

POSITION PAPER

Australasian recurrent pregnancy loss clinical management guideline 2024, part II

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Part II of the Australasian guideline for the investigation and management of recurrent pregnancy loss (RPL) provides evidence-based guidance on the management of RPL provided. The implications of inherited and acquired thrombophilia with respect to RPL and suggestions for clinical management are provided. Autoimmune factors, including human leukocyte antigen, cytokines, antinuclear antibodies and coeliac antibodies, and guidance for management are discussed. Infective, inflammatory and endometrial causes of RPL are discussed in detail. Environmental and lifestyle factors, male factor and unexplained causes are outlined. Levels of evidence and grades of consensus are provided for all evidence-based statements.

KEYWORDS

recurrent pregnancy loss, recurrent miscarriage, RPL, guideline

THROMBOPHILIA

Thrombophilias can be inherited (congenital) or acquired (Table 1). Congenital thrombophilias are associated with an increased risk of thromboembolism, yet available evidence related to recurrent pregnancy loss (RPL) is inconclusive. In comparison, acquired thrombophilias are associated with RPL.

Inherited thrombophilia

Several prospective trials have failed to demonstrate any association between congenital thrombophilia and RPL, whereas some case-control and retrospective cohort studies have found weak but positive associations.^{1–6} A Cochrane systematic literature review (2014) failed to show any benefit in treating RPL with aspirin and/or

low-molecular-weight heparin (LMWH).⁷ It has been proposed that maternal thrombophilia does not affect pregnancies <10 weeks' gestation but may be harmful later in pregnancy.^{8,9} The 2023 Heparin for women with recurrent miscarriage and inherited thrombophilia open-labelled randomised controlled trial (RCT) showed that LMWH did not improve live birth rates (LBR) in women who had two or more pregnancy losses and confirmed inherited thrombophilia.¹⁰

Factor V Leiden

Factor V Leiden (FVL) (heterozygous or homozygous) occurs in 2.7–10.9% of pregnancies, and prevalence varies between ethnic groups, with higher rates among Caucasians.^{11,12} Data on the possible link between FVL and RPL have been contradictory to date. Certain studies have found that heterozygosity for FVL is not

[†]The Australasian CREI (Certificate of Reproductive Endocrinology and Infertility) Consensus Expert Panel on Trial Evidence (ACCEPT) Group members are presented in Appendix 0.

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associated with early RPL,^{1,13} whereas other studies have suggested FVL heterozygosity may increase susceptibility for RPL.^{12,14,15}

Prothrombin gene mutation

The prevalence of prothrombin gene mutation (PGM) has been shown to not differ between women who have experienced RPL and the general pregnant population, although these findings may vary depending on the variant of mutation and population studied.^{11,12,14,16–18}

Proteins C and S deficiency

The link between protein C and protein S in RPL is controversial in both prospective and retrospective studies.^{8,9,14,19} Some studies have observed an association between protein S deficiency and stillbirth.⁸

Antithrombin deficiency

Evidence regarding the association between antithrombin deficiency and RPL is conflicting.^{9,14} Antithrombin deficiency is associated more with fetal loss (second to third trimesters) as opposed to RPL.⁸

Methylenetetrahydrofolate reductase mutations

Methylenetetrahydrofolate reductase (MTHFR) gene mutations are a common mutation with about 37% incidence of heterozygosity in the Australian population.^{20,21} No strong correlation between MTHFR mutations and RPL has been reported in prospective or retrospective studies.^{14,19,22} MTHFR in the context of a raised homocysteine level (fasting levels of >15 µmol/L) may be linked to

increased rates of fetal death and placental abruption, but there is no link between hyperhomocysteinaemia and RPL.^{23,24}

Acquired thrombophilia

Antiphospholipid syndrome

Antiphospholipid syndrome (APS) is the most common acquired thrombophilia, involving both biochemical and clinical criteria for diagnosis (Table 2).²⁵ The antibodies found in APS have been reported at rates of 11–15% within an RPL population compared with 1.5% of fertile negative control women.^{13,26} de Jong et al. demonstrated that APS has a strong correlation with RPL, particularly in the context of anticardiolipin antibody presence (odds ratio (OR): 5.1; 95% confidence interval (CI): 1.8, 14.0).²⁷

Management

Inherited thrombophilia

There are no data to support a definitive benefit from treating inherited thrombophilias with antithrombotic agents (Table 3). A meta-analysis of prospective studies on women with inherited thrombophilia (including FVL, PGM, protein C and S deficiency, antithrombin and MTHFR) failed to demonstrate any difference in LBRs with the use of LMWH compared to an untreated control group.²⁸ Similarly, there is no evidence of improved LBR in inherited thrombophilia with aspirin treatment.^{29,30}

Acquired thrombophilia

A Cochrane systematic literature review and meta-analysis including 11 trials and 1672 participants with antiphospholipid antibodies and RPL (>two pregnancy losses) demonstrated the greatest efficacy for reducing pregnancy loss with combined antithrombotic therapy (aspirin plus heparin) based on low-certainty evidence.³¹ The dosing and types of aspirin and heparin varied (aspirin, 75–100 mg/day; heparin unfractionated, 5000 IU twice a day; or enoxaparin, 20–40 mg/day).

