period. They found similar numbers of CD3$^+$ and CD8$^+$ cells in both groups. However, the staining of CD4$^+$, CD14$^+$, CD16$^+$, CD56$^+$ and MHC class II$^+$ cells in the RM group was significantly higher than in the control group. Moreover, they found that within the group of women with RM, those who had a further subsequent miscarriage after the endometrial study had greater staining of CD56$^+$ cells than women whose subsequent pregnancy resulted in a live birth.

Clifford *et al*. also examined the CD56$^+$ cells in the endometrium of 29 women with RM and compared the results with 10 control subjects. All the endometrial biopsies were taken in the luteal phase, from days LH + 7 to LH + 10. The authors employed more refined, quantitative methods of evaluation and measured the number of CD56$^+$ cells per 10 high power fields (Clifford *et al*., 1999). They confirmed an earlier finding (Quenby *et al*., 1999) that there were significantly more endometrial CD56$^+$ cells in women with RM than in the control group. Moreover, they found no correlation between the number of CD56$^+$ cells and the maternal age, the number of previous miscarriages, a past history of a live birth and the time since last miscarriage. Interestingly, they found that among women with RM, those ($n = 6$) with at least one late loss (>13 weeks) did not appear to have an increase in CD56$^+$ cells compared with the control group.

The above two studies suggest that RM is associated with an alteration in the endometrial leukocyte population, especially an increase in CD56$^+$ cells. Quack *et al*. studied the leukocyte activation in the decidua of women with RM with chromosomally normal and abnormal fetuses (Quack *et al*., 2001). They found that significantly higher numbers of activated leukocytes were detected in the decidua of women with unexplained RM who had a normal male karyotype compared to those with a trisomy miscarriage or normal pregnancies following elective termination procedures. In addition, the number of cells comprising the major leukocyte subpopulation CD56$^+$ NK cells appeared reduced in the decidua of women with unexplained RM compared with decidua from women having elective termination. There is therefore good evidence to suggest that altered cellular immunity at the fetal–maternal interface is involved in unexplained RM.

In murine models, there is evidence for a dichotomous T-helper response, in that pregnancy rejection is mediated by T-helper-1 (TH-1) cytokines, whereas a T-helper-2 (TH-2) cytokine response confers protection. Lim *et al*. examined the expression of TH-1 and TH-2 cytokines in the endometrium of mid-luteal phase of non-conception cycles of women with RM and compared the results to a group of fertile control subjects by RT–PCR (Lim *et al*., 2000). Levels of TH-1 cytokines [interferon-γ, interleukin (IL)-2, IL-12 and tumour necrosis factor-β] were significantly higher, and levels of IL-6 (TH-2 cytokines) were significantly lower in women with RM. The observations suggest that the normal dichotomous T-helper response within the normal endometrium around the time of implantation, which creates an immunological environment conducive to successful implantation by the conceptus (Lim *et al*., 1998), is disrupted in women with RM. The findings were confirmed by von Wolff *et al*. (2000) who reported abnormal expression of IL-1β and IL-6 in the mid-secretory phase of women with RM.

An interesting question is whether or not the alterations in cellular immune response in the endometrium of women with RM are hormonally dependent, i.e. secondary to hormone changes. In the small study reported by Lim *et al.* (2000), the changes do not appear to be a consequence of suboptimal progesterone production by the corpus luteum (Lim *et al.* 2000).

Cervical weakness

Cervical weakness (incompetence) commonly causes pregnancy loss in the second trimester. It may be associated with congenital uterine anomalies such as septate or bicornuate uterus. Rarely it may be congenital following in-utero exposure to diethylstilboestrol (Edmonds, 1992; Plouffe *et al.*, 1992). The typical history is that of a sudden rupture of fetal membranes in the second trimester, followed by a painless miscarriage. There is no single truly diagnostic test for cervical weakness (Medical Research Council/Royal College of Obstetricians and Gynaecologists Working Party, 1993).

The diagnosis is often based on the following: (i) a typical history of painless dilatation of the cervix in the second trimester of pregnancy, leading to the finding of bulging membranes on presentation or sudden rupture of membranes, followed by a relatively painless miscarriage or preterm delivery; (ii) during the non-pregnant stage, the cervical resistance, as measured by the cervical resistance index (Anthony *et al.*, 1982, 1997), is reduced; and (iii) during pregnancy, the cervix is found to be effaced or dilated in the mid-trimester, or serial ultrasound examination in the mid-trimester shows that the cervical length has reduced to <25 mm (Althuisius *et al.*, 2001), often associated with the funnelling sign.

Infections

Infections appear not to play a significant role in first trimester RM. Associations of RM with high titres of IgG antibody to chlamydia have been reported (Daya, 1994) but later refuted (Osser and Presson, 1996). Summers concluded that infection is an occasional cause of sporadic spontaneous abortion and, consistent with statistical probability, RM due to infection must be rare (Summers, 1994). Most patients with a history of RM will not benefit from an extensive infection work-up. Charles and Larsen also concluded that it is very unlikely that maternal infection causes recurrent abortion (Charles and Larsen, 1990). In our unit, we have recently ceased to carry out tests for toxoplasmosis, rubella, cytomegalovirus, herpes (TORCH screen) and intrauterine swab for chlamydia, after our own internal audit had shown that there was no positive result among 200 patients over a 5 year period.

Chorioamnionitis, however, may be a cause of premature labour and mid-trimester loss. Bacterial vaginosis (BV), a condition associated with a complex (quantitative) alteration in vaginal flora involving *Mobiluncus* species, *Bacteroides* species, peptostreptococci and *Mycoplasma hominis*, in addition to *Gardnerella vaginalis*. These changes are accompanied by a depletion in vaginal lactobacilli. BV is thought to be associated with sexual activity. Unlike other infections that depend primarily on bacteriological study, the diagnosis of BV is based on composite criteria (Hillier, 1993) in which three out of four should be present: (i) vaginal pH > 4.5; (ii) a grey homogeneous (malodorous, fishy-smelling) vaginal discharge; (iii) the presence

of clue cells in a wet mount preparation of vaginal fluid; and (iv) the amine test, in which a fishy odour is released after the addition of 10% potassium hydroxide to vaginal fluid.

