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# Guideline

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# Recurrent Pregnancy Loss

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# Update 2022

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*The European Society of Human Reproduction and Embryology (hereinafter referred to as 'ESHRE') developed the current clinical practice guideline, to provide clinical recommendations to improve the quality of healthcare delivery within the European field of human reproduction and embryology. This guideline represents the views of ESHRE, which were achieved after careful consideration of the scientific evidence available at the time of preparation. In the absence of scientific evidence on certain aspects, a consensus between the relevant ESHRE stakeholders has been obtained.*

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# Introduction to the Guideline

Previous evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage have been published in 2006 on behalf of the ESHRE Special Interest Group (SIG) Early Pregnancy (Jauniaux et al. 2006). However, the ESHRE SIG Implantation and Early Pregnancy believed that these guidelines were outdated and initiated the guideline, published in 2017.

The current guideline is an update of the version from 2017, with amendments to the recommendations based on recently published data. Where amendments were made, this is labelled as such (Update 2022).

The 2017 guideline and the update are developed according to a well-documented methodology, universal to ESHRE guidelines and described in the Manual for ESHRE guideline development (www.eshre.eu). Details on the methodology of the current guideline are outlined in Annex 5.

The guideline development group (GDG) for the current update consisted of the previous guideline group with minor changes. One member of the GDG (2017) decided to step down and was replaced, and one additional GDG member was added. The members of the guideline development group are listed in Annex 1.

## **GUIDELINE SCOPE**

The overall aim of this guideline is to supply healthcare providers with the best available evidence for investigation and treatment of women with recurrent pregnancy loss. Recurrent Pregnancy Loss (RPL) is defined as the loss of two or more pregnancies. It excludes ectopic pregnancy and molar pregnancy. How to handle the definition is further elaborated in part A.

The guideline provides an overview of suggested treatments for RPL, and which of those are recommended. Furthermore, recommendations are made on the investigations that could be helpful to identify the origin of the pregnancy losses and possible therapeutic targets. In addition, recommendations are written regarding organization of care for couples faced with RPL.

## **TARGET USERS OF THE GUIDELINE**

The guideline covers the care provided by secondary and tertiary healthcare professionals who have direct contact with, and make decisions concerning the care of, couples with recurrent pregnancy loss.

This guideline is of relevance to European healthcare providers and couples with recurrent pregnancy loss. For the benefit of patient education and shared decision making, a patient version of this guideline was developed.

## **References**

Jauniaux E, Farquharson RG, Christiansen OB, and Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Hum Reprod* 2006; **21**: 2216-2222.

# List of all recommendations

Chapter	Nr	Recommendation	Strength	Quality of evidence	Justification	Remarks
Recurrent Pregnancy Loss						
		A diagnosis of Recurrent Pregnancy Loss (RPL) could be considered after the loss of two or more pregnancies.	/	/	/	Conclusion
		The guideline development group (GDG) concludes to use the term Recurrent Pregnancy Loss.	/	/	/	Conclusion
		Pregnancy loss is a significant negative life event and the repetitive nature of RPL may intensify the grief experienced. Studies have mostly focused on women, and there is a need for studies on the emotional impact of RPL on men. Clinicians and clinics should take the psychosocial needs of couples faced with RPL into account when offering and organizing care for these couples.	/	/	/	Conclusion
RISK FACTOR AND HEALTH BEHAVIOR MODIFICATIONS						
1	1	Women should be sensitively informed that the risk of pregnancy loss is lowest in women aged 20 to 35 years.	Strong	⊕⊕○○	Although the evidence is of low quality (based on small but consistent observational studies), the GDG decided to strongly recommend information provision on the topic.	
1	2	Women should be informed that the risk of pregnancy loss rapidly increases after the age of 40.	Strong	⊕⊕○○		
1	3	Stress is associated with RPL, but couples should be informed that there is no evidence that stress is a direct cause of pregnancy loss.	Strong	⊕○○○	This recommendation is based on a significant concern of couples, with only very low-quality evidence on an association and no evidence for a causal relation	

2	4	Couples with RPL should be informed that smoking could have a negative impact on their chances of a live birth, and therefore cessation of smoking is recommended.	GPP		Smoking is a moderate risk factor for RPL and associated with poor obstetric outcomes. Cessation of smoking could be recommended in couples with RPL even in the absence of studies on the effect of smoking cessation.
2	5	Couples with RPL should be informed that maternal obesity or being significantly underweight is associated with obstetric complications and could have a negative impact on their chances of a live birth and on their general health.	Strong	⊕⊕○○	Maternal obesity is a strong risk factor in RPL, and weight loss in overweight women has a positive impact on fertility outcomes and reduced weight is associated with reduced complications during pregnancy and birth. Striving for a normal BMI is recommended, even in the absence of studies on the impact of weight loss on a subsequent pregnancy loss.
2	6	Striving for a healthy normal range body mass index (BMI) is recommended.	GPP		
2	7	Couples with RPL should be informed that excessive alcohol consumption is a possible risk factor for pregnancy loss and proven risk factor for foetal problems (Foetal alcohol syndrome).	Strong	⊕⊕○○	Alcohol consumption is a weak risk factor for pregnancy loss. Clinicians should provide information on alcohol, and advice to limit consumption based on the absence of harms. Women suggesting that alcohol use has caused a previous pregnancy loss can be reassured that there is no evidence for a causal association.
2	8	Couples with RPL should be advised to limit alcohol consumption.	GPP		
<b>INVESTIGATIONS IN RPL</b>					
3	9	Medical and family history could be used to tailor diagnostic investigations in RPL.	GPP		The GDG concludes that a thorough reproductive history should be taken in couples presenting with RPL and stresses that number of preceding pregnancy losses and female age provide the best available prognostic information
3	10	The guideline development group (GDG) recommends to base prognosis on woman's exact pregnancy history and female age.	Strong	⊕⊕⊕○	
4	11	Genetic analysis of pregnancy tissue is not routinely recommended but it could be performed for explanatory purposes.	Conditional	⊕⊕○○	As the impact of further clinical decision-making and the exact influence on prognosis for an individual patient is unclear, the GDG decided to formulate a conditional recommendation on genetic testing of the pregnancy tissue.
4	12	For genetic analysis of the pregnancy tissue, Array-based Comparative Genomic Hybridization (array-CGH) is recommended based on a reduced maternal contamination effect.	Strong	⊕⊕○○	
4	13	Parental karyotyping is not routinely recommended in couples with RPL. It could be carried out after individual assessment of risk.	Conditional	⊕⊕○○	Couples should primarily be informed that, even if a parental abnormality is found, the cumulative live birth rates are good. Furthermore, they should be informed of the limitations of karyotyping and the impact of the test result.

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5	14	For women with RPL, we suggest not to screen for hereditary thrombophilia unless in the context of research, or in women with additional risk factors for thrombophilia	Conditional	⊕⊕⊕○	There is no, or at best a weak, association between RPL and hereditary thrombophilia.
5	15	For women with RPL, we recommend screening for antiphospholipid antibodies (Lupus Anticoagulant [LA], and Anticardiolipin antibodies [ACA IgG and IgM]), after two pregnancy losses.	Strong	⊕⊕○○	Testing for aPL antibodies can provide a possible cause of the PL, and treatment in the next pregnancy can prevent antiphospholipid syndrome (APS)-associated pregnancy complications.
5	16	For women with RPL, screening for $\beta 2$ glycoprotein I antibodies (a $\beta 2$ GPI) can be considered after two pregnancy losses.	GPP		Based on a study showing treatment can improve LBR in women with RPL and a $\beta 2$ GPI, screening can be considered.
6	17	Human Leukocyte Antigen (HLA) determination in women with RPL is not recommended in clinical practice. Only HLA class II determination (HLA-DRB1*15:01, HLA-DRB1*07 and HLA-DQB1*05:01/05:2) could be considered in Scandinavian women with secondary RPL after the birth of a boy, for prognostic purposes.	Conditional	⊕⊕○○	Investigation of HLA genes in all women with RPL is not recommended in clinical practice but possible in a research setting. An exception could be investigation of class II HLA in women with secondary RPL after the birth of a boy.
6	19	Measurement of anti-HY antibodies in women with RPL is not recommended in clinical practice.	Conditional	⊕⊕○○	Clinicians could consider offering HLA-DRB1 typing to selected women with RPL, but the testing will provide no change in treatment offers.
6	18	Cytokine testing should not be used in women with RPL in clinical practice.	Strong	⊕⊕○○	Cytokine testing is not recommended, as it is not shown to be causative, and associated with technical challenges. For genetic testing there is good evidence that cytokine polymorphisms are not associated with RPL
6	20	Cytokine polymorphisms should not be tested in women with RPL.	Strong	⊕⊕⊕○	
6	21	Antinuclear antibodies (ANA) testing could be considered for explanatory purposes.	Conditional	⊕⊕○○	Measurement of ANA in women with RPL can be considered as the majority of case-control studies document an association to RPL and there is some evidence that ANA presence affects the prognosis negatively.
6	22	There is insufficient evidence to recommend Natural Killer (NK) cell testing of either peripheral blood or endometrial tissue in women with RPL.	Strong	⊕○○○	There seems to be a weak association between NK cells in peripheral blood and RPL, but NK cell testing cannot be used to select women with RPL for immunological treatments. Furthermore, there are significant technical challenges
6	23	Testing anti-Human Leukocyte Antigen (HLA) antibodies in women with RPL is not recommended.	Strong	⊕⊕⊕○	There is no significant effect of anti-HLA antibodies on first trimester complications /RPL.

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7	24	Thyroid screening (Thyroid stimulating hormone [TSH] and Thyroid peroxidase [TPO]-antibodies) is recommended in women with RPL.	Strong	⊕⊕⊕○	Based on a high prevalence of subclinical hypothyroidism and thyroid auto immunity in women with RPL and potential of treatment options testing for thyroid function is recommended.
7	25	Abnormal Thyroid stimulating hormone (TSH) and Thyroid peroxidase [TPO]-antibody levels should be followed up by Thyroxine (T4) testing in women with RPL.	Strong	⊕⊕⊕○	
7	26	Assessment of Polycystic ovary syndrome (PCOS), fasting insulin and fasting glucose is not recommended in women with RPL to improve next pregnancy prognosis.	Strong	⊕⊕○○	The mechanism of how insulin resistance can result in pregnancy loss is unknown, and to our knowledge has not been described. In addition, we did not find any studies on the prognostic potential.
7	27	Prolactin testing is not recommended in women with RPL in the absence of clinical symptoms of hyperprolactinemia (oligo/amenorrhea).	Conditional	⊕⊕○○	In the absence of consistent evidence on an association between prolactin and RPL, prolactin testing is not routinely recommended
7	28	Ovarian reserve testing is not routinely recommended in women with RPL.	Strong	⊕⊕○○	There is insufficient evidence to claim an association between low ovarian reserve and RPL.
7	29	Luteal phase insufficiency testing is not recommended in women with RPL.	Strong	⊕⊕○○	Based on inconsistent evidence of an association, and no value for prognosis and treatment, the GDG decided not to recommend luteal phase insufficiency testing.
7	30	Androgen testing is not recommended in women with RPL.	Strong	⊕⊕○○	Based on inconsistent evidence of an association, and no potential effect on prognosis or treatment, androgen testing is not recommended.
7	31	Luteinizing Hormone (LH) testing is not routinely recommended in women with RPL	Strong	⊕○○○	Based on inconsistent evidence
7	32	Measurement of homocysteine plasma levels is not routinely recommended in women with RPL.	Strong	⊕○○○	Based on inconsistent evidence of an association.
8	33	All women with RPL should have an assessment of the uterine anatomy	Strong	⊕⊕○○	
8	34	The preferred technique to evaluate the uterus is transvaginal 3D ultrasound (US), which has a high sensitivity and specificity, and can distinguish between septate uterus and bicorporeal uterus with normal cervix (former AFS bicornuate uterus).	Conditional	⊕⊕○○	Based on the association and impact on treatment decisions, the GDG recommends US in all women with RPL. Recommendations on preferred methods are also provided.

8	35	Sonohysterography (SHG) is more accurate than hysterosalpingography (HSG) in diagnosing uterine malformations. It can be used to evaluate uterine morphology when 3D ultrasound (US) is not available, or when tubal patency has to be investigated.	Conditional	⊕⊕○○		
8	36	If a Müllerian uterine malformation is diagnosed, further investigation (including investigation of the kidneys and urinary tract) should be considered.	Conditional	⊕⊕○○		
8	37	MRI is not recommended as first line option for the assessment of uterine malformations in women with RPL but can be used where 3D US is not available.	Conditional	⊕⊕○○	Based on the higher costs and the absence of a diagnostic benefit compared to 3D US. However, if 3D US is not available, MRI is a good alternative.	
8	38	All women with RPL should have 2D ultrasound to rule out adenomyosis	Conditional	⊕⊕○○		UPDATED (2022)
9	39	In the couples with RPL, it is recommended to assess lifestyle in the male partner (paternal age, smoking, alcohol consumption, exercise pattern, and body weight).	Conditional	⊕⊕○○	Based on suggested association between lifestyle and sperm quality.	UPDATED (2022)
9	40	Assessing sperm DNA fragmentation in couples with RPL should be considered.	Strong	⊕⊕⊕○	There is a growing body of evidence showing strong association between sperm DNA damage and RPL.	UPDATED (2022)
<b>TREATMENT TO INCREASE LIVE BIRTH RATE IN RPL</b>						
10	10	The guideline development group (GDG) recommends to base prognosis on the number of preceding pregnancy losses and female age.	Strong	⊕⊕⊕○		
10	41	Prognostic tools (Lund, Brigham, Kolte & Westergaard) can be used to provide an estimate of subsequent chance of live birth in couples with unexplained RPL.	GPP		There is consistent evidence for an impact of the number of preceding pregnancy losses and female age on the prognosis in couples with RPL, while evidence for other factors is less clear.	UPDATED (2022)
11	42	All couples with results of an abnormal foetal or parental karyotype should receive genetic counselling.	GPP		The limited evidence for preimplantation genetic testing in couples with RPL shows no clear benefit of treatment (very low quality). Therefore, the GDG strongly	

11	43	All couples with results of an abnormal foetal or parental karyotype may be informed about the possible treatment options available including their advantages and disadvantages.	GPP		recommends that all couples with RPL with abnormal genetic results from pregnancy tissue testing or parental karyotypes should be offered genetic counselling and information on the treatment options so couples can make an informed decision on treatment.
12	44	For women with hereditary thrombophilia and a history of RPL, we suggest not to use antithrombotic prophylaxis unless in the context of research, or if indicated for venous thromboembolism (VTE) prevention	Conditional	⊕⊕○○	We found no evidence of a beneficial effect of anticoagulant treatment in women with RPL and hereditary thrombophilia.
12	45	For women who fulfil the laboratory criteria of APS and a history of three or more pregnancy losses, we suggest administration with low-dose aspirin (75 to 100 mg/day) starting before conception, and a prophylactic dose heparin (Unfractionated heparin [UFH] or Low molecular weight heparin [LMWH]) starting at date of a positive pregnancy test, over no treatment.	Conditional	⊕○○○	Based on evidence suggesting that a combination of heparin and aspirin improves LBR in women with APS and RPL
12	46	The GDG suggests offering anticoagulant treatment for women with two pregnancy losses and APS, only in the context of clinical research.	GPP		
14	47	Overt hypothyroidism arising before conception or during early gestation should be treated with levothyroxine in women with RPL.	Strong	⊕⊕○○	Treatment with levothyroxine is recommended based on existing guidelines and possible maternal and foetal complications associated with untreated hypothyroidism
14	48	There is conflicting evidence regarding treatment effect of levothyroxine for women with subclinical hypothyroidism (SCH) and RPL. Treatment of women with SCH may reduce the risk of miscarriage, but the potential benefit of treatment should be balanced against the risks.	Conditional	⊕⊕○○	Treatment with levothyroxine is insufficiently evidence-based and the efficacy and safety should be further investigated.
14	49	If women with subclinical hypothyroidism and RPL are pregnant again, thyroid stimulating hormone (TSH) level should be checked in early gestation (7-9 weeks AD), and hypothyroidism should be treated with levothyroxine.	GPP		
14	50	If women with thyroid autoimmunity and RPL are pregnant again, thyroid stimulating hormone (TSH) level should be checked in early gestation (7-9 weeks AD), and	GPP		

hypothyroidism should be treated with levothyroxine									
14	51	Euthyroid women with thyroid antibodies and RPL should not be treated with levothyroxine.	Conditional	⊕⊕○○	Evidence showed that levothyroxine treatment does not increase the chance of a live birth in women with a history of RPL and thyroid autoimmunity (normal TSH and TPO Ab+).	UPDATED (2022)			
14	52	There is insufficient evidence to recommend the use of progesterone to improve live birth rate in women with RPL and luteal phase insufficiency.	Conditional	⊕⊕⊕○	The GDG recommends against progesterone in women with RPL, consistent with the recommendation in women with unexplained RPL, based on insufficient evidence of benefit.				
14	53	There is insufficient evidence to recommend the use of human chorionic gonadotrophin (hCG) to improve live birth rate in women with RPL and luteal phase insufficiency.	Conditional	⊕⊕○○	Studies are considered too limited to recommend the use of hCG in women with RPL and luteal phase insufficiency.				
14	54	There is insufficient evidence to recommend metformin supplementation in pregnancy to prevent PL in women with RPL and glucose metabolism defects.	Conditional	⊕○○○	Indirect evidence could support the use of metformin treatment to increase the live birth rate in women with PCOS, but in the absence of any substantial studies in women with RPL and PCOS, the GDG decided metformin could not be recommended.				
14	55	Bromocriptine treatment can be considered in women with RPL and hyperprolactinemia to increase live birth rate.	Conditional	⊕○○○	In women with hyperprolactinemia, bromocriptine treatment normalizes serum prolactin levels and could improve the chance of a live birth.				
14	56	Preconception counselling in women with RPL could include the general advice to consider prophylactic vitamin D supplementation	GPP		Based on the significant prevalence of vitamin D deficiency in women with RPL and the possibly associated obstetrical / foetal complications, prescribing vitamin D supplementation can be considered, even though evidence for the effectiveness is absent. Vitamin D supplementation can be considered safe.				
15	57	There is low quality evidence in favor of hysteroscopic septum resection. Reduction in risk of miscarriage, no effect on live birth.	Conditional	⊕○○○		UPDATED (2022)			
15	58	Metroplasty is not recommended for bicorporeal uterus with normal cervix (former AFS bicornuate uterus) and RPL.	Strong	⊕○○○					
15	59	Uterine reconstruction is not recommended for hemi-uterus (former AFS unicornuate uterus) and RPL.	Strong	⊕○○○	There are currently no high quality studies to support surgery for improving the live birth rate or decreasing the miscarriage rate.				
15	60	There is insufficient evidence in favor of metroplasty in women with bicorporeal uterus and double cervix (former AFS didelphic uterus) and RPL.	Conditional	⊕○○○					

15	61	There is insufficient evidence supporting hysteroscopic removal of submucosal fibroids or endometrial polyps in women with RPL.	Conditional	⊕○○○	There is no evidence that fibroids or polyps are associated with RPL, nor that surgery increases the chance of a live birth in women with RPL.
15	62	Surgical removal of intramural fibroids is not recommended in women with RPL. There is insufficient evidence to recommend removing fibroids that distort the uterine cavity.	Conditional	⊕○○○	
15	63	There is insufficient evidence of benefit for surgical removal of intrauterine adhesions for pregnancy outcome. After hysteroscopic removal of intrauterine adhesions in women with RPL, precautions have to be taken to prevent recurrence of adhesions.	Conditional	⊕○○○	Small observational studies have shown that surgery may decrease miscarriage rates in women with RPL. However, uterine surgery is a known cause for adhesions, and treatment should attempt to prevent recurrence of adhesions.
15	64	Women with a history of second-trimester pregnancy losses and suspected cervical weakness should be offered serial cervical sonographic surveillance.	Strong	⊕⊕○○	
15	65	In women with a singleton pregnancy and a history of recurrent second-trimester pregnancy loss attributable to cervical weakness, a cerclage could be considered. There is no evidence that this treatment increases perinatal survival.	Conditional	⊕⊕○○	Based on inconclusive evidence on the benefit, and the possible harms associated with any surgery, the GDG is cautious in the recommendations on cerclage for RPL, but strong in recommending ultrasound surveillance.
16	66	Couples with RPL should be informed that smoking, alcohol consumption, obesity and excessive exercise could have a negative impact on their chances of a live birth, and therefore cessation of smoking, a normal body weight, limited alcohol consumption and a normal exercise pattern is recommended.	GPP		
16	67	Sperm selection by PICSi may be considered for older women (>35 y) who have experienced RPL	GPP		Sperm selection by PICSi leads to a significant decrease in pregnancy loss rates in women >35y (not RPL) as shown by a RCT.
16	68	Antioxidants for men have not been shown to improve the chance of a live birth.	Conditional	⊕○○○	In a Cochrane review, antioxidants did improve live birth rate in subfertile men, but it did not significantly decrease the risk of a pregnancy loss
17	69	Lymphocyte immunization therapy should not be used as treatment for unexplained RPL as it has no significant effect and there may be serious adverse effects.	Strong	⊕⊕○○	LIT should not be used in clinical practice since its scientific foundation is weak, its effect to prevent miscarriage is not established and there are many proven and potential adverse effects.

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17	70	Intravenous immunoglobulin (IVIg) is not recommended as a treatment of RPL.	Strong	⊕⊕○○	Based on the available evidence, IVIG cannot be recommended for clinical use in women with RPL. Further randomized controlled trials are needed.	
17	71	Glucocorticoids are not recommended as a treatment of unexplained RPL or RPL with selected immunological biomarkers.	Strong	⊕⊕○○	The evidence points toward some beneficial effect of prednisolone in selected women with RPL. However, based on adverse events associated with the use of prednisone, the GDG decided to recommend against treatment awaiting further studies.	
17	72	Heparin or low dose aspirin are not recommended, as there is evidence that they do not improve live birth rate in women with unexplained RPL.	Strong	⊕⊕⊕○	Based on a meta-analysis and results of two subsequent large randomized controlled trials there is no evidence that heparin alone, aspirin alone, or heparin in combination with low-dose aspirin improves the live birth rate in unexplained RPL.	
17	73	Low dose folic acid is routinely started preconceptionally to prevent neural tube defects, but it has not been shown to prevent pregnancy loss in women with unexplained RPL.	Strong	⊕⊕○○	Based on the absence of evidence for a benefit, and possible harms, high-dose folic acid supplementation should not be used for women with RPL without hyperhomocysteinemia or underlying conditions (diabetes, epilepsy) associated with increased risk of neural tube defects.	
17	74	Vaginal progesterone may improve live birth rate in women with 3 or more pregnancy losses and vaginal blood loss in a subsequent pregnancy	Strong	⊕⊕⊕○	Vaginal progesterone during early pregnancy may have beneficial effect in women with unexplained RPL with vaginal bleeding. There is some evidence that oral dydrogesterone initiated when foetal heart action can be confirmed may be effective, but more trials are needed.	UPDATED (2022)
17	75	There is insufficient evidence to recommend intralipid therapy for improving live birth rate in women with unexplained RPL.	Strong	⊕○○○	There is no evidence to support the use of Intralipid therapy in the treatment of RPL and the treatment is associated with potential adverse effects.	
17	76	There is no evidence to recommended G-CSF in women with unexplained RPL.	Strong	⊕⊕⊕○	The results from a recent trial (Eapen et al. 2019) overrule those from Scarpellini and Sbarca trial (2009) due to the much larger size of the former study and its high quality	UPDATED (2022)
17	77	There is no evidence to recommended endometrial scratching in women with unexplained RPL	GPP		There is no evidence that endometrial scratching improves subsequent pregnancy outcome in women with RPL.	
18	78	If women with RPL ask about using multivitamin supplements, they should be advised on multivitamin supplements that are safe in pregnancy.	GPP		Based on frequent questions from women with RPL, it was decided to add a recommendation on vitamin supplements. As there is no conclusive evidence, they are not recommended as treatment. However, based on the possible harms associated with some vitamin supplements (vitamin A, E), the GDG recommends advice on safe options.	

## Part A: Recurrent Pregnancy Loss

### DEFINITION OF RPL

A pregnancy loss (miscarriage) is defined as the spontaneous demise of a pregnancy before the foetus reaches viability. The term therefore includes all pregnancy losses (PLs) from the time of conception until 24 weeks of gestation. It should be noted that advances in neonatal care have resulted in a small number of babies surviving birth before 24 weeks of gestation ([Green-top Guideline 2011](#)) and different definitions apply in different countries.

The distinction between primary and secondary recurrent pregnancy loss can be made. Primary RPL is described as RPL without a previous ongoing pregnancy (viable pregnancy) beyond 24 weeks' gestation, while secondary RPL is defined as an episode of RPL after one or more previous pregnancies progressing beyond 24 weeks' gestation.

By definition, "recurrent" pregnancy loss is defined as the loss of two or more pregnancies. However, to which extent this definition needs to be extended or constricted is less clear, as is shown by different definitions used in different guidelines and different countries.

For this guideline, we tried to collect all evidence / opinions with regard to the definition of RPL:

- There is no pathophysiological proof that help us in the context of the discussion of consecutive versus non-consecutive losses.
- There is some evidence from one observational study that whether the pregnancy losses are consecutive or not, or two versus three losses is not associated with the risk of APS ([van den Boogaard et al. 2013](#))
- There is some evidence from one retrospective study that distribution of associated factors in couples with two versus three or more pregnancy losses is equal ([Youssef et al. 2020](#))
- There is some evidence from one observational study that there is no difference in the probability of carrier status (of a structural chromosomal abnormality) between couples that had two or three consecutive pregnancy losses, compared to two or three non-consecutive losses ([van den Boogaard et al. 2010](#)).
- There is some evidence from one observational study that whether the pregnancy losses are consecutive or not impacts on prognosis in unexplained RPL ([Egerup et al. 2016](#)).
- Only a minority of the RPL couples (estimated to be less than 10%) experience 2 or more non-consecutive pregnancy losses ([van den Boogaard et al. 2010](#); [van den Boogaard et al. 2013](#))

With regard to the definition of RPL, and taken into account the evidence above, the GDG would like to stress the importance of the issue and the need for further scientific research (including epidemiological studies on the effect of various RPL definitions on diagnosis, prognosis and treatment).