Antithrombotic therapy in unexplained RPL

Trials (including randomised double-blind placebo-controlled trials) demonstrate that LMWH, aspirin and LMWH plus aspirin do not improve LBR in unexplained RPL.^{32–34}

TABLE 1 Congenital and acquired thrombophilias

Inherited (congenital) thrombophilias	Acquired thrombophilias
Factor V Leiden	Antiphospholipid syndrome
Prothrombin gene mutation	(anticardiolipin antibody, lupus anticoagulant, β 2-glycoprotein antibody)
Antithrombin deficiency	
Protein C and protein S deficiency	

TABLE 2 Updated International Consensus Sydney criteria for antiphospholipid syndrome, Miyakis et al.²⁵

Clinical criteria	Biochemical criteria
Previous thrombosis • Venous or arterial	Persistent presence of the following on two occasions, at least 12 weeks apart: • Anticardiolipin antibody • Lupus anticoagulant • β2 glycoprotein antibody
Pregnancy morbidity • Multiple unexplained pregnancy losses (≥ 3 losses, <10 weeks' gestation) • ≥ 1 unexplained fetal death (≥ 20 weeks' gestation) • ≥ 1 preterm birth (34 weeks' gestation) due to eclampsia, pre-eclampsia or recognised features of placental insufficiency	

TABLE 3 Recommendations pertaining to thrombophilia in RPL

Statement	Level of evidence Grade of consensus
Antiphospholipid syndrome is associated with RPL. Women with RPL should be screened for antiphospholipid syndrome.	Level III-2 Consensus grade β
The association between inherited thrombophilias and RPL is uncertain.	Level III-2 Consensus grade γ
At this stage, screening for inherited thrombophilias should not be performed for RPL.	Good practice point (GPP) Consensus grade α
Combined antithrombotic therapy in the form of low-dose aspirin and heparin should be commenced in women with RPL and APS and a positive pregnancy test.	Level I Consensus grade β
There is no definite evidence to support commencing antithrombotic therapy in any form for those with inherited thrombophilias and RPL.	Level I Consensus grade γ
There is no evidence to support antithrombotic therapy in any form for those with unexplained RPL.	Level II Consensus grade γ
Clinicians should explain the risks of commencing antithrombotic therapy with patients, including bleeding, heparin-induced thrombocytopenia (<0.1%) and local skin reactions.	GPP Consensus grade α

APS, antiphospholipid syndrome; RPL, recurrent pregnancy loss.

TABLE 4 Recommendations pertaining to autoimmune disorders in RPL

Statement	Level of evidence Grade of consensus
Uterine NK cells are believed to play an important role in implantation and placentation.	Level III-2 Consensus grade γ
Testing for NK cells in the context of RPL is not warranted unless in a research setting.	GPP Consensus grade α
Human leukocyte antigen antibody determination in women with RPL is not recommended in clinical practice outside of research settings.	GPP Consensus grade γ
A raised ANA is associated with unexplained RPL.	Level III-2 Consensus grade γ
Further research is required to evaluate specific ANA titre cut-offs and whether specific antibodies are associated with RPL.	Level III-2 Consensus grade α
Testing for coeliac antibodies should be individualised with a low threshold for testing patients who are symptomatic or have a significant family history.	Level III-2 Consensus grade β
The use of IVIg in the context of RPL is not warranted; however, it may be considered in certain populations.	Level I Consensus grade γ
Patients should be counselled regarding the evidence behind immunotherapy as well as potential associated adverse effects.	GPP Consensus grade γ

ANA, antinuclear antibody; IVIg, intravenous immunoglobulin; NK, natural killer; RPL, recurrent pregnancy loss.

AUTOIMMUNE FACTORS

Immunogenic factors are a key area of research, and it has been hypothesised that there is a potential relationship with RPL. Research to date has focused primarily on human leukocyte antigen (HLA) antibodies, killer cell immunoglobulin-like receptors (KIRs), natural killer (NK) cells and cytokines. Immunomodulation therapies, including intravenous immunoglobulin (IVIg), corticosteroids, intralipid therapy and granulocyte colony-stimulating factor (G-CSF), have all been proposed as therapies for the management of RPL. Recommendations pertaining to autoimmune disorders in RPL are presented in [Table 4](#).

