Aust N Z J Obstet Gynaecol 2024; 1–14

ANZJOG

DOI: 10.1111/ajo.13820

POSITION PAPER

Australasian recurrent pregnancy loss clinical management guideline 2024, part II

Adriana SUKER[1], Ying LI[2], Danielle ROBSON[2], Anthony MARREN[2] and on behalf of the Australasian CREI (Certificate of Reproductive Endocrinology and Infertility) Consensus Expert Panel on Trial Evidence (ACCEPT) Group[†]

[1]Department of Obstetrics and Gynaecology, Liverpool Hospital, Sydney, New South Wales, Australia

[2]Department of Reproductive Endocrinology and Infertility, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia

Correspondence: Dr. Anthony Marren, Department of Reproductive Endocrinology and Infertility, Royal Prince Alfred Hospital, Sydney, NSW, Australia. Email: anthony@marrencampbellmackie.com.au

Conflict of Interest: The authors report no conflicts of interest.

Received: 28 September 2023; Accepted: 3 April 2024

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.

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]

TABLE 1 Congenital and acquired thrombophilias

Inherited (congenital) thrombophilias	Acquired thrombophilias
Factor V Leiden Prothrombin gene mutation Antithrombin deficiency Protein C and protein S deficiency	Antiphospholipid syndrome (anticardiolipin antibody, lupus anticoagulant, ß2-glycoprotein antibody)

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

Clinical criteria	Biochemical criteria
Previous thrombosis • Venous or arterial 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	Persistent presence of the following on two occasions, at least 12 weeks apart: • Anticardiolipin antibody • Lupus anticoagulant • ß2 glycoprotein antibody

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 cells[38] 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-y 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 (≥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*, *Klebisiella 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