The GDG believes that defining RPL as two or more pregnancy losses will facilitate research, shared decision-making and psychological support to couples. In addition, testing for APS, a treatable sub-diagnosis of RPL, can be performed after two losses.

The GDG acknowledges that the definition of RPL may in the future be further restricted but currently the data are lacking to do so.

There was some discrepancy in opinions among the guideline group members regarding the definition. Some guideline group members would like to stress that they disagree with the suggested definition and will keep a definition of three or more consecutive losses in their clinical practice

In conclusion and after significant debate in the GDG, the definition of RPL is set as follows:

**A diagnosis of Recurrent Pregnancy Loss (RPL) could be considered after the loss of two or more pregnancies.**

For this guideline, we have based our recommendations on offering investigations and/or treatments on the best available evidence. Where available from the studies, we have added details on whether investigations should be performed after 2 pregnancy losses, or whether they can be postponed. However, for most investigations, the decision on when to start investigations will have to be decided by the doctor and the couple, as the result of shared decision-making, and be compliant with available resources.

A pregnancy in the definition is confirmed at least by either serum or urine b-hCG, i.e. including non-visualized pregnancy losses (biochemical pregnancy losses and/or resolved and treated pregnancies of unknown location). In the non-visualized pregnancy loss group, pregnancy losses after gestational week 6 are included, where an ultrasound examination was only done after complete expulsion of the embryo and trophoblast or no ultrasound was done after heavy bleeding: it includes pregnancies that would have been classified as clinical miscarriages in case an earlier ultrasound scan had been done.

If identified as such, ectopic and molar pregnancies should not be included in the definition. Implantation failure is also excluded from the definition. Pregnancy losses both after spontaneous conception and after ART treatments should be included in the definition.

Recurrent “Early” Pregnancy Loss (REPL) is the loss of two or more pregnancies before 10 weeks of gestational age ([Kolte et al. 2015a](#)).

## TERMINOLOGY

The terminology used for pregnancy loss needs to be clear, consistent and patient-sensitive. For the purposes of this guideline, the GDG recommends the use of ‘pregnancy loss’ as a general term, and ‘early embryo loss’, ‘first trimester pregnancy loss’ and ‘second trimester pregnancy loss’ when gestation-specific reference is needed.

We recommend the use of ‘*recurrent pregnancy loss*’ to describe repeated pregnancy demise and to reserve ‘*recurrent miscarriage*’ to describe cases where all pregnancy losses have been confirmed as intrauterine miscarriages.

The terms spontaneous abortion, chemical pregnancy and blighted ovum are ambiguous and should be avoided ([Kolte et al. 2015a](#)).

The use of consistent terminology and careful description of couples’ reproductive history is of the utmost importance in RPL research as it is a prerequisite for comparison of studies ([Kolte et al. 2015a](#)).

The GDG concludes to use the term Recurrent Pregnancy Loss.

DRAFT for REVIEW

## PREVALENCE OF RPL

Pregnancy loss is a common complication in early pregnancy. The data of the Scottish registry show a prevalence of miscarriage of 5% (<http://www.isdscotland.org/Health-Topics/Maternity-and-Births/Publications/data-tables.asp>). These data are based on clinical losses, after the missed menstrual period, or a positive pregnancy test, excluding biochemical pregnancy losses. Other studies have shown a higher prevalence of pregnancy loss, ranging from 10 to 15%. A population based register study showed that 13.5% of the pregnancies intended to be carried to term ended with foetal loss ([Nybo Andersen et al. 2000](#))

Recurrent pregnancy loss is less prevalent. It has been reported that RPL affects approximately 1% to 2% of women, when defined as three consecutive pregnancy losses prior to 20 weeks from the last menstrual period ([Ford, Schust 2009](#)). Larsen reported a prevalence of 0.8% to 1.4% if only clinical miscarriages (confirmed by ultrasound and/ or histology) are included; adding biochemical losses increases the prevalence to 2% to 3% ([Larsen et al. 2013](#)). However, these and other similar reviews often do not quote original sources of their data.

The exact prevalence of RPL is very difficult to estimate, as both the numbers in the numerator (experienced RPL) and the denominator (women at risk of RPL, all women at fertile age, or all women who try to get pregnant), are difficult to obtain.

In one study amongst female doctors who retrospectively reported about their previous pregnancies, 0.8% had experienced RPL among those who had attempted pregnancy  $\geq 3$  times ([Alberman 1988](#)). In another study, 1.4% of women with  $\geq 2$  previous pregnancies had experienced RPL ([Stray-Pedersen, Lorentzen-Styr 1979](#)) and in a Danish questionnaire-based study, 0.8% had experienced RPL among women with  $\geq 2$  pregnancies ([Fertility and employment](#)). These studies, which all include a well-defined population in the denominator, thus find that the RPL prevalence is between 0.8% and 1.4% among women with  $\geq 2$  pregnancies. In a Japanese questionnaire-based study among an unselected group of women aged 35-79 years, 0.88% reported a history of  $\geq 3$  consecutive miscarriages ([Sugiura-Ogasawara et al. 2013b](#)). Except for the latter, these studies are old (or include women being pregnant many years ago) and from a time where the methods for detection of very early pregnancy loss were uncertain. The RPL prevalence would probably be larger if these studies were repeated today.

## PSYCHOLOGICAL IMPACT OF RPL

Recurrent pregnancy loss has a significant emotional impact on women and their partners. Studies in general focused primarily on women with RPL ([Rowell et al. 2001](#); [Craig et al. 2002](#); [Andalib et al. 2006](#); [Kagami et al. 2012](#); [Mevorach-Zussman et al. 2012](#); [Sugiura-Ogasawara et al. 2013a](#); [Toffol et al. 2013](#); [Kolte et al. 2015b](#)). With regards to the emotional impact on the partner, there are studies on (usually male) partners' reactions to sporadic pregnancy loss ([www.miscarriageassociation.org.uk](http://www.miscarriageassociation.org.uk) ; [Boynton 2015](#)). A recent study in RPL specifically suggests the impact for (usually male) partners maybe less significant ([Hedegaard et al. 2021](#)), however the preferences in the need of supportive care may differ for men compared to women ([du Fossé et al. 2021](#))

For most women and their partners, pregnancy loss represents the loss of a baby and the hopes and plans invested in that child. Feelings of loss and grief, common after a single pregnancy loss, can intensify with repeated losses, as can a sense of personal failure ([Stirtzinger, Robinson 1989](#); [Brier 2008](#); [Bardos et al. 2015](#)). Some losses may weigh more heavily than others do, irrespective of gestation or

pregnancy order. However, it should be noted that even a single pregnancy loss can have a significant psychological impact on women and their partners (Farren et al. 2020, 2021)

Support and understanding, along with acknowledgement that these reactions are normal and understandable, can help most couples, but some will require referral for professional counselling or support.

The delivery and organization of care can also affect the individual's experience. In addition to medical expertise, couples want the medical team to know their obstetric history and to provide compassionate care (show understanding, take them seriously, show empathy), and clear information (on RPL and progress) (Musters et al. 2013) and recognition that RPL is a significant life event (based on survey results of the Miscarriage Association; www.miscarriageassociation.org.uk).

### Pregnancy after RPL

Anxiety about pregnancy after RPL is both normal and understandable. Before trying to conceive, most couples want an explanation for their losses and treatment that will prevent a recurrence. Without one or both of these, many couples are vulnerable to offers of tests and treatments that are not evidence-based. The same may be true for couples whose treatment plan has not resulted in a live baby. Some couples will decide to stop trying.

With or without specific treatment, couples value a plan for the pregnancy after RPL, with the care of a dedicated and supportive individual physician or team (Musters et al. 2013). There is only limited and weak evidence that this approach in itself improves pregnancy outcome (Stray-Pedersen, Stray-Pedersen 1984; Clifford et al. 1997; Liddell et al. 1997) but even if not, it is hard to argue against this approach.

Pregnancy loss is a significant negative life event and the repetitive nature of RPL may intensify the grief experienced. Studies have mostly focused on women, and there is a need for studies on the emotional impact of RPL on men.

Clinicians and clinics should take the psychosocial needs of couples faced with RPL into account when offering and organizing care for these couples.

More information on caring for the RPL couple is presented in PART B.

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## PART B: Organization of care

### KEY QUESTION: HOW SHOULD CARE FOR THE RPL PATIENT BE ORGANIZED?

#### ACCESS TO CARE

A dedicated Recurrent Pregnancy Loss (RPL) clinic is an outpatient clinic that offers specialist investigations, support and if possible, treatment of couples with RPL. These consultant-led clinics provide a dedicated and focused service to couples who have experienced RPL. It is a non-acute service, and the couples should preferably be seen and tested prior to a new pregnancy. Couples with two or more pregnancy losses could be referred to a RPL clinic.

Information provision is one of the important aims of a RPL clinic. Investigations do not necessarily lead to treatment options, and this should be clear from the beginning. New unproven interventions should be tested through clinical evaluation studies ([Van den Berg et al. 2014](#)).

#### THE RPL CLINIC

The following elements are required in a RPL clinic:

##### ○ *Staffing*

Experienced staff members (gynecologists/ fertility doctors/specialized nurses) appropriately trained in ultrasound, and with appropriate listening skills are part of the RPL team. Ideally there should also be trained and qualified staff (e.g., psychologists/ social workers/counsellors) either onsite or accessible, who offer support tailored to the psychological needs of the couples.

##### ○ *First Visit*

The first visit should allow time for the clinician to review the patient's history, to answer questions and to propose a plan for investigations and, perhaps, treatment. In advance of the appointment, providing written information for couples about what to expect can help to reduce anxiety and manage expectations.

##### ○ *Equipment/ Location*

The clinic should have excellent ultrasound provision and offer 3D ultrasound or additional saline or gel infusion sonography (*see also chapter 8*). The team should have close contact with the appropriate laboratories for further testing. The outpatient clinic is preferably not located next to an antenatal clinic, maternity unit, or obstetrics department ward.

##### ○ *Provision of information*

The first visit is the opportunity to provide general information about RPL incidence, causes and investigations and to link it to the patient's history. Staff should be aware that many women with RPL will already have information from a variety of sources, and some explanation and re-education may be needed. Patient information leaflets from professional and/or reputable societies or the clinic should be offered. (*See also the ESHRE patient information leaflet for couples with RPL*) In addition, clinics can organize information sessions for RPL couples.

○ ***Appropriate evaluation (testing)***

There should be individual evaluation of the investigations appropriate to each woman or couple, based on age, fertility/sub-fertility, pregnancy history, family history, previous investigations and/or treatments. This should include discussion of wishes or views that the patient already has regarding the investigations she wants or does not want.

It is crucial to explain before testing that investigations may not identify a likely cause or causes for previous losses, and what this means for the future. It is equally important to explain that there are some causes for which there is little or no or known treatment or where treatment outcomes are uncertain. (*See below 'research'*)

Couples will want to know the timeframe for investigations and discussion of results. They may also have questions on whether they should delay conception in the meantime.

○ ***Care tailored to psychological needs of the couples***

Providing the time and opportunity to discuss pregnancy history, provide information and discuss options can be supportive in itself, especially if it is done well, with good listening, sensitive terminology and consideration of the patient's experience and feelings.

○ ***Treatment plan***

Most couples want investigations to show an identifiable problem that has a recognized treatment protocol. If results show no obvious cause, couples may be very distressed, even if statistics suggest that the prognosis is good. They may need a plan for additional support in a subsequent pregnancy, such as regular visits and scans. They may also be willing to consider taking part in a clinical trial.

When diagnosed with a problem for which treatment is uncertain, couples will need more information about possible benefits and disadvantages.

○ ***Research***

Some couples may be willing to consider taking part in research into RPL treatments/trials or in qualitative research. This can feel like a positive step forward, both for themselves and for others. This may be suggested during a routine clinic visit, but any discussions should take place at a separate visit (e.g. with a research nurse).

## **TREATMENT PLAN, SUPPORTIVE CARE AND PSYCHOLOGICAL CARE**

Couples coming to the RPL clinic primarily seek expertise, investigations and a treatment plan that will reduce the risk of further losses. A plan for the subsequent pregnancy involves potential treatments, lifestyle advice where appropriate and the patient's individual choices regarding seeing the same doctor each time, having ultrasound scans, and the frequency of visits. It is important to show understanding, good listening skills, awareness of the patient's obstetric history and respect towards her previous pregnancy losses ([Musters et al. 2013](#)).

Couples' psychological states and needs will vary, so there is no single model of care that will suit everyone, but the following elements will be appreciated:

- Recognition of the patient as an **individual**: this woman/couple, this history, this pregnancy, this time
- **Time** for questions, information, repetition and discussion, especially when the patient is distressed or anxious.
- **Good listening**: to the facts and the feelings
- **Respect**: for the patient, her partner (male or female), and the pregnancies (or babies) she has lost; and for her wishes and choices (even if they are not possible/advisable)
- **Clear and sensitive language**: explaining terminology, avoiding insensitive terms (recurrent abortion, products of conception, blighted ovum, incompetent cervix, pregnancy failure), and mirroring the patient's preferred terms (baby, foetus, pregnancy etc.)
- **Honesty**: about processes, likely outcomes, prognoses; avoid false reassurance
- **Shared planning**: a partnership approach, enabling some element of control, can boost patient confidence
- **Supportive care** in the next pregnancy/ies: access to the team (actual, by phone or online), additional/early scans if wanted
- **Kindness**: concern, empathy, compassion as appropriate for that patient

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## PART C: Risk factors and health behavior

For some lifestyle behaviors and environmental exposures, an association with the risk of pregnancy complications and/or neonatal malformations is suggested. Some of these factors have been proposed as a risk factor for pregnancy loss, and therefore in theory, modification of these behaviors or reduction of the exposures could reduce the risk of pregnancy loss. This section summarizes the evidence on risk factors for recurrent pregnancy loss, and on health behavior modifications that could reduce the risk of pregnancy loss in couples with recurrent pregnancy loss (RPL).

### 1. Risk factors for recurrent pregnancy loss

**KEY QUESTION: WHAT ARE THE KNOWN RISK FACTORS OF RECURRENT PREGNANCY LOSS?**

#### 1.1 Age

Advanced female age is a well-established risk factor for female subfertility, foetal anomalies, stillbirth, and obstetric complications (Nybo Andersen et al. 2000; Sauer 2015). Based on a computer simulation fertility model that included data on the chance of age-dependent pregnancy loss after conception, couples should start trying to conceive when the female partner is 31 years of age or younger to have a chance of at least 90% to realize a family with two children. If IVF is not an option, couples should start no later than age 27 years. To achieve a one-child family, couples should start trying before age 32, or age 35 if IVF is an option (Habbema et al. 2015).

#### Evidence

##### Female age

An association between advanced female age and RPL has been consistently shown in several studies. Based on 2 cohorts (n=119+165), Cauchi and colleagues concluded that female age less than 30 years correlated significantly with success rate in subsequent pregnancy in women with RPL and that female age above 30 years is a risk factor for pregnancy loss in women with RPL (Cauchi et al. 1991).

A descriptive cohort study assessing the chance of live birth in 987 RPL couples during a 5-year follow-up period found a significant decrease in the chance of live birth with increasing female age (Lund et al. 2012).

In a cohort study investigating factors associated with PL in 696 women with RPL, and a female age  $\geq$  35 years was found to double the risk of another PL, compared to women  $<$  35 years (OR 1.99; 95% CI 1.45-2.73) (Lo et al. 2012)

In an epidemiological study in Scotland (n=151,021) the risk of miscarriage increased dramatically after the age of 30, irrespective of previous obstetric history (Bhattacharya et al. 2010).

Finally, a retrospective cohort study concluded that female age (older than 35 years) was the only statistically significant predictor of the chromosomal anomaly risk in sporadic and recurrent PL (Grande et al. 2012). Cytogenetic abnormalities are further addressed in Chapter 4 (Screening for genetic factors).

#### Male age

Most studies evaluating male age have reported a significant association between increasing male age and the incidence of miscarriage (Sharma et al. 2015). To our knowledge, there are no studies on the impact of male age on RPL.

#### Recommendations

Women should be sensitively informed that the risk of pregnancy loss is lowest in women aged 20 to 35 years.

Strong

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Women should be sensitively informed that the risk of pregnancy loss rapidly increases after the age of 40.

Strong

⊕⊕○○

#### Justification

Female age is an important risk factor for RPL; women older than 40 years have a higher risk of RPL, and have worse prognosis compared to younger women. In couples diagnosed with RPL, the information that age is a risk factor is still important as it may affect the diagnostic procedures, and the decision-making of treatment or expectant management.

Although the evidence is of low quality (based on observational studies), the GDG decided to strongly recommend information provision on the topic, but it has to be explained sensitively.

### 1.2 STRESS

#### Evidence

Studies have suggested that maternal stress during pregnancy is possibly associated with an increased risk of several adverse pregnancy and birth outcomes, but there are currently no high-quality studies available. The impact of stress on the risk of miscarriage or recurrent pregnancy loss is unclear.

We found two studies assessing stress in women with RPL. From a case–control study it was concluded that stress is a risk factor for RPL based on the finding of a significantly higher total score on the perceived stress scale (PSS) among 45 women with unexplained RPL compared with 40 controls (Li et al. 2012). In another study, stress and depression were assessed in 301 RPL patients and 1813 women without RPL trying to conceive. A high stress level, defined as  $\geq 19$  on the PSS scale, was more prevalent in women with RPL (41.2%) as compared to controls (23.2%). In addition, the odds of moderate to severe depression was more than five times higher in women with RPL (Kolte et al. 2015).

An association between RPL and stress can be assumed based on these studies, but it is unclear whether stress results from RPL, or whether stress is a causing factor for the next pregnancy loss.

One small study (22 pregnancies) on pregnancy loss and stress during pregnancy showed an association between maternal stress and pregnancy loss, possibly mediated through higher cortisol levels (Nepomnaschy et al. 2006). Other studies however found no evidence for stress as a factor leading to pregnancy loss (Nelson et al. 2003; Plana-Ripoll et al. 2016).

## Recommendation

Stress is associated with RPL, but couples should be informed that there is no evidence that stress is a direct cause of pregnancy loss.

Strong

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## Justification

Whether stress increases the chance of another pregnancy loss in the next pregnancy is a major concern of patients with RPL.

The studies to date on stress in couples with pregnancy loss have significant limitations with regard to quality, different scales are used, and stress and distress are mixed.

Overall, the studies indicate that there is an association between stress and pregnancy loss, but they provide no information on whether the stress is a result of RPL, or whether stress could be a causal factor in RPL. Ideally, prospective studies should be performed assessing the impact of high stress on the outcome of a subsequent pregnancy.

## 1.3 OCCUPATIONAL OR ENVIRONMENTAL EXPOSURE

### Evidence

We found only two small studies evaluating occupational or environmental exposure as risk factor for RPL. In the first study serum zinc, copper, and vitamin E levels were significantly lower in 35 women with RPL and serum selenium, lead, and cadmium were significantly higher compared with 34 controls, which could indicate that heavy metals and a lack of micronutrients could cause pregnancy loss in women with RPL (Ajayi et al. 2012). In the second study, higher levels of organochlorine pesticides were detected in blood of 30 women with RPL compared to 30 controls, which could indicate an association between organochlorine pesticides and RPL (Pathak et al. 2010).

A descriptive review, summarizing studies on occupational exposures associated with pregnancy loss, reported that the evidence was inconclusive for video display terminals and electromagnetic field (Gold, Tomich 1994). An association was consistently reported by studies evaluating exposure to organic solvents and pregnancy loss. The review did not include RPL as an outcome and most studies described in the review were small and poor quality. Another study reported an increased risk of pregnancy loss in personnel exposed to anaesthetic gases in operating and recovery rooms (n=8032) as compared to non-exposed hospital staff (n=2525) (OR 1.98; 95% CI 1.53-2.56). The authors recommend minimizing exposure to waste anaesthetic gases (Guirguis et al. 1990).

### Conclusion

Based on only a few small studies, exposure to occupational and environmental factors (heavy metals, pesticide, lack of micronutrients) seems to be associated with an increased risk of pregnancy loss in

women with RPL. Although exposure to possible hazardous substances should be avoided during pregnancy (for all pregnant women), there are insufficient data to recommend protection against a certain occupational or environmental factor in women with RPL.

#### 1.4 CHRONIC ENDOMETRITIS

##### Evidence

Chronic endometritis is characterized by a plasma cell infiltrate in the endometrium associated with a range of pathogenic organisms. There have been a series of papers suggesting a 7-58% prevalence of chronic endometritis in women with RPL ([Cicinelli et al. 2014](#); [McQueen et al. 2014](#); [McQueen et al. 2015](#)). The prevalence is dependent on the method of detection with high rates reported when hysteroscopy and/or immunohistochemistry with antibodies to CD138 are used ([Kitaya 2011](#); [Russell et al. 2013](#); [Cicinelli et al. 2014](#); [McQueen et al. 2014](#); [McQueen et al. 2015](#); [Bouet et al. 2016](#)). However, the only study comparing the prevalence of chronic endometritis to fertile controls found no statistical difference in prevalence rates ([Liu et al. 2018](#)). In contrast, increasing numbers of endometrial CD138+ cells have been associated with an increasing risk of future pregnancy losses ([Rimmer et al. 2021](#))

Chronic endometritis has been associated with endometrial and vaginal dysbiosis using sequencing of 16S ribosomal RNA ([Lozano et al. 2021](#)). In turn, dysbiosis of vaginal microbiome has been associated with recurrent pregnancy loss. This dysbiosis is characterised by increased diversity of bacteria species and a lack of lactobacilli ([Zhang et al. 2019](#); [Al-Memar et al. 2020](#); [Fan et al. 2020](#)). To date, there are no studies on the predictive value of vaginal or endometrial dysbiosis in recurrent pregnancy loss.

Antibiotics were found to reduce the endometritis with an apparent improvement in live birth rate ([Cicinelli et al. 2014](#); [McQueen et al. 2014](#)). However, this concept has not been tested in randomized controlled trials.

##### Conclusion

Further research is needed including prospective observational studies and randomized controlled trials before screening women for endometritis can be recommended.

#### 1.5 ENDOMETRIAL DECIDUALIZATION AND SENESCENCE

A growing number of endometrial studies have focused on the role of decidualization in recurrent pregnancy loss. Decidualization denotes differentiation of resident stromal cells into specialist decidual cells, which transform the endometrial mucosa into a robust, tolerogenic matrix to accommodate invading trophoblast ([Gellersen, Brosens 2014](#)). Decidualization not only leads to the emergence of progesterone-dependent, anti-inflammatory decidual cells but also progesterone-independent senescent decidual cells ([Brighton et al. 2017](#); [Lucas et al. 2020](#)). Senescent decidual cells, which secrete an abundance of inflammatory mediators and extracellular matrix proteases, are implicated in endometrial remodelling for embryo implantation. They are subsequently cleared by activated uterine natural killer (uNK) cells, a process believed essential to prevent chronic sterile inflammation and breakdown of the emerging maternal-foetal interface in pregnancy ([Brighton et al. 2017](#); [Lucas et al. 2020](#); [Kong et al. 2021](#)). In parallel, bone marrow-derived decidual progenitor cells are recruited, enabling rapid expansion of the decidua ([Tal et al. 2019](#); [Diniz-da-Costa et al. 2021](#)). Recurrent pregnancy loss is associated with lack of bone marrow-derived decidual progenitor cells and increased

frequency of menstrual cycles characterised by excessive senescent decidual cells (Lucas et al. 2016; Lucas et al. 2020; Diniz-da-Costa et al. 2021). The abundance of uNK cells varies markedly throughout the luteal phase and between cycles (Brighton et al. 2017), likely accounting for inconsistent findings in different studies. Nevertheless, there is evidence that lower uNK cell activity is associated with higher miscarriage rates (Hiby et al. 2008; Katano et al. 2013; Fukui et al. 2017; Lucas et al. 2020). Further, the frequency abnormal cycles appear to correlate with the recurrence risk of miscarriage (Lucas et al. 2016; Lucas et al. 2020; Diniz-da-Costa et al. 2021). At present, however, biomarkers of senescent decidual cells have not been validated for clinical use.

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## 2. Health behavior modifications

**KEY QUESTION: ARE HEALTH BEHAVIOR MODIFICATIONS RELEVANT FOR REDUCING THE RISK OF PREGNANCY LOSS IN WOMEN WITH A HISTORY OF RPL?**

### 2.1 SMOKING CESSATION

#### Evidence

Smoking is strongly associated with adverse obstetric and neonatal outcomes, including ectopic pregnancy, stillbirth, placenta praevia, preterm birth, low birth weight, and congenital anomalies. Studies have also reported associations between maternal smoking during pregnancy and problems during childhood, including sudden infant death syndrome, obesity, psychosocial problems and malignancies (Leung, Davies 2015). Smoking cessation is therefore recommended to all pregnant women.

The impact of smoking or smoking cessation on pregnancy loss in women with RPL is less clear. In a retrospective study, comparing lifestyle behavior in 326 women with RPL and 400 controls who had at least one live birth, environmental exposure to tobacco smoke (passive smoking) significantly increased the risk of RPL compared with tobacco-free controls. The risk increased with the daily duration of exposure (adjusted OR 2.30; 95% CI 1.50-3.52 for short exposure of <1h/day; adjusted OR 4.75; 95% CI 3.23-6.99 for long exposure of ≥1 h/day). Female smoking, consumption of alcohol or coffee intake were not associated with the risk of RPL (Zhang et al. 2010).

