Preventing recurrent miscarriage of unknown aetiology

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Key content
• One to three per cent of couples are affected by recurrent miscarriage (defined as more than three consecutive pregnancy losses).
• Current interventions are centred on known causes of aetiology.
• Recent research on miscarriage of unknown cause has investigated the requirements for successful embryo implantation.
• Treatment of immunological risk factors with immunotherapy does not have a strong evidence base.
• The overall evidence supporting the use of human chorionic gonadotrophin supplementation during pregnancy is inconclusive.
• The efficacy of progesterone as an intervention remains empirical, with further trials under way.

Learning objectives
• To understand the aetiology of recurrent miscarriage and patient risk factors.
• To understand the theoretical pathophysiology underlying miscarriage of unknown aetiology.
• To be aware of the potential agents of intervention for recurrent miscarriage and their efficacy.
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Ethical issues
• Treating patients with recurrent miscarriage can be emotive; should women be treated with interventions that have limited evidence-based clinical efficacy?
• How do we manage patients with the psychological impact of recurrent miscarriage?

Keywords: human chorionic gonadotrophin / pathophysiology / progesterone / recurrent miscarriage

Introduction

Miscarriage is defined by the World Health Organization as the loss of a viable pregnancy before 24 weeks of gestation.¹ It has been estimated that 15–20% of all clinical pregnancies end in miscarriage.² These pregnancy losses are usually sporadic, often unavoidable and may be due to underlying chromosomal or structural abnormalities.³ However, 1–3% of couples are affected by recurrent miscarriage, defined as having three or more consecutive early pregnancy losses.²,⁴

The mechanisms underlying recurrent miscarriage remain poorly understood; after thorough investigation, the cause remains elusive in 50% of cases.² Recurrent miscarriage of unknown aetiology presents a challenge to the clinician since the psychological impact for affected couples can be profound, with increased depression, anxiety and lowered self-esteem being reported.³ A variety of potential therapeutic interventions have been explored, although the evidence base remains equivocal, warranting further research into the pathophysiology of recurrent miscarriage.

Known aetiology of recurrent miscarriage

Chromosomal abnormalities are thought to account for 70% of sporadic first trimester miscarriages.⁵ These defects may be structural, such as Robertsonian translocations, or be caused by single gene abnormalities.⁶ A 2005 study found 4% of couples with recurrent miscarriage to have abnormal karyotypes.⁷ In couples with low maternal age, multiple miscarriages and a family history of recurrent miscarriage, the presence of carrier status for a structural chromosomal abnormality should be suspected.⁶ In a case–control study, Franssen et al.⁶ found the risk of carrier status to be 4.2% in women under 23 years, 3.7% in women aged 23–33 years, 2.4% in women aged 34–36 years, reducing to 1.7% in women 37–38 years of age. Increasing pregnancy losses caused by fetal aneuploidy are seen with advancing maternal age, where the rate of karyotypic abnormality is 52% in women under 35 years, rising to 82% in women older than 35 years.³ Consanguinity among couples presents a theoretical potential risk for recurrent miscarriage. However, one retrospective and one prospective study in communities...
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with a high prevalence of consanguineous marriage\textsuperscript{7,8} found no relationship between consanguinity and the aetiology of recurrent miscarriage or risk of subsequent pregnancy loss.

Recurrent miscarriage caused by embryos with chromosomal abnormalities cannot be targeted with therapeutics. Patients with recurrent miscarriage could potentially undergo pre-implantation genetic screening. This aims to identify and transfer only genetically normal embryos to women, within the context of an in vitro fertilisation (IVF) cycle. However, this demands that the couple undergo an IVF cycle. The Royal College of Obstetricians and Gynaecologists (RCOG) states that genetic screening does not improve live birth rates in women with recurrent miscarriage.\textsuperscript{9}