HLA, KIRs and uterine NK cells

HLAs are major histocompatibility complexes, and it has been suggested that HLAs or the absence of maternal blocking antibodies could cause RPL. In theory, if a blastocyst is developmentally intact, then the embryo should be completely encased in trophoblastic cells and have no exposure to the maternal immune system. However, at times a transient exchange of cells may occur. As such, paternally derived antigens are exposed to the maternal immune system, which may lead to an immune response. Early studies have also implicated the loss of molecular immunosuppressive factors at the decidual-placental interface as a possible contributor to miscarriage.³⁵

Uterine NK cells promote implantation by regulating trophoblast invasion and enhancing vascular remodelling by extra-villous trophoblasts. They are the dominant cells at the maternal-fetal interface.³⁶ The exact origin of uterine NK cells remains unknown; theories include that they are derived from haemopoietic progenitor cells in bone marrow,³⁷ arise from peripheral NK cells³⁸ or already reside within the uterus.³⁹

KIRs determine the NK cell function in the context of other receptor-ligand interactions. Uterine NK cells are thought to be immunotolerant, which is different from peripheral NK cells which are cytotoxic. Extra-villous trophoblastic cells express class I HLA-C and non-classic HLA-G and HLA-E antigens; HLA-C molecules are polymorphic; and ligands for KIRs are expressed by uterine NK cells.⁴⁰ Maternal and paternal HLA-C allotypes are expressed on the trophoblastic cell surface, and the KIR cell receptors are variable with high levels of diversity. The maternal KIR genotype has been deemed activating (AB or BB) or non-activating (AA), whereas fetal HLA-C ligands have two ordered groups, HLA-C1 and HLA-C2; placentation is regulated by these interactions, and it has been suggested that certain expressions have a strong association with RPL, such as KIR AA/HLA-C2.³⁶

There is no agreed-upon method of reporting the level of NK cells both peripherally and within the uterus, although there has been some research in this area. A meta-analysis of observational studies demonstrated little difference between NK cell levels in women with RPL and fertile controls.⁴¹ Analysis of uterine NK cells in the context of RPL reported no difference between RPL and fertile controls; however, it was acknowledged that significant statistical heterogeneity existed across all studies. In comparison, peripheral NK cell levels differed significantly between RPL and controls (mean difference: 1.36; 95% CI: 0.04, 2.69; $P=0.04$).⁴¹ Conflicting results between the included studies were reported due to the variety of methods used for NK cell evaluation and differences in assays.

Cytokines

Cytokines are proteins important for intracellular communication, and some studies have found an association between increased expression of cytokines and RPL.⁴² Commonly studied cytokines include IL-1, IL-4, IL-10, IL-6, IL-8, IFN- γ and TNF- α . Studies in this area are often small and observational and have failed to show a strong association with RPL. As such, further research in this area is warranted.

Antinuclear antibodies

Antinuclear antibodies (ANA) are autoantibodies that bind nuclear and cytoplasmic antigens, serving as biomarkers for autoimmune disorders. The incidence of ANA within the RPL population has been reported as 20.6–22% compared to 6.7–8.3% in women without RPL.^{43,44} There is evidence of a significant association between the presence of ANA and unexplained RPL (OR: 3.27; 95%

CI: 1.91, 4.64; $P<0.00001$).⁴⁴ This association remained significant regardless of whether the patient had an autoimmune disorder (OR: 2.23; 95% CI: 1.40, 3.55; $P=0.0007$) and was specific to a higher titre. A low titre was not associated with RPL, whereas a higher titre ($\geq 1:160$) was found to have a significant association (OR: 45.89; 95% CI: 8.44, 249.45; $P<0.00001$). It is uncertain which ANA pattern of immunofluorescence is associated with RPL.⁴³

Coeliac antibodies

Poorly controlled coeliac disease or undiagnosed coeliac disease has been associated with infertility and RPL.⁴⁵ This association is reduced in patients with well-controlled disease. However, the utility of routine screening of tissue transglutaminase antibodies (IgA + IgG) and endomysial antibodies (IgA and IgG) in women with RPL remains unclear as several studies have demonstrated a low incidence and a low yield in testing.^{46,47} Screening of patients for coeliac antibodies should be individualised, with a lower threshold for screening patients who are symptomatic or who have a significant family history.

Intravenous immunoglobulin

IVIg is thought to produce anti-inflammatory effects due to cytokine modulation, in addition to reducing peripheral NK cell activity.⁵⁰ A systematic review and meta-analysis involving 11 studies and 582 patients found a marginally significant benefit of IVIg in women with RPL.⁵¹ Further subgroup analysis found differing outcomes depending on the timing of IVIg treatment, with administration prior to conception associated with an increased LBR. However, all included studies were small and underpowered. More recently, a small high-quality RCT ($n=102$) looking at administering repeated doses of IVIg in early pregnancy found a moderate increase in LBR, in women who had experienced four or more unexplained pregnancy losses.⁵² As such, there is some emerging evidence to suggest that IVIg administration in early pregnancy may be beneficial.