Other studies have evaluated the effect of maternal and paternal smoking on the risk of sporadic pregnancy loss. A small study investigating the association of several lifestyle factors with early pregnancy loss (EPL) in 128 pregnancies found no evidence for any risk factors including maternal and paternal smoking (Wilcox et al. 1990). A similar larger study looked at risk factors for EPL in 1196 IVF pregnancies of which 195 resulted in EPL. In their study, smoking was associated with a significant increased risk of EPL after adjusting for other factors (OR 2.00; 95% CI 1.27-3.15). Body mass index (BMI) and female age were not associated with EPL (Winter et al. 2002). In a prospective study, the impact of paternal smoking on the risk of pregnancy loss was evaluated in 526 couples. The adjusted odds ratio for total pregnancy loss (early pregnancy loss or spontaneous miscarriage) for women of heavy smokers (≥20 cigarettes/day) was 1.17 in the first conception cycle, 1.22 in the first 2 cycles, 1.39 in the first 3 cycles, and 1.45 in all conceptions. The impact of male smoking was more significant in heavy smokers compared to moderate smokers (<20 cigarettes/day) (Venners et al. 2004).

We found no studies on the effect of smoking cessation on the chance of a live birth in couples with RPL.

#### Recommendation

Couples with RPL should be informed that smoking could have a negative impact on their chances of a live birth, and therefore cessation of smoking is recommended.

GPP

## Justification

Smoking has not been conclusively shown to be a risk factor for RPL. However, based on an established association between smoking and poor obstetric outcomes, and between smoking and general health, cessation of smoking could be recommended in couples with RPL even in the absence of prospective studies on smoking cessation and chance of live birth.

## 2.2 STRIVING FOR A HEALTHY, NORMAL RANGE BODY MASS INDEX

### Evidence

#### Weight loss

Obesity has a significant impact on female reproductive health. Increased body mass index (BMI) is associated with subfertility, poorer outcomes following fertility treatment, and pregnancy loss ([Metwally et al. 2008](#); [Pandey et al. 2010](#)).

A normal BMI for a Caucasian population is considered 20-30 kg/m<sup>2</sup>. As such a BMI of 25-30 kg/m<sup>2</sup> is classified as overweight, although the adverse effects on reproduction and early pregnancy loss in overweight people are minimal ([Metwally et al. 2010](#)). Ethnicity interacts with the health risks posed by obesity so that a BMI of less than 27 kg/m<sup>2</sup> is recommended for people of Asian origin rather than 30 kg/m<sup>2</sup> ([Misra et al. 2009](#)).

Obesity (BMI >30 kg/m<sup>2</sup> according to WHO) has also been evaluated as a risk factor for RPL. A systematic review reported a higher prevalence of RPL in obese women as compared to women with a normal BMI (0.4% versus 0.1%; OR 3.51; 95% CI 1.03-12.01) based on 1644 obese women and 3288 controls ([Lashen et al. 2004](#); [Boots, Stephenson 2011](#)). In women with RPL (n=491), there was a higher miscarriage rate in the obese versus non-obese women (OR 1.71; 95% CI 1.05-2.8) ([Metwally et al. 2010](#)). The latter study also reported that an increased BMI was the second-most significant factor predicting early pregnancy loss (after advanced female age). The presence of PCOS or the number of previous losses did not predict a pregnancy loss in the next pregnancy ([Metwally et al. 2010](#)).

More recent studies on obesity and RPL also found an association. Boots and colleagues assessed the frequency of a euploid miscarriage in 372 women with RPL. There were 117 subsequent miscarriages and the frequency of a euploid miscarriage among obese women was 58% compared with 37% of non-obese women (relative risk RR 1.63; 95% CI 1.08-2.47) ([Boots et al. 2014](#)). In the retrospective study of Zhang, mentioned above, evaluating the impact of lifestyle factors on the risk of RPL, a BMI of 24.0 or greater was associated with an increased risk of RPL (adjusted OR 1.55; 95% CI 1.12-2.14) ([Zhang et al. 2010](#)). Lo and colleagues assessed the relationship between maternal BMI and the future outcomes of pregnancy in 696 couples with unexplained RPL. They found that BMI, female age, number of previous pregnancy losses, and ethnicity were significantly associated with pregnancy outcome. Logistic regression demonstrated that maternal obesity (BMI ≥ 30 kg/m<sup>2</sup>) significantly increased the risk of miscarriage in couples with unexplained RPL (OR 1.73; 95% CI 1.06- 2.83). There was no increased risk in women with overweight (OR 1.27; 95% CI 0.89-1.83) ([Lo et al. 2012](#)).

Gradual weight loss has been shown to improve fertility and the outcomes of fertility treatments. ([Pandey et al. 2010](#))). We found no studies on the effect of weight loss on recurrent pregnancy loss.

### Gaining weight

Being underweight (BMI <18.5) was found to be significantly associated with sporadic first trimester miscarriage in a large case-control study (OR 1.72; 95% CI 1.17-2.53) (Maconochie et al. 2007). The evidence of an association of maternal underweight and RPL is scarce and does not support an increased risk of RPL in women with low BMI. In a study assessing risk factors for PL in 696 women with RPL, Lo and colleagues found no increased risk of subsequent PL in women that are underweight as compared to women with normal BMI (OR 0.12; 95% CI 0.15-1.00) (Lo et al. 2012).

The impact of maternal BMI on the risk of early pregnancy loss was assessed in an oocyte donation model. The miscarriage rate was 18.2% in lean women (BMI <20kg/m<sup>2</sup>), which was not significantly different from women with normal BMI (13.3%) ((Bellver et al. 2003)

### Male weight

To our knowledge there are no studies evaluating the impact of male weight on RPL. Indirect evidence of the impact of male factors, including obesity, on pregnancy loss through sperm DNA damage is discussed in chapter 9.

### Recommendation

Couples with RPL should be informed that maternal obesity or being significantly underweight is associated with obstetric complications and could have a negative impact on their chances of a live birth and on their general health.

Strong

⊕⊕○○

Striving for a healthy normal range BMI is recommended.

GPP

### Justification

Maternal obesity is a strong risk factor in RPL, but there are no studies evaluating the impact of weight loss on subsequent PL. However, weight loss has a positive impact on fertility outcomes and reduced weight is associated with reduced complications during pregnancy and birth and reduced cardiovascular and diabetic morbidity and mortality. The GDG formulated a strong recommendation for information provision and for striving for a healthy normal BMI (20-25 kg/m<sup>2</sup> for Caucasians).

## 2.3 REDUCING CAFFEINE INTAKE

### Evidence

Observational studies have reported a dose-dependent association between caffeine intake and late pregnancy loss (Greenwood et al. 2010). At least one large case-control study did not find an effect of caffeine when adjusting for nausea. They compared 603 cases with 6116 controls, and found a strong trend of increased prevalence of pregnancy loss (late miscarriage and stillbirth) with increasing daily caffeine consumption, but they also found that the effect of caffeine was almost entirely due to the effect of nausea (women who felt sick did not tend to drink coffee, the main source of caffeine) (Maconochie et al. 2007).

From a retrospective case-control study, caffeine was suggested as a risk factor for RPL. The odds ratio for RPL in women with moderate (150-300 mg/day) or high (>300 mg/day) caffeine intake during the periconceptional period and early gestation as compared to mild (<150 mg/day) consumption were 3.045 (95% CI 1.23-7.28) and 16.016 (95% CI 6.54-39.61). There was a linear association between the amount of daily caffeine intake and the risk of multiple pregnancy losses. The effect of reducing caffeine intake on the pregnancy outcome was not evaluated ([Stefanidou et al. 2011](#)).

CYP1A2 is an enzyme primarily responsible for caffeine metabolism and was assessed as a susceptibility gene for the effect of caffeine intake on RPL. They reported a significantly increased risk of RPL only among women who had homozygous CYP1A2\*1F alleles with a dosage effect of daily caffeine intake. Caffeine intake had no effect on the RPL risk among women who had other CYP1A2 genotypes. ([Sata et al. 2005](#))

## Conclusion

Some studies have also suggested caffeine intake as a risk factor for RPL, but not all studies reported an association. An association has been described between caffeine intake and late pregnancy loss. Based on the evidence, it is unclear whether caffeine intake is a risk factor for RPL.

## 2.4 EXERCISE

### Evidence

To our knowledge there are no studies investigating the impact of exercise on the chances of a live birth in women with recurrent pregnancy loss.

Exercise during pregnancy is generally advocated, as it is believed to provide various benefits for the women's health. A review of 2008 assessing the effects of physical activity during pregnancy on several outcomes concluded that physical activity does appear to reduce the risk of preeclampsia and gestational diabetes. The results for miscarriage were less clear. The reviewers found one study showing a beneficial effect of leisure-time physical activity; however, four studies found no effect ([Schlussel et al. 2008](#)). Another, more recent review, also reported diverging results concerning the association between exercise during early pregnancy and miscarriage. Two case-control studies found that exercise was associated with a lower risk of miscarriage, one large cohort study reported a graded association between exercise and higher risk of miscarriage, and two studies (of which one was also included in the review of Schlussel) showed the same risk for miscarriage in exercising versus non-exercising pregnant women ([Hegaard et al. 2016](#)). With regards to occupational physical activity, three studies reported no effect, while two high quality studies pointed to high-intensity occupational activity as a risk factor for miscarriage ([Schlussel et al. 2008](#)).

## 2.5 AVOIDING ALCOHOL

### Evidence

Alcohol has a clear negative impact on pregnancy and neonatal outcomes, not the least of which are foetal alcohol spectrum disorders. Therefore, it is advisable that women avoid consumption of alcohol during the pregnancy.

With regard to pregnancy loss, the evidence is not consistent, but a large proportion of the studies have shown that alcohol consumption during pregnancy increases risk of pregnancy loss, with a threshold between two to four drinks<sup>1</sup> per week (Andersen et al. 2012; Avalos et al. 2014). A case-control study reported a dose-dependent association between alcohol consumption and miscarriage. An increasing risk of miscarriage was found in women who drink regularly (at least once a week) (OR 1.46; 95% CI 1.16-1.85) and those who drink more than 14 units of alcohol per week (OR 1.64; 95% CI 1.09-2.47) compared to controls who do not drink alcohol at all (Maconochie et al. 2007).

We did not find any studies on the impact of consuming alcohol on the chance of a live birth in couples with RPL.

Studies have explored the impact of paternal alcohol consumption on the outcome of ART pregnancies, including semen parameters and pregnancy loss. Paternal alcohol consumption of more than five drinks a week was shown to be associated with a reduction in sperm count and in reproductive potential in a cross-sectional study (Jensen et al. 2014). In a longitudinal cohort study of the impact of several fertility treatments on the chance of early pregnancy loss, Brandes and colleagues found an association of paternal alcohol consumption with early pregnancy loss after fertility treatment (Brandes et al. 2011).

### Recommendation

Couples with RPL should be informed that excessive alcohol consumption is a possible risk factor for pregnancy loss and proven risk factor for foetal problems (Foetal alcohol syndrome).

Strong ⊕⊕○○

Couples with RPL should be advised to limit alcohol consumption.

GPP

### Justification

Alcohol consumption is a weak risk factor for obstetric and neonatal complications, including pregnancy loss. We found no studies evaluating alcohol consumption in women with RPL. The GDG recommends clinicians to provide information on alcohol, and to advise women to limit consumption based on the absence of harms and similar to other pregnant women. Women suggesting that alcohol use has caused their previous pregnancy loss can be informed that there is no evidence for a causal association. From clinical experience, it was noted that women with RPL generally avoid alcohol consumption.

<sup>1</sup> Women were asked the “total number of alcohol consumptions” with one beer is equal to 12 ounces; one glass of wine or champagne is equal to 4 ounces, and one mixed drink is equal to 1 ounce of hard liquor.

## 2.6 OTHER LIFESTYLE CHANGES

Whether intercourse during pregnancy can cause an early pregnancy loss is a matter of debate as there are no studies on the topic for sporadic early pregnancy loss or RPL ([Moscrop 2012](#)). Women with threatened early pregnancy loss are often advised to refrain from intercourse at least until the bleeding/pain have stopped, but this advice is based on presumptions of the doctor, not clinical evidence. Furthermore, such advice may cause guilt in couples experiencing pregnancy loss. Until evidence is available, clinicians are recommended to inform women asking about intercourse during pregnancy and pregnancy loss, that there is no evidence on the topic.

Similarly, we found no evidence that using soft drugs (e.g., cannabis) could be a risk factor for pregnancy loss in women with RPL. However, avoiding soft drugs is in general recommended, and especially during pregnancy.

Exposure to high dose radiation during pregnancy can potentially induce deleterious effects to the embryo or foetus, including congenital malformations, mental retardation and foetal death ([Brent 2015](#)). The extent of the damage is dependent on the stage of development, and the absorbed dose of radiation. However, most radiological diagnostic procedures will use ionizing radiation at low doses, below the no-adverse-effect level. Evidence to date suggests that there is no increased risk of the offspring, nor is there increased risk of pregnancy loss in parents who have been exposed to diagnostic radiological procedures ([Brent 2015](#)).

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## PART D: Investigations in RPL

### 3. Medical and family history

#### Evidence

The first visit after referral for RPL should allow time for the clinician to review the patient's history, which includes medical, obstetric, and family history, but also information on lifestyle of both the male and female partner.

We have summarized the evidence for known and suspected lifestyle risk factors in RPL in part C of this document. Studies have suggested an impact of the following lifestyle factors on the risk of RPL: smoking, excessive alcohol consumption, excessive exercise and being overweight or underweight. Assessment of these lifestyle factors in both the male and female partner is recommended.

In addition to lifestyle factors, information should be collected on a previous diagnosis of medical conditions that may be associated with RPL, including thrombophilia, PCOS, and diabetes, or a family history of hereditary thrombophilia.

A large registry-based study showed that a complete pregnancy history (i.e. the number of previous pregnancy losses, live births and their sequence) is important in estimating the chance of live birth in the next pregnancy, and more informative than only the total number of preceding pregnancy losses and live births ([Kolte et al. 2021](#)). Similarly, it has been shown, in a large cohort study, that a longer time to pregnancy TTP in a prior pregnancy was associated with a higher risk of pregnancy loss, in particular among women with a history of subfertility ([Arge et al. 2022](#)). From the evidence and recommendations in this guideline, some diagnostic tests, although not recommended for all couples, can be relevant only in selected RPL couples, for instance:

- prolactin testing in women with clinical symptoms of hyperprolactinemia (oligo-amenorrhea)
- HLA class II determination in women with secondary RPL after the birth of a boy ([Nielsen et al. 2009](#))
- sperm DNA fragmentation assessment can be more relevant in males with unhealthy lifestyles (smoking, alcohol, excessive exercise, unhealthy body weight) (indirect evidence from infertile couples)

Other investigations could be less relevant in specific couples. For instance, it has been shown that parental karyotyping is less relevant in couples with female age above 39, less than 3 pregnancy losses and a negative family history, as in these couples the chance of being a carrier of a translocation is very low (below 2.2 %) ([Franssen et al. 2005](#)).

There were no studies linking family or medical history to genetic analysis of pregnancy tissue, testing for antiphospholipid syndrome (APS), thyroid screening, antinuclear antibodies (ANA) testing, or assessment of uterine anatomy.

Female age and number of previous losses are the only known factors consistently shown to impact prognosis. This has been described in detail in chapter 10 on prognosis.

950 Recommendation (updated in 2022)

Medical and family history could be used to tailor diagnostic investigations in RPL.

GPP

951

The GDG recommends to base prognosis on woman's exact pregnancy history and female age.

Strong

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952

### 953 Justification

954 The GDG concludes that a thorough reproductive history should be taken in couples presenting with  
955 RPL. Based on recent data, the second recommendation was adapted stating that the prognosis should  
956 be based on the woman's pregnancy history rather than the number of preceding pregnancy losses.

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969

## 4. Screening for genetic factors

**KEY QUESTION: WHAT IS THE VALUE OF SCREENING FOR GENETIC FACTORS IN THE DIAGNOSIS OF RECURRENT PREGNANCY LOSS?**

### 4.1 GENETIC ANALYSIS OF PREGNANCY TISSUE

There are two common types of abnormalities that occur in early pregnancy losses: developmental and genetic abnormalities. Most pregnancies that miscarry early are morphologically abnormal ([Philipp et al. 2003](#)). The use of embryoscopy, direct visualization of the embryo or early foetus in utero has shown that these abnormalities occur in 86-91% of miscarriages where an embryo is present. Some of these phenotypically abnormal embryos will also be genetically abnormal, as will some phenotypically normal embryos. This chapter will address the genetic analysis of both pregnancy tissue and parental blood.

#### Evidence

Genetic abnormalities of the conceptus are a recognized cause of sporadic and recurrent pregnancy loss (RPL). In a systematic review, the prevalence of chromosome abnormalities in a single sporadic miscarriage was 45% (95% CI 38-52; 13 studies; 7012 samples). The prevalence of chromosome abnormalities in a subsequent miscarriage after preceding RPL was comparable (prevalence 39%; 95% CI 29-50; 6 studies; 1359 samples) ([van den Berg et al. 2012](#)).

It is possible to ascertain whether an early pregnancy loss is due to a genetically abnormal embryo or foetus (aneuploidy) by analysing the pregnancy or foetal tissue ([Mathur et al. 2014](#)). Published studies have used a variety of genetic techniques (conventional karyotyping, fluorescence *in situ* hybridization [FISH], or array-based comparative genomic hybridization [array-CGH]). Analysis by conventional karyotyping is limited by the failure of tissue culture and the fact that it does not distinguish between maternal contamination and a normal (euploid) female foetus ([Robberecht et al. 2009](#)). FISH is limited as it only uses probes for certain chromosomes, and therefore does not necessarily detect the chromosomal cause of the miscarriage. Array CGH is a better technique, and currently preferred technique, looking at all chromosomes and avoiding the limitations associated with karyotype and FISH, but may identify clinically irrelevant findings ([Kudesia et al. 2014](#); [Mathur et al. 2014](#); [Smits et al. 2020](#)). New techniques such as next generation sequencing (NGS), SNP arrays, whole genome screening (WGS) and whole exome screening (WES) have not yet been extensively investigated in genetic analysis of pregnancy tissue but may be useful in the near future ([Shamseldin et al. 2013](#); [Wang et al. 2017](#); [Colley et al. 2019](#); [Quintero-Ronderos, Laissue 2020](#); [Rajcan-Separovic 2020](#)).

Several authors have suggested a strategy of karyotyping the pregnancy tissue of the second miscarriage and only proceeding to further maternal investigations (for thrombophilia, thyroid dysfunction, uterine malformations) for the cause of the recurrent pregnancy loss if the result is euploid ([Hogge et al. 2003](#); [Bernardi et al. 2012](#); [Foyouzi et al. 2012](#); [Petracchi et al. 2017](#); [Popescu et al. 2018](#)).

Determining the chromosomal status of pregnancy tissue from women with recurrent pregnancy loss may provide them with a cause or reason for the particular loss being investigated, but it does not necessarily rule out other underlying conditions. No clear effect of genetic testing of the pregnancy

tissue on prognosis (subsequent live birth) has been described so far and the role of genetic analysis of pregnancy tissue should be further elaborated within a prognostic model.

If women are offered genetic analysis of pregnancy tissue, they should be aware of the issues as mentioned.

#### Recommendation

Genetic analysis of pregnancy tissue is not routinely recommended but it could be performed for explanatory purposes.

Conditional ⊕⊕○○

For genetic analysis of the pregnancy tissue, array-CGH is recommended based on a reduced maternal contamination effect.

Strong ⊕⊕○○

#### Justification

	Association	Contributing factor	Prognosis	Treatment
Karyotyping of the pregnancy tissue	Yes	Yes	No	No

Aneuploidy is a recognized cause of pregnancy loss, and the frequency of aneuploid early pregnancy losses increases with female age. Aneuploidies occur in comparable frequencies in both women with sporadic and recurrent pregnancy loss. Genetic analysis of pregnancy tissue has the benefit of providing the patient with a reason for the pregnancy loss and may help to determine whether further investigations or treatments are required. As the impact of further clinical decision making and the exact influence on prognosis for an individual patient is unclear. The GDG decided to formulate a conditional recommendation on genetic testing of the pregnancy tissue.

The preferred method of genetic analysis is array-CGH, as this is not limited by tissue culture failure or false negative results due to maternal cell contamination. However, array-CGH has some limitations with regard to not being able to detect balanced rearrangements and low-level mosaicism (<10–15%) (Sahoo et al. 2017) and low sensitivity for minor copy number variants (Freeman et al. 2006). A more recent study suggests that array-CGH can also be used for cytogenetic analysis of spontaneously discharged pregnancy tissue, although high incidence of maternal contamination needs to be taken into account (Ozawa et al. 2016). New techniques such as next generation sequencing (NGS), whole genome screening (WGS) and whole exome screening (WES) may be useful in the near future (Shamseldin et al. 2013; Colley et al. 2019; Quintero-Ronderos, Laissue 2020; Rajcan-Separovic 2020).

## 4.2 PARENTAL GENETIC ANALYSIS

### Evidence

Abnormal parental karyotypes were found in around 1.9% of couples (n=20432) referred for genetic testing after recurrent pregnancy loss in a large retrospective cohort study ([Franssen et al. 2006](#); [Barber et al. 2010](#)). In another retrospective study of 795 couples with two or more pregnancy losses, chromosomal abnormalities were found in 3.5% of the couples. The subsequent miscarriage rate was higher and the live birth rate was lower in carrier couples, although the cumulative live birth rate was 64% ([Flynn et al. 2014](#)). Another cohort study reported a lower live birth rate in carrier couples (63.0%) compared to women with a normal karyotype (78.7%). This study did not mention the number of carrier couples deciding not to attempt to conceive again ([Sugiura-Ogasawara et al. 2008](#)).

The subsequent pregnancy loss has been shown to be dependent on the nature of the parental karyotype abnormality with more pregnancy losses in carriers of reciprocal translocations and inversions as compared to Robertsonian translocations or other types of abnormalities ([Sugiura-Ogasawara et al. 2004](#); [Franssen et al. 2006](#); [Stephenson, Sierra 2006](#)). For example, in one case-control study 85 of 157 (54%) with reciprocal translocations had one or more miscarriages compared with 18 of 37 (49%) with inversions, 13 of 38 (34%) with Robertsonian translocations, and four of 15 (27%) with other types of abnormality ([Franssen et al. 2006](#)).

Ongoing pregnancies with unbalanced translocations were detected in less than 1% in carrier couples seen for prenatal diagnosis in a large retrospective study ([Barber et al. 2010](#)), and in 2.9% of 34 pregnancies in carrier couples in a smaller study ([Sugiura-Ogasawara et al. 2004](#)). These numbers are in contrast with a case-control study showing that couples have a high-perceived risk of receiving an abnormal result and a suboptimal understanding of the tests carried out ([Vansenne et al. 2011](#)). Deduction from two large nationwide studies reveals a negligible chance, an estimated 0.02%, of a live born handicapped child with unbalanced chromosome abnormalities in the unselected RPL population ([Franssen et al. 2006](#); [Barber et al. 2010](#)).

Although parental karyotyping could provide relevant information for those couples whose karyotypical abnormality put them at high risk of a subsequent pregnancy loss, the benefit is limited in other couples. In a nested case-control study with 279 carrier couples and 428 controls, it was reported that the probability of carrier status is very low in couples with higher female age ( $\geq 39$  years), fewer than 3 pregnancy losses and no indication for an abnormal parental karyotype from the family history, and therefore testing may be of limited value in these couples ([Franssen et al. 2005](#)).

A proportion (15.1%/17.8%) of carrier couples opt not to try to conceive again following an abnormal parental karyotype result ([Franssen et al. 2006](#); [Flynn et al. 2014](#)). In non-carriers, the proportion was only 6% ([Franssen et al. 2006](#)). In carrier couples the main reasons to not try to conceive were the risk of having a child with congenital abnormalities and not wanting to have more miscarriages, in non-carrier couples the main reasons were advanced maternal age and fear of further miscarriages ([Franssen et al. 2006](#)).

## Recommendations

Parental karyotyping is not routinely recommended in couples with RPL. It could be carried out after individual assessment of risk.

Conditional ⊕⊕○○

## Justification

	Association	Contributing factor	Prognosis	Treatment
Parental genetic testing	Yes	Yes <sup>1</sup>	Yes <sup>2</sup>	No

<sup>1</sup> For couples with a parental chromosome abnormality, about one third of pregnancy losses are caused by parental chromosome abnormality; the other losses are aneuploidies, unexplained or a contribution of another underlying factor might exist.

<sup>2</sup> Increased chance of a subsequent pregnancy loss in case of carrier status; Negligible chance of a live born child with an unbalanced chromosome abnormality for the whole RPL population

It was decided to recommend parental karyotyping in RPL couples only after an individual risk assessment. Parental karyotyping can be recommended based on genetic history (for instance in case of the previous birth of a child with congenital abnormalities, offspring with unbalanced chromosome abnormalities in the family, or detection of a translocation in the pregnancy tissue). For other couples, the benefit of the test is limited as the chances of finding an abnormality are very low: in couples with female age above 39, less than three pregnancy losses and a negative family history, the chance of being a carrier of a translocation is very low (Franssen et al. 2005).

Parental karyotyping may provide couples with a possible contributing factor and prognostic information for the subsequent pregnancy. Regarding prognosis, couples should be informed that, even if a parental abnormality is found after karyotyping, the cumulative live birth rates are good, as are the chances of a healthy child, despite a higher risk of a subsequent pregnancy loss. Furthermore, they should be informed of the limitations of karyotyping, including that karyotyping does not predict unbalanced translocation in next pregnancy.

Information provision will aid couples in decision making regarding continuing to try to conceive, stop trying, or choose invasive tests like prenatal diagnosis or preimplantation genetic testing (PGT) (for instance PGT-SR in case of a balanced translocation) (see also chapter 11).

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## 5. Thrombophilia screening

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Thrombophilia is a hereditary or acquired condition that predisposes women with RPL to venous thromboembolism (i.e. venous thrombosis and pulmonary embolism).