Current interventions to prevent further pregnancy losses are centred upon known causes of pathology. Standard investigations for recurrent miscarriage are given in Table 1. Women should only be diagnosed with antiphospholipid syndrome if antiphospholipid antibodies are persistently positive (two readings over 12 weeks apart),\textsuperscript{9} given that their presence is variable over time and may occur as a response to pregnancy loss rather than being the cause. Recurrent miscarriage is commonly linked to systemic maternal diseases, such as poorly controlled diabetes mellitus, thyroid disease and obesity. Gynaecological factors such as amenorrhoea prior to pregnancy, polycystic ovary syndrome and luteal phase defects are implicated in an increased risk of miscarriage.\textsuperscript{10} Congenital uterine abnormalities have been found in 5–30% of women with a history of recurrent miscarriage.\textsuperscript{11} The müllerian variants include canalisation defects, such as subseptate or septate uteri or unification of the müllerian ducts resulting in a unicornuate or bicornuate uterus or uterus didelphys. A large systematic review of 3805 women\textsuperscript{11} found a significant increase in first trimester miscarriage with canalisation defects ($P < 0.001$) but no increase in risk with duct unification anomalies ($P = 0.08$). Conversely, a significant association was seen between müllerian duct anomalies and second trimester miscarriage ($P = 0.003$), which was not present for the septate uteri ($P = 0.15$).\textsuperscript{11}

Fibroids have been implicated as a cause of second trimester recurrent miscarriage. The risk associated with fibroids is increased with intracavity distortion and this group of women may therefore benefit from myomectomy.\textsuperscript{12} Genital infection and cervical incompetence are particularly associated with second trimester pregnancy loss.\textsuperscript{13}

Inherited thrombophilias, including activated protein C resistance, deficiency in protein C/S, deficiency in antithrombin III, hyperhomocysteinuria and prothrombin gene mutations, have been associated with recurrent miscarriage.\textsuperscript{2} Antiphopholipid antibodies have multiple potential mechanisms of action, including immune modulation and endothelial dysfunction. This results in an increased risk of pregnancy loss due to anti-trophoblastic effects and decidual thrombosis.\textsuperscript{14,15}

Recurrent miscarriage is multifactorial in aetiology, with considerable heterogeneity in causation within both the known and unknown aetiology groups. Maternal smoking, alcohol consumption, obesity, illicit drug use, caffeine ingestion and certain prescription medications, such as non-steroidal anti-inflammatory drugs, may also contribute.\textsuperscript{16}

\textbf{Potential aetiology of recurrent miscarriage}

\textbf{Luteal insufficiency}

A long-standing concept in recurrent miscarriage is that a deficiency in the hormones progesterone and/or human chorionic gonadotrophin hormone (hCG) will lead to pregnancy loss. However, a definitive diagnosis of luteal phase deficiency requires endometrial biopsy, which is rarely done in practice.\textsuperscript{17}

When embryo implantation is successful, trophoblast cells secrete hCG.\textsuperscript{18} The rapid rise in serum hCG increases estrogen and progesterone levels, necessary for maintaining the corpus luteum and decidua basalis, until the placenta can support the pregnancy alone by approximately 7 weeks of gestation.\textsuperscript{19} The vital importance of corpus luteum function was demonstrated in 1978,\textsuperscript{20} when luteectomy prior to 7 weeks of gestation consistently resulted in miscarriage. Indeed, the abortifacient drug mifepristone, is a progesterone receptor antagonist.

hCG is one of the earliest endocrine products to be produced and levels are in excess of the requirements for corpus luteum maintenance, leading to speculation that hCG may have other functions.\textsuperscript{21,22}