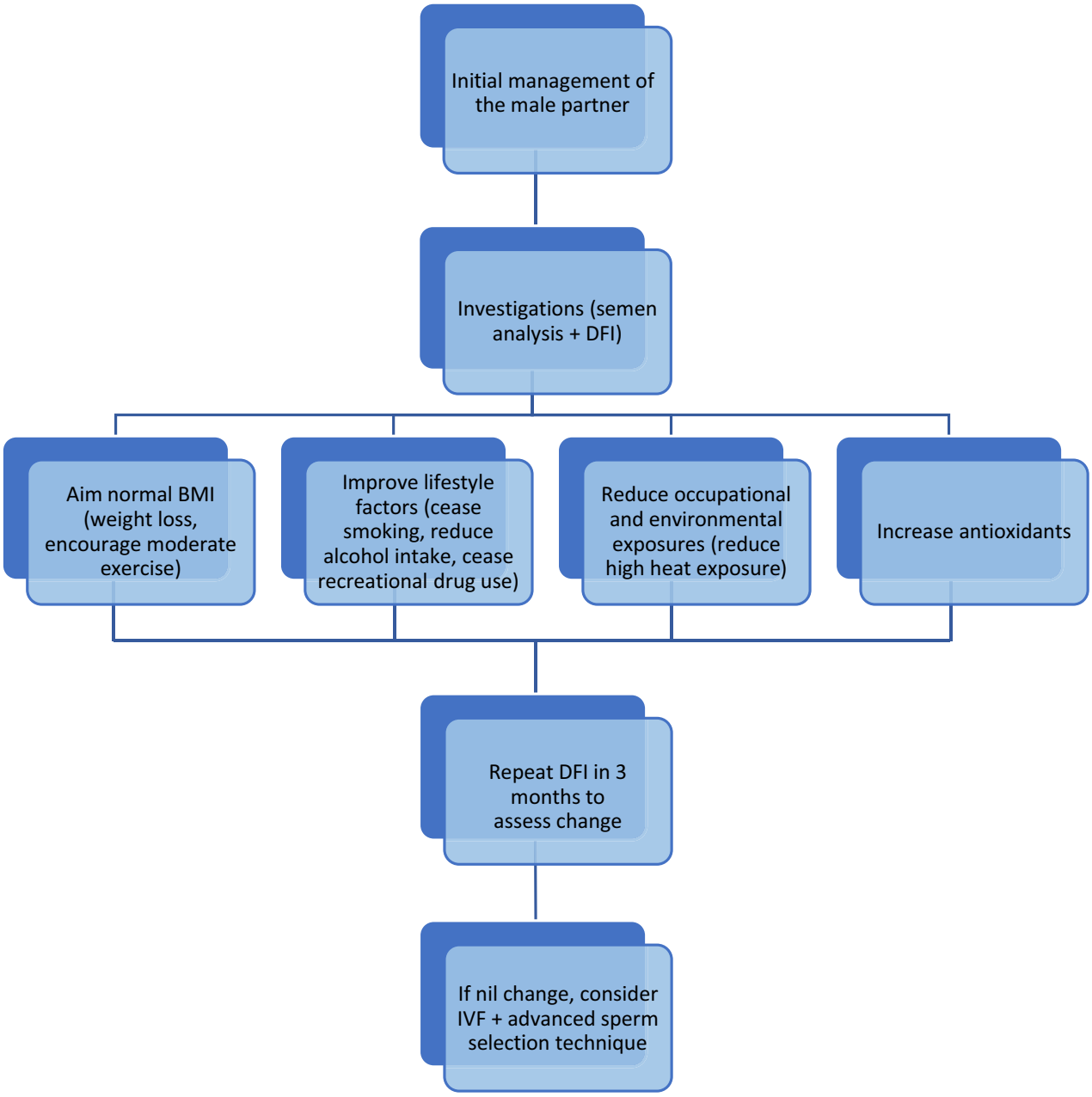

FIGURE 1 Summary for management of the male partner. BMI, body mass index; DFI, DNA fragmentation index.

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.

ACKNOWLEDGEMENT

Open access publishing facilitated by The University of Sydney, as part of the Wiley - The University of Sydney agreement via the Council of Australian University Librarians.

REFERENCES

1. Dizon-Townson DMC, Sibai B, Spong CY *et al*. The relationship between the factor V leiden mutation and pregnancy outcomes for mother and fetus. *Obstet Gynecol* 2005; **106**(3): 517–524.
2. Said JM, Higgins JR, Moses EK *et al*. Inherited thrombophilia polymorphisms and pregnancy outcomes in nulliparous women. *Obstet Gynecol* 2010; **115**(1): 5–13.
3. Murphy RP, Donoghue C, Nallen RJ *et al*. Prospective evaluation of the risk conferred by factor V Leiden and thermolabile methylenetetrahydrofolate reductase polymorphisms in pregnancy. *Arterioscler Thromb Vasc Biol* 2000; **20**(1): 266–270.
4. Clark P, Walker ID, Govan L *et al*. The GOAL study: A prospective examination of the impact of factor V Leiden and ABO(H) blood groups on haemorrhagic and thrombotic pregnancy outcomes. *Br J Haematol* 2008; **140**(2): 236–240.
5. Linqvist PSP, Marsaal K, Grennert L *et al*. Activated protein C resistance (FV: Q506) and pregnancy. *Thromb Haemost* 1999; **81**(4): 532–537.
6. Silver RM, Zhao Y, Spong CY *et al*. Prothrombin gene G20210A mutation and obstetric complications. *Obstet Gynecol* 2010; **115**(1): 14–20.
7. de Jong PG, Kaandorp S, Di Nisio M *et al*. Aspirin and/or heparin for women with unexplained recurrent miscarriage with or without inherited thrombophilia. *Cochrane Database Syst Rev* 2014; **2014**(7): CD004734.
8. Preston FE, Rosendaal FR, Walker ID *et al*. Increased fetal loss in women with heritable thrombophilia. *Lancet* 1996; **348**(9032): 913–916.
9. Roque H, Paidas MJ, Funai EF *et al*. Maternal thrombophilias are not associated with early pregnancy loss. *Thromb Haemost* 2004; **91**(2): 290–295.
10. de Jong PG, Quenby S, Bloemenkamp KWM *et al*. ALIFE2 study: Low-molecular-weight heparin for women with recurrent miscarriage and inherited thrombophilia—study protocol for a randomized controlled trial. *Trials* 2015; **16**(1): 208.
11. Kocher O, Cirovic C, Malynn E *et al*. Obstetric complications in patients with hereditary thrombophilia identified using the LCx microparticle enzyme immunoassay: A controlled study of 5,000 patients. *Am J Clin Pathol* 2007; **127**(1): 68–75.