**KEY QUESTION: WHAT IS THE VALUE OF THROMBOPHILIA SCREENING IN WOMEN WITH RPL?**

### 5.1 HEREDITARY THROMBOPHILIA

Several genetic causes predisposing patients to venous thromboembolism (VTE) have been identified and are often tested among patients presenting with a thromboembolic event, or their family members. Even in the setting of venous thromboembolism, the value of testing and treatment is controversial ([Bates et al. 2016](#)). Genetic thrombophilia factors have been evaluated in women with RPL, as they are presumed to be a causing factor of RPL and could be associated with severe obstetric complications. This includes Factor V Leiden mutation, Prothrombin mutation, Protein C, Protein S and Antithrombin deficiency.

The prevalence of hereditary thrombophilia in women with RPL is unclear.

#### Evidence

##### Factor V Leiden variant

The factor V Leiden variant (1691G→A) renders factor V resistant to cleavage by activated protein C (also termed Activated Protein C resistance).

Studies on the Factor V Leiden variant and RPL were summarized and analysed for analytical validity, clinical validity and clinical utility ([Bradley et al. 2012](#)). The reviewers concluded that the test for the Factor V Leiden was of adequate quality with high sensitivity and specificity (98.8% and 99.3%, respectively). Regarding the clinical validity, the reviewers reported a significant association between the factor V Leiden (F5 c.1691G>A) genotype and RPL (OR 2.02; 95% CI 1.60-2.55; based on 33 case-control studies), and between the factor V Leiden mutation and the risk of a pregnancy loss in the next pregnancy (OR 1.93; 95% CI 1.21–3.09; based on 4 prospective cohort studies). Carriers of the Factor V Leiden mutation were more likely to have a subsequent loss as compared to non-carriers (OR 2.03; 95% CI 1.29-3.17; based on eight cohort studies) ([Bradley et al. 2012](#)).

With regard to the clinical utility, the reviewers concluded that a positive test result was not associated with improved outcomes for the couples based on the lack of an effect of treatments on pregnancy outcome (*see chapter 12*) and the lack of evidence for non-health related benefits (for example information on a cause for RPL). In addition, there were several harms in testing, including anticoagulant-related maternal risks, costs, and unneeded treatment after a false-positive result.

In addition to a congenital form (caused by a factor V Leiden variant), activated protein C resistance can also be acquired. Acquired activated protein C resistance was associated with a higher risk of RPL in the first trimester (OR 2.60; 95% CI 1.21-5.59) based on two studies ([Robertson et al. 2006](#)).

### Prothrombin variant

The 20210G→A mutation in the gene encoding prothrombin raises plasma concentrations of prothrombin and thereby increases the risk of thrombosis.

A significant association between the Prothrombin variant and RPL was reported by the reviews on the topic, although the details were inconsistent. A review from 2015 reported an overall 2-fold increased risk of RPL in women with G20210A (pooled OR 1.81; 95% CI 1.26-2.60; based on 37 case-control studies). They found this association in European studies, among older women and for foetal loss (>10 weeks) (rather than embryonic loss i.e. <10 weeks) (Gao,Tao 2015). Bradley and colleagues also reported a significant association (OR 2.07; 95% CI 1.59-2.70; based on 29 case-control studies), but they did not find any diagnostic criteria associated with the prothrombin mutation and RPL (Bradley et al. 2012). Finally, Rey and colleagues reported an association between prothrombin mutation and RPL (OR 2.05; 95% CI 1.18-3.54; 9 studies; n=2087) and between the mutation and RPL before 13 weeks (OR 2.32; 95% CI 1.12-4.79; 4 studies; n=979). The association was found for women with two or more pregnancy losses, but not for three or more pregnancy losses (Rey et al. 2003).

Bradley and colleagues also analysed the relevance of testing for the prothrombin G20210A mutation. Again, they found adequate analytic validity (sensitivity 98.3%, specificity 99.6%). The association between the variant and the risk of a next pregnancy loss was not significant (OR 3.29; 95%CI 0.594-18.19, 1 study), nor was the occurrence rate (OR 1.77; 95%CI 0.87-3.61; four cohort studies). Similar to Factor V Leiden, the clinical utility was judged as minimal and the harms of testing outweigh the benefits (Bradley et al. 2012).

### Protein C, Protein S and Antithrombin deficiency

Inherited deficiencies of anticoagulant proteins, e.g. protein C, protein S and Antithrombin are less common, but more strongly associated with venous thromboembolism than factor V Leiden and the prothrombin mutation. In a review, they reported no strong or significant association between deficiencies in these proteins and RPL (Protein C: OR 1.57; 95% CI 0.23-10.54; 2 studies; n=633 - Protein S: 14.72; 95%CI 0.99-217.01; 2 studies; n=624 – Antithrombin: OR 0.88; 95% CI 0.17-4.48; 1 study; n=204) (Rey et al. 2003). A more recent cross-sectional study on protein S found no difference in the frequency of the protein S missense variant (PS-Tokushima) between 355 women with RPL and 101 parous controls. They also reported that there was no difference in live birth rate between women with RPL with low PS activity or normal PS activity (Matsukawa et al. 2017).

### Methylenetetrahydrofolate reductase (MTHFR) mutation

MTHFR gene polymorphisms have historically been classified as a hereditary thrombophilia factor but the mutations are no longer considered for routine assessment of thrombosis risk (Levin,Varga 2016).

Two mutations of the MTHFR gene have been studied. The 677C→T mutation results in a thermolabile variant of MTHFR that can cause mild to moderate hyperhomocysteinemia. An association between 677C→T MTHFR and RPL has been reported by some reviews (Nelen et al. 2000; Govindaiah et al. 2009; Chen et al. 2016), while others did not find evidence of an association (Rey et al. 2003). Although less well studies, no significant associations were found between other mutations of the MTHFR gene and RPL (Hickey et al. 2013; Chen et al. 2016)

## Recommendation

For women with RPL, we suggest not to screen for hereditary thrombophilia unless in the context of research, or in women with additional risk factors for thrombophilia.

Conditional ⊕⊕⊕○

## Justification

	Association	Contributing factor	Prognosis	Treatment
Hereditary thrombophilia*	No/weak	Unclear	Yes	No

\* this includes Factor V Leiden mutation - Prothrombin mutation - MTHFR mutation - Protein C, Protein S and Antithrombin deficiency

There is no, or a weak association at best, between RPL and hereditary thrombophilia. The recommendation not to screen for hereditary thrombophilia in women experiencing RPL is similar to the recommendations of the guideline on VTE, thrombophilia, antithrombotic therapy and pregnancy of the American College of Chest Physicians (Bates et al. 2012). If additional risk factors for hereditary thrombophilia are present (for instance family members with hereditary thrombophilia, or previous VTE), screening can be considered. Also in a research setting, screening can be considered to provide further data on the impact of thrombophilia in women experiencing RPL.

Due to physiological changes, thrombophilia markers increase or decrease during pregnancy (Kristoffersen et al. 2017). Correct interpretation of results and diagnosis of hereditary thrombophilia is possible for the DNA mutations factor V Leiden and prothrombin 20210A, but can be problematic for antithrombin, protein C, and most notably protein S. Therefore, it is recommended to postpone screening for hereditary thrombophilia until 6 weeks after the pregnancy loss.

## 5.2 ACQUIRED THROMBOPHILIA

Acquired thrombophilia refers to antiphospholipid syndrome (APS). APS is diagnosed based on the persistent presence of antiphospholipid antibodies and vascular thrombosis and/or pregnancy complications (Miyakis et al. 2006).

Three clinically relevant and well-characterized antiphospholipid antibodies (i.e. antibodies associated with thrombosis) are lupus anticoagulant (LA), anticardiolipin antibodies (ACA, IgG and IgM), and  $\beta$ 2 glycoprotein I antibodies ( $\alpha\beta$ 2GPI, IgG and IgM).

The Miyakis criteria, an update of the Sapporo classification of 1999, have been determined by consensus. The clinical criterion 'three or more unexplained consecutive spontaneous miscarriages before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.' is one of the clinical criteria which may lead to the diagnosis APS (Miyakis et al. 2006). After the Miyakis criteria have been published, new evidence has appeared. In a retrospective cohort study, there was no difference in the number of pregnancy losses, the sequence of pregnancies, or maternal age between women with RPL and APS and women with unexplained RPL. Therefore, the authors concluded that it is justifiable to offer testing for APS to all

women with a history of two or more, consecutive or non-consecutive, pregnancy losses ([van den Boogaard et al. 2013](#)).

## Evidence

### Lupus anticoagulant

In a meta-analysis, a strong, consistent and significant association was reported of Lupus anticoagulant (LA) with late RPL (prior to 24 weeks' gestation with (OR 7.79; 95% CI 2.30-26.45; based on 9 case-control studies; n = 2195). There were no data available to pool RPL prior to 13 weeks' gestation ([Opatrny et al. 2006](#)). The direct relationship between LA and RPL was also demonstrated in a more recent meta-analysis ([Santos et al. 2017](#)).

### Anticardiolipin Antibodies

Anticardiolipin IgG antibodies (ACA) were found to be associated with RPL prior to 13 weeks' gestation (OR 3.56; 95% CI 1.48–8.59; 2 studies; n=907; all titers) and with RPL prior to 24 weeks' gestation (OR 3.57; 95% CI 2.26-5.65; 10 studies; n=3631) ([Opatrny et al. 2006](#)). A further analysis of studies only including moderate and high ACA titers increased the strength of the association (OR 4.68; 95% CI 2.96-7.40; 6 studies; n = 2724).

In the same meta-analysis, an association was reported between ACA IgM with RPL prior to 24 weeks' gestation (OR 5.61, 95% CI 1.26-25.03; 4 studies; n=1822). This association was no longer found if only moderate and high ACA IgM titers were included (OR 4.03; 95% CI 0.84-19.34; 3 studies; n=1579). There were no data for women exclusively positive for ACA IgM, nor did the authors find any studies in women with RPL prior to 13 weeks' gestation ([Opatrny et al. 2006](#)).

An association between both positive ACA IgG and IgM and RPL prior to 24 weeks' gestation was found (OR 5.39; 95% CI 3.72-7.82; 10 studies; n=3534) when restricting the analysis to 10 homogeneous studies using an a priori definition for moderate to high antibody titers ([Opatrny et al. 2006](#)).

Similar association was found in four of nine studies included in another meta-analysis ([Santos et al. 2017](#)).

### $\beta 2$ glycoprotein I antibodies

Based on five studies included in a meta-analysis from 2006, no statistically significant association was found between  $\alpha\beta 2$ GPI antibodies and RPL prior to 13 weeks' gestation (OR 2.12; 95% CI 0.69-6.53; 5 studies; n=1788). However, the risk appears increased and the upper boundary of the 95% CI may indicate a large effect ([Opatrny et al. 2006](#)).

In a more recent meta-analysis, one of the three studies included found a significant association between  $\alpha\beta 2$ GPI antibodies and RPL ([Santos et al. 2017](#)). The authors explained that this controversy in the literature can be explained by interlaboratory variability in the study of  $\alpha\beta 2$ GPI antibodies.

### Other Antibodies

Several studies have been evaluating the diagnostic potential of new antibodies against phospholipids. In general the added clinical value of these antibodies, alone or in panel, in addition to LA, ACA and  $\alpha\beta 2$ GPI antibodies is limited and inconsistent, and should be confirmed before applied in clinical practice ([Aoki et al. 1993](#); [Subrt et al. 2008](#); [Tebo et al. 2008](#); [Sater et al. 2012](#); [Subrt et al. 2013](#)).

A similar conclusion can be drawn for anti-Annexin V ([Bizzaro et al. 2005](#); [Galli et al. 2007](#); [Vora et al. 2008](#); [Sater et al. 2011](#)).

## Recommendations

For women with RPL we recommend screening for antiphospholipid antibodies (LA and ACA [IgG and IgM]), after two pregnancy losses.

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For women with RPL screening for aβ2GPI can be considered after two pregnancy losses.

GPP

## Justification

	Association	Contributing factor	Prognosis	Treatment
Antiphospholipid antibodies: LA and ACA (IgG and IgM)	Yes	Yes	Yes	Weak evidence
aβ2GPI	Possible (not statistically significant)	Possible	No data	No data

Screening of antiphospholipid antibodies can provide information for a diagnosis of APS and possible treatment. In addition, screening is of value in women with RPL with regard to providing them with a possible cause (as aPL have been suggested to play a role in the pathogenesis of RPL via complement activation ([Arachchillage et al. 2015](#))), and to possibly prevent pregnancy complications associated with APS (pre-eclampsia, placenta-mediated complications, neonatal mortality) ([Bouvier et al. 2014](#)).

Screening for aβ2GPI antibodies could be considered in women with RPL to improve future knowledge. The results of a recent prospective study, although needing confirmation, suggests that a decrease in aβ2GPI antibodies (IgM) with anticoagulant treatment was correlated with better pregnancy outcomes ([Song et al. 2017](#)).

Although the time interval for reliable testing of LA, ACA and aβ2GPI antibodies after a pregnancy (loss) is not known, generally a time interval of 6 weeks is considered appropriate. Confirmation of the test results after at least 12 weeks is necessary in the Miyakis criteria for APS diagnosis ([Miyakis et al. 2006](#)).

The GDG group reached consensus that it can be recommended to screen for antiphospholipid antibodies after two pregnancy losses and recommends further study of clinical criteria for the diagnosis of APS (e.g. female age, number of pregnancy losses, consecutive or non-consecutive losses).

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## 6. Immunological screening

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**KEY QUESTION: WHAT IS THE VALUE OF IMMUNOLOGICAL SCREENING IN THE DIAGNOSIS OF RPL?**

### 6.1 HUMAN LEUKOCYTE ANTIGEN (HLA)

#### Evidence

Due to the different ways HLA<sup>2</sup> can influence immune reactions, studies of HLA in RPL can be divided into three main categories: 1) studies of HLA allele compatibility (sharing) between partners with RPL, 2) studies of HLA allele prevalence in women with RPL and 3) studies of HLA-C and -G alleles in partners with RPL.

#### HLA compatibility

Increased HLA compatibility between partners was originally thought to decrease the probability of the mother to produce so-called blocking antibodies that were suggested to protect against foetal rejection. A meta-analysis reported that allele sharing in the HLA-A, -B and -C loci was not found with different frequencies in RPL and control couples whereas sharing in the HLA-DR locus was borderline significantly increased (Beydoun, Saftlas 2005). In a subsequent large case-control study using up-to-date DNA-based HLA determination no increased HLA-DR sharing was found in RPL couples (Aruna et al. 2011).

#### HLA allele prevalence in women with RPL

In one case-control study of 588 Caucasian women with RPL and 562 Caucasian controls, the HLA-DRB1\*03 allele was found significantly more often in women with RPL than controls also after correction for multiple comparisons (Kruse et al. 2004). The association to HLA-DRB1\*03 was stronger in women with  $\geq 4$  previous pregnancy losses or women with secondary RPL (OR = 1.8, 95% CI 1.3-2.5). This dose response effect supports a causative role for HLA-DRB1\*03 (or a gene variant in LD with this allele) in RPL (or at least secondary RPL) (Kruse et al. 2004). In a recent large case-control study including 1078 Caucasian women with RPL and 2066 controls it was found that the HLA-DRB1\*07 allele was highly significantly associated to RPL (OR = 1.29, 95% CI 1.09-1.52 in heterozygous RPL patients and OR = 2.27, 95% CI 1.31-3.93 in homozygous patients) (Thomsen et al. 2021). In this study, the association did not become stronger with increased number of miscarriages or in patients with secondary RPL and the association to HLA-DRB1\*03 was not confirmed. Other studies have been conducted on the HLA class II genes (HLA-DRB1 or -DQB1) but they included insufficient numbers of patients and controls to have sufficient power after correction for multiple testing, which is essential when studying multiple HLA alleles. There are no prospective studies investigating the prognostic impact of carrying HLA-DRB1\*03,

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<sup>2</sup> The HLA region comprises several genetic loci located on chromosome 6 and it contains the most polymorphic genes known in humans. Dependent on the genetic distance between the various HLA loci, the alleles of the genes in each locus display various degrees of linkage disequilibrium (LD), which means that alleles in different loci are inherited together more or less often than expected by chance. LD to genetic variants in other loci in the HLA region must be considered when finding a specific allele associated with RPL.

or other HLA genes in patients with primary RPL whereas several studies of the impact of carriage of maternal HLA class II have been performed in women with secondary RPL.

In a cohort study, it has been suggested that the prior birth of a boy in women with secondary RPL can affect subsequent pregnancy outcome negatively (for birth after a firstborn boy vs. a firstborn girl; adjusted OR 0.37; 95% CI 0.2-0.7) (Nielsen et al. 2008). A prospective study (n=358) provided evidence that women with secondary RPL after the birth of a boy have a significantly lower (22%) subsequent live birth rate when they carried one of three HLA class II alleles DRB1\*15:01; -DQB1\*05:01/05:02 and -DRB3\*03:01 known to predispose to clinically relevant anti-HY immune reactions (Nielsen et al. 2009). Carrying two of these HLA alleles was associated with a significantly higher risk than carrying zero or one allele suggesting a dose-response relationship. In a subsequent cohort study of long-term outcome (n=585) the negative prognostic effect of HLA-DRB1\*15 and -\*DQB1\*05:01/02 was confirmed. Furthermore, HLA-DRB1\*07:01 and HLA-DRB3\*03:01 also seemed to have a negative prognostic effect, though probably weaker. As in the Nielsen study, the negative prognostic effect of maternal carriage of HY-restricting HLA class II alleles on subsequent live birth was only observed for women with a firstborn boy (Kolte et al. 2016).

#### *HLA-C and -G alleles in couples*

Reactions of NK cells (cytotoxicity and cytokine production) in pregnant women are suggested to be modified by interactions between specific receptors (Killer immunoglobulin-like receptors or KIRs) on the NK cells and HLA-C or HLA-G, which are the only HLA genes expressed on the trophoblast.<sup>3</sup> Hiby and colleagues reported that the combination of the woman carrying KIR genes that are mainly inhibitory and the man carrying C2 allotypes is more frequent among RPL than control couples (Hiby et al. 2008). Another case-control study reported that maternal inhibitory KIRs in combination with C2 homozygosity in both partners was found significantly more often in controls (Faridi, Agrawal 2011). In a third study no association between maternal activating or inhibitory KIR and RPL could be detected in 52 women with RPL (Witt et al. 2004), whereas smaller studies found a significant increase in activating or decrease of inhibitory KIRs in women with RPL (Varla-Leftherioti et al. 2003; Vargas et al. 2009). Due to the contradictive findings concerning KIR genotyping in couples with RPL, KIR and HLA-C typing is not suitable for diagnostic and therapeutic purposes at present.

Another set of studies have investigated HLA-G polymorphisms in RPL. Soluble HLA-G is suggested to modulate NK cytotoxicity and cytokine secretion at the feto-maternal interface. Low plasma soluble HLA-G levels may be associated with homozygosity for a HLA-G14 bp insertion in the HLA-G gene. Two meta-analyses reported that the HLA-G14 bp insertion frequency was significantly increased in women with RPL (OR 1.27 (1.04-1.55) and 1.47 (1.13-1.91), respectively) (Wang et al. 2013; Fan et al. 2014). Since the HLA-G14 bp insertion is in strong positive linkage disequilibrium with the HLA-DRB1\*03 allele (Hviid, Christiansen 2005), the question remains whether the association of RPL to the HLA-G14 bp insertion is secondary to a primary association to the HLA-DRB1\*03 allele.

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<sup>3</sup> HLA-C alleles can be divided into C1 and C2 groups according to a genetic dimorphism leading to changes in the segment of HLA-C molecule that can bind KIR. This binding between KIR and HLA-C will ultimately result in either inhibition or activation of NK cell function.

## Recommendation (updated in 2022)

Human Leukocyte Antigen (HLA) determination in women with RPL is not recommended in clinical practice. Only HLA class II determination (HLA-DRB1\*15:01, HLA-DRB1\*07 and HLA-DQB1\*05:01/05:02) could be considered in Scandinavian women with secondary RPL after the birth of a boy, for explanatory and prognostic purposes.

Conditional ⊕⊕○○

## Justification

	Association	Contributing factor	Prognosis	Treatment
HLA-compatibility	Controversial evidence	NA	No prognostic potential	NA
HLA class II: HLA-DR and HLA-DQ (maternal)	Strong, but only shown in Scandinavian women	YES, especially for secondary RPL after first born boy	Negative impact on future live birth	None available
HLA-G	Significant but weak	No data	No data	NA
KIR and HLA-C	Controversial evidence	No data	No data	NA

The association between subsequent pregnancy outcome and HLA polymorphisms in women or couples with RPL is not sufficiently studied. For HLA compatibility and HLA-C alleles in couples, the evidence for an association with RPL is inconsistent, while a weak association is reported for specific HLA-G alleles in RPL women. Investigation of HLA-DR (or other classical HLA genes) in women with RPL is not recommended in clinical practice but could be performed in a research setting. An exception could be investigation of class II HLA in women with secondary RPL after the birth of a boy, even though this has only been shown in a large Scandinavian study and needs further confirmation in non-Scandinavian women. With the availability of additional data, the information on the specific HLA alleles to be determined was adapted in the recommendation.

## 6.2 ANTI-HY ANTIBODIES

Anti-HY antibodies are antibodies directed against male-specific minor histocompatibility (HY) antigens expressed on most or all nucleated cells from males.

## Evidence

Detection of anti-HY antibodies in the serum of women with RPL may display some negative prognostic impact; women without these antibodies had a subsequent 61% livebirth rate compared with 48% in

anti-HY antibody positive women in an observational study ([Nielsen et al. 2010b](#)), but confirmatory studies are needed.

## Recommendation

Measurement of anti-HY antibodies in women with RPL is not recommended in clinical practice.

Conditional ⊕⊕○○

## Justification

	Association	Contributing factor	Prognosis	Treatment
<b>Anti-HY immunity</b>	Moderate (only shown in Scandinavian women)	YES, especially for sec RPL after first born boy	Negative impact on future live birth*	None available

\* Prognostic impact is stronger for women with secondary RPL with a first-born boy and HLA class II alleles predisposing to anti-HY immunity

Since the risk increment conferred by carrying these HLA alleles is substantial in women with secondary RPL after a birth of a boy, clinicians could consider offering HLA-DRB1 typing to these patients for clarification of the pathogenesis and assessment of prognosis. However, so far, the testing will provide no change in treatment offers.

## 6.3 CYTOKINES

### Evidence

In general, investigation of the cytokine levels in peripheral blood is not informative except for TNF- $\alpha$ , a marker for the degree of systemic inflammation. High plasma TNF- $\alpha$  levels are reported to increase the risk of miscarriage in women with RPL ([Mueller-Eckhardt et al. 1994](#)) and high TNF- $\alpha$  and TNF- $\alpha$ /IL10 ratios characterize women with euploid compared to aneuploid miscarriages ([Calleja-Agius et al. 2012](#)). Women with secondary RPL seem to have significantly higher plasma levels of TNF- $\alpha$  in early pregnancy than women with primary RPL ([Piosik et al. 2013](#)). Lee and colleagues found a significantly increased percentage of Th1 cells expressing intracellular TNF- $\alpha$  in peripheral blood lymphocytes and a significantly increased TNF- $\alpha$ /IL10 Th-cell ratio in RPL patients compared to controls ([Lee et al. 2013](#)).

In a study of mitogen-stimulated peripheral blood lymphocytes, Th2 cytokine secretion was significantly higher in pregnant fertile controls and RPL women who later gave birth compared with RPL women who miscarried ([Makhseed et al. 2001](#)). However, the fact that some samples were taken at time of miscarriage and some at time of birth may flaw the results. In another small study, it was found that mitogen-stimulated lymphocytes from women with RPL who later went on to miscarry produce more TNF- $\alpha$  than those of patients who gave birth ([Kruse et al. 2003](#)).

The plasma levels or in-vitro production of many cytokines are influenced by polymorphisms in the cytokine genes, which has also been explored in women experiencing RPL. In two studies an association between TGF $\beta$ 1 or TNF- $\alpha$  gene polymorphisms and RPL was reported ([Amani et al. 2005](#); [Zhang et al. 2012](#)). However, meta-analyses have not been able to find polymorphisms in relevant cytokine genes

associated with RPL, except for a weak association to a -1082 IL10 genotype ([Choi, Kwak-Kim 2008](#); [Medica et al. 2009](#)).

## Recommendations

Cytokine testing should not be used in women with RPL in clinical practice.

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Cytokine polymorphisms should not be tested in women with RPL.

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## Justification

	Association	Contributing factor	Prognosis	Treatment
Cytokines	Yes	Unclear	Unknown	NA
Polymorphisms in cytokine genes	No association	NA	NA	NA

Research into the role of cytokines in RPL is complex since the function of cytokines may change according to length of gestation and cytokine production of blood lymphocytes. Furthermore, plasma cytokine concentrations may be completely different from that in the uterus and measurement of cytokines in endometrial tissue, decidual tissue or endometrial flushing is subject to technical difficulties.

Although studies have shown an association between TNF- $\alpha$  and RPL, the relevance of routine testing is unclear. Measuring cytokine levels or evaluating cytokine gene polymorphisms in women with RPL are so far only useful in the context of research projects.

## 6.4 ANTINUCLEAR ANTIBODIES (ANA)

Antinuclear antibodies (ANA) are antibodies directed against various components of the cell nuclei, often detected in patients with autoimmune diseases.

## Evidence

Two independent meta-analyses concluded that there is an association between RPL and ANA, when the latter is determined by immunofluorescence ([Cavalcante et al. 2020](#); [Chen et al. 2020](#)). The presence of ANA was associated with a significantly increased ORs for RPL (3.30 ([Cavalcante et al. 2020](#)) and 2.97 ([Chen et al. 2020](#))). With regards to ANA assessed with the ELISA technique, which is not standard clinical practice, a single study showed no association with RPL ([Chen et al 2020](#)).

Some studies reported that ANA positivity was more prevalent in women with RPL with a new miscarriage (n=24) as compared to those who gave birth (n=82) ([Cavalcante et al. 2014](#)). Similarly, a higher miscarriage rate was reported in ANA-positive as compared to ANA-negative women with RPL

in a small prospective study ([Harger et al. 1983](#)). However, the study by Ogasawara did not find that the presence of ANA could predict new pregnancy loss ([Ogasawara et al. 1996](#)).