\begin{table}[h]
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\begin{tabular}{|l|l|}
\hline
\textbf{Aetiology} & \textbf{Investigations} \\
\hline
Antiphospholipid syndrome & Lupus anticoagulant \hspace{1cm} Anticardiolipin antibodies \\
Chromosomal abnormality & Cyto genetic analysis of the products of conception for third and subsequent miscarriages \hspace{1cm} Karyotyping if cytogenetic analysis reveals a chromosomal abnormality \\
Uterine anatomical defects & Pelvic ultrasound if recurrent first trimester losses or any second trimester miscarriage \hspace{1cm} MRI of the pelvis \hspace{1cm} Endometrial biopsy for recurrent miscarriage \\
Inherited thrombophilias & Factor V Leiden \hspace{1cm} Prothrombin gene mutation \hspace{1cm} Protein S deficiency \\
\hline
Note that routine screening for occult diabetes and thyroid disease is not recommended
\end{tabular}
\caption{Standard investigations to determine the aetiology of recurrent miscarriage\textsuperscript{9}}
\end{table}
hCG binds to the endometrium at surface receptors, which are expressed during the implantation window. Insulin-like growth factor binding protein-I (IGFBP-I) is secreted by endometrial stromal cells, peaking just prior to the end of the fertile period. IGFBP-I is therefore a good marker for decidualisation. In experimental work, Licht et al. used an in vivo intrauterine model to measure the paracrine response when hCG is added. They found multiple effects of hCG on the endometrium, including significant inhibition of IGFBP-1. Therefore, hCG may be secreted by the blastocyst to forestall the production of IGFBP-I, thus prolonging the time available for implantation. hCG hormone may directly impact on implantation through stimulating proteases implicated in endometrial breakdown.

During decidualisation, cytokines stimulate the neoangiogenesis process involved in placental development. At a molecular level hCG shares sequence homology with these cytokines, including vascular endothelial growth factor (VEGF). In vitro, hCG can induce significant increases in VEGF levels.

A deficiency in either progesterone or hCG may therefore impact on the corpus luteum, preparation for implantation and endometrial support, resulting in miscarriage. Although given that a pregnancy with an embryo with no villus circulation will not secrete progesterone from the corpus luteum, it may be feasible that low progesterone levels may be a result of, rather than a cause of, miscarriage.25

**Immunology**

The role of the complex immunological adaptation of pregnancy in recurrent miscarriage has been the subject of debate. In normal pregnancy, there is an upregulation of the type 2 antibody-mediated response and corresponding downregulation of the type 1 cell-mediated response. One study of recurrent miscarriage found an increase in maternal serum concentrations of cytokines associated with both type 1 and type 2 arms of the immune response. However, this remains controversial, since there are many contradictory studies, providing no consistent evidence for the role of cytokines in recurrent miscarriage. Additionally, the raised levels of C-reactive protein in normal early pregnancy suggest that an immune-mediated reaction to the fetus is not necessarily harmful.

A recent area of debate is the potential involvement of natural killer cells in recurrent miscarriage. Natural killer cells are lymphocytes present in peripheral blood and in the endometrium. The natural killer cells found in the endometrium are referred to as decidual natural killer cells. Decidual natural killer cells are the dominant immune cells in the endometrium during the luteal phase and early pregnancy, and they double in number between the follicular and luteal phases of the cycle. These lymphocytes may therefore have a role in trophoblast invasion, aiding implantation through the secretion of cytokines. The production of cytokines, chemokines and growth factors contributes to placentation through maternal spiral artery remodelling.

Endometrial natural killer cells express the surface antigen CD56 and have been categorised into CD56Bright and CD56Dim, based on their appearance in vitro. The CD56Bright cells promote proliferation, whereas CD56Dim cells are associated with cytotoxicity. Endometrial biopsies have shown that women with recurrent miscarriage have greater numbers of decidual natural killer cells compared with control biopsies obtained from women attending for sterilisation after achieving live births. Of these decidual natural killer cells, women with recurrent miscarriage have fewer CD56Bright cells and more CD56Dim cells than control samples.

It is unknown whether decidual natural killer cells are derived from their own cell lineage or recruited from peripheral blood, where 90% of natural killer cells are CD56Dim. Beer et al. first reported an increase in the peripheral blood natural killer cells of women with recurrent miscarriage. However, within a normal population, levels of natural killer cells vary widely from person to person and during the menstrual cycle, and levels are altered by the timing and method of collection of the venous sample. There is therefore no useful assay to quantify levels of peripheral natural killer cells meaningfully. Future studies need to investigate the levels of peripheral natural killer cells depending on, for example, the day of menstruation, time of day and exercise.