Whilst BV is associated with second trimester loss, preterm premature rupture of membranes and preterm labour (Hay *et al.*, 1994a,b), it is generally considered to be unrelated to first trimester RM, although a recent study (Ralph *et al.*, 1999) showed that BV was associated with an increased risk of miscarriage in the first trimester of women undergoing IVF treatment.

Nutrition/environmental factors

Coffee consumption. A recent study involving 782 women with spontaneous (not recurrent) miscarriage in the first trimester and 1543 controls suggested that the odds ratios of spontaneous miscarriage, in comparison with non-drinkers, were 1.2, 1.8 and 4.0 respectively for drinkers of 1, 2–3 and ≥4 cups of coffee per day (Parazzini *et al.*, 1998). Similar findings have been reported previously (Dominguez-Rojas *et al.*, 1994).

Smoking and alcohol. Smoking has been reported to be associated with RM (Dominguez-Rojas *et al.*, 1994). Although one study (Parazzini *et al.*, 1994) found no association between alcohol consumption and miscarriage, two other studies found a positive association between alcohol consumption and miscarriage. Florey *et al.* found that drinking >3 units per week during the first trimester increases the relative risk of spontaneous miscarriage (RR = 2.3; 95% confidence interval = 1.1–4.5) (Florey *et al.*, 1992). Another study (Harlap and Shiono, 1980) found that whereas alcohol consumption did not increase the risk of first trimester miscarriage, drinking ≥1 unit per day resulted in a significant RR (1–2 units, RR = 1.98; ≥3 units, RR = 3.53) of second trimester miscarriage.

Hyperhomocysteinaemia. Homocysteine is a sulphydryl nonessential amino acid whose metabolism is normally tightly regulated. In a number of inherited and acquired conditions the levels of homocysteine are increased.

Hyperhomocysteinaemia is associated with the development of venous and arterial thrombosis (Makris, 2000). Homocysteine levels fall during normal pregnancy and this fall is independent of folate intake during the pregnancy (Bonnette *et al.*, 1999). High homocysteine levels have been associated with a number of pregnancy-related complications including neural tube defects, placental infarcts, fetal growth retardation as well as placental abruption.

Nelen *et al.* recently published a meta-analysis showing a significant association between hyperhomocysteinaemia and recurrent early pregnancy loss (Nelen *et al.*, 2000a). It has been suggested that maternal hyperhomocysteinaemia interferes with embryonic development through defective chorionic villous vascularization (Nelen *et al.*, 2000b).

Folate deficiency is one of the commonest acquired causes of hyperhomocysteinaemia. Among the genetic causes a common one is polymorphism at position 677 in the methyl tetrahydrofolate reductase (MTHFR) gene which in the homozygous form leads to a thermolabile enzyme variant. The thermolabile variant is found in 15% of the normal population and in association with folate levels in the lower part of the normal range leads to hyperhomocysteinaemia (Makris, 2000).

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When taking blood for homocysteine estimation, it is important that fasting samples are obtained and the laboratory should process these samples within 1 h of collection.

Exposure to pentachlorophenol (PCP). This compound, present in certain types of timber preservative, has been reported as a possible cause of miscarriage. Serum PCP level may be measured and should not exceed 25 µg/ml (De Maeyer *et al*., 1995).

Selenium deficiency. This is detected by a reduction of serum selenium level, and has been reported to be associated with first trimester miscarriage in one study (Barrington *et al*., 1996), but a subsequent study (Zachara *et al*., 2001) was unable to confirm the findings. Al-Kunani *et al*. found no significant difference in the serum level of selenium between women with RM and control subjects, but there was a significant reduction in the hair selenium level in the RM group compared with the control group (Al-Kunani *et al*., 2001). The authors concluded that although there appeared to be selenium deficiency in women with RM, the reduction in selenium level was seen only in hair samples but not in serum samples, suggesting that the observation does not represent a simple nutritional deficiency.

Coeliac disease. A recent study (Kolho *et al*., 1999) showed that there was no association between (subclinical) coeliac disease and RM.

Stress. It is unclear to what extent stress is responsible for miscarriage. Psycho-neuro-endocrinological or psycho-neuro-immunological pathways have been proposed as possible mechanisms in which stress may contribute to miscarriage. The relationship between stress and corpus luteal dysfunction has been reviewed (Li and Cooke, 1991), whereas the relationship between stress and immune mediators in miscarriage was examined recently (Arck *et al*., 2001). These investigators studied the decidual tissue of women with first trimester miscarriage and found that women with high stress scores had a different pattern of immunocompetent cells than women with low stress scores. Specifically, women with high stress scores had higher numbers of tryptase$^+$ mast cells, CD8$^+$ T-cells and TNF-α^+ cells. It seems that stress responses may be linked to immunological imbalances.

Maternal diseases

Maternal diseases including diabetes mellitus, systemic lupus erythematosus, thyroid disease, chronic essential hypertension and renal diseases appear to increase the risk for RM. A case of prolonged hypoparathyroidism presenting as second trimester miscarriage has been reported (Eastall *et al*., 1985). However, current evidence suggests that well-controlled diabetes mellitus or treated thyroid dysfunction is not associated with RM (Clifford *et al*., 1994). Routine screening for occult diabetes is not necessary in asymptomatic women presenting with RM (Royal College of Obstetricians and Gynaecologists, 1998). However, the value of a routine thyroid function test is still uncertain as a recent study showed that 2% of women with a mid-trimester loss were found to be hypothyroid (Drakeley *et al*., 1998).