Corticosteroids

Corticosteroids are known to produce anti-inflammatory and immunosuppressive effects. There is evidence that in women with RPL and an increased uterine NK cell count, pre-conception treatment with prednisolone can decrease the number of NK cells.⁵³ Although studies are limited, a meta-analysis reported a favourable effect of prednisolone on women with RPL and high uterine NK cell density ($>5\%$) and improved the LBR by 58%.⁵⁴ Similarly, an RCT that compared groups receiving prednisolone in conjunction with aspirin and heparin and a control group receiving only aspirin and heparin demonstrated increased rates of ongoing pregnancy at 20 weeks' gestation; however, LBRs and adverse effects such as birth defects were not reported on.⁵⁵ Studies in this area remain small and the methodology is varied,

and therefore, routine use of corticosteroids for women with RPL is not recommended.

Intralipid therapy

Intralipid therapy is proposed to modulate the immune function of NK cytotoxicity and pro-inflammatory cytokine generation. There are currently no RCTs investigating intralipid/lipid emulsion therapy that evaluates the effect on LBR in RPL. However, a double-blind RCT involving 296 women with RPL and elevated NK cells (>12%) undergoing IVF demonstrated no significant difference in chemical pregnancy rates when compared to placebo.⁵⁶ Further research on the use of intralipid therapy for women with RPL is required.

Granulocyte colony-stimulating factor

G-CSF is a growth factor that stimulates the proliferation and differentiation of haematopoietic cells of neutrophil lineage. The evidence for the role of G-CSF in the management of RPL is conflicting. In an RCT, G-CSF was associated with an improved LBR in women with RPL (greater than or equal to three losses) when compared with placebo.⁵⁷ Conversely, a larger ($n=150$) double-blind RCT showed no benefit of G-CSF in an RPL population when compared with placebo (greater than or equal to three losses).⁵⁸ Further research on the use of G-CSF for women with RPL is required.

Management

Prospective and randomised trials on immunotherapies such as IVIg, corticosteroids, intralipid therapy and G-CSF are limited, and results remain conflicting. A 2014 Cochrane review assessed immunotherapy for RPL and did not identify any one beneficial therapy or improvement in LBRs.⁴⁸

Similar findings were present in a more recent systematic review and meta-analysis of RCTs focusing on immunotherapy for RPL in the context of in vitro fertilisation (IVF).⁴⁹ When the efficacy of a range of immune therapies, including IVIg, lymphocyte and intralipid immunotherapy and intrauterine infusion of G-CSF and peripheral blood mononuclear cells, was assessed, or when

TNF- α inhibitors, leukaemia inhibitory factor or glucocorticoids were administered, the authors concluded that there was a lack of evidence to support the use of any of the immunotherapies for IVF outcomes.

INFECTIVE, INFLAMMATORY AND ENDOMETRIAL CAUSES

Endometritis

Overt infection can result in pregnancy loss. However, there is no robust evidence to suggest that RPL is associated with acute or chronic infection (Table 5).^{59–62} Bacterial vaginosis, as a dysbiotic condition involving an imbalance of various bacteria, has been associated with second-trimester loss.^{63,64} Further research is currently being conducted to understand the role of the uterine microbiome in fertility, with the interplay between commensal and pathogenic microorganisms.⁶⁵

Chronic endometritis

It is thought that microorganisms are a causal factor for chronic endometritis, an inflammatory condition characterised by infiltration of plasma cells into the endometrial stroma.⁶⁶ Women are usually asymptomatic or experience subtle symptoms, making the condition at times difficult to diagnose. The prevalence of chronic endometritis in RPL has been reported at 7–27%, confirmed by endometrial biopsies in prospective studies.^{67,68} However, no study has compared the occurrence of endometritis in RPL to fertile control groups.

McQueen *et al.* performed a retrospective analysis of women with RPL, identifying a greater trend in miscarriage rate in women with chronic endometritis versus no endometritis (32.3 vs 12.9%, $P=0.08$).⁶⁸ Additionally, chronic endometritis was associated with a lower LBR compared to no chronic endometritis (67.6 vs 87.1%, $P=0.08$).

The most commonly associated organisms found in women with chronic endometritis undergoing fertility workup include *Staphylococcus* spp., *Enterococcus*, *Streptococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Mycoplasma*, *Ureaplasma*, *Chlamydia* and *Corynebacterium*.⁶⁶

TABLE 5 Recommendations pertaining to infective, inflammatory and endometrial causes in RPL

Statement	Level of evidence Grade of consensus
There is some evidence to suggest increased prevalence of chronic endometritis in patients with RPL.	Level III-3 Consensus grade γ
There is some evidence that demonstrates that treatment of chronic endometritis with antibiotics improves live birth rates, but randomised control trials are needed.	Level III-3 Consensus grade γ
An endometrial biopsy to screen for chronic endometritis should be considered in women with unexplained RPL.	GPP Consensus grade γ

RPL, recurrent pregnancy loss.