Recurrent miscarriage has been associated with an increased prevalence of thyroid peroxidase antibodies, with the hypothesis that the presence of these antibodies is indicative of subclinical thyroid dysfunction. However, a cohort study of 496 women with recurrent miscarriage of unknown aetiology and 220 women with a diagnosed aetiology of recurrent miscarriage found that thyroid antibodies had no prognostic value regarding the outcome of future pregnancies in women with any cause of recurrent miscarriage. A large double-blind placebo controlled trial is being carried out to investigate the efficacy of administering thyroxine to women with normal thyroid function who have thyroid peroxidase antibodies and a history of recurrent miscarriage (Thyroid AntiBodies and LEvoThyroxine study [TABLET]).

Conformity between maternal and paternal HLA antigens has also been reported in couples with recurrent miscarriage, with ‘HLA-sharing’ being a postulated aetiology. Other potential risk factors for recurrent miscarriage currently under investigation include nuclear hormone receptors, such as peroxisome proliferator-activated receptors, and the presence of circulating microparticles that have already been associated with pre-eclampsia.
Prognosis

The strongest independent risk factors for having further miscarriages due to any aetiology are increasing maternal age and number of previous miscarriages. Risch et al.\textsuperscript{38} reported a doubling in the risk of miscarriage when maternal age doubles from 20 to 40 years. A 1997 longitudinal study\textsuperscript{39} found rates of miscarriage in the next pregnancy to be 25% in women under 30, 28% in women aged 31–36 years, 33% in women aged 36–39 years and 52% in women over 40 years. Increased paternal age is also associated with miscarriage, with 1.6 times the risk when comparing a paternal age of 40 to that of a 25–29-year-old man.\textsuperscript{40} However, recurrent miscarriage has not been linked to any specific paternal health factors.

A poorer prognostic outcome with increasing numbers of miscarriages has been widely documented, with a 53% chance of further miscarriage with six or more miscarriages versus 29% with three pregnancy losses (Table 2).\textsuperscript{39} Although, based on these data, 48% of women over 40 years of age and 47% of women with more than six previous miscarriages did achieve a live birth in the next pregnancy without having treatment. More recent data corroborate these findings, where a large cohort study of women (\(n = 978\)) with recurrent miscarriage found that 66.7% (95% confidence interval [CI] 63.7–69.7) achieved a live birth within 5 years of the first clinic consultation.\textsuperscript{40} The presence of concurrent subfertility also increases the likelihood of another pregnancy loss, although having had a previous live birth does not appear to confer any reduced risk of subsequent miscarriage.\textsuperscript{39,42}

There are therefore subgroups of women attending a recurrent miscarriage clinic: those with an excellent prognosis – only 21% likelihood of miscarriage when less than 40 years of age and fewer than six previous losses – and those with a poorer prognosis, so patients should be counselled accordingly.\textsuperscript{39}

Management of recurrent miscarriage of unknown aetiology

Human chorionic gonadotrophin hormone

A 2013 meta-analysis investigating the efficacy of hCG included five studies, with 151 participants in the intervention arm and 151 in the control arm.\textsuperscript{43–45} A significant reduction in the odds of subsequent miscarriage was seen in the hCG group (OR 0.38; 95% CI 0.22–0.67).\textsuperscript{47} The mean number needed to treat to obtain a benefit from hCG was 5.9. Each of the studies indicated that hCG is safe, with no adverse events reported as a direct result of hCG. However, there was significant heterogeneity in the meta-analysis (\(I^2 = 50\%\)), suggesting a lack of combinability in the data. This may reflect the range of sample sizes within the included studies (\(n = 20–98\)) and the disparate years of study, from 1982 to 2005. The regimens and duration of hCG treatment varied widely across the studies, with doses ranging from 5000 to 10 000 units/day.

A sensitivity analysis removing the two oldest studies (1982, 1985) with the smallest sample sizes reduced the heterogeneity to 0%. These results revealed no significant difference in miscarriage rate (odds ratio [OR] 0.57, 95% CI 0.31–1.06).\textsuperscript{44,45,47} Removing these studies does reduce the data from which conclusions can be drawn, therefore limiting the power of this meta-analysis to exclude a treatment effect.