12. Rodger MA, Langlois NJ. Is thrombophilia associated with placenta-mediated pregnancy complications? A prospective cohort study: Reply. *J Thromb Haemost* 2014; **12**(8): 1378–1379.
13. Rai R, Backos M, Elgaddal S *et al*. Factor V Leiden and recurrent miscarriage-prospective outcome of untreated pregnancies. *Hum Reprod* 2002; **17**(2): 442–445.
14. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: A meta-analysis. *Lancet* 2003; **361**(9361): 901–908.
15. Sergi C, Al Jishi T, Walker M. Factor V Leiden mutation in women with early recurrent pregnancy loss: A meta-analysis and systematic review of the causal association. *Arch Gynecol Obstet* 2015; **291**(3): 671–679.
16. Bouvier S, Cochery-Nouvellon E, Lavigne-Lissalde G *et al*. Comparative incidence of pregnancy outcomes in thrombophilia-positive women from the NOH-APS observational study. *Blood* 2014; **123**(3): 414–421.
17. Pickering W, Marriott K, Regan L. G20210A prothrombin gene mutation: Prevalence in a recurrent miscarriage population. *Clin Appl Thromb Hemost* 2001; **7**(1): 25–28.
18. Sottilotta G, Oriana V, Latella C *et al*. Genetic prothrombotic risk factors in women with unexplained pregnancy loss. *Thromb Res* 2006; **117**(6): 681–684.
19. Dilley A, Benito C, Hooper WC *et al*. Mutations in the factor V, prothrombin and MTHFR genes are not risk factors for recurrent fetal loss. *J Matern Fetal Neonatal Med* 2002; **11**(3): 176–182.
20. Domagala TB, Adamek L, Nizankowska E *et al*. Mutations C677T and A1298C of the 5,10-methylenetetrahydrofolate reductase gene and fasting plasma homocysteine levels are not associated with the increased risk of venous thromboembolic disease. *Blood Coagul Fibrinolysis* 2002; **13**(5): 423–431.
21. McColl MD, Ellison J, Reid F *et al*. Prothrombin 20210 G→a, MTHFR C677T mutations in women with venous thromboembolism associated with pregnancy. *BJOG* 2000; **107**(4): 565–569.
22. Foka ZJ, Lambropoulos AF, Saravelos H *et al*. Factor V leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. *Hum Reprod* 2000; **15**(2): 458–462.
23. Nurk E, Tell GS, Refsum H *et al*. Factor V Leiden, pregnancy complications and adverse outcomes: The Hordaland homocysteine study. *QJM* 2006; **99**(5): 289–298.
24. Vollset SE, Refsum H, Irgens LM *et al*. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: The Hordaland homocysteine study. *Am J Clin Nutr* 2000; **71**(4): 962–968.
25. Miyakis S, Lockshin MD, Atsumi T *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; **4**(2): 295–306.
26. Sauer R, Roussev R, Jeyendran RS, Coulam CB. Prevalence of antiphospholipid antibodies among women experiencing unexplained infertility and recurrent implantation failure. *Fertil Steril* 2010; **93**(7): 2441–2443.
27. de Jong PG, Goddijn M, Middeldorp S. Antithrombotic therapy for pregnancy loss. *Hum Reprod Update* 2013; **19**(6): 656–673.
28. Skeith L, Carrier M, Kaaja R *et al*. A meta-analysis of low-molecular-weight heparin to prevent pregnancy loss in women with inherited thrombophilia. *Blood* 2016; **127**(13): 1650–1655.
29. Kaandorp SP, Goddijn M, van der Post JA *et al*. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. *N Engl J Med* 2010; **362**(17): 1586–1596.
30. Visser J, Ulander VM, Helmerhorst FM *et al*. Thromboprophylaxis for recurrent miscarriage in women with or without thrombophilia. HABENOX: A randomised multicentre trial. *Thromb Haemost* 2011; **105**(2): 295–301.
31. Hamulyak EN, Scheres LJ, Marijnen MC *et al*. Aspirin or heparin or both for improving pregnancy outcomes in women with persistent antiphospholipid antibodies and recurrent pregnancy loss. *Cochrane Database Syst Rev* 2020; **5**(5): CD012852.