A direct pathophysiological link between the presence of autoantibodies such as ANA in women with RPL and foetal death has not yet been documented. A known genetic predisposing factor is the HLA-DRB1\*03 allele, which is associated with both production of various autoantibodies including ANA and the risk of RPL ([Christiansen 1996](#)).

## Recommendation

**Antinuclear antibodies (ANA) testing could be considered for explanatory purposes.**

Conditional ⊕⊕○○○

## Justification

	Association	Contributing factor	Prognosis	Treatment
<b>ANA antibodies</b>	Yes	Probably not – no documentation	Unclear	NA

Measurement of ANA in women with RPL could be considered two independent meta-analyses document an association to RPL and there is some evidence (from small prospective studies) that ANA presence affects the prognosis negatively ([Harger et al. 1983](#); [Cavalcante et al. 2014](#)). Whether ANA positivity can identify a subset of women with RPL that responds beneficially to various forms of immunotherapy is unknown and can only be shown in randomized controlled trials.

## 6.5 NATURAL KILLER CELLS (NK CELLS)

### Evidence

Investigations of NK cells in RPL can be divided into (1) flow-cytometric analyses or tests of NK cell cytotoxicity of peripheral blood lymphocytes before or during pregnancy and (2) studies of NK cells in pre-pregnancy endometrial biopsies or decidual tissue from miscarriages and terminated pregnancies.

#### NK cells in peripheral blood

In several large studies of good or acceptable quality it was found that the percentage of CD56+ NK cells in peripheral blood taken prior to pregnancy is significantly higher in RPL women than controls ([Kwak et al. 1995](#); [Shakhar et al. 2003](#); [Perricone et al. 2007](#); [Prado-Drayer et al. 2008](#); [King et al. 2010](#); [Karami et al. 2012](#); [Yoo et al. 2012](#); [Lee et al. 2013](#)), or had predictive value for subsequent pregnancy outcome ([Emmer et al. 1999](#); [Emmer et al. 2000](#)) whereas other studies did not find NK cell numbers or percentages associated to RPL ([Chao et al. 1995](#); [Wang et al. 2008](#); [Carbone et al. 2009](#)) or predictive for outcome ([Morikawa et al. 2001](#); [Yamada et al. 2003](#); [Liang et al. 2012](#)). In many of these case-control studies most of the RPL women were nulliparous and most controls were multiparous; which can flaw the results since a previous successful pregnancy can induce permanent changes in lymphocyte subsets including NK cells ([Shakhar et al. 2003](#); [Toth et al. 2019](#)).

Several of the studies of pre-pregnancy blood samples found significantly increased NK cell cytotoxicity in women with RPL compared to controls ([Shakhar et al. 2006](#); [Hadinedoushan et al. 2007](#); [Karami et al. 2012](#); [Lee et al. 2013](#)) whereas a study performed during pregnancy did not find such a difference ([Chao et al. 1995](#)). One small prospective study found significantly reduced NK cytotoxicity in women with RPL compared with controls ([Souza et al. 2002](#)).

Aoki and colleagues reported that RPL patients with high pre-pregnancy peripheral blood NK cell cytotoxicity had a significantly higher subsequent rate of miscarriage compared with those with lower NK cytotoxicity (71% versus 20%) ([Aoki et al. 1995](#)). Smaller studies found a higher or similar NK cell cytotoxicity in patients with a subsequent euploid miscarriage compared with those with a live birth ([Morikawa et al. 2001](#); [Yamada et al. 2003](#)). However, in prospective studies it was reported that high NK cell cytotoxicity before pregnancy had no impact on subsequent miscarriage rates; in the study of Katano there was no impact of NK cytotoxicity even after adjustment for recognized risk factors for miscarriage ([Emmer et al. 1999](#); [Liang et al. 2012](#); [Katano et al. 2013](#)).

#### ***NK cells in endometrial biopsies or decidual tissue***

One small case-control study reported that the CD56<sup>bright</sup> NK cell subset was significantly lower in endometrial biopsies of women with RPL than in controls ([Lachapelle et al. 1996](#)) whereas other studies found that the frequency of CD56+ (or unspecified NK cells) cells was significantly higher in RPL than in controls ([Clifford et al. 1999](#); [Quenby et al. 2005](#); [Tuckerman et al. 2007](#)). In two case-control studies, no difference was found in NK cell subsets in the endometrium between women with RPL and controls ([Michimata et al. 2002](#); [Shimada et al. 2004](#)). Importantly, no relationship between CD56+ NK cell count in the endometrium and subsequent pregnancy outcome was found in a blind retrospective study ([Tuckerman et al. 2007](#)).

Studies comparing NK cell subsets in decidual tissue from miscarriages of women with RPL with tissue from women having a termination of pregnancy found differences in NK cell subsets between the two groups ([Vassiliadou, Bulmer 1996](#); [Ozcimen et al. 2009](#); [Bao et al. 2012](#)). However, since the tissue in the former cases is necrotic and often inflamed and the latter cases is fresh and vital, these kind of studies provide limited valid information.

In a series of studies, combinations of maternal KIR gene polymorphisms and parental HLA-C allotypes have been investigated in RPL and controls couples as a measure of the potential for maternal NK cell activation ([Varla-Leftherioti et al. 2003](#); [Witt et al. 2004](#); [Hiby et al. 2008](#); [Vargas et al. 2009](#); [Faridi, Agrawal 2011](#)). These studies have been previously discussed and evaluated in the HLA section.

#### **Recommendation**

**There is insufficient evidence to recommend NK cell testing of either peripheral blood or endometrial tissue in women with RPL.**

Strong

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#### **Justification**

	Association	Contributing factor	Prognosis	Treatment
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NK in Peripheral blood: numbers	Weak	No	Unclear – No	No
NK cell cytotoxicity in peripheral blood	Unclear	/	No	No
NK in endometrium / uterine	Weak	/	Unclear	No

From studies analysing NK cells in peripheral blood lymphocytes before or during pregnancy, there seems to be a weak association with RPL, but NK cell testing cannot be used to select women with RPL for immunological treatments.

Furthermore, there are significant technical challenges; the frequencies of NK cell subsets between the endometrium and peripheral blood are extremely different. NK cells can be measured in endometrial biopsies taken in non-pregnant cycles by immunohistochemistry or flow cytometry of homogenized tissue. The former technique is prone to subjective evaluation and using the latter can change surface marker expression since the tissue undergoes enzymatic digestion. Furthermore, endometrial and peripheral blood NK cell numbers fluctuate hugely in the menstrual cycle so exact timing of samples is crucial but has rarely been done. Last, previous live births seem to exhibit a long term impact on NK cell frequencies in the blood and endometrium and therefore patients and controls in future studies of NK cells should have comparable parities ([Toth et al. 2019](#)).

The measurement of uterine NK cells, although in theory a better approach, is also unfit for clinical practice due to lack of consensus about ranges of normal values and lack of standardization in the measurement of NK cells. A long-awaited study that establish normal values for endometrial NK cells and recommends standardized cell counting techniques has been published ([Chen et al. 2017](#)). Adherence to these recommendations can hopefully improve the quality of future studies of endometrial NK cells in RPL.

## 6.6 OTHER IMMUNOLOGICAL TESTS

### Evidence

#### Anti-HLA antibodies

In a large retrospective cohort study, anti-HLA class I or II antibodies could be detected with significantly increased frequency in multiparous controls compared with women with RPL, which can be explained by the higher number of previous deliveries in the former group ([Bartel et al. 2011](#)). However, women with “unexplained” RPL had the same prevalence of these antibodies as the women in whom the cause of RPL was considered known. In a small study on the prospective impact of antibodies blocking mixed lymphocyte reactions (which may be similar to anti-HLA antibodies), these antibodies were not predictive of subsequent pregnancy outcome ([Jablonowska et al. 2001](#)). Another study reported that in pregnant women with RPL, those that were HLA-antibody positive had lower live birth rate (41%) as compared to HLA-antibody negative RPL women (76%) (Adjusted OR 0.22; 95% CI 0.07-0.68) ([Nielsen et al. 2010a](#)). A meta-analysis found no significant effect of anti-HLA antibodies (class I and II) on first trimester complications (RPL) but the included studies showed significant heterogeneity ([Lashley et al. 2013](#)).

### Celiac disease serum markers

A case-control study measured tissue transglutaminase (tTG) antibodies (IgA + IgG) and endomysial antibodies (IgA + IgG) in 116 women with unexplained RPL and 116 age-matched controls. Although women with RPL had significantly higher serum levels of IgG tTG antibodies compared with controls, the proportion of women with antibodies indicative of celiac disease was very low and similar in both groups ([Sharshiner et al. 2013](#)). Therefore, testing for celiac disease serum markers is not indicated in women with RPL in absence of symptoms of celiac disease.

### Antisperm antibodies

Antisperm antibodies have also been described in women with RPL, although the results are inconsistent, and the relevance is unclear. Al-Hussein and colleagues concluded that there was no significant difference with respect to elevated antiparental antibodies and pregnancy outcome based on flow cytometric analysis of maternal antipaternal antibodies in the sera of 24 women with RPL, and 6 controls with no history of RPL ([Al-Hussein et al. 2002](#)). In another case-control study, anti-sperm antibodies (measured by ELISA) were found in 22.6% of 155 women with RPL, which was significantly more compared to controls (8%, n=50) ([Motak-Pochrzest, Malinowski 2013](#)). However, in a study without control group anti-sperm antibodies were found in only 4.8% of 123 women with RPL ([Christiansen et al. 1998](#)).

Other immune biomarkers such as IL2 receptor levels ([Wilson et al. 2003](#)), anti-protein Z presence ([Sater et al. 2011](#)) and anti-complementary activity ([Quinn, Petric 1988](#)) have only been studied in a single study and it is impossible to assess their clinical impact.

### Recommendation

Testing anti-HLA antibodies in women with RPL is not recommended.

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### Justification

Overall, there is no documentation for the value of measuring anti-HLA antibodies in the screening of women with RPL and it is not recommended to measure it in these women. Several other immunological tests were described in a single study, but until further data, they are not recommended in clinical practice.

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## 7. Metabolic and endocrinologic factors

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**KEY QUESTION: WHAT IS THE VALUE OF SCREENING FOR METABOLIC/ENDOCRINOLOGICAL ABNORMALITIES IN THE DIAGNOSIS OF RPL?**

### 7.1 THYROID DYSFUNCTION

Thyroid hormones are essential for foetal development. A review on the thyroid function and reproduction concluded that thyroid hormone disorders and increased Thyroid peroxidase (TPO) antibodies (TPO-Ab) are associated with disturbed folliculogenesis, spermatogenesis, fertilization and embryogenesis, supporting an important role for thyroid hormone disorders and thyroid autoimmunity in subfertility and pregnancy loss ([Vissenberg et al. 2015](#)).

#### Evidence

##### Hyperthyroidism

Hyperthyroidism, most often Graves' disease, is found in 0.1-0.4% of pregnant women ([Bahn et al. 2011](#)). Those women have an increased risk of several pregnancy complications including sporadic pregnancy loss, pre-eclampsia, preterm delivery, and congestive heart failure. However, no studies were found that *described or searched* for an association between hyperthyroidism and recurrent pregnancy loss (RPL).

##### Hypothyroidism

We did not identify any high-quality studies on an association between overt hypothyroidism and RPL. One moderate quality study assessed of thyroid function in 163 non-pregnant women with a history of RPL and 170 age-matched controls. The prevalence of hypothyroidism, based on serum T3 (triiodothyronine), T4 (thyroxine) and TSH (thyroid stimulating hormone) levels, was higher in RPL women (4.29%) compared to the controls (0.61%), but there was no evidence for a difference in risk of RPL between 8 hypothyroid and 325 euthyroid women (OR 7.6; 95% CI 0.92-62) ([Rao et al. 2008](#); [van den Boogaard et al. 2011](#)).

Three studies investigated a possible association between subclinical hypothyroidism (SCH) and RPL. In the cohort study of Bernardi and colleagues, 19% of 286 women with RPL ( $\geq 2$  pregnancy losses  $<10$  weeks) showed subclinical hypothyroidism, i.e. TSH  $>2.5$  mIU/L with a normal free thyroxine or free thyroxine index. They detected a similar cumulative LBR in women with SCH and euthyroid women (27/39 (69%) versus 104/141 (74%)) ([Bernardi et al. 2013](#)). Similar results were reported by van Dijk and colleagues who detected subclinical hypothyroidism in only 2.4% of 848 women with RPL and found no differences in live birth or miscarriage rate between women with subclinical hypothyroidism and euthyroid women ([van Dijk et al. 2016](#)). In the third study, subclinical hypothyroidism was detected in 27% of 100 pregnant women with a history of RPL, which was similar to the prevalence in the control group of 100 pregnant women without a history of pregnancy loss (24%). In the RPL group, the incidence of subclinical hypothyroidism was significantly higher in the TPOAb positive group compared

to the TPOAb negative group (52 vs 16%). There was no difference in the prevalence of miscarriage or obstetric outcomes between RPL and controls irrespective of TPO status ([Lata et al. 2013](#)).

### Isolated hypothyroxinaemia

Isolated hypothyroxinaemia is defined as a normal female TSH concentration in conjunction with FT4 concentrations in the lower 5th or 10th percentile of the reference range ([Stagnaro-Green et al. 2011](#)). Isolated hypothyroxinaemia (low Free T4) in pregnancy has been associated with an increased risk of obstetric complications and child neurocognitive impairment, although other studies reported no association ([Lazarus et al. 2014](#)). A meta-analysis found an association of isolated hypothyroxinaemia with placental abruption, but not with pregnancy loss ([Chan,Boelaert 2015](#)).

### Thyroid autoantibodies

In women with RPL, thyroid peroxidase autoantibodies (TPOAb) are mostly studied, and shown to be more relevant than antibodies against the thyroid gland ([Marai et al. 2004](#)).

The prevalence of TPOAb is 8-14% in women of reproductive age. TPOAb predispose to hypothyroidism, but the majority of women having TPOAb is euthyroid.

An association between TPOAb and RPL was found in a meta-analysis of 13 studies (3 cohort, 10 case-control studies). The odds of miscarriage with thyroid autoantibodies was increased for RPL women (OR 4.22; 95% CI 0.97-18.44; 3 studies; n=221). The reviewers noted that there was an unexplained heterogeneity in the analysis ( $I^2 = 75\%$ ). Furthermore, they found an increase in the odds of miscarriage in RPL women with thyroid autoantibodies but normal thyroid function (OR 1.86; 95% CI 1.18-2.94; 10 studies; n=2753) ([Thangaratinam et al. 2011](#)). Based on similar studies, another review also reported an association between the thyroid antibodies and increased risk of RPL (OR 2.3; 95% CI 1.5-3.5) ([van den Boogaard et al. 2011](#)).

A more recent case-control study detected thyroid autoantibodies (anti-thyroglobulin (TGAb), TPOAb or anti-TSH receptor (TSHr-Ab) autoantibodies) in 28.75% of 160 women with RPL and in 13% of 100 women of the control group. There was no difference in the prevalence or titers of thyroid autoantibodies in women with two losses compared to those with three or more losses. Among the women of RPL group, 91.3% of women positive for thyroid autoantibodies were positive also for other autoantibodies (mostly ANA), compared to only 53.1% of RPL women without thyroid autoantibodies. Most of the women included in the study were euthyroid (96.3% of women with RPL and 93% of the controls) ([Ticconi et al. 2011](#)).

In conclusion, a clear association between thyroid auto immunity and RPL has been found.

### Recommendations

Thyroid screening (TSH and TPO antibodies) is recommended in women with RPL.

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Abnormal TSH and TPO-antibody levels should be followed up by T4 testing in women with RPL.

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## 1965 Justification

	Association	Contributing factor	Prognosis	Treatment
Hypothyroidism	Only sporadic PL	Only for sporadic PL	Yes	Supplementation of Levothyroxine
Subclinical hypothyroidism	Yes	Yes	No clear effect as of yet.	Unknown if effective
Hyperthyroidism	No	No	No clear effect as of yet.	Yes: Propylthiouracil
TPO-antibodies	Yes	Yes	Yes	Need for treatment studies
TG antibodies	No	Mostly detected combined with TPO antibodies	Yes	Need for treatment studies

1966

1967 Based on a high prevalence of subclinical hypothyroidism and thyroid auto immunity in women with  
 1968 RPL and potential of treatment options testing for thyroid function is recommended.

1969 **7.2 PCOS AND DISTURBANCES OF THE INSULIN METABOLISM**1970 **Evidence**1971 Polycystic ovary syndrome (PCOS)

1972 Polycystic ovary syndrome (PCOS) is associated with several pregnancy complications, including  
 1973 gestational diabetes, pre-eclamptic toxemia, pregnancy-induced hypertension and probably pregnancy  
 1974 loss ([Homburg 2006](#)). The uncertainty for an association between PCOS and pregnancy loss could be  
 1975 explained by several factors suggested to be associated with both PCOS and pregnancy loss, including  
 1976 obesity, hyperinsulinemia, LH hypersecretion, hyperandrogenism, and thrombophilia ([Homburg 2006](#);  
 1977 [Kazerooni et al. 2013](#); [Ke 2014](#)).

1978 In the cohort study of Sagie, PCOS was significantly more prevalent in 56 women with RPL had polycystic  
 1979 ovaries compared with 11 parous women (82% versus 18%) ([Sagie et al. 1988](#)) In the study by Watson,  
 1980 81% of the women showed PCO morphology compared to 10% of 10 multiparous controls ([Watson et  
 1981 al. 1993](#)). In another small study, no difference was found in the prevalence of PCOS morphology  
 1982 between 42 women with RPL and 18 fertile controls (16.3% versus 0%) ([Okon et al. 1998](#)).

1983 In the cohort study of Rai, PCOS morphology was not predictive of live birth in women with RPL, live  
 1984 birth rate was 60.9% in women with PCOS and 58.5% in women without PCOS ([Rai et al. 2000](#)). Similar  
 1985 findings were reported in a smaller cohort study of 17 women with PCOS and 31 women without PCOS  
 1986 ([Liddell et al. 1997](#)).

1987 Insulin metabolism

1988 Several markers for insulin metabolism have been assessed in women with RPL and controls, including  
 1989 fasting insulin (FI), fasting glucose (FG), the fasting glucose to insulin ratio (FG/FI), and insulin resistance  
 1990 (IR). Insulin resistance is a condition in which the efficacy of insulin in promoting the absorption and

utilization of glucose by organs, tissues, and cells is lower than normal. Individuals with IR show glucose levels that are either normal or high, and insulin levels that are more or no less than normal ([Wang et al. 2011](#)). Studies have used different definitions for Insulin resistance, including a fasting insulin level  $>20 \mu\text{U/ml}$  or a fasting glucose to insulin ratio of  $<4.5$ . The homeostatic model assessment insulin resistance (HOMA-IR) index is a quantitative assessment of the contributions of insulin resistance and deficient  $\beta$ -cell function to the fasting hyperglycemia, calculated by comparing the patient's fasting values with the model's predictions.

Insulin resistance, calculated via the HOMA-IR index, FI and FG were evaluated in 65 women with idiopathic RPL and 53 fertile controls with no pregnancy losses. HOMA-IR index (2.98 versus 2.69) and FI (15.24 versus 12.83) were significantly higher in the RPL patients, FG was significantly higher in the control group (85.6 versus 79.8) ([Ispasoiu et al. 2013](#)).

In the case-control study of Maryam, FG, FI, FG to FI ratio and IR were measured in 50 women with RPL and compared to 50 controls. The differences in the frequency of FG, FI and FG to FI ratio were not significantly different between women with RPL and controls. IR was detected in 24% of the women with RPL as compared to 8% of the controls (OR 3.6; 95% CI 1.1-12.3) ([Maryam et al. 2012](#)).

In another case-control study, insulin resistance was also more prevalent in 74 women non-pregnant, nondiabetic women with RPL as compared to 74 parous women with no RPL (27.0% versus 9.5%; OR 2.55; 95% CI 1.40-90.1). The groups had similar FG levels, FI levels and FG/FI ratios. ([Craig et al. 2002](#)).

Another test used for glucose metabolism is the glucose tolerance test. The prevalence of an abnormal test result for the oral glucose tolerance test was higher in 164 women with RPL compared to 74 controls who had previously at least two normal full term pregnancies (17.6% versus 5.4%). Two women had a GTT result of more than 200 mg/dl and were diagnosed with diabetes mellitus ([Zolghadri et al. 2008](#)). Similarly, Wang and colleagues showed that the 1-, 2-, and 3-hour plasma glucose and insulin levels after OGTT (measured in early pregnancy) were significantly higher in women with RPL (more than 2 PLs) as compared to controls who were early in their pregnancy and who did not have a history of an unhealthy pregnancy ([Wang et al. 2011](#)). No statistically significant differences were found in the FG, FI, HOMA-IR, and HOMA- beta between the patient and control groups.

### PCOS and insulin metabolism

A retrospective case-control study comparing the characteristics of RPL women with PCOS (n=126) and without PCOS (n=117) described significantly higher BMI, LH/FSH ratio, post-prandial blood sugar, HOMA-IR and homocysteine levels in women with PCOS compared to those without PCOS. There was no difference in prolactin, TSH, or FG ([Chakraborty et al. 2013](#)).

Another case-control study by Kazerooni compared several parameters in four groups of 60 women: PCOS with RPL, RPL without PCOS, PCOS without RPL, and women without RPL or PCOS. They found the highest levels for fasting insulin in women with PCOS and RPL; and significantly lower levels in all other groups. For the Quantitative Insulin Sensitivity Check Index (calculated  $1/\log(\text{FI})+\log(\text{FG})$ ), the lowest index was found in women with PCOS with RPL, with significantly higher levels in all other groups. There was no significant difference in fasting insulin or the Quantitative Insulin Sensitivity Check Index between women with RPL and without RPL (both without PCOS). For women with PCOS, FI was higher and the Quantitative Insulin Sensitivity Check Index was lower in women with RPL compared to those without RPL ([Kazerooni et al. 2013](#)).

A recent case-control study found higher levels of maternal serum fructosamine (a marker of glycemic control) in women with RPL (n=117) as compared to controls, which could indicate an association between subclinical glucose intolerance and RPL, although this needs confirmation (Romero et al. 2016).

## Recommendation

Assessment of PCOS, fasting insulin and fasting glucose is not recommended in women with RPL to improve next pregnancy prognosis.

Strong

⊕⊕○○

## Justification

	Association	Contributing factor	Prognosis	Treatment
PCOS	YES	YES	NO	Metformin for sporadic PL no studies for RPL
Insulin resistance*	YES (OR 3.6)	Unclear	No studies	No studies
Fasting insulin	Inconsistent (2 YES, 1 NO)	Unclear	No studies	No studies
Fasting glucose	NO	NO	No studies	No studies
<i>* IR calculated based on fasting insulin and fasting glucose</i>				

Insulin resistance is shown to be more prevalent in women with a history of RPL than in women without RPL. The mechanism of how insulin resistance can result in pregnancy loss is unknown, and to our knowledge has not been described. In addition, we did not find any studies on the prognostic potential.

## 7.3 HYPERPROLACTINEMIA

Prolactin is a hormone, essential to female reproduction. Prolactin may play an important role in maintaining corpus luteum function and progesterone secretion, although the mechanism is still unclear (Li et al. 2013).

## Evidence

One case-control study reported RPL to be associated with abnormalities in prolactin secretion during the follicular phase, after finding higher mean concentrations of prolactin in 42 non-pregnant women with a history of RPL as compared to 42 nulligravid females with tubal or male factor infertility without miscarriage ( $14.2 \pm 6.7$  ng/ml versus  $10.5 \pm 3.5$  ng/ml; 95% CI 0.8-6.1) (Bussen et al. 1999).

In contrast, a cross-sectional descriptive study found no difference in basal serum prolactin (evaluated with the thyrotrophin-releasing hormone (TRH) test) in 69 women with RPL compared to 31 women with primary infertility or 30 fertile women. Also the prevalence of hyperprolactinemia, defined as basal

serum prolactin  $\geq 15$  ng/ml was similar in RPL women (15/69; 21.7%) as compared to infertile women (13/31; 41.9%) and fertile controls (5/30; 16.7%) ([Triggianese et al. 2015](#)).

Li and colleagues found hyperprolactinemia in three of 174 women with unexplained RPL, the other women had prolactin levels within the normal range ( $<660$  mIU/l). In the same study, the prognostic potential of prolactin was evaluated in 109 RPL women; those who miscarried had significantly lower serum prolactin concentrations (adjusted OR 0.99; 95% CI 0.97-0.99 after adjustment for age) compared to those who had a live birth. They concluded that lower basal serum prolactin concentrations were associated with an increased risk of miscarriage in a subsequent pregnancy in women with unexplained RPL ([Li et al. 2013](#)).

Prolactin levels are often measured for assessment of ovulatory dysfunction.

## Recommendation

**Prolactin testing is not recommended in women with RPL in the absence of clinical symptoms of hyperprolactinemia (oligo/amenorrhea).**

Conditional ⊕⊕○○

## Justification

	Association	Contributing factor	Prognosis	Treatment
<b>Prolactin</b>	Inconsistent results	No data	Possible	Yes

Studies have been performed on serum and endometrial prolactin with the aim of clarifying the association with RPL. However, most of the studies retrieved were of poor quality and many did not include a control group. It was concluded that in the absence of consistent evidence on an association between prolactin and RPL, prolactin testing is not routinely recommended.

Prolactin disorders are possibly associated with PCOS, luteal phase deficiency, stress and obesity, which further complicates studies attempting to find a direct link between prolactin and RPL.

## 7.4 OVARIAN RESERVE TESTING

### Evidence

From the association between advanced maternal age and RPL, it is suggested that diminished ovarian reserve could be a causative or prognostic factor in RPL.

Ovarian reserve can be assessed with measurements of FSH, estrogen (E2), inhibin B, and anti-Müllerian hormone (AMH), or ultrasound investigation to determine antral follicle count (AFC) and ovarian volume.