Management

Chronic endometritis

There are limited data to suggest that treating chronic endometritis with appropriate antibiotic regimen may improve pregnancy outcomes and LBR.^{68,69} Nevertheless, antibiotic therapy may prove to be a simple treatment option in reversing some of these negative effects.

Repeat endometrial sampling to determine the test of cure for chronic endometritis has supported antibiotics as an effective treatment resulting in cure of the condition.^{66,68} Antibiotic regimens have varied between studies, with a combination of doxycycline, ciprofloxacin, metronidazole, ofloxacin, amoxicillin + clavulanate and ceftriaxone used.⁶⁶ These regimens have led to some studies observing an improved LBR within an RPL population.

Observational studies have demonstrated improved LBRs after treatment of chronic endometritis in women with RPL.^{68,69} A systematic review and meta-analysis involving treatment for chronic endometritis in women undergoing IVF with repeated implantation failure found a significantly higher rate of ongoing pregnancy/LBR in patients with cured chronic endometritis compared to those with persistent chronic endometritis (OR: 6.82; 95% CI: 2.08, 22.24; $P = 0.001$).⁷⁰ The authors proposed that these data may suggest that chronic endometritis is a reversible factor for infertility.

Endometriosis and adenomyosis

There is weak evidence to suggest that endometriosis and adenomyosis are associated with RPL. Endometriosis via its potential impacts on oocyte quality and endometrial receptivity has been postulated to impair early pregnancy outcomes.⁷¹ Epidemiological data have demonstrated an association between endometriosis and RPL, strengthened by the number of losses (greater than or equal to three losses; OR: 1.44; 95% CI: 1.31, 1.59).⁷² A similar relationship with adenomyosis and RPL is less clear.⁷²

Management

Endometriosis and adenomyosis

Research investigating the management of endometriosis for fertility outcomes has explored surgical laparoscopy, medical treatment or a combination of the two (Table 6). Medical treatments studied have included gonadotropin-releasing hormone (GnRH) agonists, letrozole, danazol, pentoxifylline and dydrogesterone.⁷³ Although there is evidence to support the use of surgical laparoscopy, GnRH agonists or surgical laparoscopy with pentoxifylline to increase the odds of a clinical pregnancy, these treatments do not appear to improve the rates of pregnancy loss.^{73–75}

Although assisted reproductive technology is often used to treat infertility in women with endometriosis, findings regarding the outcome of pregnancy loss are inconsistent.^{76,77}

TABLE 6 Recommendations pertaining to endometriosis and adenomyosis in RPL

Statement	Level of evidence Grade of consensus
There is a weak association between endometriosis and RPL.	Level III-2 Consensus grade γ
The relationship between adenomyosis and RPL is unclear.	Level III-2 Consensus grade α
There is no evidence that the treatment of endometriosis or adenomyosis is beneficial for RPL.	Level I Consensus grade γ

RPL, recurrent pregnancy loss.

Given the lack of a clear association between adenomyosis and RPL, there are currently no studies addressing the treatment of adenomyosis in the RPL population.

ENVIRONMENTAL AND LIFESTYLE FACTORS

The effects of environmental and lifestyle factors on pregnancy loss have mostly been studied in the context of spontaneous pregnancy loss. There is some evidence to suggest an increased risk of spontaneous pregnancy loss with exposure to certain substances. As most of these factors are exposure related and therefore modifiable, previous guidelines have consistently recommended to cease smoking and alcohol consumption, while limiting caffeine intake.^{78–80} The most common environmental and lifestyle factors are summarised in Table 7, and recommendations are provided in Table 8.

Management

Environmental exposures

There are no high-quality studies or RCTs to support treatment or management recommendations. Therefore, management for mitigating these potential risks includes encouraging couples to adhere to general health recommendations; eliminating modifiable risk factors such as smoking cessation and alcohol avoidance; decreasing caffeine intake; and reducing unnecessary exposure to heavy metals, plastics and chemicals.⁸¹

Psychological stress

Despite an unclear relationship, it is imperative to ensure this burden is minimised for couples. Couples value a sensitive, empathetic and holistic approach to managing RPL.⁸² Some older studies have demonstrated positive outcomes based on a ‘tender loving care’ approach alone. A small Australian study by Liddell et al. found a beneficial effect of formal emotional support in women with RPL (86% LBR vs 33% in RPL control group).⁸³