32. Clark P, Walker ID, Langhorne P *et al*. SPIN (Scottish pregnancy intervention) study: A multicenter, randomized controlled trial of low-molecular-weight heparin and low-dose aspirin in women with recurrent miscarriage. *Blood* 2010; **115**(21): 4162–4167.
33. Dolitzky M, Inbal A, Segal Y *et al*. A randomized study of thromboprophylaxis in women with unexplained consecutive recurrent miscarriages. *Fertil Steril* 2006; **86**(2): 362–366.
34. Pasquier E, de Saint Martin L, Bohec C *et al*. Enoxaparin for prevention of unexplained recurrent miscarriage: A multicenter randomized double-blind placebo-controlled trial. *Blood* 2015; **125**(14): 2200–2205.
35. Johnson PM, Ramsden GH, Chia KV *et al*. A combined randomised double-blind and open study of trophoblast membrane infusion (TMI) in unexplained recurrent miscarriage. *Cell Mol Biol Materno-Fetal Relation* 1991; **212**: 277–284.
36. Hiby SE, Apps R, Sharkey AM *et al*. Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. *J Clin Invest* 2010; **120**(11): 4102–4110.
37. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer* 1975; **16**(2): 216–229.
38. Male V, Hughes T, McClory S *et al*. Immature NK cells, capable of producing IL-22, are present in human uterine mucosa. *J Immunol* 2010; **185**(7): 3913–3918.
39. Vacca P, Moretta L, Moretta A, Mingari MC. Origin, phenotype and function of human natural killer cells in pregnancy. *Trends Immunol* 2011; **32**(11): 517–523.
40. King A, Hiby SE, Gardner L *et al*. Surface expression of HLA-C antigen by human extravillous trophoblast. *Placenta* 2000; **21**(Suppl A): S81–S85.
41. Seshadri S, Sunkara SK. Natural killer cells in female infertility and recurrent miscarriage: A systematic review and meta-analysis. *Hum Reprod Update* 2014; **20**(3): 429–438.
42. Grimstad F, Krieg S. Immunogenetic contributions to recurrent pregnancy loss. *J Assist Reprod Genet* 2016; **33**(7): 833–847.
43. Cavalcante MB, Cavalcante C, Sarno M *et al*. Antinuclear antibodies and recurrent miscarriage: Systematic review and meta-analysis. *Am J Reprod Immunol* 2020; **83**(3): e13215.
44. Chen S, Yang G, Wu P *et al*. Antinuclear antibodies positivity is a risk factor of recurrent pregnancy loss: A meta-analysis. *Semin Arthritis Rheum* 2020; **50**(4): 534–543.
45. Tersigni C, Castellani R, de Waure C *et al*. Celiac disease and reproductive disorders: Meta-analysis of epidemiologic associations and potential pathogenic mechanisms. *Hum Reprod Update* 2014; **20**(4): 582–593.
46. Kutteh MA, Abiad M, Norman GL, Kutteh WH. Comparison of celiac disease markers in women with early recurrent pregnancy loss and normal controls. *Am J Reprod Immunol* 2019; **82**(1): e13127.
47. Sharshiner R, Romero ST, Bardsley TR *et al*. Celiac disease serum markers and recurrent pregnancy loss. *J Reprod Immunol* 2013; **100**(2): 104–108.
48. Wong LF, Porter TF, Scott JR. Immunotherapy for recurrent miscarriage. *Cochrane Database Syst Rev* 2014; **2014**(10): CD000112.
49. Achilli C, Duran-Retamal M, Saab W *et al*. The role of immunotherapy in in vitro fertilization and recurrent pregnancy loss: A systematic review and meta-analysis. *Fertil Steril* 2018; **110**(6): 1089–1100.
50. Carp H. Immunotherapy for recurrent pregnancy loss. *Best Pract Res Clin Obstet Gynaecol* 2019; **60**: 77–86.
51. Wang SW, Zhong SY, Lou LJ *et al*. The effect of intravenous immunoglobulin passive immunotherapy on unexplained recurrent