In a meta-analysis, 15 studies (3082 women) investigating an association between ovarian reserve – measured by AMH, AFC, FSH, LH, estradiol or FSH:LH ratio – and RPL were summarised ([Bunnewell et al. 2020](#)). The reviewers found that more women with RPL seemed to have diminished ovarian reserve as compared to controls (AMH, OR = 2.77, 95% CI 1.41–5.46; AFC, OR = 2.45, 95% CI, 1.16–5.19),

suggesting an association between diminished ovarian reserve and RPL. The reviewers further concluded that more studies are needed to make any conclusions on the prognostic value in the management of women with RPL

There is growing evidence that diminished ovarian reserve is associated with RPL. In a meta-analysis, ovarian reserve tests were evaluated to screen for an association between diminished ovarian reserve and higher odds for RPL, but due to the lack of standardized reporting thresholds, conclusions are difficult to make (Bunnewell et al. 2020).

A cohort study compared the results of ovarian reserve tests (FSH and E2 on Day 3, FSH on Day 10, and clomiphene citrate challenge test (CCCT)) between 44 women with RPL and 648 infertile controls (without a history of RPL). Day 3 FSH was lower in women with RPL compared to the controls, while the results for the CCCT, E2 and FSH on Day 10 were similar between the groups. The incidence of diminished ovarian reserve in women with RPL was 18%. Delivery rates after 1-year follow-up were similar between the groups and poor in women with an abnormal CCCT test (0/8 RPL women and 5/117 controls) (Hofmann et al. 2000). Incidence of diminished ovarian reserve in RPL was 18%.

In contrast, no difference was found in FSH levels, measured in early follicular phase, between 42 women with RPL and 42 controls with male or tubal infertility (Bussen et al. 1999).

No difference for AMH, inhibin B, FSH, LH, E2 (day 2-3) or FSH, LH, E2 and P (day 8-9) was found in a study comparing 34 women with RPL (both explained and unexplained) with 10 controls with no history of pregnancy loss and a normal menstrual cycle (Prakash et al. 2006).

## Recommendation

**Ovarian reserve testing is not routinely recommended in women with RPL.**

Strong

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## Justification

	Association	Contributing factor	Prognosis	Treatment
<b>Ovarian reserve</b>	Yes	Unclear	Abnormal CCCT = poor LBR	No studies

To date, there is still no universally accepted definition of diminished ovarian failure, hindering the improvement of knowledge for women with RPL.

Several studies have attempted to evaluate ovarian reserve. Based on a recent meta-analysis (Bunnewell et al. 2020), low ovarian reserve could be indicative of a less favorable prognosis in women with RPL. However, there is no good test for diminished ovarian reserve and conclusions are difficult to make.

Even though there are no studies showing an association between RPL (or miscarriage) and Premature Ovarian Insufficiency (POI), a proportion of women referred for RPL show signs of POI. In case of a suspicion of POI, ovarian reserve testing can be used for diagnosis of POI and for estimating the future chances of a live birth for these couples.

## 7.5 LUTEAL PHASE INSUFFICIENCY

Luteal phase insufficiency is described as a condition of insufficient progesterone exposure to maintain a regular secretory endometrium and is allowed for normal embryo implantation and growth ([Palomba et al. 2015](#)). Progesterone is essential for secretory transformation of the endometrium that permits implantation as well as maintenance of early pregnancy. Luteal phase insufficiency can be caused by several endocrinopathies, including stress, PCOS, and prolactin disorders ([Ke 2014](#)).

### Evidence

The assessment of a possible association between luteal phase insufficiency and RPL is hampered by the diagnostic criteria for luteal phase insufficiency. The sensitivity and specificity of common clinical tests used for the diagnosis of luteal phase insufficiency were compared in 19 women with infertility or RPL and 15 normal controls. The recommended test for the determination of luteal phase insufficiency is a midluteal phase single serum Progesterone (P) level  $<10$  ng/mL or the sum of three serum P levels that is  $<30$  ng/mL. Timed endometrial biopsy (performed at late luteal phase) was found to have marginally acceptable sensitivity and specificity. Low sensitivity and/or specificity were found for the appearance of basal body temperature charts, luteal phase length  $\leq 11$  days, and preovulatory follicle diameter ([Jordan et al. 1994](#)). Other authors have questioned midluteal phase progesterone level as the recommended test for luteal phase insufficiency, as secretion is pulsatile and levels vary significantly over a short amount of time ([Shah, Nagarajan 2013](#)). Salivary P assay was unable to diagnose LPD ([Tulppala et al. 1991](#)).

The frequency of luteal phase insufficiency as an etiologic factor has been assessed in uncontrolled studies. In a cohort study, a luteal phase defect, measured by endometrial biopsy, was detected in 38.6% (32/83) of the women with RPL ([Badawy, Westpfal 2000](#)). In a prospective cohort study, a luteal phase defect, defined as two late luteal phase endometrial biopsies with maturation delay of  $>3$  days, was detected in 17.2% (34/197) of women with three or more consecutive and euploid PLs ( $<20$  weeks) ([Stephenson 1996](#)).

Despite the diagnostic problems and different tests available, research has attempted to assess a possible link between luteal phase insufficiency and RPL. Two out of three controlled studies of acceptable quality failed to confirm an association between luteal phase insufficiency and RPL. Jordan and colleagues found a luteal phase defect, defined as integrated P  $<80$  ng x days/mL, in one of three women with RPL and two of 15 (13%) normal controls ([Jordan et al. 1994](#)). Li and colleagues found a luteal phase defect, defined as midluteal P  $<30$  nmol/L, in 27% of 122 women with RPL and in 11% of 18 fertile controls ([Li et al. 2000](#)). Balasch and colleagues found luteal phase insufficiency, diagnosed by endometrial biopsy, in 28.3% of 60 women with RPL, which was significantly more than in controls (4% in 25 fertile women and 12.9% in 355 infertile patients) ([Balasch et al. 1986](#)).

Finally, luteal phase insufficiency, defined as midluteal phase single serum P level  $<10$  ng/mL, was found to be not associated with the outcome of the next pregnancy. Of the 197 women with a history of two consecutive first trimester pregnancy losses, 38 (19.3%) suffered another pregnancy loss. There was no difference in the incidence of another PL between women without or with luteal phase deficiency (20.5% (31/151) and 15.2% (7/46), respectively) ([Ogasawara et al. 1997](#)).

## 2155 Recommendation

Luteal phase insufficiency testing is not recommended in women with RPL.

Strong

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## 2156 Justification

	Association	Contributing factor	Prognosis	Treatment
Luteal phase insufficiency testing*	Inconsistent	No data	No	possible

\* *midluteal progesterone or endometrial biopsy*

2157 Based on inconsistent evidence of an association, and no clear value for prognosis and treatment, the  
 2158 GDG decided not to recommend luteal phase insufficiency testing. The only study evidence for benefit  
 2159 of treatment of women with RPL and luteal phase insufficiency was small, not designed to evaluate  
 2160 treatment, and used different treatments ([Balasch et al. 1986](#)).

## 2161 7.6 ANDROGENS

2162 Elevated androgen levels are associated with the retardation of endometrial development in luteal  
 2163 phase, and have been assessed as a possible cause of (recurrent) pregnancy loss.

## 2164 Evidence

2165 Three case-control studies of acceptable quality show inconsistent results for an association of  
 2166 testosterone and RPL. Testosterone and androstenedione levels were significantly higher in 42  
 2167 women with RPL compared to 18 fertile controls without a history of RPL ([Okon et al. 1998](#)). Similarly,  
 2168 testosterone levels were significantly higher in 21 women with unexplained RPL compared to 10  
 2169 multiparous women ([Watson et al. 1993](#)). However, in the study of Kazerooni, testosterone levels were  
 2170 not significantly different in 60 women with RPL and 60 healthy controls without a history of pregnancy  
 2171 loss ([Kazerooni et al. 2013](#)).

2172 Two prognostic studies found no association between testosterone levels and the pregnancy outcome  
 2173 (LBR) in the next pregnancy ([Rai et al. 2000](#); [Nardo et al. 2002](#)).

2174 One study showed a prognostic relevance for the free androgen index (FAI = testosterone\*100/ sex  
 2175 hormone-binding globulin [SHBG]). An elevated FAI (>5) was detected in 49 of 437 women with RPL  
 2176 (11%). The miscarriage rate was significantly increased in RPL women with elevated FAI as compared  
 2177 to women with normal FAI (68% [23/34] vs 40% [91/229]) ([Cocksedge et al. 2008](#)).

## 2178 Recommendation

Androgen testing is not recommended in women with RPL.

Strong

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2179

2180

2181

## Justification

	Association	Contributing factor	Prognosis	Treatment
Androgens (Testosterone)	Inconsistent (2 YES vs 1 NO)	/	No	/
Elevated FAI*	/	/	Possible	/

\*Free androgen index

Based on inconsistent evidence of an association, and no potential effect on prognosis or treatment, androgen testing is not recommended.

## 7.7 VITAMIN D

## Evidence

Vitamin D deficiency has been studied extensively in relation to obstetric complications and was described as a risk factor for gestational diabetes, small for gestational age infants and preeclampsia in systematic reviews ([Aghajafari et al. 2013](#)).

Very few studies have assessed vitamin D in women with RPL and the results for an association between vitamin D deficiency and pregnancy loss are less consistent.

In a case-control study, evidence for vitamin D deficiency (<30 ng/ml) was detected in 47.4% of 133 women with RPL. In addition, decreased vitamin D level was associated with the increased prevalence of antiphospholipid antibody, antinuclear antigen antibody (ANA), anti-ssDNA, and anti-thyroid peroxidase antibody (TPOAb), and with higher peripheral blood CD19+ B and CD56+ NK cell levels and NK cytotoxicity ([Ota et al. 2014](#)). A study of the same research team suggest that vitamin D has immune regulatory effects on NK cell cytotoxicity, cytokine secretion and degranulation ([Ota et al. 2015](#)).

In an attempt to clarify the role of vitamin D in the complex immunoregulation at the foetal-maternal interface and the potential benefit of vitamin D supplementation in RPL, two studies have explored differences in the expression of Vitamin D Receptor and 25-hydroxyvitamin D<sub>3</sub>-1 $\alpha$ -hydroxylase (CYP27B1) (mRNA and protein) in chorionic villi and decidua of women with RPL. They reported a lower expression of Vitamin D Receptor and 25-hydroxyvitamin D<sub>3</sub>-1 $\alpha$ -hydroxylase in women with RPL compared with the normal pregnant women ([Wang et al. 2016](#); [Yan et al. 2016](#)).

## Conclusion

Even though one study showed a significant prevalence of vitamin D deficiency in women with RPL, there are no indications that vitamin D status is a contributing factor for RPL. Moreover, vitamin D deficiency was shown to be associated with several obstetric and foetal complications, but there is no report of an association between vitamin D status and miscarriage, and hence testing of vitamin D status is not recommended for women with RPL. Irrespective of RPL, vitamin D supplementation is nowadays frequently prescribed in pregnant women (see chapter 14.6 for more details).

	Association	Contributing factor	Prognosis	Treatment
Vitamin D	Possible	Possible	/	Vitamin D supplementation

2212

2213 **7.8 LUTEINIZING HORMONE (LH)**

2214 High serum concentrations of luteinizing hormone (LH) ( $\geq 10$  IU/L) in the early to mid-follicular phase,  
 2215 with or without PCOS, have been associated with an increased prevalence of pregnancy loss in several  
 2216 reports, both after spontaneous conception and ART ([Kaur, Gupta 2016](#)).

2217 **Evidence**

2218 An association between pre-pregnant elevated LH and pregnancy loss was found in a small  
 2219 observational study of 30 women with RPL and 17 women with at least one successful pregnancy and  
 2220 no history of PL. Elevated LH serum ( $\geq 10$  IU/l) was found in nine (30%) women with RPL, compared to  
 2221 one (1.8%) of the controls. Furthermore, the live birth rate was significantly lower in women with  
 2222 elevated LH (2/6; 33%) compared to women with normal LH (15/16; 71%) ([Regan et al. 1990](#)).

2223 In the comparative case-control study of Kazerooni, several parameters were assessed in four groups  
 2224 of 60 patients: PCOS with RPL, RPL without PCOS, PCOS without RPL, and women without RPL or PCOS.  
 2225 LH serum levels, FSH serum levels and LH/FSH ratio were significantly higher in women with RPL and  
 2226 PCOS as compared to women without RPL or PCOS, or women with RPL without PCOS. Serum levels  
 2227 were similar in women with RPL without PCOS and controls (women without RPL or PCOS), indicating  
 2228 an association of LH, FSH and LH/FSH with PCOS rather than with RPL ([Kazerooni et al. 2013](#)). Similarly,  
 2229 no differences for LH (day 2-3) or LH (day 8-9) were found between 34 women with RPL (both explained  
 2230 and unexplained) and 10 controls with no history of pregnancy loss and a normal menstrual cycle  
 2231 ([Prakash et al. 2006](#)).

2232 Urinary LH levels exceeding the normal range at one or more stages of the cycle were detected in 16  
 2233 of 21 (76%) women with RPL. The excessive secretion of LH in the pregnancy loss group was most  
 2234 marked in the early luteal phase (days +3 to +6),  $249 \pm 135$  IU/l versus  $126 \pm 62$  in 10 multiparous women.  
 2235 Serum LH or FSH levels were not different at either stage of the cycle ([Watson et al. 1993](#)).

2236 In the cohort study of Sagle, 46 (82%) of the 56 women with RPL had polycystic ovaries compared with  
 2237 two (18%) of the 11 parous women. None of the RPL women or controls showed elevated serum LH  
 2238 levels ([Sagle et al. 1988](#)).

2239 In contrast to the study of Regan, no prognostic potential for elevated LH was detected in two other  
 2240 studies. Rai and colleagues found no difference in the live birth rate for RPL women with elevated LH  
 2241 ( $\geq 10$  IU/l), compared to women with normal serum LH levels (72% [38/53] versus 58% [252/433]) ([Rai](#)  
 2242 [et al. 2000](#)). Similar results were found in a cohort of 37 women with RPL (LBR 39% versus 42%) ([Carp](#)  
 2243 [et al. 1995](#)).

## 2244 Recommendation

LH testing is not routinely recommended in women with RPL

Strong

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## 2245 Justification

	Association	Contributing factor	Prognosis	Treatment
<b>Elevated LH (serum)</b>	Inconsistent (1 YES vs 3 NO)	/	Inconsistent (1 YES vs 2 NO)	No studies

2246

2247 There is inconsistent evidence, and therefore it is not recommended to routine perform LH testing in  
2248 women with RPL.

2249 **7.9 HYPERHOMOCYSTEINEMIA**

2250 Hyperhomocysteinemia (HHcy), defined as elevated plasma levels of homocysteine (Hcy), is described  
2251 as a risk factor for venous thromboembolism, and adverse pregnancy outcomes (neural tube defects,  
2252 pre-eclampsia, and placental abruption).

2253 Plasma homocysteine levels are determined by several factors, including blood levels of vitamin B6,  
2254 vitamin B12, folate, MTHFR mutations, increased age, and hypothyroidism ([Hague 2003](#)), which have  
2255 all been suggested to be associated with RPL.

## 2256 Evidence

2257 Hyperhomocysteinemia was found to be associated with RPL. In a meta-analysis of case-control studies,  
2258 and association was found between RPL and fasting plasma homocysteine (Hcy) levels (OR 2.7; 95% CI  
2259 1.4-5.2; 3 studies; n=652) and afterload Hcy (measured after methionine loading) (OR 4.2; 95% CI 2.0-  
2260 8.8; 4 studies; n=580) ([Nelen et al. 2000](#)).

2261 Several studies have reported conflicting results. In a small study, fasting total plasma Hcy levels were  
2262 higher in 20 women with RPL ( $19.2 \pm 6.14 \mu\text{M}$ ) and 20 women with unexplained infertility ( $21.05 \pm$   
2263  $8.78 \mu\text{M}$ ) compared to healthy controls ( $7.85 \pm 3.31 \mu\text{M}$ ;  $p < 0.05$ ). The same study reported similar levels  
2264 of vitamin B12 and reduced folate concentrations in patients versus controls ([D'Uva et al. 2007](#)). In a  
2265 case-control study including 107 women with unexplained RPL and 343 fertile controls, HHcy was found  
2266 to be significant risk factors for RPL (OR=7.02; 95% CI 3.85-12.80). However, this study found also an  
2267 association for vitamin B12 deficiency with RPL (OR 16.39; 95% CI 7.71-34.80), while folate deficiency  
2268 was more common in controls (63.47%) as compared to the women with RPL (2.56%) (OR 0.015; 95%  
2269 CI 0.0036-0.064) ([Puri et al. 2013](#)).

2270 In a large case-control study of postpartum patients who had a history of vascular-related pregnancy  
2271 complications, 569 patients experienced recurrent early pregnancy loss. Associations were detected of  
2272 Hcy levels with pregnancy-induced hypertension, abruption placentae and Intrauterine growth  
2273 retardation, but these associations were no longer significant after correction for time interval  
2274 (between delivery and testing) and maternal age ([Steegers-Theunissen et al. 2004](#)). In another case-  
2275 control study, no significant differences were observed neither in plasma Hcy levels, red blood cell,

folate or vitamin B12 serum levels between 60 women with unexplained RPL and 30 healthy, fertile controls ([Creus et al. 2013](#)). Similar results were reported by Zammiti and colleagues, concentrations of total plasma Hcy were comparable in 350 women with RPL and 200 healthy controls ( $10.80 \pm 7.94$  versus  $8.72 \pm 6.86$   $\mu\text{mol/ml}$ ) ([Zammiti et al. 2008](#)). Alonso and colleagues diagnosed 2 out of 75 women with RPL and HHcy without an MTHFR mutation and without vitamin defects (vitamin B6, B12, and folic acid), while HHcy was not detected in 75 controls ([Alonso et al. 2002](#)).

Also, no difference was detected in the prevalence of elevated Hcy levels ( $<12$   $\mu\text{mol/l}$ ) when comparing women with primary versus secondary RPL (2.1% versus 3.0%), or when comparing women with 2PLs to women with 3 or more PLs (3.0% versus 1.3%) ([Lee et al. 2016](#)).

Hyperhomocysteinemia has also been suggested as a factor in the link between PCOS and RPL. Two recent studies reported that HHcy was associated with RPL in patients with PCOS. The incidence of HHcy was significantly higher in RPL-affected PCOS (70.63%,  $n=126$ ) patients, compared to in women with RPL without PCOS (57.26%,  $n=117$ ;  $p<0.04$ ) ([Chakraborty et al. 2013](#)). In the study of Kazerooni, mentioned before, women with RPL and PCOS had significantly higher levels of Hcy ( $12.4 \pm 1.6$ ;  $n=60$ ) compared to women with PCOS and without RPL ( $7.3 \pm 1.1$ ;  $n=60$ ), women with RPL and without PCOS ( $9.65 \pm 0.9$ ;  $n=60$ ), and controls ( $6.7 \pm 1.9$ ;  $n=60$ ) ([Kazerooni et al. 2013](#)). In contrast, the prevalence of elevated Hcy levels was comparable between 92 women with RPL and PCOS (8.7%), compared to 92 women with RPL without PCOS (7.6%) ([Moini et al. 2012](#)).

Finally, one case-control study explored paternal homocysteine levels, and reported an association between paternal HHcy and RPL with mean concentrations of  $19.6 \pm 9.5$   $\mu\text{mol/l}$  in 140 men of couples with RPL and  $14.2 \pm 7.4$   $\mu\text{mol/l}$  in 140 fathers of healthy controls couples (OR 6.92; 95% CI 3.90–12.29). The risk of RPL associated with paternal HHcy could be due to its effect on sperm quality by increasing DNA damage ([Govindaiah et al. 2009](#)).

## Recommendation

Measurement of homocysteine plasma levels is not routinely recommended in women with RPL.

Strong ⊕○○○

## Justification

	Association	Contributing factor	Prognosis	Treatment
Hyperhomocysteinemia	Inconsistent	Possible in PCOS	No data	(high-dose) folic acid and vit B6 LMWH + aspirin

There is inconsistent evidence for an association of elevated Hcy levels with RPL. The impact of pregnancy and several lifestyle factors (vitamin intake and deficiency (vitamin B6, B12, folate), smoking, coffee and alcohol consumption, physical activity) on plasma Hcy levels further complicates research on the topic. Furthermore, we realize that there is a geographical and ethnic variation in the genetic pathways of the homocysteine metabolism ([Wilcken et al. 2003](#); [Binia et al. 2014](#)).

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## 8. Anatomical investigations

### KEY QUESTION: WHAT IS THE VALUE OF ANATOMICAL INVESTIGATIONS IN THE DIAGNOSIS OF RPL?

#### 8.1 CONGENITAL UTERINE MALFORMATIONS

##### Evidence

An association between congenital uterine malformations and recurrent pregnancy loss (RPL) has been well documented, but the exact prevalence in this population has not been clearly defined ([Saravolos et al. 2008](#)). Potentially relevant congenital Müllerian tract malformations include septate uterus, bicornuate uterus with normal cervix (AFS bicornuate uterus), bicornuate uterus with double cervix (AFS didelphic uterus) and hemi-uterus (AFS unicornuate uterus). The prevalence of uterine malformations is higher in women having a history of RPL (13.3%; 95% CI 8.9-20) than in the general/fertile population (5.5%; 95% CI 3.5-8.5). The prevalence of uterine malformations diagnosed with optimal test was similar in women with three or more losses (15.4%; 95% CI 10.3- 23) compared to women with two or more losses (10.9%; 95% CI 3.6-33.3) ([Saravolos et al. 2008](#); [Chan et al. 2011b](#)).

Two systematic reviews have also reported a higher prevalence of miscarriage in women with congenital uterine malformations compared to controls ([Chan et al. 2011a](#); [Venetis et al. 2014](#)). In a meta-analysis of comparative studies, women with septate uterus (RR 2.65, 95% CI 1.39-5.09, based on 6 studies,  $I^2=93\%$ ) and bicornuate uterus (RR 2.32, 95% CI 1.05-5.13,  $I^2=87\%$ ) had an increased probability of first-trimester PL, compared to their controls. Women with arcuate uterus (RR 2.27, 95% CI 0.64-7.96, based in 4 studies,  $I^2=0\%$ ), septate uterus (RR 2.95, 95% CI 1.51-5.77, based on 5 studies,  $I^2=39\%$ ) and bicornuate uterus (RR 2.90, 95% CI 1.56-5.41, based on 4 studies,  $I^2=0\%$ ) had an increased probability of second-trimester PL, compared to their controls ([Venetis et al. 2014](#)).

##### *Diagnosis of congenital uterine malformations*

Based on the higher prevalence of uterine malformations in women with RPL, diagnostic imaging of the uterus should be considered in women with RPL (primary or secondary) ([Jaslow, Kutteh 2013](#); [Elsokkary et al. 2018](#)).

Imaging for detection of uterine malformations has been performed with a range of different techniques, all with different potential and limitations for diagnosing the various types of malformations. An ESHRE consensus for diagnosis of congenital uterine malformations was published in 2016 ([Grimbizis et al. 2016](#)).

In the review by Saravolos, combined hysteroscopy and laparoscopy have been considered the gold standard in diagnosing uterine malformations, because they allow for a direct visualization of the internal and external contour of the uterus ([Saravolos et al. 2008](#)). The main disadvantage of hysteroscopy is the invasiveness of the procedure, although nowadays it can be performed in an office setting under local anaesthetics.

Sonohysterography (or hysterosonography) (SHG) appears a safe procedure which provides more information about uterine abnormalities than hysterosalpingography (HSG) or ultrasound (US) alone ([Tur-Kaspa et al. 2006](#)). SHG is accurate in diagnosing and classifying congenital uterine malformations

([Ventolini et al. 2004](#); [Valenzano et al. 2006](#)). In addition, SHG has a higher sensitivity and specificity than HSG or diagnostic hysteroscopy to diagnose uterine malformations in general ([Ludwin et al. 2011](#)). SHG uses the introduction of fluid (saline or contrast) into the uterine cavity to enhance US imaging studies, which could be uncomfortable for women. The diagnosis of septate uterus by SHG eliminates the need to perform laparoscopy prior to hysteroscopic metroplasty ([Ludwin et al. 2011](#)).

Three-dimensional US allows visualization of the internal and external contour of the uterus, has high sensitivity and specificity, and it is non-invasive ([Saravolos et al. 2008](#); [Caliskan et al. 2010](#)). It appears to be very accurate for the diagnosis and classification of congenital uterine malformations and may conveniently become the only mandatory step in the assessment of the uterine cavity in women with a history of RPL, although further studies are required for confirmation ([Ghi et al. 2009](#)).

Two-dimensional US and hysterosalpingography (HSG) are non-invasive and widely available. Two-dimensional US has a low sensitivity, but a high specificity for diagnosis of malformations. HSG has a good sensitivity for diagnosing more pronounced uterine malformations, but it is limited in differentiating between the types of malformations ([Saravolos et al. 2008](#)). Overall, 2D transvaginal ultrasound (TV-US) and HSG are suboptimal to diagnose uterine malformations, based on a poor accuracy and limited potential in classifying malformations, especially when used as the only diagnostic technique ([Saravolos et al. 2008](#)). We found no data on differences between contrasts (gel and saline) used during ultrasound.

Magnetic resonance imaging (MRI) has been proposed as an optimal test that allows a simultaneous assessment of the cavity and fundus of the uterus, although controversy exist in whether MRI can replace combined hysteroscopy and laparoscopy ([Chan et al. 2011b](#)). The accuracy and practicality of MRI has not yet been determined for the diagnosis of uterine malformations ([Oppelt et al. 2007](#); [Saravolos et al. 2008](#)). MRI can be used to extend the examination to the abdomen, which could be helpful in the detecting renal malformations that are frequently associated with uterine malformations ([Oppelt et al. 2007](#); [Hall-Craggs et al. 2013](#)).