Sperm and RM

Earlier studies using conventional semen analysis showed that the male partners of women with RM were not significantly different from control subjects (Hill *et al*., 1994; Sbracia *et al*., 1996). A more recent study of two cases found that the aneuploidy rates in Percoll-processed samples were higher than those found in whole specimens. Whilst the observations were clearly preliminary, the authors suggested the need for evaluating sperm aneuploidy in couples with RM. Furthermore, it is also known that chromosomal abnormalities limited to spermatogenic cells and not detected in somatic chromosomes are found in ~6% of patients in whom meiotic studies are performed (Egozcue *et al*., 1983; De Braekeleer and Dao, 1991).

Advances in molecular biology have, however, brought a better understanding of the interactions between the male and female genomes during the time of both natural and assisted conception. Evidence is now accumulating regarding the important role that the paternal genome may play during early embryonic development by providing the centrosome in the first mitotic division (Palermo *et al*., 1994). This role was recently highlighted in natural conception, using the sperm chromatic structure assay (SCSA), which measures increased sperm chromatin susceptibility to acid denaturation (Evenson *et al*., 1999; Spano *et al*., 2000). These authors observed a significant delay in the time from unprotected intercourse to conception in men with high SCSA values. It is not quite clear whether this is due to lack of fertilization or poor implantation arising from a poorly formed zygote, even though higher values of the SCSA were able to predict 39% of the miscarriages (Evenson *et al*., 1999). Similarly, experience from assisted conception techniques has provided invaluable information on the contribution of the male gamete to the developing embryo. Various studies looking at the outcome of micromanipulation IVF treatment cycles have shown that correlations exist between sperm DNA integrity and outcomes of these cycles (Lopes *et al*., 1998; Sakkas *et al*., 1998). Furthermore, increased rate of spontaneous miscarriages (33.3 versus 7.1%) in ICSI cycles was observed in patients known to have meiotic disorders associated with increased frequencies of diploidy, compared with controls with normal meiosis (Aran *et al*., 1999).

At present, the role of the paternal genome in RM has yet to be fully investigated. The first report in the literature was probably that of Rosenbusch and Sterzik. They compared 15 male partners of patients with RM with 10 controls and found no significant differences in their rate of chromosomal hypohaploidy and hyperhaploidy. However, they reported a high rate of chromosomal breaks and accentric fragments in the RM group (Rosenbusch and Sterzik, 1991). A more recent report (Rubio *et al*., 1999), looking at sperm chromosome anomalies, found a significant increase in sex disomy of couples with RM compared with controls (0.84 versus 0.37%). These reports suggest that abnormal sperm chromosomes may be associated with RM.

There are few data on the role of damaged sperm DNA in RM. A DNA flow cytometry investigation of the ejaculates of 21 partners of patients with the condition found only a negative correlation between median and peak values of DNA fluorescence with motility (Drudy and McCaffrey, 1996). It is difficult to interpret the significance of this finding as no comparison was made with controls, although this study appears to suggest an association between poor motility and RM. However, a study involving 20 male partners of women with RM compared with 20 prospective semen donors, reported significantly lower hypo-

osmotic swelling test scores in the RM group (Buckett *et al.*, 1997). In summary, preliminary data mainly from assisted conception treatment appear to suggest that a paternal component may be implicated in pregnancy loss.

Unexplained RM

The cause of miscarriage in ~50% of women with RM remains unexplained despite thorough investigations. A number of possible aetiologies have been proposed to explain the occurrence of RM in patients in whom there does not appear to be any obvious cause. These include the following.

Fetal chromosomal anomalies

Repeated sporadic fetal chromosomal anomalies occurring by chance, such as repetitive fetal aneuploidies due to increasing maternal age, may be responsible for RM (Stirrat, 1990). Several studies suggest that fetal chromosomal anomalies account for ~50% of sporadic first trimester miscarriage, and possibly a similar proportion of RM. However, cytogenetic study of the products of conception may be misleading. Contamination by maternal cells, intermingled with anchoring villus trophoblast at the implantation site, make it impossible to rule out maternal contamination when a diagnosis of '46,XX' is encountered (Bell *et al.*, 1999). There is therefore a need for refinement in the genetic assessment of pregnancy loss tissues. Whilst it is routine practice to send products of conception for cytogenetic study in women with RM, the usefulness of cytogenetic investigation of fetal tissues seems rather limited as it seldom influences clinical practice. Miscarriages due to abnormal karyotypes often occur before 10 of weeks gestation, including cases with fetal heart beats which suddenly disappear at ~8–10 weeks. Miscarriage occurring after 10 weeks gestation is less likely to be associated with significant chromosomal anomalies. Women over the age of 37 years are more likely to have miscarriages associated with fetal chromosomal anomalies, as a result of age-related decline in oocyte quality.

Ogasawara *et al.* found that the frequency of normal embryonic karyotypes significantly increases with the number of miscarriages (Ogasawara *et al.*, 2000). The observation indirectly suggests that as the number of miscarriages increases, maternal factors involved in embryo–endometrial dialogue may become increasingly responsible for pregnancy failure.

In a recent study of 125 miscarriage tissues from women with RM successfully karyotyped, only 36 (29%) had chromosomal aberrations; 94% of the aberrations were aneuploid, and 6% were structural (Carp *et al.*, 2001). Interestingly, after an aneuploid miscarriage, there was a 68% subsequent live birth rate compared with 41% after a euploid miscarriage. They concluded that alternative mechanisms (other than chromosomal anomalies) may be responsible for the majority of RM and that patients with karyotypically aneuploid fetuses have a good prognosis.