All of the trials were underpowered, demonstrating the need for an up-to-date randomised controlled trial of adequate sample size. However, studies thus far have failed to recruit adequate numbers of women. A large, international multicentre study was discontinued after an interim analysis showed lack of efficacy of hCG alongside ‘escalating costs and diminishing participation’.\textsuperscript{46} Therefore, data are only available for the 75 patients recruited before the trial was halted.\textsuperscript{46}

Higher powered studies would enable subgroup analysis, for example to stratify against maternal age and number of miscarriages. Quenby and Farquharson\textsuperscript{48} found a statistically significant benefit with hCG supplementation compared with placebo in a subgroup of women with oligomenorrhoea (miscarriage rate of 86% versus 40%, respectively). This effect was not seen in women with regular menstrual cycles. It is therefore prudent to ascertain prognostic groups of women who are at higher risk of recurrent miscarriage that may benefit from hCG, such as those with polycystic ovary syndrome.\textsuperscript{39}

Progesterone

Progesterone supplementation has been used for many years in women with recurrent miscarriage.\textsuperscript{50} A Cochrane review\textsuperscript{51} of progesterone to prevent miscarriage included four trials investigating recurrent miscarriage. It found a statistically significant benefit of using progesterone versus placebo or no treatment (OR 0.38, 95% CI 0.2–0.7). However, this needs to be interpreted with caution given the small sample size (\(n = 223\)) and three of the studies are over 40 years old. Although there are 41 years between the oldest and most recent study (1964 versus 2005), there are narrow confidence intervals and low heterogeneity (\(I^2\) of 0%) within this data pool. A previous meta-analysis including only the three older

<table>
<thead>
<tr>
<th>Number of previous miscarriages</th>
<th>Miscarriage rate (%)</th>
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<tbody>
<tr>
<td>3</td>
<td>34/119 (29)</td>
</tr>
<tr>
<td>4</td>
<td>13/49 (27)</td>
</tr>
<tr>
<td>5</td>
<td>7/16 (44)</td>
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<tr>
<td>≥ 6</td>
<td>9/17 (53)</td>
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Anticoagulation
A 2010 randomised controlled trial\(^55\) compared 364 women randomly assigned to receive aspirin combined with low-molecular-weight heparin, aspirin alone or placebo. Of 299 women who became pregnant, live birth rates were 69.1% in the combination group, 61.6% with aspirin alone and 67.0% in the placebo group.\(^55\) Therefore neither of these intervention strategies produced a significant impact on subsequent miscarriage.

Immunotherapy
Active immunotherapy involves injecting leucocytes derived from paternal blood with the aim of boosting the ‘subsequent maternal recognition of the developing conceptus’.\(^37\) The evidence supporting paternal leucocyte immunisation has been conflicting. A 2004 meta-analysis found a significant benefit when using this intervention in recurrent miscarriage patients.\(^56\) However, non-blinded studies were included and assessment of study methodology was not stringent.\(^56\) Passive immunisation with intravenous immunoglobulin is designed to neutralise maternal autoantibodies and inhibit cytotoxicity.\(^37\)

A 2006 Cochrane review analysed 20 trials (\(n = 1137\)) using immunotherapy and found no interventions to produce a statistically significant benefit.\(^57\) The overall odds ratios of live birth rate were: OR 1.23 (95% CI 0.89–1.70) following paternal leucocyte immunisation, OR 0.98 (95% CI 0.61–1.58) post-immunoglobulin, OR 1.39 (95% CI 0.68–2.82) after third party donor leucocytes and OR 0.40 (95% CI 0.11–1.45) with trophoblast membrane immunisation. However, only one trial investigated trophoblast membrane immunisation and only three investigated third party donor immunisation.