Sono-Embryoscopy and Uterine Doppler US have been suggested for the investigation of uterine malformations in women with RPL, but there is not enough evidence to support these techniques in the routine investigation of RPL ([Frates et al. 1996](#); [Ferreira et al. 2007](#); [Robberecht et al. 2012](#)).

Cervical weakness is a recognized cause of second-trimester pregnancy loss, but the true incidence is unknown, since the diagnosis is essentially a clinical one ([Kassanos et al. 2001](#); [Harger 2002](#); [Liddell, Lo 2008](#)). The diagnosis is usually based on a history of second-trimester miscarriage preceded by spontaneous rupture of membranes or painless cervical dilatation. There is currently no objective test able to identify women with cervical weakness in the non-pregnant state.

## 8.2 ACQUIRED UTERINE MALFORMATIONS

Acquired uterine malformations (submucous myomas, endometrial polyps and uterine adhesions) have been found prevalent in women that suffered pregnancy loss, but the clinical relevance is unclear ([Hooker et al. 2014](#)).

In a study of Jaslow, acquired defects were found in 113 women with RPL (12.9%), congenital defects in 61 women (7.0%), and 5 women (0.6%) had both congenital and acquired defects ([Jaslow, Kuttteh 2013](#)). Saravolos and colleagues reported fibroids in 8.2% (79/966) of women with RPL ([Saravolos et al. 2011](#)).

Other uterine pathologies should also be assessed in anatomical investigations such as chronic endometritis and adenomyosis. Several papers showed that chronic endometritis (CE) is more prevalent in infertile patients and may be another reason for RPL (see chapter 1.4). Adenomyosis was shown to be associated with higher rates of pregnancy loss (Younes,Tulandi 2017; Stanekova et al. 2018).

#### *Diagnosis of acquired uterine malformations*

Although the relevance of acquired uterine malformations and uterine pathologies in RPL is unclear, these malformations can be diagnosed with imaging techniques used in the detection of congenital malformations.

2D US is not a sensitive method to detect uterine adhesions. When suspected, a hysteroscopy has to be performed (Bohlmann et al. 2010).

Submucosal fibroids and endometrial polyps can be detected with 3D US, SHG, 2D US, or HSG. There is no strong evidence on which technique is preferred. Hysteroscopy is considered the gold standard (Makris et al. 2007).

Both magnetic resonance imaging and ultra-sound are non-invasive tests with equivalent accuracy in diagnosing adenomyosis (area under curve 0.91 and 0.88, respectively) (Maheshwari et al. 2012).

Hysteroscopy can show some suspicious signs for the diagnosis of chronic endometritis, but gold standard for diagnostic of chronic endometritis seems to be immunohistochemistry (CD 138) (Rimmer et al. 2021).

#### Recommendations (updated 2022)

All women with RPL should have an assessment of the uterine anatomy.

Strong

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The preferred technique to evaluate the uterus is transvaginal 3D US, which has a high sensitivity and specificity, and can distinguish between septate uterus and bicornuportal uterus with normal cervix (former AFS bicornuate uterus).

Conditional

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Sonohysterography (SHG) is more accurate than HSG in diagnosing uterine malformations. It can be used to evaluate uterine morphology when 3D US is not available, or when tubal patency has to be investigated.

Conditional

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If a Müllerian uterine malformation is diagnosed, further investigations (including investigation of the kidneys and urinary tract) should be considered.

Conditional

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MRI is not recommended as first line option for the assessment of uterine malformations in women with RPL, but can be used where 3D US is not available.

Conditional ⊕⊕○○

All women with RPL should have 2D-ultrasound to rule out adenomyosis.

Conditional ⊕⊕○○

#### Justification

From the evidence, it can be concluded that congenital uterine malformations are more prevalent in women with RPL, as compared to controls. However, the exact contribution that congenital uterine malformations make to RPL remains unclear; the reported variability in the prevalence reflects the differences in the diagnostic criteria and techniques, and the lack of homogeneity in the definition of RPL. Growing evidence showed that chronic endometritis and adenomyosis are associated with pregnancy loss and need to be rule out in all women with RPL.

	Association	Contributing factor	Prognosis	Treatment
<b>Congenital uterine malformations</b>	Yes	Suggested some malformations	/	Surgical trials in case of a septate uterus
<b>Acquired uterine malformations</b>	Yes	Unclear	/	Unclear

The recommendation of uterine assessment in all women with RPL is consistent with the Thessaloniki ESHRE/ESGE consensus on diagnosis of female genital malformations, which classifies women with RPL as 'high risk' for the presence of a female genital anomaly ([Grimbizis et al. 2016](#)). Transvaginal 3D Ultrasound was reported to have the highest sensitivity and specificity for diagnosing congenital malformations. Based on the higher costs and the absence of a diagnostic benefit compared to 3D US, MRI is not recommended as a first line option, but it can be used in the absence of 3D US, and for surgical planning. Apart from availability, local expertise could be relevant in selecting the diagnostic approach, as most techniques are highly dependent on operator skills.

Data from well-controlled prospective trials are needed to clarify the role of congenital uterine malformations in RPL and predict live birth rates per type of congenital uterine abnormality. Executing such studies is further complicated by difficulties to recruit a high number of eligible patients in a short period of time.

In a study of 202 women with uterine malformations (not RPL), 36% of the women had associated abnormalities, mostly renal, but also cardiac, skeleton and neurological abnormalities were detected ([Oppelt et al. 2007](#)). Another study suggested ultrasound for screening and MRI or CT (computed tomography) scan for confirmation of congenital malformations of the kidneys and upper urinary tract

(Ramanathan et al. 2016). Based on the high prevalence, further investigations should be considered in women with uterine malformations.

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## 9. Male factors

Recurrent pregnancy loss has long been considered an issue stemming exclusively from female causes. If a man achieved a pregnancy, his gametes were deemed normal and any loss of the pregnancy was believed to be from female anomalies, ranging from genetic, endocrinologic or anatomical factors to autoimmune diseases. Although together, these factors only account for an estimated 50-60% of RPL, leaving 40-50% of RPL remaining unexplained. Possible male factors have not been satisfactorily addressed or taken into account in these numbers.

### **KEY QUESTION: DOES THE QUALITY OF THE MALE GAMETES CONTRIBUTE TO RPL?**

#### **Evidence**

A meta-analysis investigating the association of advanced paternal age with spontaneous miscarriage during the first trimester of pregnancy showed that there is an increased risk for miscarriage for male age categories 30-34, 35-39 and 40-44 and this risk was higher for the  $\geq 45$  age category (du Fossé et al. 2020). A meta-analysis investigating the association of advanced paternal age with spontaneous miscarriage during the first trimester of pregnancy showed that there is an increased risk for miscarriage for male age categories 30-34, 35-39 and 40-44 and this risk was higher for the  $\geq 45$  age category (du Fossé et al. 2020). Furthermore, more accurate prediction of future pregnancy outcome in couples with unexplained recurrent pregnancy loss is possible by adding additional (male) predictors. (du Fossé et al. 2022) In a large multicentered, parallel two group randomized trial (HABSelect) study of 2752 couples, Miller and colleagues found that the proportion of couples with clinical pregnancy that ended in miscarriage was lower in the group where sperm for ICSI with less DNA damage had been chosen using hyaluronan adherence (HA selection by PICSI) than in the standard ICSI group. The confidence interval for the absolute difference between groups was narrow (Miller et al, 2019). This reduction in miscarriage by selecting sperm with less DNA damage by PICSI has been observed in previous studies (Worilow et al. 2013; Mokánszki et al. 2014). As miscarriage reduction was a significant secondary outcome of the full HABSelect trial, samples 84 (n=1,247) selected for the mechanistic analysis were deliberately enriched for miscarriage 85 outcomes (n=92 or 7.7%) from a total of 154 miscarriages (5.6%) among all (n=2752) 86 couples randomized by stratified random sampling (West et al, 2022). Older women (>35 years) randomized to the trial's experimental arm had the same live birth rates as younger women, with no increase in miscarriage rates with maternal age. This mechanism for this was most probably the avoidance of sperm with damaged DNA by using HA selection by PICSI (West et al. 2022). Moreover, the effects of male semen quality, occupational exposure, and lifestyle on RPL were examined based on semen analyses and detailed questionnaires from 68 RPL couples and 63 randomly selected healthy controls (Ruixue et al. 2013). Semen from men in the RPL group had significantly reduced viability, normal morphology and total progressive sperm motility and a higher mean percentage of DNA damaged sperm compared with those of controls. In addition, a distinct seminal plasma cytokine profile is associated with male age and lifestyle characteristics in RPL. There is a less favourable pro-inflammatory cytokine expression profile in seminal plasma of men affected by RPL and this is associated with advanced male age and lifestyle risk factors (du Fossé et al. 2022). Furthermore, the risk of RPL was significantly increased when smoking, drinking and occupational exposure to environmental factors were superimposed (OR 11.965; 95% CI 1.49-95.62). It was concluded that in couples with RPL, male factors such as paternal age, sperm quality,

occupational exposure, and lifestyle (smoking, alcohol consumption and soft drugs) should be assessed in addition to female factors ([Anifandis et al. 2014](#); [Jensen et al. 2014](#); [Pacey et al. 2014](#); [Miller et al. 2019](#); [Smits et al. 2019](#); [du Fossé et al. 2020](#); [Montagnoli et al. 2021](#); [du Fossé et al. 2022](#); [West et al. 2022](#))

A systematic review and meta-analysis of 24 case-control and cohort studies on Chinese couples including 1,690 male partners of women with RPL, and 1,337 male partners of fertile control women showed that male partners of women with RPL had a significantly lower level of sperm density (SMD=-0.53, 95%CI: -0.75 to -0.30), sperm viability (standard mean deviation SMD=-1.03, 95%CI: -1.52 to -0.54), sperm progressive motility rate (SMD=-0.76, 95%CI:-1.06 – -0.46), and normal sperm morphology rate (SMD= -0.56, 95%CI: -0.99 to -0.12), and had a significantly higher rate of sperm deformity rate (SMD=1.29, 95%CI: 0.60 – 1.97), and sperm DNA fragmentation index DFI (SMD=1.60, 95% CI: 1.04 to 2.17), when compared with the reference group. The 2 groups had no significant difference of semen volume (SMD=-0.03, 95%CI: -0.14 – 0.08) and semen pH value (SMD= -0.23, 95% CI: -0.50 to 0.05) ([Li et al. 2021](#)). Several other smaller studies have also compared sperm parameters of couples with RPL to fertile controls and found no differences in sperm volume or sperm count ([Sbracia et al. 1996](#); [Gopalkrishnan et al. 2000](#); [Bhattacharya 2008](#); [Brahem et al. 2011](#); [Imam et al. 2011](#); [Talebi et al. 2012](#); [Khadem et al. 2014](#)). The percentage of motile sperm and percentage of sperm with normal morphology were reported to be lower in RPL men in some studies, while others found no difference. Three studies consistently reported higher DNA fragmentation in RPL men. Some studies have suggested a difference in sperm parameters between RPL couples that achieve a successful pregnancy and live birth rate, and couples that experienced an additional pregnancy loss, or failed to achieve pregnancy. One study reported differences in sperm concentration and motility between successful and unsuccessful couples ([Sbracia et al. 1996](#)).

Several studies addressing male factors and RPL have focused on male genetic defects. These range from markers of Y chromosomal deletions, sperm aneuploidy, sperm imprinted gene methylation, chromatin integrity and DNA damage. A systematic review and meta-analysis showed that the RPL patients' partners had significantly higher rates of total sperm aneuploidy compared with the control group including partner of fertile women ([Pu et al. 2020](#)). The few studies on chromosomal anomalies were poorly powered and overall indicated no relationship with miscarriage ([Bernardini et al. 2004](#); [Carp et al. 2006](#); [Bronet et al. 2012](#)). Similarly, Y chromosome microdeletions were not associated with increased miscarriage rates in RPL couples ([Kaare et al. 2008](#); [Wettasinghe et al. 2010](#); [Pereza et al. 2013](#)). Sperm DNA shows more promise. Of the systematic reviews with meta-analysis, Robinson and colleagues interrogated 16 cohort studies (2969 couples) of which 14 were prospective ([Robinson et al. 2012](#)). In 15 out of 16 included studies, sperm DNA damage was assessed in couples undergoing IVF or ICSI, while one study focused on spontaneous conception. The meta-analysis showed a significant increase in miscarriage rates in men with high sperm DNA damage compared with those with low sperm DNA damage (RR 2.16; 95% CI 1.54-3.03). A subgroup analysis showed that the miscarriage association is strongest for the TUNEL assay (RR 3.94; 95% CI 2.45-6.32) ([Robinson et al. 2012](#)). Similarly, Zhao and colleagues performed a systematic review in 2014 also including 16 cohort studies (3106 couples) showing that sperm DNA fragmentation had a detrimental effect on clinical outcomes (decreased pregnancy and increased miscarriage rates) after IVF/ICSI ([Zhao et al. 2014](#)). The studies used different sperm DNA damage test assays, endpoints were for different treatment types (IVF/ICSI/IUI), different aspects of DNA damage were measured and different thresholds for DNA damage were used. Further, female inclusion and exclusion criteria were imposed, and the definitions of miscarriage were not

always coherent. Given a significantly increased RR despite these numerous confounding factors, both Robinson and Jing Zhao concluded that sperm DNA damage testing should be offered to couples following even a single miscarriage after fertility treatment ([Robinson et al. 2012](#); [Zhao et al. 2014](#)).

Similar results were shown more recently. A meta-analysis of studies including 3992 ART patients showed a higher miscarriage rate in high-DFI group compared to the low-DFI group (RR=1.57 [1.18, 2.09],  $P<0.01$ ) ([Deng et al. 2019](#)). Another systematic review and meta-analysis of thirteen prospective studies showed that male partners of women with RPL have significantly higher SDF rates than male partners of fertile control women (mean difference MD: 11.9%, 95% CI 4.97 –18.86) ([McQueen et al. 2019](#)). Similarly, it was shown in a systematic review and meta-analysis including 2 prospective and 12 retrospective studies on RPL couples that the SDF levels were higher in RPL couples compared to fertile controls (MD: 11.98%, 95%CI 6.64- 17.32,  $p<0.001$ ) ([Tan et al. 2019](#)). An altered methylation of sperm-imprinted genes (mainly, H19/IGF2 genes) was shown to be associated with SDF and pregnancy loss rates in a meta-analysis including 10 observational studies ([Cannarella et al. 2021](#)).

There have also been original observational studies ([Rogenhofer et al. 2017](#); [Esquerré-Lamare et al. 2018](#); [Dhawan et al. 2019](#); [Ribas-Maynou et al. 2020](#); [Zhu et al. 2020](#); [Haddock et al. 2021](#); [Le et al. 2021](#)) reporting a relationship between sperm DNA damage and pregnancy loss and a large multi-centre randomized control study ([Miller et al. 2019](#)) that reported an inverse relationship between sperm isolated based on hyaluronan binding and pregnancy loss.

Further there have been four other international societies including the association between sperm DNA fragmentation and recurrent pregnancy loss with recommendations for testing of male partners presenting with this condition (American Society of Reproductive Medicine 2020 and American Urological Society, 2020 ([Schlegel et al. 2021a, 2021b](#)), European Association of Urology, Sexual and Reproduction Health ([Salonia et al. 2021](#)), and Society for Translational Medicine ([Agarwal et al. 2017](#))). In addition, the most recent World Health Organisation manual for semen analysis recommends extended testing using sperm DNA fragmentation, where indicated ([WHO laboratory manual 2021](#)).

A major cause of DNA damage is oxidative stress and this seems to be exacerbated by smoking, obesity and excessive exercise ([Aitken et al. 2009](#); [Hsu et al. 2009](#); [Du Plessis et al. 2010](#); [Aitken, Bakos 2021](#)). Clinicians should advise male partners of couples presenting with RPL of these connections and suggest ways to prevent sperm DNA damage caused by unhealthy lifestyles ([Sharma et al. 2013](#); [Wright et al. 2014](#); [Smits et al. 2019](#)).

#### Recommendations (updated 2022)

In the couples with RPL, it is recommended to assess lifestyle factors in the male partner (paternal age, smoking, alcohol consumption, exercise pattern, and body weight).

Conditional ⊕⊕○○

Assessing sperm DNA fragmentation in couples with RPL should be considered.

Strong ⊕⊕⊕○

## Justification

	Association	Contributing factor	Prognosis	Treatment
<b>Sperm DNA damage</b>	Yes	Yes	requires further clarification	For couples having ICSI, the use of hyaluronan selection looks promising. Further studies are needed to confirm this benefit.

Since 2017, several meta-analysis and observational studies showed that recurrent pregnancy loss rates were increased with abnormal SDF levels. Several assays have been described to measure sperm DNA damage and are available worldwide. Therefore, the GDG recommend assessing sperm DNA fragmentation in order to screen for male factor in couples with RPL. Sperm DNA damage is associated with advanced paternal age and caused by unhealthy lifestyles (such as smoking, obesity and excessive exercise). It is recommended that clinicians make couples aware of these risks and encourage the man addresses his unhealthy habits. Evidence shows that lifestyle modifications of the male partner could improve the clinical outcomes of couples experiencing RPL.

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## Part E: Prognosis and treatment

### 10. Assessing prognosis of a couple with RPL

#### KEY QUESTION: WHAT IS THE VALUE OF INFORMATION ON MEDICAL AND FAMILY HISTORY IN ESTABLISHING THE PROGNOSIS OF RPL?

Several studies were identified that have evaluated the impact of medical and family history on the prognosis in RPL couples. The chance of a live birth, time to live birth and the risk of a pregnancy loss in the next pregnancy are considered relevant outcomes for prognosis in RPL.

In the absence of any interventions proven to ameliorate the chances of a live birth in couples with unexplained RPL, investigators have attempted to develop prognostic tools, based on the identified factors affecting prognosis. Although not an intervention as such, informing couples confronted with RPL about their individual prognosis in a next pregnancy and in the long term is an essential part of the management of couples and allows the couples to decide for or against further pregnancy attempts (Lund et al. 2012).

#### 10.1 FACTORS AFFECTING PROGNOSIS

##### Evidence

##### Reproductive history

The impact of the number of prior pregnancy losses for the chance of live birth has been investigated in a number of cohort studies. The authors consistently find that the number of prior pregnancy losses is an important prognostic factor for chance of live birth in both the first pregnancy after referral and in the long term (Parazzini et al. 1988; Knudsen et al. 1991; Quenby, Farquharson 1993; Brigham et al. 1999; Bhattacharya et al. 2010; Lund et al. 2012; Kolte et al. 2014; Greenberg et al. 2015; Kling et al. 2016).

In a nested cohort study of 251 women with two or more miscarriages from the ALIFE trial, it was demonstrated that the number of prior miscarriages was a determinant both for time to live birth and cumulative incidence of live birth. Follow-up was limited to 24 months after enrolment in the trial (Kaandorp et al. 2014).

One retrospective cohort study of 587 women with unexplained RPL ( $\geq 3$  PLs) following spontaneous conception showed that among the 499 women who subsequently became pregnant, the relative risk of live birth in the first pregnancy after referral was the same for miscarriages and non-visualized pregnancy losses (Kolté et al. 2014). This suggests that the type of pregnancy loss is less important for chance of live birth but needs corroboration in independent cohorts.

For secondary unexplained RPL, a recent cohort study suggested that only consecutive pregnancy losses after the birth influenced the subsequent prognosis, while the number of losses prior to the birth did not affect the prognosis in the next pregnancy ([Egerup et al. 2016](#)).

In a multicentre study on 777 patients, subsequent pregnancy success rate was found to be significantly associated with pregnancy loss history (i.e. time (in years) between first and last miscarriage prior to assessment) and subfertility index (i.e. the product of the number of PLs and the pregnancy loss history), suggesting an effect of the time needed to conceive ([Cauchi et al. 1995](#)). In this study, the maternal age was only borderline significant associated with the subsequent pregnancy success rate, but only if treated as a dichotomous variable (< 30 years or ≥ 30 years). The number of spontaneous pregnancy losses was significantly associated with the subsequent pregnancy success rate.

### Sex of firstborn

In secondary RPL, the sex of the firstborn may be important for prognosis. In a study of 358 Danish women with unexplained secondary RPL compared to the Danish general population, sex ratios were shown to be significantly skewed in the RPL population: sex ratio (boy/girl) of the children born prior to secondary RPL was 1.49 compared to 1.05 in the general population. The sex-ratio of live born children in the first pregnancy after referral was 0.76, and thus the sex ratio significantly changed from firstborn (more boys) to the first pregnancy after referral (more girls) in couples with secondary RPL ([Nielsen et al. 2010](#)). In an Irish cohort study of 85 women with secondary RPL, sex-ratios prior to secondary RPL was 1.66, but there were no significant differences in chances of live birth according to sex of the firstborn ([Ooi et al. 2011](#)). In a study of 170 women with secondary RPL, another observational study reported a skewed sex ratio for first stillborn children, but not live born children ([Li et al. 2014](#)).

### Family history

The results from a recent register-based study of 2138 women with RPL indicate that pregnancy losses among first-degree family members is not an important risk factor for outcome of the first pregnancy after referral among women with RPL ([Kolte et al. 2021](#)).

A number of studies have reported that sporadic or recurrent (≥2) pregnancy loss is more common among RPL patients' first-degree relatives than controls, approximately a doubled incidence or per pregnancy loss rate ([Alexander et al. 1988](#); [Johnson et al. 1988](#); [Christiansen et al. 1990](#); [Ho et al. 1991](#); [Zhang et al. 2010](#); [Kolte et al. 2011](#)). While this may suggest a familial or hereditary component to RPL, none of the abovementioned studies investigated whether affected family members are important for the prognosis of an individual patient. Furthermore, it should be remembered that studies evaluating risk of pregnancy loss among patients' relatives may be subject to information bias, especially if information on relatives' pregnancy losses is derived from the patients. In families where one person suffers from RPL, there may be more openness about reproductive history than in other families and women are referred earlier than women without family members with pregnancy losses ([Kolte et al. 2021](#)).

## 10.2 PROGNOSTIC TOOLS

### Evidence

In a descriptive cohort study, prognosis was evaluated in 987 women with primary or secondary RPL referred to a tertiary center in Denmark ([Lund et al. 2012](#)). Five years after the first consultation, 66.7% (95% CI 63.7-69.7) had achieved a live birth, increasing to 71.1% (95% CI 68.0-74.2) after 15 years. There

was a significantly decreased chance of at least one subsequent live birth with increasing maternal age; of women aged 40 years or older, 41.7% (95% CI 29.8-56.1) achieved a live birth within 5 years compared to 81.3% (95% CI 69.2-90.7) of women aged 20–24 years. There was also a significant decrease in chance of a live birth by increasing number of miscarriages before first consultation ranging from 71.9% (95% CI 67.5-76.1) in women with 3 miscarriages to 50.2% (95% CI 40.5-60.8) in women with 6 or more previous miscarriages. There was no evidence of an interaction between maternal age and the number of previous miscarriages. The Lund model was not designed for individual risk assessment, given the descriptive scope of the study. Furthermore, the study does not discriminate between unexplained and explained RPL.

Another longitudinal study prospectively collected data of 716 RPL patients (325 idiopathic) attending a referral clinic in Liverpool over a 10-year period (Brigham et al. 1999). Of the patients achieving a further pregnancy, 167/222 (75%) had a successful outcome with survival beyond 24 weeks. There was no statistically significant difference in outcome between primary (77%) and secondary losers (74%). From a survival curve, it was shown that the most perilous time for women with idiopathic RPL was between 6 and 8 weeks' gestation. By 8 weeks' gestation, if a foetal heartbeat had been identified, the chances of a successful outcome in a subsequent pregnancy were 98%, climbing to 99.4% at 10 weeks' gestation. Previous miscarriage history and age of the patient significantly affected the chances of a successful outcome, age being slightly more significant than previous number of miscarriages.

A cohort study aimed to explore whether predicting the chance of a subsequent ongoing pregnancy in couples with unexplained RPL could be improved by taking, besides maternal age and the number of previous pregnancy losses, additional candidate predictors into account (du Fossé et al. 2022). Indeed, the authors showed that predicting the chance of a subsequent ongoing pregnancy beyond 24 weeks of gestation in couples with RPL becomes more accurate when, besides the conventional predictors maternal age and number of previous pregnancy losses, more variables are incorporated into the model. The additional predicting variables include both male and female characteristics, advocating a couple-focused rather than a female-focused approach in RPL. However, the predictive ability of the current model remains limited, and we emphasize that more research is needed to develop a model that can be used in clinical practice.

Although the Brigham model and the Lund model were both reviewed with high methodological quality and both studies have consistent results, these models did not follow the nowadays recommended TRIPOD guideline in the development and reporting of a prediction model. Both models were never internally nor externally validated, which leaves their predictive performance unknown. Youssef et al externally validated the Brigham model in a Dutch population of 739 couples and showed that there is poor predictive performance of this model on a new population (Youssef et al. 2022). The model overestimates has too extreme predictions and has a poor discriminative ability.

#### Recommendation (updated 2022)

The GDG recommends to base prognosis on the woman's exact pregnancy history and female age.

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Prognostic tools (Lund, Brigham, Kolte & Westergaard) can be used to provide an estimate of subsequent chance of live birth in couples with unexplained RPL.

GPP

#### Justification

The number of pregnancy losses before referral for RPL is of prognostic importance for future chance of a live birth. Although the studies are of high quality and consistent, evidence on the prognostic potential of reproductive history can only be obtained by observational studies, which is reflected in the low evidence level. The GDG concludes that a thorough reproductive history should be taken in couples presenting with RPL and stresses that number of preceding pregnancy losses and female age provide the best available prognostic information.

The studies of Lund and Brigham show that RPL couples have a good prognosis for a next live birth, especially if female age and the number of previous miscarriages are low. However, one should be aware that the model might overestimate ([Youssef et al. 2022](#)). There is a need for revising the prediction model in order to estimate the chance of a successful pregnancy in couples with unexplained RPL more accurately. Until new studies have been performed, the information from the studies (*summarized in the graphs and tables below*) can be used by clinicians to estimate the chance of a live birth in a next pregnancy in couples with RPL. Optionally, the new prognostic tool Kolte & Westergaard could be used to predict a live birth in the next pregnancy ([Kolte et al. 2021](#)). However, it should be noticed that this tool had not been externally validated.