In another study (Stephenson *et al.*, 2002), 420 miscarriage specimens from 285 couples with RM were karyotyped. Overall, 54% specimens were euploid, and the remaining 46% were cytogenetically abnormal. Among the abnormal results, 66.5% were trisomic, 19% polypoid, 9% monosomy X, 4% unbalanced translocations and 0.5% were a combination of trisomy 21 and monosomy X. Furthermore, the frequency of euploid miscarriages was significantly higher in women <36 years of age with RM,

compared with controls; whereas in women aged ≥36 years there was no difference between women with RM and controls. The authors concluded that there are non-cytogenic factors associated with RM, particularly in women aged <36 years.

Pellicer *et al.* found that the incidence of numerical chromosomal abnormalities in preimplantation embryos from women with unexplained RM was 53%, which was significantly higher than a control group (19.3%) (Pellicer *et al.*, 1999). The concept of preimplantation diagnosis, in conjunction with IVF to treat women with RM, by selecting only embryos without any obvious chromosomal abnormalities, is a novel one and worthy of further studies. According to a recent report (European Society of Human Reproduction and Embryology Preimplantation Genetic Diagnosis Consortium, 2002), 45 treatment cycles were carried out in 2001 for aneuploidy screening in women with RM.

Endometrial receptivity

Histological examination of endometrial biopsy in the luteal phase is the classic method used to evaluate endometrial receptivity. A number of studies have shown that RM is associated with abnormal morphological development of the endometrium in the luteal phase (Table II).

Moreover, there is also biochemical evidence to suggest the existence of an endometrial factor in RM (Tulppala *et al.*, 1995). The levels of endometrial protein glycodelin A were found to be reduced in plasma and in uterine flushings of women with RM (Dalton *et al.*, 1995). In addition, the amount of glycoprotein MUC-1 was also found to be reduced in the endometrium of women with RM (Hey *et al.*, 1995).

Genetic factors

HLA sharing in couples, especially HLA B and DR, has been proposed as a possible explanation for unexplained RM (Edmonds, 1992). Two recent studies suggested a link between HLA class II phenotypes and antiphospholipid antibodies in women with RM (Christiansen *et al.*, 1998; Hataya *et al.*, 1998). Further studies are required to establish the role played by genetic factors in RM.

Mid-trimester miscarriage

In women who have had three or more miscarriages, of which at least one occurred in the mid-trimester, the underlying aetiology may be quite different to those with only first trimester losses. Among 656 consecutive couples with two or more spontaneous miscarriages referred to the Liverpool Unit (Drakeley *et al.*, 1998), 158 (25%) had experienced a mid-trimester loss. The results of investigation showed that 33% of subjects tested positive for antiphospholipid syndrome, 8% fulfilled the criteria for cervical incompetence, 4% had uterine anomaly, 3% were thought to have pregnancy loss related to infection and 2% were hypothyroid. Of these subjects, 5% had dual pathology.

Management

The treatment of RM is directed at the cause. Thorough investigations according to a structured protocol are therefore advisable prior to the initiation of treatment.

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Table II. The prevalence of abnormal endometrial morphology in women with recurrent miscarriage[a]

Study	*n*	Definition of recurrent miscarriage used	No. of cycles in which endometrial biopsy taken	Method used for chronological dating	Prevalence of abnormality (%)
Grant *et al*. (1959)	75	3	1	BBT	65
Llusia (1962)	50	3	2	NS	38
Jones (1976)	NS	NS	2	BBT, NMP	25
Balasch *et al*. (1986)	60	2	2	BBT, NMP	32
Vanrell and Balasch (1986)	50	2	2	BBT, NMP	30
Balasch and Vanrell (1986)	43	2	2	BBT, NMP	32.5
Davidson *et al*. (1987)	25	2	NS	NMP	28
Daya *et al*. (1988)	65	NS	2	NMP	40
Tulppala *et al*. (1991)	46	3	2	LH surge	17.4
Li *et al*. (2000)	122	3	1	LH surge	27

[a]All studies used methods reported by Noyes *et al*. (1950) to determine histological dating.
BBT = basal body temperature; NS = not stated; NMP = next menstrual period. NS = not stated.

Chromosome anomalies

Patients with chromosomal problems should be referred to a clinical geneticist for genetic counselling. Donor gametes, adoption or acceptance may be considered. Preimplantation diagnosis or prenatal diagnosis in subsequent pregnancies is recommended.

Uterine anomalies

Treatment of congenital uterine anomalies usually involves metroplasty. The commonest type of congenital uterine anomaly associated with RM is the septate uterus. Historically the removal of a uterine septum involved laparotomy and metroplasty by either the Jones or Tomkin's technique. However, with the introduction of hysteroscopic surgery, the septum may now be easily and safely removed via the resectoscope. Whilst there is as yet no prospectively conducted randomized controlled study to confirm the value of hysteroscopic metroplasty in the septate uterus, an important point highlighted in one review (Matts *et al*., 2000), the current literature data suggest a benefit in those with three or more, and possibly two, miscarriages (Homer *et al*., 2000) (Table III). Laparoscopic guidance is preferable during hysteroscopic metroplasty. On the other hand, hysteroscopic metroplasty is inappropriate for bicornuate uterus, the correction of which is best carried out with the conventional Straussman's metroplasty. Acquired anatomical uterine factors include uterine fibroids and synechiae. From a number of retrospective and cohort studies, there is good evidence that removal of submucous fibroids reduces miscarriage rate, and some evidence that removal of intramural fibroids also reduces miscarriage rate (Li *et al*., 1999; Bajekal and Li, 2000).