spontaneous abortion: A meta-analysis. *Reprod Biomed Online* 2016; **33**(6): 720–736.
52. Yamada H, Deguchi M, Saito S *et al*. Intravenous immunoglobulin treatment in women with four or more recurrent pregnancy losses: A double-blind, randomised, placebo-controlled trial. *EClinicalMedicine* 2022; **50**: 101527.
53. Quenby S, Kalumbi C, Bates M *et al*. Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. *Fertil Steril* 2005; **84**(4): 980–984.
54. Dan S, Wei W, Yichao S *et al*. Effect of prednisolone administration on patients with unexplained recurrent miscarriage and in routine intracytoplasmic sperm injection: A meta-analysis. *Am J Reprod Immunol* 2015; **74**(1): 89–97.
55. Gomaa MF, Elkholy AG, El-Said MM, Abdel-Salam NE. Combined oral prednisolone and heparin versus heparin: The effect on peripheral NK cells and clinical outcome in patients with unexplained recurrent miscarriage. A double-blind placebo randomized controlled trial. *Arch Gynecol Obstet* 2014; **290**(4): 757–762.
56. Dakhly DM, Bayoumi YA, Sharkawy M *et al*. Intralipid supplementation in women with recurrent spontaneous abortion and elevated levels of natural killer cells. *Int J Gynaecol Obstet* 2016; **135**(3): 324–327.
57. Scarpellini F, Sbracia M. Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: A randomised controlled trial. *Hum Reprod* 2009; **24**(11): 2703–2708.
58. Eapen A, Joing M, Kwon P *et al*. Recombinant human granulocyte-colony stimulating factor in women with unexplained recurrent pregnancy losses: A randomized clinical trial. *Hum Reprod* 2019; **34**(3): 424–432.
59. Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. *Fertil Steril* 2010; **93**(4): 1234–1243.
60. Paukku M, Tulppala M, Puolakkainen M *et al*. Lack of association between serum antibodies to chlamydia trachomatis and a history of recurrent pregnancy loss. *Fertil Steril* 1999; **72**(3): 427–430.
61. Rehman F, Shah M, Ali A *et al*. Unpasteurised milk consumption as a potential risk factor for toxoplasmosis in females with recurrent pregnancy loss. *J Obstet Gynaecol* 2020; **40**(8): 1106–1110.
62. Sherkat R, Meidani M, Zarabian H *et al*. Seropositivity of cytomegalovirus in patients with recurrent pregnancy loss. *J Res Med Sci* 2014; **19**(Suppl 1): S22–S25.
63. Llahi-Camp JM, Rai R, Ison C *et al*. Association of bacterial vaginosis with a history of second trimester miscarriage. *Hum Reprod* 1996; **11**(7): 1575–1578.
64. Oakeshott P, Hay P, Hay S *et al*. Association between bacterial vaginosis or chlamydial infection and miscarriage before 16 weeks' gestation: Prospective community based cohort study. *BMJ* 2002; **325**(7376): 1334.
65. Benner M, Ferwerda G, Joosten I, van der Molen RG. How uterine microbiota might be responsible for a receptive, fertile endometrium. *Hum Reprod Update* 2018; **24**(4): 393–415.
66. Kimura F, Takebayashi A, Ishida M *et al*. Review: Chronic endometritis and its effect on reproduction. *J Obstet Gynaecol Res* 2019; **45**(5): 951–960.
67. Bouet PE, El Hachem H, Monceau E *et al*. Chronic endometritis in women with recurrent pregnancy loss and recurrent implantation failure: Prevalence and role of office hysteroscopy and immunohistochemistry in diagnosis. *Fertil Steril* 2016; **105**(1): 106–110.
68. McQueen DB, Bernardi LA, Stephenson MD. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. *Fertil Steril* 2014; **101**(4): 1026–1030.
69. Cicinelli E, Matteo M, Tinelli R *et al*. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reprod Sci* 2014; **21**(5): 640–647.
70. Vitagliano A, Saccardi C, Noventa M *et al*. Effects of chronic endometritis therapy on in vitro fertilization outcome in women with repeated implantation failure: A systematic review and meta-analysis. *Fertil Steril* 2018; **110**(1): 103–112. e1.
71. Pirtea P, Cicinelli E, De Nola R *et al*. Endometrial causes of recurrent pregnancy losses: Endometriosis, adenomyosis, and chronic endometritis. *Fertil Steril* 2021; **115**(3): 546–560.
72. Boje AD, Egerup P, Westergaard D *et al*. Endometriosis is associated with pregnancy loss: A nationwide historical cohort study. *Fertil Steril* 2023; **119**(5): 826–835.
73. Hodgson RM, Lee HL, Wang R *et al*. Interventions for endometriosis-related infertility: A systematic review and network meta-analysis. *Fertil Steril* 2020; **113**(2): 374–382. e2.
74. Bafort C, Beebeejaun Y, Tomassetti C *et al*. Laparoscopic surgery for endometriosis. *Cochrane Database Syst Rev* 2020; **10**(10): CD011031.
75. Grammatis AL, Georgiou EX, Becker CM. Pentoxifylline for the treatment of endometriosis-associated pain and infertility. *Cochrane Database Syst Rev* 2021; **8**(8): CD007677.
76. Barbosa MA, Teixeira DM, Navarro PA *et al*. Impact of endometriosis and its staging on assisted reproduction outcome: Systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2014; **44**(3): 261–278.
77. Hamdan M, Omar SZ, Dunselman G, Cheong Y. Influence of endometriosis on assisted reproductive technology outcomes: A systematic review and meta-analysis. *Obstet Gynecol* 2015; **125**(1): 79–88.
78. Practice Committee of the American Society for Reproductive M. Evaluation and treatment of recurrent pregnancy loss: A committee opinion. *Fertil Steril* 2012; **98**(5): 1103–1111.
79. European Society of Human Reproduction & Embryology (ESHRE). Guideline on the management of recurrent pregnancy loss. 2022; at https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Recurrent-pregnancy-loss [accessed 20 June 2023].
80. Royal College of Obstetricians & Gynaecologists (RCOG). The Investigation and Treatment of Couples with Recurrent First Trimester and Second Trimester Miscarriage, Green-top guideline no. 17. 2011; available at: https://ranzcog.edu.au/wp-content/uploads/2022/05/The-Investigation-and-Treatment-of-Couples-with-Recurrent-First-trimester-and-Second-trimester-Miscarriage.pdf [accessed 20 June 2023].
81. Cochrane Australia & South Australian Health and Medical Research Institute. Report for systematic reviews of the association between different levels and patterns of maternal alcohol consumption during pregnancy and while breastfeeding and selected health outcomes for fetuses and children (up to age five). 2018 [online report available at: https://www.nhmrc.gov.au/file/14978/download?token=-BTzojYa accessed July 2023].
82. Koert E, Malling GMH, Sylvest R *et al*. Recurrent pregnancy loss: couples' perspectives on their need for treatment, support and follow up. *Hum Reprod* 2019; **34**(2): 291–296.
83. Liddell HS, Pattison NS, Zanderigo A. Recurrent miscarriage—outcome after supportive Care in Early Pregnancy. *Aust N Z J Obstet Gynaecol* 1991; **31**(4): 320–322.
84. Clifford K, Rai R, Regan L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum Reprod* 1997; **12**(2): 387–389.
85. Ricci E, Al Beitawi S, Cipriani S *et al*. Semen quality and alcohol intake: A systematic review and meta-analysis. *Reprod Biomed Online* 2017; **34**(1): 38–47.
86. Ruixue W, Hongli Z, Zhihong Z *et al*. The impact of semen quality, occupational exposure to environmental factors and lifestyle on recurrent pregnancy loss. *J Assist Reprod Genet* 2013; **30**(11): 1513–1518.

87. Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: A new meta-analysis examining the effect of the 2010 World Health Organization Laboratory methods for the examination of human semen. *Eur Urol* 2016; **70**(4): 635–645.
88. Cao X, Cui Y, Zhang X *et al*. The correlation of sperm morphology with unexplained recurrent spontaneous abortion: A systematic review and meta-analysis. *Oncotarget* 2017; **8**(33): 55646–55656.
89. Hill JA, Abbott AF, Politch JA. Sperm morphology and recurrent abortion. *Fertil Steril* 1994; **61**(4): 776–778.
90. Zidi-Jrah I, Hajlaoui A, Mougou-Zerelli S *et al*. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. *Fertil Steril* 2016; **105**(1): 58–64.
91. Pourfahraji Fakhrabadi M, Kalantar SM, Montazeri F *et al*. FISH-based sperm aneuploidy screening in male partner of women with a history of recurrent pregnancy loss. *Middle East Fertil Soc J* 2020; **25**(1): 23.
92. Sakkas D, Alvarez JG. Sperm DNA fragmentation: Mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril* 2010; **93**(4): 1027–1036.
93. Robinson L, Gallos ID, Conner SJ *et al*. The effect of sperm DNA fragmentation on miscarriage rates: A systematic review and meta-analysis. *Hum Reprod* 2012; **27**(10): 2908–2917.
94. McQueen DB, Zhang J, Robins JC. Sperm DNA fragmentation and recurrent pregnancy loss: A systematic review and meta-analysis. *Fertil Steril* 2019; **112**(1): 54–60. e3.
95. Samavat J, Cantini G, Lotti F *et al*. Massive weight loss obtained by bariatric surgery affects semen quality in morbid Male obesity: A preliminary prospective double-armed study. *Obes Surg* 2018; **28**(1): 69–76.
96. Sepidarkish M, Maleki-Hajiagha A, Maroufizadeh S *et al*. The effect of body mass index on sperm DNA fragmentation: A systematic review and meta-analysis. *Int J Obes (Lond)* 2020; **44**(3): 549–558.
97. Wood GJA, Tiseo BC, Paluello DV *et al*. Bariatric surgery impact on reproductive hormones, semen analysis, and sperm DNA fragmentation in men with severe obesity: Prospective study. *Obes Surg* 2020; **30**(12): 4840–4851.
98. Roque M, Esteves SC. Effect of varicocele repair on sperm DNA fragmentation: A review. *Int Urol Nephrol* 2018; **50**(4): 583–603.
99. Li F, Yamaguchi K, Okada K *et al*. Significant improvement of sperm DNA quality after microsurgical repair of varicocele. *Syst Biol Reprod Med* 2012; **58**(5): 274–277.
100. Smits RM, Mackenzie-Proctor R, Yazdani A *et al*. Antioxidants for male subfertility. *Cochrane Database Syst Rev* 2019; **3**(3): CD007411.
101. Tremellen K. Oxidative stress and male infertility—A clinical perspective. *Hum Reprod Update* 2008; **14**(3): 243–258.
102. Lepine S, McDowell S, Searle LM *et al*. Advanced sperm selection techniques for assisted reproduction. *Cochrane Database Syst Rev* 2019; **7**(7): CD010461.
103. Haas DM, Hathaway TJ, Ramsey PS. Progestogen for preventing miscarriage in women with recurrent miscarriage of unclear etiology. *Cochrane Database Syst Rev* 2019; **2019**(11): CD003511.
104. Murugappan G, Shahine LK, Perfetto CO *et al*. Intent to treat analysis of in vitro fertilization and preimplantation genetic screening versus expectant management in patients with recurrent pregnancy loss. *Hum Reprod* 2016; **31**(8): 1668–1674.
105. Lund M, Kamper-Jørgensen M, Nielsen HS *et al*. Prognosis for live birth in women with recurrent miscarriage: What is the best measure of success? *Obstet Gynecol* 2012; **119**(1): 37–43.
106. Rashtian J, Chavkin DE, Merhi Z. Water and soil pollution as determinant of water and food quality/contamination and its impact on female fertility. *Reprod Biol Endocrinol* 2019; **17**(1): 5.