TABLE 7 Summary of environmental and lifestyle factors on RPL

	Definition/presence	Pathophysiology mechanism in RPL	Evidence of association with RPL
Endocrine-disrupting chemicals†			
Bisphenol A (BPA)	Found in food and beverage packaging, processed foods and medical devices. It is a synthetic chemical used in polycarbonate plastics and epoxy resins, possessing both oestrogenic and androgenic properties. ¹⁰⁶	Possible downregulation of uterine progesterone receptors and differentiation of endometrial stromal tissue to decidua as observed in animal models. ¹⁰⁷	Possible association between BPA and RPL as well as spontaneous loss in small observational studies. ^{108–110}
Phthalates	Found in plastics, such as food containers, adhesives, detergents, pharmaceuticals, solvents, soap, shampoo and nail polish. ¹⁰⁶	Phthalates have been demonstrated to affect oestrogen and androgen syntheses. ¹⁰⁶	Limited evidence suggesting an association with the metabolite dibutyl phthalate and RPL. ¹¹¹
Heavy metal exposure	Heavy metals are metallic elements with high density, with the potential to cause toxicity. Some of these include lead, cadmium, zinc, copper and mercury. Exposure may include through ingestion, inhalation or absorption through skin.	Heavy metals may induce immunological changes, which may increase the risk of pregnancy loss. ¹¹² Lower levels of serum zinc and copper have been observed in RPL and spontaneous loss. ^{113,114}	Studies investigating the effect of heavy metal exposure on RPL are contradictory and inconclusive. ^{115–119} Micronutrient deficiency (zinc and copper) may play a role in RPL and spontaneous loss. ^{113,114}
Cigarette smoking	Cigarette smoke contains nicotine, carbon monoxide and cyanide.	Negative effects on reproductive health and pregnancy outcomes are well documented. The exact mechanism is unknown, but components may lead to trophoblastic dysfunction and embryonic/fetal growth restriction and demise. ^{78,120}	Limited studies to suggest increased risk of RPL with smoking and passive smoking. ^{121,122} There is good evidence to demonstrate associations with infertility, ^{123,124} and increased rates of spontaneous pregnancy loss in smokers compared with non-smokers. ¹²⁵
Caffeine intake	Caffeine is a psychoactive substance found in coffee, tea, soft drink and cocoa.	Various potential mechanisms for caffeine exposure and pregnancy loss have been postulated. These include increased vasoconstriction in the uteroplacental unit, ¹²⁶ direct influence on metabolism in fetal development ¹²⁷ and downregulation of the corpus luteum function resulting in lower hCG and oestradiol. ¹²⁷	Studies have reported inconclusive findings on the association between caffeine and RPL. ^{121,122,127} There is some evidence to suggest increased risk of spontaneous loss with caffeine in a dose-dependent manner. ¹²⁶
Alcohol consumption		Alcohol acts as a teratogenic agent in pregnancy; however, exact mechanisms in RPL are unclear.	Nil association between alcohol and RPL in small case-control studies. ^{121,122} There is some evidence demonstrating women who drink alcohol during pregnancy have a higher risk of spontaneous loss ¹²⁸ ; however, the exact dose response is unknown. ¹²⁹
Psychological stress	Psychological stress includes perceived distress as well as depression.	Disturbs the hypothalamic-pituitary axis, consequently leading to increased cortisol and immunological changes affecting reproductive pathways.	There is evidence of high emotional and psychological stress in women with RPL compared to controls; however, evidence of causation is lacking. ^{130–132} It has been demonstrated that some of this stress is reversed after a live birth. ¹³⁰

†Endocrine-disrupting chemicals are substances that interfere with hormone synthesis, metabolism or action, resulting in deviation from normal homeostatic control.¹³³

RPL, recurrent pregnancy loss.

TABLE 8 Recommendations pertaining to lifestyle factors in RPL

Statement	Level of evidence Grade of consensus
There is no strong evidence to suggest investigation of serum or urinary heavy metal levels outside of a research setting in women with RPL.	Level III-2/3 Consensus grade α
Intending parents should be encouraged to limit their exposure to endocrine disruptors (eg plastics) and caffeine, in line with general health recommendations.	GPP Consensus grade γ
Alcohol consumption and smoking are associated with poor reproductive, obstetric and long-term outcomes. Couples should be encouraged to cease alcohol consumption and smoking.	Level III-2, GPP Consensus grade γ
Behavioural and lifestyle modifications should be managed using a specialised and multidisciplinary approach.	GPP Consensus grade γ

RPL, recurrent pregnancy loss.

Similarly, a larger study reported a reduced pregnancy loss rate among women with RPL (greater than or equal to three losses) who received supportive care compared to those who did not (pregnancy loss rate 26 and 51%, respectively; $n = 201$).⁸⁴

MALE FACTOR

Male health and well-being plays a significant role in the management of RPL. Overweight and obesity, smoking, alcohol consumption and environmental and occupational exposures have been associated with RPL.^{85–87} Medical history and examination of the male partner are important first steps in assessing the couple. Assessment of the association between semen analyses and RPL shows inconsistent findings.^{88,89}

Sperm aneuploidy

Several studies have observed increased sperm aneuploidy in men with RPL.^{90,91} However, the authors acknowledge the testing of sperm aneuploidy is not readily available.