Endometrial defect

The treatment of endometrial defect is a controversial subject. Many different forms of treatment have been proposed but none of them has been evaluated carefully in women with RM. A recent observation on the artificial cycle suggests that appropriate estrogen priming in the follicular phase is of particular importance for normal endometrial development in the luteal phase and that inadequate estrogenic stimulation may lead to abnormally retarded endometrium despite the administration of an adequate

Table III. Meta-analysis of 16 published series of the reproductive outcome before and after hysteroscopic metroplasty for septate uterus (adapted from Homer *et al*., 2000)

	Before metroplasty	After metroplasty
Pregnancy	1062	491
Miscarriage[a]	833 (88)	67 (14)
Preterm delivery[b]	95 (9)	29 (6)
Term delivery[a]	34 (3)	395 (80)

Values in parentheses are percentages.
[a]$P < 0.01$.
[b]Not significant.

amount of progesterone (Li *et al*., 1992). Previous observations also suggest that the majority of cases of luteal phase defect are associated not with suboptimal progesterone but with an abnormal response of the endometrium to progesterone (Li and Cooke, 1991). It seems therefore that a logical treatment is targeted at improving the responsiveness of the endometrium to progesterone by enhancing priming of the endometrium in the follicular phase. In a recent study (Li *et al*., 2001), we employed ovarian stimulation with gonadotrophins to increase estrogen production in the follicular phase and enhance estrogen priming in women with a history of RM and retarded endometrium. Of 13 women who had a repeat biopsy in the treatment cycle, 11 (85%) biopsies were normal. In addition, the miscarriage rate in the treatment group (2/13) was significantly ($P < 0.05$) lower than in the non-treatment group (7/12).

Prothrombotic states

Antiphospholipid antibodies

The optimal treatment for women with RM and antiphospholipid syndrome is low dose aspirin and subcutaneous heparin. Two prospective randomized studies have shown that this combination results in a successful outcome in 70% of cases and is superior to the administration of aspirin alone (Kutteh, 1996; Rai *et al*., 1997). Although both of these studies used unfractionated

heparin, low molecular weight heparins are now preferred due to their reduced risk of heparin-induced thrombocytopoenia and osteoporosis. Aspirin (75 mg once daily) should start at the first positive pregnancy test and heparin/low molecular weight heparin should be commenced when the fetal heart is seen on ultrasonography (Greaves *et al.*, 2000). The ideal treatment length has not been defined, and, although it is reasonable to stop at 34 weeks gestation in women with only a history of early pregnancy losses, it should normally be continued until the time of delivery. Post-partum thromboprophylaxis is only required for women with a personal history of thrombosis or who have had a Caesarean section. In any pregnant woman starting on heparin, the platelet count should be monitored weekly for the first 3 weeks and every 4–6 weeks thereafter (Greaves *et al.*, 2000).

Although the use of a combination of aspirin and heparin improves the chances of a live birth in women with antiphospholipid syndrome, these treated pregnancies are frequently complicated by fetal growth retardation, pregnancy-induced hypertension and premature delivery (Backos *et al.*, 1999).

Recently, a double-blind randomized, placebo-controlled trial (Pattison *et al.*, 2000) involving 40 women (20 women in each arm) to assess the efficiency of low dose aspirin therapy for antiphospholipid syndrome reported a live birth rate of 85% in the placebo group and 80% in the aspirin-treated group, which brings into question the need for pharmacological intervention for women with antiphospholipid syndrome for whom RM is the only feature.

Heritable thrombophilia

Although the association between heritable thrombophilia and RM is still disputed, at least one intervention study has already been published. Brenner *et al.* treated 50 women with thrombophilia and fetal loss with 40–120 mg enoxaparin daily and found that the chance of a successful outcome was 75%—significantly better than the historical outcome in these women before the diagnosis of thrombophilia was made (20%) (Brenner *et al.*, 2000). There is an urgent need for large prospective studies on the both the association of thrombophilia and miscarriage as well as its treatment. For now, routine treatment of women with thrombophilia and fetal loss with heparin or low molecular weight heparin is not indicated outside clinical trials.

Other haematological conditions

Women with essential thrombocythaemia and RM should be managed jointly with a haematologist. They are likely to require higher doses of aspirin, and if cytoreductive therapy is required, interferon is usually preferred to chemotherapeutic agents when used in the first trimester (Griesshammer *et al.*, 1998).

Polycystic ovarian syndrome (PCOS), hypersecretion of LH and hyperandrogenaemia

Pituitary suppression with GnRH analogues, followed by ovulation induction with pure FSH was initially thought to be capable of reducing the risk of spontaneous miscarriage in women with PCOS. However, a randomized controlled trial and showed that down-regulation treatment (GnRH agonist + HMG) did not reduce the miscarriage rate of women with 'hypersecretion of LH' (Clifford *et al.*, 1996).

Table IV. Results of a randomized, controlled trial on the outcome of treatment with laparoscopic ovarian drilling, hMG and FSH by Abdel Gadir *et al.* (1990)

	Laparoscopic ovarian drilling (%)	hMG (%)	FSH (%)
Conception (6m)	55	55	38
Multiple pregnancy	0	20	20
Miscarriage	21	53	40

Recent data suggest that laparoscopic ovarian drilling is an effective treatment for PCOS in women with anovulatory infertility (Li *et al.*, 1998a). A randomized controlled trial (Abdel Gadir *et al.*, 1990) analysed the results of laparoscopic ovarian drilling versus gonadotrophins (Table IV). It seems that laparoscopic ovarian drilling treatment is associated with a reduced miscarriage rate, and appears to be a logical treatment for PCOS associated with RM, although randomized controlled studies are required to confirm the preliminary findings.