107. Li Q, Davila J, Kannan A *et al*. Chronic exposure to bisphenol a affects uterine function during early pregnancy in mice. *Endocrinology* 2016; **157**(5): 1764–1774.
108. Lathi RB, Liebert CA, Brookfield KF *et al*. Conjugated bisphenol a in maternal serum in relation to miscarriage risk. *Fertil Steril* 2014; **102**(1): 123–128.
109. Shen Y, Zheng Y, Jiang J *et al*. Higher urinary bisphenol a concentration is associated with unexplained recurrent miscarriage risk: Evidence from a case-control study in eastern China. *PLoS One* 2015; **10**(5): e0127886.
110. Sugiura-Ogasawara M, Ozaki Y, Sonta S *et al*. Exposure to bisphenol a is associated with recurrent miscarriage. *Hum Reprod* 2005; **20**(8): 2325–2329.
111. Liao KW, Kuo PL, Huang HB *et al*. Increased risk of phthalates exposure for recurrent pregnancy loss in reproductive-aged women. *Environ Pollut* 2018; **241**: 969–977.
112. Gerhard I, Waibel S, Daniel V, Runnebaum B. Impact of heavy metals on hormonal and immunological factors in women with repeated miscarriages. *Hum Reprod Update* 1998; **4**(3): 301–309.
113. Ajayi OO, Charles-Davies MA, Arinola OG. Progesterone, selected heavy metals and micronutrients in pregnant Nigerian women with a history of recurrent spontaneous abortion. *Afr Health Sci* 2012; **12**(2): 153–159.
114. Thaker R, Oza H, Shaikh I, Kumar S. Correlation of copper and zinc in spontaneous abortion. *Int J Fertil Steril* 2019; **13**(2): 97–101.
115. Bjorklund G, Chirumbolo S, Dadar M *et al*. Mercury exposure and its effects on fertility and pregnancy outcome. *Basic Clin Pharmacol Toxicol* 2019; **125**(4): 317–327.
116. El-Badry A, Rezk M, El-Sayed H. Mercury-induced oxidative stress may adversely affect pregnancy outcome among dental staff: A cohort study. *Int J Occup Environ Med* 2018; **9**(3): 113–119.
117. Gerhard I, Monga B, Waldbrenner A, Runnebaum B. Heavy metals and fertility. *J Toxicol Environ Health A* 1998; **54**(8): 593–611.
118. Buck Louis GM, Smarr MM, Sundaram R *et al*. Low-level environmental metals and metalloids and incident pregnancy loss. *Reprod Toxicol* 2017; **69**: 68–74.
119. Yildirim E, Derici MK, Demir E *et al*. Is the concentration of cadmium, Lead, mercury, and selenium related to preterm birth? *Biol Trace Elem Res* 2019; **191**(2): 306–312.
120. van Oppenraaij RH, Koning AH, van den Hoff MJ *et al*. The effect of smoking on early chorionic villous vascularisation. *Placenta* 2012; **33**(8): 645–651.
121. George L, Granath F, Johansson AL *et al*. Risks of repeated miscarriage. *Paediatr Perinat Epidemiol* 2006; **20**(2): 119–126.
122. Zhang BY, Wei YS, Niu JM *et al*. Risk factors for unexplained recurrent spontaneous abortion in a population from southern China. *Int J Gynaecol Obstet* 2010; **108**(2): 135–138.
123. Augood C, Duckitt K, Templeton AA. Smoking and female infertility: A systematic review and meta-analysis. *Hum Reprod* 1998; **13**(6): 1532–1539.
124. Pineles BL, Park E, Samet JM. Systematic review and meta-analysis of miscarriage and maternal exposure to tobacco smoke during pregnancy. *Am J Epidemiol* 2014; **179**(7): 807–823.
125. Meeker JD, Missmer SA, Cramer DW, Hauser R. Maternal exposure to second-hand tobacco smoke and pregnancy outcome among couples undergoing assisted reproduction. *Hum Reprod* 2007; **22**(2): 337–345.
126. Chen LW, Wu Y, Neelakantan N *et al*. Maternal caffeine intake during pregnancy and risk of pregnancy loss: A categorical and dose-response meta-analysis of prospective studies. *Public Health Nutr* 2016; **19**(7): 1233–1244.
127. Stefanidou EM, Caramellino L, Patriarca A, Menato G. Maternal caffeine consumption and sine causa recurrent miscarriage. *Eur J Obstet Gynecol Reprod Biol* 2011; **158**(2): 220–224.