Sperm deoxyribonucleic acid fragmentation

Deoxyribonucleic acid (DNA) fragmentation in sperm can occur either during spermatogenesis or during transport through the reproductive tract.⁹² Mechanisms may include (i) apoptosis during spermatogenesis, (ii) defective chromatin remodelling during spermiogenesis, (iii) oxidative DNA damage during transportation in the reproductive tract, (iv) activation of caspases and endonucleases, (v) induction of radiation and chemotherapy (vi) and environmental causes such as smoke and pollution.⁹² There is evidence to suggest that sperm DNA fragmentation is associated with a significant increase in spontaneous pregnancy loss (relative risk (RR): 2.16, 95% CI: 1.54, 3.03)⁹³ and RPL.⁹⁴

Management

The main aim of management is to improve the level of DNA fragmentation of sperm. It is recommended for men to maintain a

healthy weight, cease smoking, reduce alcohol intake and limit occupational exposures while incorporating moderate exercise as a part of a healthy lifestyle (Fig. 1).^{95–97} Recommendations pertaining to male factor in RPL are provided in Table 9.

In the context of an elevated DNA fragmentation index, testicular ultrasound is recommended to assess for the presence of varicoceles. If varicocele is noted, then urological opinion should be sought. Varicoceles have been associated with increased sperm DNA fragmentation, with oxidative stress hypothesised to play a key component in the pathophysiological process.⁹⁸ Heat, hypoxia and increased metabolites have also been postulated to play a role.⁹⁸ Several studies have demonstrated improvement in DNA fragmentation after surgical repair of varicocele.^{98,99} There is currently no literature on the effects of varicocele repair and reproductive outcomes in couples with RPL.

Studies looking at DNA fragmentation in the context of infertility show that antioxidants are effective at improving DNA fragmentation.^{100,101} Given this is a low-risk intervention, there is support to include antioxidants as a part of management.

In the event that the aforementioned lifestyle measures do not lead to an improvement in DNA fragmentation, IVF with advanced sperm selection technique (eg hyaluronic acid-intracytoplasmic sperm injection (HA-ICSI)) may be warranted. HA-ICSI has been associated with a lower rate of miscarriage (compared to ICSI) per clinical pregnancy (RR: 0.62; 95% CI: 0.46, 0.82) but no difference in LBRs (RR: 1.09; 95% CI: 0.97, 1.23), according to a meta-analysis on selective sperm techniques within the general population.¹⁰²

UNEXPLAINED RPL

For couples experiencing RPL, 50–75% of cases remain unexplained.⁷⁸ The nature-unknown aetiology poses a challenge to treating these couples (Table 10).

Management

The initial management of couples with unexplained RPL is to investigate and treat the identifiable causes. Once these have been