Cervical incompetence (weakness)

Cervical cerclage is used for cervical incompetence. Cervical cerclage should be offered to women with a clear history of cervical incompetence or those at high risk of mid-trimester loss, such as those with a history of three or more pregnancies ending before 37 weeks gestation (Medical Research Council/Royal College of Obstricians and Gynaecologists, Working Party, 1993). D'Addato *et al.* reported a successful pregnancy rate of 73.3% following the insertion of a MacDonald cervical cerclage in patients between the 8th and 34th weeks of pregnancy in a study involving 198 patients (D'Addato *et al.*, 1992). Transabdominal cervico-isthmic cerclage (TCC) may be used in a highly selective group of women with anatomical defects (e.g. following large/repeated cone biopsy) of the cervix and previously failed transvaginal cerclage associated with cervical damage. This method has a very encouraging fetal survival rate post-procedure [85.2% (Gibb and Salaria, 1995); 86.6% (Anthony *et al.*, 1997)]. Two recent studies described the techniques of laparoscopic TCC (Brolmann and Oei *et al.*, 2000; Ind and Mason, 2000). In women who are at risk of mid-trimester loss but the diagnosis of cervical incompetence is uncertain, serial transvaginal ultrasonography from 16–20 weeks onwards may be performed to measure the length of the cervix (normal >2.5 cm) and to exclude funnelling of the upper cervical canal which may precede shortening.

The results of a prospective randomized trial, the Cervical Incompetence Prevention Randomised Cerclage Trial (CIPRACT) (Althuisius *et al.*, 2001), found that cervical cerclage with bed-rest reduced pre-term delivery before 34 weeks of gestation and neonatal morbidity in women with risk factors or symptoms of cervical incompetence and a cervical length of <25 mm before 27 weeks of gestation. Similar conclusions were reached in a retrospective cohort study (Novy *et al.*, 2001). In those with an unclear history of cervical weakness, however, early transvaginal ultrasonography is to be preferred to empirical cervical cerclage; especially as one study found that early

(empirical) cerclage in this group of women did not offer significant benefit over early transvaginal ultrasonography (before 20 weeks gestation) (Kelly *et al*., 2001).

Unexplained RM

Various treatment options have been tried in this category of patients without any obvious cause for the RM. These include the following.

Tender loving care (TLC)

Women with RM who conceive again do worry that it will end up in yet another miscarriage. They welcome reassurance, close monitoring, including serial ultrasonography to reassure them that the pregnancy is ongoing. Two studies compared TLC versus 'ordinary' or 'general practitioner' care (Stray Pederson and Stray Pederson, 1984; Liddell *et al*., 1991). In both studies the treatment group was associated with a higher successful pregnancy outcome (~80%) than the 'control' group (~30%). However, TLC is not a well-defined entity. Further studies are required to define what exactly constitutes TLC and the mechanism through which it operates.

At present, TLC in the form of continued psychological support and serial first trimester ultrasonography should be offered in a dedicated clinic (MacDonald, 1989; Edmonds, 1992; Rai *et al*., 1996). Serial ultrasonography and hCG measurement in early pregnancy of women with a history of RM are also useful in predicting the pregnancy outcome in this group of subjects (Li *et al*., 1998b).

Hormone treatment

Progestagens. Progestagens were used some years ago to treat women with RM. One recent meta-analysis showed that such a treatment confers no benefit (Goldstein *et al*., 1989) whereas another meta-analysis suggested that it may confer benefit (Daya, 1989). Whilst further prospective research is required to resolve the controversy, in the absence of clear evidence of benefit, the use of progestogen in pregnancy should be abandoned (MacDonald, 1989; Royal College of Obstetricians and Gynaecologists, 1998).

hCG. Similarly, there is no evidence to suggest that serial hCG injections are of benefit in women with unexplained RM. However, in a subgroup of women ($n = 23$) with oligomenorrhoea, hCG support is associated with a significantly more successful outcome (86%) than in the placebo group (40%) (Quenby and Farquharson 1993).

Immunological therapy

Immunotherapy is based on the hypothesis that miscarriage occurs due to a failure of maternal immunological adaptation to the developing semiallogenic conceptus resulting in a form of transplantation rejection. Immunotherapy for RM may be either active or passive. In active immunotherapy either paternal leukocyte injection or trophoblast membrane infusion is used with a view to boost the subsequent maternal immune recognition of a developing conceptus. In passive immunotherapy, regular intravenous infusion of immunoglobulins is employed to produce beneficial immunomodulatory effects including the neutralization of circulating autoantibodies, the inhibition of complement-mediated cytotoxicity, and the modulation of release of cytokines from lymphocytes.

Paternal leukocyte immunization. The first reported randomized immunotherapy trial comparing paternal leukocyte immunization with control autologous maternal leukocyte immunization (Mowbray *et al*., 1985) suggested a significant improvement in pregnancy success rate in the treatment group (77%) compared with the control group (37%). It was unclear why the success in the control group was unexpectedly low. Several subsequent randomized controlled trials of lymphocyte immunization failed to show a benefit of treatment (Ho *et al*., 1991; Cauchi *et al*., 1991; Ober *et al*., 1999). The use of paternal leukocyte immunization in the treatment of RM remains a highly controversial subject. Clarke *et al*. recently critically re-analysed the published and unpublished data on leukocyte immunotherapy and highlighted the various difficulties of meta-analysis (Clarke *et al*., 2001). It seems that careful selection of subjects may be the key to finally resolving the controversy. For now, the value of this particular form of treatment should be considered unproven—perhaps treatment should only be considered within the context of a carefully designed randomized controlled trial (Li, 1998).

Trophoblast membrane infusion. Ramsden and Johnson, in a combined randomized double-blind and open study, reported that trophoblast membrane infusion, another form of active immunization, did not confer any benefit in women with apparently unexplained RM (Ramsden and Johnson, 1992).

Intravenous immunoglobulin infusion (IVIG). There have so far been five double-blind randomized controlled trials on the use of intravenous immunoglobin to treat women with RM (German RSA/IVIG Group, 1994; Christiansen *et al*., 1995; Coulam *et al*., 1995; Stephenson *et al*., 1998; Jablonowska *et al*., 1999). Two of the trials showed an increase in successful pregnancy outcome, but the other three did not. A more recent prospective study (Stricker *et al*., 2000) showed that in 'older' women (≥ 28 years) IVIG treatment appeared to improve outcome. However, the study was not randomized, nor double-blinded. Overall, current evidence suggest that there is as yet no firm evidence to suggest benefit with this form of treatment.