128. Sundermann AC, Zhao S, Young CL *et al*. Alcohol use in pregnancy and miscarriage: A systematic review and meta-analysis. *Alcohol Clin Exp Res* 2019; **43**(8): 1606–1616.
129. Oostingh EC, Hall J, Koster MPH *et al*. The impact of maternal lifestyle factors on periconception outcomes: A systematic review of observational studies. *Reprod Biomed Online* 2019; **38**(1): 77–94.
130. Kolte AM, Olsen LR, Mikkelsen EM *et al*. Depression and emotional stress is highly prevalent among women with recurrent pregnancy loss. *Hum Reprod* 2015; **30**(4): 777–782.
131. Li W, Newell-Price J, Jones GL *et al*. Relationship between psychological stress and recurrent miscarriage. *Reprod Biomed Online* 2012; **25**(2): 180–189.
132. Tavoli Z, Mohammadi M, Tavoli A *et al*. Quality of life and psychological distress in women with recurrent miscarriage: A comparative study. *Health Qual Life Outcomes* 2018; **16**(1): 150.
133. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC *et al*. Endocrine-disrupting chemicals: An Endocrine Society scientific statement. *Endocr Rev* 2009; **30**(4): 293–342.

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 McChessney, Clare Boothroyd, Neerja Kamal, Anne Clark, Violet Kieu, Rituparna Dutta, Lynn Burmeister, Ashleigh Smith, Louise Hull, Vamsee Thalluri, Giselle Crawford, Beverley Vollenhoven Sarah Hunt, Nicole Hope, Sameer Jakar, Jinny Foo, Iris Wang, Rabia Shaik, Seema Mohiuddin, Fleur Cattrall, Peter Leung, Roger Hart, Alison Gee, Katrina Rowan, Michele Kwik, Iris Wang, Jinny Foo, Rabia Shaik, Tamara Hunter, Nalini Gayer, Raelia Lew, Rebecca Mackenzie-Proctor, Anusch Yazdani, Rob Lahoud, Cheryl Phua, Shannon Zawada, Gabrielle Dezarnaulds, Sonal Karia, Devini Ameratunga, Vanessa Ross, Manny Mangat, Raewyn Tierney, Shadi Khashaba, David Greening, Janelle McDonald, Myvanwy McAveen, Ying Li, Anthony Marren and Danielle Robson.