Distinct disadvantages with these therapies include risk of virus transmission, allergic reaction and high cost. A randomised controlled trial investigating glucocorticoids in recurrent miscarriage (\(n = 773\)) found increased rates of prematurity, diabetes mellitus and hypertension in the treatment group.\(^58\) The live birth rate was not significantly affected by the administration of prednisone (\(P = 0.19\)). Therefore, these interventions can only be used in the context of a randomised controlled trial.\(^57\) The 2006 Cochrane review concluded that the development of a specific assay to diagnose immune-mediated cases of recurrent miscarriage could identify a subgroup of women who would potentially benefit from immunotherapy.\(^57\)

Psychological care
Couples with recurrent miscarriage experience greater levels of depression, anxiety and feelings of guilt compared with the general population.\(^5\) Fertl et al.\(^59\) correlated poor pregnancy outcomes with fear during early pregnancy. Therefore, patients may benefit from regular contact time, regular reassurance scans and potentially psychotherapy. During clinic appointments, women should be given preconceptual advice regarding modifiable factors such as smoking, alcohol consumption and taking folic acid, in preparation for pregnancy. However, there are no specific recommendations to be given that can affect the success of a subsequent pregnancy regarding counselling couples with recurrent miscarriage. Patients should be given reassurance about their chances of a successful pregnancy in the future. In women with miscarriage due to any cause, Clifford et al.\(^39\) reported miscarriage rates to be significantly lower (\(P = 0.002\)) in those attending a specialised early pregnancy clinic than in non-attenders (26% versus 51%, respectively). The time and cost of running a specialised, dedicated, truly multidisciplinary team-led recurrent miscarriage service is therefore justified. The RCOG has published an information leaflet which may be useful for patients: Recurrent and Late Miscarriage – Tests and Treatment of Couples: Information for You. In this, the RCOG recommends the Miscarriage Association as a source of support (www.miscarriageassociation.org.uk).\(^60\)

Ethics and implications for practice
A cornerstone of the current drive towards clinical governance is the practice of evidence-based medicine. It is clear that the evidence base supporting the above interventions is either equivocal or not shown to be effective. This gives rise to the RCOG recurrent miscarriage guideline stating that the use of empirical treatment in women with recurrent miscarriage is ‘unnecessary and should
be resisted. However, recurrent miscarriage is a particularly emotive condition and patients may be desperate to try any strategy. Therefore patient autonomy has to be considered, with the onus of decision making no longer resting solely with the doctor. Couples also have access to a wealth of resources through the media and Internet and may therefore be aware of potential treatments, such as aspirin and hCG, as well as acupuncture and other homeopathic remedies. Additionally, hCG and progesterone have been shown to be safe in clinical practice, with little scope for harm to patients. The ethics of deciding whether to treat women with these evidence-limited interventions hinge on respecting patient autonomy, weighing up the beneficence and non-maleficence, and deciding if the action and its cost are fair overall. To answer this question definitively, better evidence is needed, in the form of adequately powered randomised controlled trials, enabling stratification for maternal age and previous number of miscarriages. Future studies should carefully consider patient selection to investigate particular prognostic groups, for example hCG and progesterone in women with luteal phase defects. Ideally, the results should be corrected to exclude fetal chromosomal abnormalities, since no therapeutics can overcome inherently abnormal embryos. Until such studies are available, a frank and open discussion with patients should address their wishes and the relative pros and cons of intervening. Couples should be counselled about their high chances of having a live birth without any treatments, even after multiple miscarriages.

**Conclusion**

If investigations reveal a specific cause of recurrent miscarriage, directed treatment is warranted. However, the evidence for supporting the use of current interventions to prevent pregnancy loss in women with a history of unexplained recurrent miscarriage remains equivocal.

The current RCOG guideline on the investigation and treatment of couples with recurrent miscarriage states that the use of empirical treatment in these patients is ‘unnecessary and should be resisted’. Patients should be counselled about their prognosis for future pregnancies, with an emphasis on supportive care, with the provision of dedicated miscarriage services.

However, the emotive difficulties of managing couples who have experienced recurrent miscarriage may, in certain cases, warrant the use of interventions without proven clinical efficacy, particularly in patients with poor prognostic factors.

**Disclosure of interests**

None to declare.

**References**


Gregersen in Recurrent Miscarriages (PROMISE) Study [www.controlled-trials.com/ISRCTN92644181].