Aspirin treatment

Rai *et al*. showed that the empirical use of aspirin in women with unexplained RM has no benefit and is therefore not justified (Rai *et al*., 2000b). However, they also observed that in a subgroup of women with at least one or more mid-trimester loss, low dose (75 mg daily) aspirin treatment produced a higher live birth rate (64.6%) than no aspirin (49.2%).

Thyroid autoantibodies

It is now recognized that women with thyroid autoantibodies are at risk of developing subclinical hypothyroidism (Glinoer *et al*., 1994). Hence, close monitoring with thyroxine function test and TSH levels is necessary. Thyroxine treatment should be considered in those with a TSH level in the high end of the normal range.

Nutrition/environmental factors

In view of the possible association between coffee, alcohol consumption, smoking and RM, it would be reasonable at this stage to recommend reduction and if possible avoidance of these activities.

Hyperhomocysteinaemia

Patients found to have elevated homocysteine levels should have their red cell folic acid and serum vitamin B_{12} levels estimated and if deficient corrected by appropriate supplementation. Patients with raised homocysteine levels but normal red cell folic acid levels should still receive folic acid supplements because this has been shown to reduce homocysteine levels (Homocysteine Lowering Trialists' Collaboration, 1998). For patients who fail to normalize their plasma homocysteine, vitamin B_{12} and vitamin B_6 should also be administered.

There is no evidence that the administration of low molecular weight heparin or aspirin to patients with hyperhomocysteinaemia and RM improves outcome.

Prognostic features

Detection of fetal heartbeats

In our recent study of pregnancies in women with a history of RM following referral (T.C.Li et al., manuscript submitted), 105/480 (21.9%) of all pregnancies resulted in a very early loss, i.e. prior to the detection of fetal heartbeats; in contrast to 49/480 (10.2%) of loss later on in the first trimester, after the detection of fetal heartbeats. Later losses, in second and third trimester, occurred in 20/480 cases, i.e. 4.2%. In other words, among the population studied, the likelihood of a pregnancy loss after the detection of fetal heartbeats, excluding ectopic pregnancy and termination of pregnancy, is as high as 69/359 (14.2%). This is quite different to a low risk population, in which the likelihood of pregnancy loss after the demonstration of fetal heartbeats was reported to vary from 2 to 6% (Achiron et al., 1991; Nazari et al., 1991).

Moreover, Deaton et al. showed that in women aged >35 years, the detection of fetal cardiac activity is not as reassuring as found in younger subjects, with a miscarriage rate of 10% in women 36–39 years, and 29% in women ⩾40 years of age (Deaton et al., 1997). On the other hand, our results are consistent with a miscarriage risk of 21.7% after detection of fetal heartbeats in a small study of 23 pregnancies of women with RM (Opsahl and Pettit, 1993) and of 32% among 113 pregnancies from women with unexplained RM (Li et al., 1998b). It is therefore important that, in a high risk population such as women with a history of RM, undue optimism should not be conveyed after detection of fetal heartbeats. Further, serial ultrasonography later on in the first trimester, which constitutes part of the TLC ought to be offered to establish whether or not the pregnancy is progressing normally.

An earlier study (Brigham et al., 1999) examined pregnancy outcome following idiopathic RM. They found that, among 222 pregnancies, 55 resulted in miscarriage before 24 weeks. Only 6/222 pregnancies (2.7%) miscarried after detection of fetal cardiac activity, which was significantly lower than in previous studies. It is quite possible that the inclusion of a significant number of women with only two previous miscarriages in their study reduced the overall risk of miscarriage in their population.

The number of previous miscarriages

A number of studies in women with RM showed that the higher the number of previous losses, the lower the live birth rate in a subsequent pregnancy (Quenby and Farquharson, 1993; Clifford et al., 1997; Brigham et al., 1999; T.C.Li et al., manuscript submitted). In our own series, the live birth rate gradually dropped from 64% among women with two previous miscarriages to 43.2% among women with six or more miscarriages.

Age

Age has been shown repeatedly to have a profound impact on pregnancy outcome (Quenby and Farquaharson, 1993; Brigham et al., 1999). In our recent study (T.C.Li et al., manuscript submitted), among pregnancies after referral, we noted that the outcome was similar in three age groups, ⩽30, 31–35 and 36–40 years with a live birth rate of ~60%, whereas in the age group ⩾41 years, the live birth rate was significantly reduced to 36.6%. More interestingly, analysis of the pattern of loss in the older age group (⩾41 years) showed that the rate of very early loss (19/41, 46.3%) was significantly higher than that of the younger groups (86/439, 19.6%). A recent prospective register linkage study in Denmark showed that the high fetal loss rate in older women occurred irrespective of reproductive history (Anderson et al., 2000).

Underlying aetiology

The underlying aetiological factors and treatment, if any, clearly affect the pregnancy outcome; for example, in women with prothrombotic state, the live birth rate without treatment (28.6%) was significantly lower than those with treatment (77.5%) (Li et al., 2002), which is consistent with previous literature reports (Granger and Farquharson, 1997; Rai et al., 1997; Backos et al., 1999) of the favourable impact of treatment with unfractionated or low molecular weight heparin and/or aspirin with this condition.