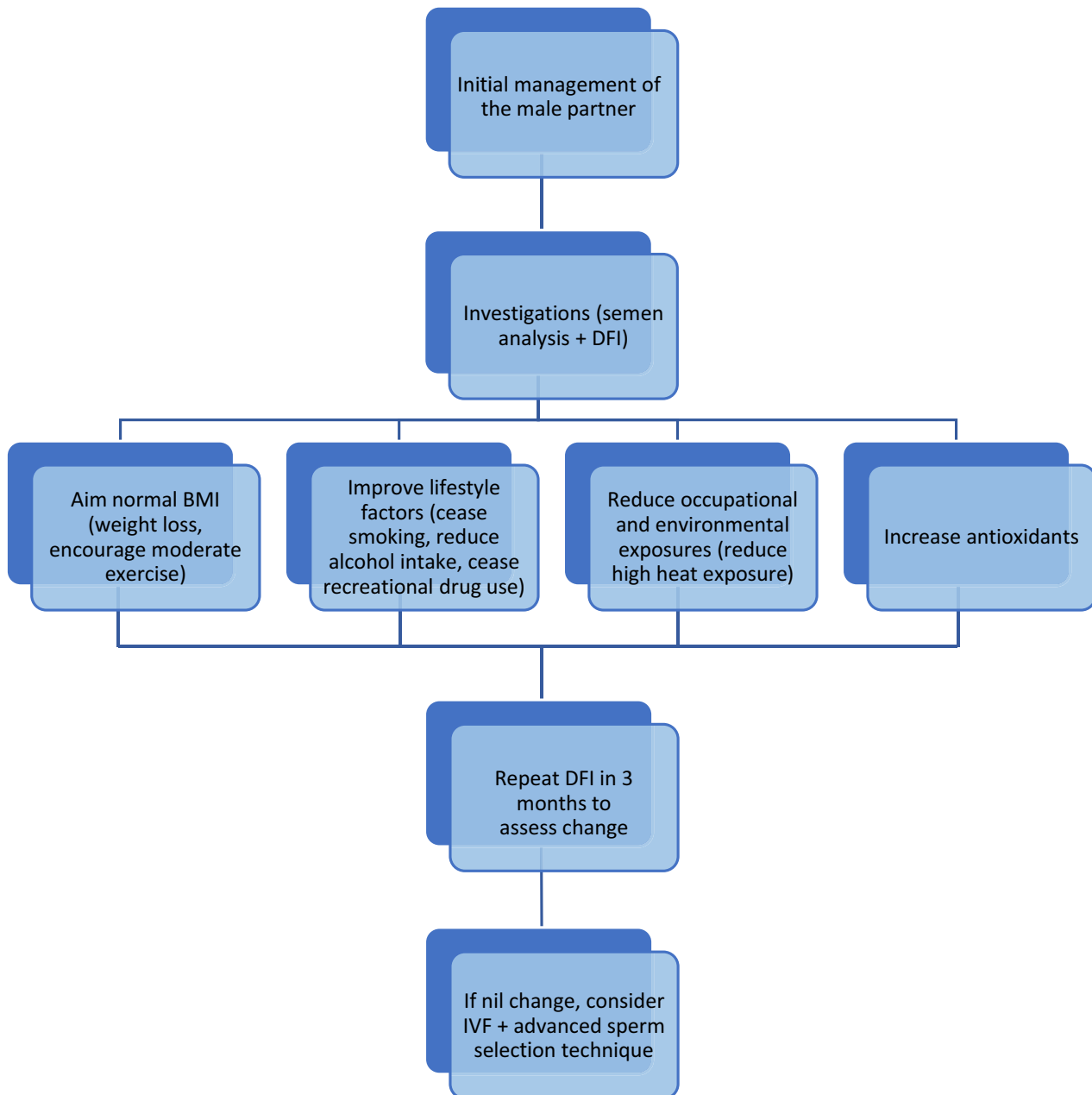


FIGURE 1 Summary for management of the male partner. BMI, body mass index; DFI, DNA fragmentation index.

excluded, empirical treatment may involve progesterone supplementation, IVF with preimplantation genetic testing (PGT) and close monitoring with supportive care.

There is some evidence for the use of empirical progesterone from early pregnancy. A recent Cochrane review demonstrated a non-significant improvement in LBR for women who receive progesterone from the first trimester (RR: 1.07; 95% CI: 1.00, 1.13; $n = 1411$).¹⁰³ Subgroup analysis demonstrated no difference in pregnancy loss rate based on the route of administration (oral, intramuscular and vaginal).

As previously discussed, chromosomal aneuploidy is the most common cause of RPL (see part 1 – chromosomal factors). There is evidence that PGT-A with euploid transfer results in a higher LBR compared to controls.¹⁰⁴ Therefore, there may be a role for PGT in unexplained RPL.

The diagnosis of RPL, particularly without a known aetiology, is a difficult diagnosis for couples to receive. Care within a supportive multidisciplinary and specialised unit has shown beneficial outcomes.^{83,84} Couples should also be counselled on the natural course of RPL.¹⁰⁵

TABLE 9 Recommendations pertaining to male factor in RPL

Statement	Level of evidence Grade of consensus
There is evidence that links lifestyle to sperm DNA fragmentation.	Level III-2/3 Consensus grade γ
There is indirect evidence that links DNA damage in sperm to RPL.	Level III-3 Consensus grade β
Lifestyle assessment, including smoking, exercise, recreational substance use, alcohol consumption and body weight, should be performed.	GPP Consensus grade α
Assessment for varicocele in setting of high sperm DNA fragmentation should be offered.	Level III-2, GPP Consensus grade β
Cessation of smoking, limiting alcohol consumption, controlled normalisation of body weight and the uptake of a normal exercise program should be recommended to males with RPL.	GPP Consensus grade α
The use of antioxidants is considered low risk and is therefore reasonable.	Level I Consensus grade γ
IVF with advanced sperm selection technique could be considered for cases where more conservative treatment options have failed.	Level I Consensus grade γ

IVF, in vitro fertilisation; RPL, recurrent pregnancy loss.

TABLE 10 Recommendations pertaining to unexplained RPL

Statement	Level of evidence Grade of consensus
There is some evidence to suggest the use of progesterone from early pregnancy.	Level I Consensus grade γ
There is some evidence to support the use of PGT-A and subsequent euploid embryo transfer, as a means of reducing further pregnancy loss due to aneuploidy.	Level III-2 Consensus grade α
Couples experiencing unexplained RPL should be managed by a supportive care team.	GPP Consensus grade α

RPL, recurrent pregnancy loss.

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APPENDIX

The Australasian CREI (Certificate of Reproductive Endocrinology and Infertility) Consensus Expert Panel on Trial Evidence (ACCEPT) group: Prof. Rob Norman, Gab Kovacs, Lucy Prentice, Elizabeth Glanville, Leigh Searle, Olivia Stuart, Vicki Nisenblat, Neil Johnson, Yousif Alyousif, Maree Lee, Kate Burston, Michael Chapman, Sebastian Leathersich, Phill McChesney, Clare Boothroyd, Neerja Kamal, Anne Clark, Violet Kieu, Rituparna Dutta, Lynn Burmeister, Ashleigh Smith, Louise Hull, Vamsee Thalluri, Giselle Crawford,

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