On the other hand, in the uterine anomaly group, the overall live birth rate was only 35.8%. The results of treatment on the outcome in the uterine anomaly group appears somewhat disappointing, with only a slight difference in live birth rate between treatment (41.7%) and no treatment (31.0%) (T.C.Li et al., manuscript submitted). However, it is difficult to draw a meaningful conclusion in this group of subjects, as the uterine anomaly group is rather heterogeneous, with congenital anomalies ranging from septate, arcuate, bicornuate, unicornate and acquired anomalies including fibroids and intrauterine adhesions. Some cases, e.g. unicornuate, are not amenable to surgery. The decision to proceed with surgery in those who are potentially amenable to surgery is often a result of individual assessment and counselling, mainly because there is no consensus or accepted criteria as to when treatment is to be recommended. There is an urgent need to address the potential beneficial value of surgery in this group of subjects.

In general, in women with a diagnosis of unexplained RM the prognosis is good.

History of live birth

In our series (T.C.Li et al., manuscript submitted), the pregnancy outcomes including the live birth rate and the pattern of loss were

very similar between those who did or did not have a previous live birth. It confirms two recent reports that a history of previous live birth did not confer benefit and improve subsequent obstetric performance (Clifford *et al*., 1997; Brigham *et al*., 1999), despite an earlier report that a previous live birth was associated with a higher live birth rate in a subsequent pregnancy among women with a history of RM (Quenby and Farquharson, 1993).

History of infertility

In a previous study, Whitley *et al*. reported that a history of infertility did result in a fourfold increase in risk of miscarriage/stillbirth (Whitley *et al*., 1999). The population studied was drawn from children born to radiographers and was considered at low risk of miscarriage. In our study of women at high risk of miscarriage, among pregnancies following referral, it appeared that the live birth rate in women with a history of infertility (50.6%) was significantly lower than women without a history of infertility (61.3%) (T.C.Li *et al*., manuscript submitted).

Body mass index (BMI)

In our study (Li *et al*., 2002), BMI did not have a significant impact on the live birth rate, both prior to and after referral. Although a very high BMI has been recognized to be associated with subfertility and a reduced probability of achieving pregnancy in assisted reproduction treatment, the reduced pregnancy rate observed in assisted reproductive treatment programmes is deemed not related to embryo quality, but possibly a consequence of disturbed endometrial function (Wang *et al*., 2000); direct evidence to support such a hypothesis is not yet available.

Menstrual cycle length

In an earlier study (Quenby and Farquharson 1993), menstrual regularity appeared to have a significant impact on the outcome of a subsequent pregnancy: live birth rate among women with regular cycles (87%) was higher than those with oligomenorrhoea (64%). They further noted, in their mathematical mode predicting live birth in a subsequent pregnancy, that among all factors examined oligomenorrhoea was the most critical one. However, in our study (T.C.Li *et al*., manuscript submitted), we were unable to confirm their findings.

Leptin

Previous studies in humans and mice have suggested the importance of leptin in fetal growth. In a recent study, leptin and leptin-binding activity were measured in blood obtained from women who had a history of RM between weeks 5 and 8 of the pregnancy (Laird *et al*., 2001). It was found that women who subsequently miscarried had significantly lower plasma leptin concentration than women who subsequently had a term birth. However, as there was considerable overlap between the values the two groups, the measurement of leptin is of limited use in the prediction of pregnancy outcome in women with RM.

Obstetric and neonatal outcome

Whilst the risk of miscarriage in a subsequent pregnancy has now been well documented (Quenby and Farquharson, 1993; Clifford *et al*., 1997), the obstetric and neonatal outcomes of pregnancies

which progressed to beyond 24 weeks in this group of women are still far from clear.

Reginald *et al*. examined 97 women who had had three or more miscarriages, and who had at least a singleton birth that had reached 28 weeks gestation (Reginald *et al*., 1987). They found that the small-for-gestational-age rate (30%), preterm delivery rate (28%) and perinatal mortality rate (1.6%) were higher than expected. However, the underlying causes of RM and the treatment, if any, of this group of women were not documented, and the results were not compared to a local obstetric population (control group).

In contrast, Hughes *et al*., who examined the obstetric outcome in 88 women with a past history of three or more consecutive pregnancy losses and compared the results to their local obstetric population (control group), found that rates for small-for-gestational-age infants (3.4%), preterm delivery (12.5%) and perinatal mortality (0%) were no different to the control group (Hughes *et al*., 1991). As in the study of Reginald *et al*. (1987), the investigation and treatment of these subjects with RM appeared incomplete, e.g. there was no mention of antiphospholipid syndrome and its treatment at all.

Another prospective study of 63 women with a history of recurrent (three or more) spontaneous miscarriage presented the results of a detailed investigative protocol including antiphospholipid syndrome in the population studies (Tulppala *et al*., 1993a). The obstetric outcomes of 32 deliveries were analysed. The authors found that the rates for growth retardation (20%), preterm delivery (9.7%) and impaired glucose tolerance (22.8%) appeared to be increased. Unfortunately, the results were not compared with any control population, partly because the small number of deliveries in the study did not leave much room for formal statistical analysis.

In our own study (Jivraj *et al*., 2001), we examined the obstetric and neonatal outcome of 162 pregnancies which progressed to beyond 24 weeks in a cohort of women with recurrent (three or more) miscarriages, and compared the results to a local, control obstetric population who had deliveries during the study period. We found that the rate of preterm delivery (13.3%), small-for-gestational-age (13%), Caesarean section (36%) and perinatal mortality (2.5%) in women with RM were higher than those of the control population (3.9, 2.1, 16.7 and 1% respectively), although there were no differences in the rates of hypertensive disorders and diabetes between the two groups.

Conclusions

RM is a heterogeneous condition. There are still many unresolved questions about its cause and treatment. The number of publications in the literature has substantially increased over the last 10 years, reflecting a growing interest among clinicians and scientists in this condition. As a consequence, the prognosis of RM is now better understood. Hopefully, the additional information and knowledge provided will, before long, lead to more successful treatment and improved outcome in women with RM.

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