Unproven therapeutic interventions for unexplained RPL should only be applied in a research setting with relying on reasonable pathophysiological hypotheses.

Additional information:

*Effect of female age and number of previous pregnancy losses on live birth in RPL*

Figure 1: Kaplan-Meier plot showing percentage of women in the recurrent miscarriage cohort who have had at least one live birth after first consultation by age at first consultation. (Lund et al. 2012) (reproduced with permission).

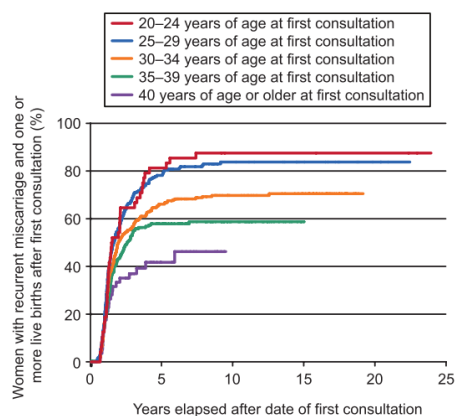


Figure 2: Kaplan-Meier plot showing percentage of women in the recurrent miscarriage cohort who have had at least one live birth after first consultation by number of miscarriages before first consultation (Lund et al. 2012) (reproduced with permission).

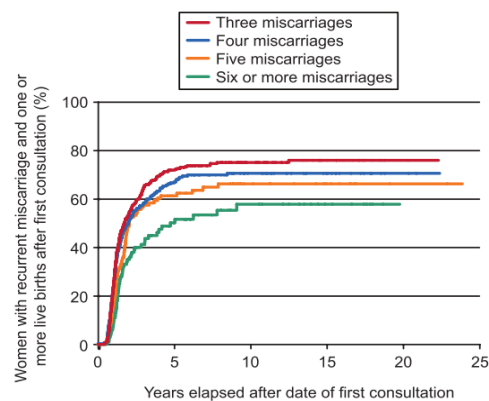


Table 1: Hazard Ratio (95% CI) of Achieving a Live Birth After Referral According to Age at First Consultation and Number of Previous Miscarriages (Lund et al. 2012) (reproduced with permission).

No. of Previous Miscarriages	Age at First Consultation (y)				
	20–24	25–29	30–34	35–39	40 or Older
3	1.28 (0.78–2.11)	1.50 (1.15–1.96)	1 (reference group)	0.81 (0.60–1.10)	0.48 (0.26–0.89)
4	1.93 (1.20–3.11)	0.99 (0.72–1.36)	0.95 (0.72–1.26)	0.67 (0.47–0.95)	0.88 (0.46–1.68)
5	0.48 (0.18–1.29)	1.51 (0.92–2.48)	0.79 (0.53–1.18)	0.76 (0.50–1.17)	0.32 (0.10–1.00)
6 or more	NE	0.80 (0.49–1.30)	0.55 (0.34–0.88)	0.51 (0.29–0.91)	NE

NE, not estimable.

Table 2: Predicted percentage success rate of subsequent pregnancy according to age and previous miscarriage history (Brigham et al. 1999) (reproduced with permission).

Age (years)	Number of previous miscarriages			
	2	3	4	5
20	<b>92</b> (86–98)	<b>90</b> (83–97)	<b>88</b> (79–96)	85 (74–96)
25	<b>89</b> (82–95)	<b>86</b> (79–93)	<b>82</b> (75–91)	79 (68–90)
30	<b>84</b> (77–90)	<b>80</b> (74–86)	<b>76</b> (69–83)	71 (61–81)
35	77 (69–85)	<b>73</b> (66–80)	<b>68</b> (60–75)	62 (51–74)
40	69 (57–82)	64 (52–76)	58 (45–71)	52 (37–67)
45	60 (41–79)	54 (35–72)	48 (29–67)	42 (22–62)

Values are percentages with 95% confidence intervals (CI) shown in parentheses. Where the CI <20%, the values are shown in bold print.

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# 11. Treatment for RPL with genetic background

## KEY QUESTION: WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO COUPLES WITH RPL DUE TO GENETIC/CHROMOSOMAL CAUSES TO INCREASE LIVE BIRTH RATE?

### Evidence

A number of interventions and treatments have been explored for couples with RPL due to genetic/chromosomal causes. Genetic counselling, including a family history the outcomes following further attempts to conceive, and any relevant prenatal diagnostic tests should be offered to all couples with RPL with a known parental karyotype abnormality.

### 11.1 PREIMPLANTATION GENETIC TESTING (PGT) FOR UNEXPLAINED RPL

Preimplantation genetic testing for aneuploidy (PGT-A) (previously preimplantation genetic screening [PGS] or preimplantation diagnosis of aneuploidy [PGD-A]), where an IVF cycle creates embryos which are biopsied and screened for chromosomal anomalies prior to implantation, has been proposed as a potential treatment for RPL. The data from published studies is limited by the PGS (PGT-A) technique used, as the vast majority have employed FISH with an embryo biopsy at Day 3, which only looks at a specific number of chromosomes at an early stage of embryo development where mosaicism is higher. Whole genome techniques such as array-CGH or Next Generation Sequencing (NGS) with a biopsy taken at blastocyst stage, looking at all chromosomes, are recognized to be more accurate screening techniques. To date the two relatively small studies have explored the use of the array-CGH technique, the first of which included only 40 women with RPL but focused on the value of morphokinetic analysis (Basile et al. 2014). The second prospective study compared the outcomes of women with RPL and recurrent implantation failure (RIF) undergoing IVF-ET. 41 women with RPL undergoing PGT-A and 38 women with RPL but no PGT-A were compared with 42 and 50 women with RIF (Sato et al. 2019). PGT-A was shown to reduce the biochemical pregnancy loss and increase the live birth rate per embryo transfer in both groups. However, there were no significant difference in the live birth rates per patient given or not given PGT-A (26.8% vs 21.1% in the RPL group and 35.7% vs 26.0% in the RIF group, respectively). A systematic reviews looking at PGS (PGT-A) for those couples with no known chromosomal abnormality concluded that there is no improvement in live birth rate with PGS (PGT-A), however FISH was used, the numbers were relatively small and the end points different (Musters et al. 2011). Two studies of the same group compared PGS (PGT-A) and expectant management (EM). Clinical outcomes improved in RPL couples undergoing IVF and PGS (PGT-A) compared with couples who received expectant management. Among all attempts at PGS (PGT-A) or EM among couples with RPL, clinical outcomes (pregnancy rate, live birth rate, clinical miscarriage rate) were similar. Median time to pregnancy was 6.5 months in the PGS (PGT-A) group and 3.0 months in the EM group. However those couples whose intended PGS (PGT-A) was cancelled had a lower live birth rate and higher clinical miscarriage rate as opposed to those who underwent PGS (PGT-A) despite similar maternal age (Murugappan et al. 2016). In addition, IVF/PGS (PGT-A) was not a cost-effective strategy for increasing live birth (Murugappan et al. 2015).

Information giving and counselling to couples with RPL is key as shown in a study of 384 patients with recurrent pregnancy loss (Takeda et al. 2020). The majority had either no opinion or poor knowledge of PGT-A and tend to want PGT-A to insure a live birth or to avoid pregnancy loss. Accurate information on advantages and disadvantages of PGT-A such as errors in diagnosis and the lack of evidence that it improves the live birth rate are needed.

## 11.2 PREIMPLANTATION GENETIC TESTING FOR RPL WITH GENETIC BACKGROUND

Preimplantation genetic testing for monogenic/single gene defects (PGT-M) or chromosomal structural rearrangements (PGT-SR), previously PGD, is an established alternative to invasive prenatal diagnosis and as such may avoid termination of pregnancy in couples with a high risk of transmitting genetic disorders such as various monogenic diseases and for structural chromosome abnormalities, the latter being found in the RPL population.

A systematic review was conducted on PGD (PGT-SR) for couples with carrier status of a structural chromosomal abnormality and RPL. The reviewers concluded that there is no improvement in live birth rate with PGD (PGT-SR) (Franssen et al. 2011), but no RCTs were found, the now invalid technique of FISH was used and the numbers were relatively small.

Recent data on PGD (PGT-SR) versus expectant management for couples with translocations reports a live birth rate of 37.8% on the first pregnancy after PGD (PGT-SR) and 53.8% on the first natural pregnancy after ascertainment of the carrier status (OR 0.52, 95% CI 0.22-1.23). PGD (PGT-SR) reduced the miscarriage rate, but cumulative live birth rate (OR 1.10; 95% CI 0.45-2.70) and time to pregnancy (12.4 months versus 11.4 months) were similar between both groups (Ikuma et al. 2015).

In a cohort study, it was found that 76.9% (206/268) of couples with a translocation opted for PGD (PGT-SR) following genetic counselling (De Krom et al. 2015). However another smaller cohort study of couples with a structural chromosomal rearrangement seen in a specialised Recurrent pregnancy loss service found that they were twice as likely to pursue natural conception than PGD (Maithripala et al. 2018).

Some studies have suggested that miscarriage rates may be lower using PGD (PGT-SR) (Ikuma et al. 2015) whilst others have shown that even with natural conception miscarriage rates do not differ from non-carrier couples (Dong et al. 2014).

### Recommendations

All couples with results of an abnormal foetal or parental karyotype should receive genetic counselling.

GPP

All couples with results of an abnormal foetal or parental karyotype may be informed about the possible treatment options available including their advantages and disadvantages.

GPP

## Justification

The limited evidence for preimplantation genetic testing in couples with RPL shows no clear benefit of treatment. The overall quality of the evidence is very low. Therefore, the GDG strongly recommends that all couples with abnormal genetic results from pregnancy tissue testing or parental karyotypes should be offered genetic counselling to discuss likely prognosis and further diagnostic options. Couples may also receive information on the treatment options so they can make an informed decision on treatment. Clinicians are encouraged to elaborate on the advantages and disadvantages of PGT, depending on the techniques used ([Brezina et al. 2016](#)). In addition, couples should be informed that PGT-SR could reduce the miscarriage rate but will not improve live birth rate or time to pregnancy. Finally, PGT is not permitted in some countries.

Further good quality trials with modern technology and methodology are therefore needed to look at the value of PGT for couples with RPL due to chromosomal abnormalities.

A recent study reported a higher percentage of aneuploidy in blastocysts and a higher incidence of IVF cycles with no embryo transfer in couples with unexplained RPL with diminished ovarian reserve, compared to those with normal ovarian reserve ([Shahine et al. 2016](#)).

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DRAFT for REVIEW

## 12. Treatment for RPL and Thrombophilia

In some women with thrombophilia, anticoagulant treatment is prescribed with the aim to prevent venous thromboembolism, according to evidence-based clinical guidelines ([Bates et al. 2018](#))

In women with thrombophilia and RPL, treatment is presumed to prevent placental thrombosis (antithrombotic agents including aspirin and anticoagulants) and/or by suppress the immune system (immunological treatments), which is suggested to increase the chance of a successful pregnancy outcome.

Antithrombotic agents investigated as treatment for RPL are aspirin and/or heparin (either unfractionated heparin (UFH) or low molecular weight heparin (LMWH)).

**KEY QUESTION: WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO COUPLES WITH RPL AND THROMBOPHILIA TO INCREASE THE CHANCE OF A LIVE BIRTH?**

### 12.1 TREATMENT FOR WOMEN WITH RPL AND HEREDITARY THROMBOPHILIA

#### Evidence

##### Anticoagulants

A systematic review reported no benefit of low molecular weight heparin (LMWH) for prevention of pregnancy loss in women with hereditary thrombophilia and prior late ( $\geq 10$  weeks) pregnancy loss (LBR LMWH versus no LMWH: RR 0.81; 95% CI 0.38-1.72; 5 RCTs; n=308) or recurrent early ( $< 10$  weeks) pregnancy loss (LBR LMWH versus no LMWH: RR 0.97; 95% CI 0.80-1.19; 2 RCTs; n=66) ([Skeith et al. 2016](#)).

A Cochrane review on anticoagulant treatment for women with RPL with or without hereditary thrombophilia combined nine RCTs including 1228 women. The reviewers reported no significant effect of treatment (aspirin, LMWH, LMWH + aspirin) compared to placebo. The risk ratio for live birth was 0.94 (95% CI 0.80-1.11; n=256) in the comparison of aspirin versus placebo, 1.23 (95% CI 0.84-1.81; n=453; studies at high risk of bias included) for LMWH versus no treatment, and 1.01 (95% CI 0.87-1.16; n=322) for LMWH and aspirin compared to no treatment. In the comparison of LMWH versus aspirin the risk ratio for live birth was 1.08 (95% CI 0.93-1.26; n=239), in the comparison of LMWH and aspirin versus aspirin alone it was 1.11 (95% CI 0.94-1.30; n=327) ([de Jong et al. 2014](#)).

##### Steroids

No studies regarding steroids for hereditary thrombophilia and RPL have been found.

##### Intravenous immunoglobulins

No studies regarding treatment with Intravenous immunoglobulins (IVIg) for hereditary thrombophilia and RPL were retrieved.

### 3331 Folic acid and vitamins

3332 Most studies on treatment with folic acid and vitamins have focused on RPL women with a mutation in  
 3333 the MTHFR gene and/or hyperhomocysteinemia. One study showed that treatment with L-methyl  
 3334 folate, vitamin B6 and vitamin B12 could reduce the homocysteine levels, and even normalize them in  
 3335 76% of patients. The impact on the next pregnancy was however not discussed ([Glueck et al. 2015](#)).  
 3336 Another study reported that 22 out of 25 women with RPL initiated a pregnancy after normalization of  
 3337 their homocysteine levels; 20 pregnancies resulted in a live birth, of which four were preterm and two  
 3338 had non-severe foetal growth retardation. No malformations, bleeding in the mother, or  
 3339 thromboembolic complications were reported.

### 3340 Recommendation

For women with hereditary thrombophilia and a history of RPL, we suggest not to use antithrombotic prophylaxis unless in the context of research, or if indicated for VTE prevention.

Conditional ⊕⊕○○

3341

### 3342 Justification

3343 We found no evidence of a beneficial effect of anticoagulant treatment in women with hereditary  
 3344 thrombophilia. An international RCT in women with RPL and hereditary thrombophilia has completed  
 3345 recruiting in 2021 and results are expected in 2022 (ALIFE2 trial/ trialreg nr NTR 3361).

## 3346 12.2 TREATMENT FOR WOMEN WITH RPL AND ANTIPHOSPHOLIPID SYNDROME (APS)

### 3347 Evidence ).

#### 3348 Anticoagulants

3349 Antithrombotic therapy (aspirin, UFH or LMWH) was summarized in a recent Cochrane review  
 3350 summarizing eleven RCTs of 1672 women with RPL and APS ([Hamulyák et al. 2020](#)). A benefit of heparin  
 3351 (UFH or LMWH) and aspirin, as compared to aspirin alone, with regard to live birth was reported (RR  
 3352 1.27, 95% CI 1.09 to 1.49, 5 studies, 1295 women, low-certainty evidence). Heparin plus aspirin may  
 3353 reduce the risk of pregnancy loss (RR 0.48, 95% CI 0.32 to 0.71, 5 studies, 1295 women, low-certainty  
 3354 evidence). It should be noted that there is significant risk of bias in the included studies, and that  
 3355 adverse effects were frequently not, or not uniformly, reported in the included studies.

3356 In women with APS, almost no data are available to support the use of aspirin only to prevent recurrent  
 3357 pregnancy loss. The pooled results of 3 very small trials (total number of 71 participants) showed no  
 3358 effect of aspirin only compared with no treatment (RR of pregnancy loss 1.05, 95% CI 0.66-1.68), but  
 3359 from the confidence interval it can be concluded that neither benefit nor harm can be ruled out  
 3360 ([Empson et al. 2005](#)). In this most recent Cochrane review, more stringent inclusion criteria were used,  
 3361 and only 1 trial with 40 women was included with no effect of aspirin on live birth compared to placebo  
 3362 (risk ratio (RR) 0.94, 95% confidence interval (CI) 0.71 to 1.25, 1 trial, 40 women, very low-certainty  
 3363 evidence). ([Hamulyák et al. 2020](#))

3364 For thrombosis prophylaxis, LMWH is preferred over UFH, because of a lower risk of osteoporosis and  
 3365 heparin-induced thrombocytopenia ([Bates et al. 2012](#)). In clinical practice, women with APS and RPL

are prescribed LMWH. When comparing LMWH plus aspirin versus aspirin alone, the pooled RR for live birth was 1.20 (95% CI 1.04 to 1.38, 3 trials, 1155 women). In the comparison of UFH plus aspirin versus aspirin alone, the RR for live birth was 1.74 (95% CI 1.28 to 2.35, 2 trials, 140 women). The observed beneficial effect of heparin was driven by one large study in which LMWH plus aspirin was compared with aspirin alone ([Bao et al. 2017](#)).

### Steroids

Steroids (prednisone) have been evaluated as treatment for women with RPL and presence of antiphospholipid antibodies. In two RCTs, no evidence was found for a benefit of prednisone combined with aspirin in comparison to placebo or aspirin only in reducing pregnancy loss in women with RPL (RR 0.85; 95% CI 0.53-1.36; n=122) ([Empson et al. 2005](#)). In addition, no benefit was found for prednisone combined with aspirin compared to heparin/aspirin (RR 1.17; 95% CI 0.47-2.93; one RCT; n=45). Furthermore, several adverse outcomes were reported associated with prednisone; there was a significant increase in premature delivery, neonatal intensive care unit admission, rate of pre-eclampsia and hypertension, risk of gestational diabetes and birthweight was significantly lower ([Empson et al. 2005](#)).

### Intravenous immunoglobulin

Based on three RCTs, a review concluded that treatment with intravenous immunoglobulin (IVIg) did not reduce the chance of pregnancy loss in women with RPL and antiphospholipid antibodies (RR 1.47; 95% CI 0.52-4.14; n=138) ([Empson et al. 2005](#)).

In 24 patients with SLE and RPL, pregnancy outcomes were compared between women who received high dose IVIg and those who received prednisone and NSAIDs. IVIg was superior to prednisone with regard to LBR (100% versus 75%), number of miscarriages (0 versus 3) and preterm delivery (25% versus 55.6%). Furthermore, there was evidence of a clinical response; a significant decrease in the lupus activity index-pregnancy (LAI-P) was reported in the IVIg treated patients, but not the prednisone group, when comparing measurement at the end versus the beginning of the pregnancy ([Perricone et al. 2008](#)).

### Recommendations

For women who fulfill the laboratory criteria of APS and a history of three or more pregnancy losses, we suggest administration with low-dose aspirin (75 to 100 mg/day) starting before conception, and a prophylactic dose heparin (UFH or LMWH) starting at date of a positive pregnancy test, over no treatment.

Conditional ⊕○○○

The GDG suggests offering anticoagulant treatment for women with two pregnancy losses and APS, only in the context of clinical research.

GPP

## Justification

Although several reviews have been published, the overall quality of evidence for live birth rate and miscarriage rate is low to very low. The existing evidence suggests that a combination of heparin (more for UFH than for LMWH) and aspirin improves LBR in women with APS and RPL (three or more PLs, no evidence for two or more PLs). It should be noted that there is significant risk of bias in the included studies. Furthermore, there appears to be large clinical heterogeneity in study population between studies; in the UFH studies that showed an effect of the intervention, the live birth rate in the comparator arm was around 44%, whereas in the LMWH studies that showed no effect, the live birth rate was close to 80% ([Middeldorp 2014](#)). There is no evidence of effect of aspirin only when compared to placebo. The GDG group recommends to further study the effectiveness of treatment for APS and clinical criteria for treatment of APS (e.g. female age, number of pregnancy losses, consecutive or non-consecutive losses), although there are major challenges in undertaking RCTs in this population ([Skeith et al. 2020](#)).

The recommendations for treatment of women with RPL and hereditary thrombophilia or APS are consistent with the recommendations from the American College of Chest Physicians ([Bates et al. 2012](#)).

The GDG decided not to formulate any recommendations for the other interventions described, except for a research recommendation on hydroxychloroquine, which has been found safe and effective for preventing obstetric complications in women with APS, but has not been investigated in women with RPL and APS.

## Additional information

In most of the included studies, UFH/LMWH combined with low-dose aspirin treatment was started as soon as pregnancy was confirmed (6 weeks' gestation), except for Kutteh and colleagues who started aspirin before conception, and added heparin treatment after foetal heart activity (6.7 weeks) ([Kutteh 1996](#)). Although not stated in all studies, aspirin/heparin treatment was continued until 35 weeks' gestation or delivery ([Farquharson et al. 2002](#); [Laskin et al. 2009](#)). Other studies provided less details on when treatment was discontinued.

Administration of low-dose aspirin (75 to 100 mg/day) starting before conception, with a prophylactic dose of heparin (UFH or LMWH) starting at the date of a positive pregnancy test until delivery is suggested.

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## 13. Treatment for RPL with immunological background

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**KEY QUESTION: WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO COUPLES WITH RPL WITH SUSPICION OF IMMUNOLOGICAL BACKGROUND TO INCREASE LIVE BIRTH RATE?**

### Evidence

As discussed in chapter 6, no immunological biomarkers have been definitively documented to cause RPL. There is quite strong evidence that presence of some autoantibodies (anticardiolipin antibodies and antithyroid antibodies) negatively affects the future live birth rate in women with or without RPL. (Nielsen, Christiansen 2005; Thangaratinam et al. 2011); whereas the impact of other autoantibodies such as antinuclear antibodies is more controversial.

In contrast, we found insufficient documentation for the impact of natural killer abnormalities and cytokine abnormalities in the blood or endometrium in RPL. It is therefore questionable to select patients to specific treatments due to the presence or absence of specific immune biomarkers outside clinical trials.

Unfortunately, very few high quality controlled trials have been undertaken in women with RPL selected due to the presence of immune biomarkers.

The majority of studies in this category comprise trials of anticoagulation therapies in women with antiphospholipid antibodies, which in these studies are considered thrombophilia factors rather than immunological biomarkers. These trials are considered in chapter 12.2. Trials attempting to treat women with RPL with antithyroid antibodies with levothyroxine are discussed in chapter 14.1.

In the overwhelming number of trials testing other treatment options: lymphocyte immunization, intravenous immunoglobulin infusions, prednisone etc. patients were not selected due to the presence of specific immune factors and they are discussed in chapter 17 (unexplained RPL). A few trials have tested intravenous immunoglobulin in women with RPL with various autoantibodies or NK cell aberrations (Stricker, Winger 2005) or NK cell/cytokine aberrations (Winger, Reed 2008; Moraru et al. 2012) but these trials are only of moderate/low quality, primarily because they were not placebo-controlled and thus not blinded. Two good placebo-controlled trials have tested prednisone in patients selected due to presence of auto- or alloantibodies (Laskin et al. 1997) or endometrial NK cell abnormalities (Tang et al. 2013). However, since the importance of these immune biomarkers is uncertain, we have chosen to include these trials in chapter 17 where they can be put into the best context

### Conclusion

No immunological biomarker, except for high-titer antiphospholipid antibodies (*see chapter 12*) can be used for selecting couples with RPL for specific treatments.

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## 14. Treatment of RPL with metabolic or endocrinologic abnormalities

**KEY QUESTION: WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO COUPLES WITH RPL AND METABOLIC OR HORMONAL ABNORMALITIES TO INCREASE LIVE BIRTH RATE?**

### 14.1 TREATMENT FOR THYROID ABNORMALITIES ASSOCIATED WITH RPL

#### Evidence

##### Overt hypothyroidism

Hypothyroidism in pregnancy is associated with adverse pregnancy complications (increased risk of premature birth, low birth weight, and miscarriage) as well as detrimental effects on foetal neurocognitive development. Treatment is indicated to avoid maternal hypothyroidism wherever possible ([Stagnaro-Green et al. 2011](#)). In addition, pregnancy presents a series of physiological changes which increase T4 requirements, therefore it is needed to increase the daily dose ([Khan et al. 2017](#)). TSH levels should be compared to local trimester-specific reference ranges, or recommended upper limits: e.g. first trimester, 2.5 mU/l; second trimester, 3.0 mU/l; third trimester, 3.5 mU/l ([Lazarus et al. 2014](#)).

##### Subclinical hypothyroidism

Conflicting advices have appeared with regard to levothyroxine treatment in women with RPL and subclinical hypothyroidism (SCH).

The European Thyroid Association Guidelines for the Management of Subclinical Hypothyroidism in Pregnancy and in Children, SCH arising before conception or during gestation should be treated with levothyroxine ([Lazarus et al. 2014](#)), based on two studies showing that levothyroxine treatment decreased the occurrence of adverse events in the mother and foetus and reduced miscarriage rates [based on ([Negro et al. 2010](#)) and ([Lepoutre et al. 2012](#))]. The American Thyroid Association recommends levothyroxine treatment for pregnant women with SCH (TSH above trimester specific ranges) and TPOAb, or SCH (with TSH levels above 10.0 mU/L), and recommends to consider treatment for pregnant women with TSH concentrations >2.5 mU/L and TPOAb, or TSH >10.0 mU/L. Levothyroxine treatment is not recommended for TPOAb negative women with normal TSH ([Alexander et al. 2017](#)).

In an observational cohort study of women with recurrent early pregnancy loss ( $\geq 2$  pregnancy losses <10 weeks), the impact of subclinical hypothyroidism (SCH) and the effect of levothyroxine treatment were assessed. Subclinical hypothyroidism, i.e. TSH >2.5 mU/l with a normal free thyroxine or free thyroxine index, was detected in 19% (n=55) of the patients. In the study, the cumulative live birth rate was compared in patients treated before 2008 (when SCH was not treated) and after 2008, when SCH patients received levothyroxine treatment pre-pregnancy to maintain TSH  $\leq 2.5$  mU/l. The per-pregnancy LBR for SCH treated (n=24) versus untreated (n=15) women was 22/46 (48%) versus 12/23 (52%), respectively ([Bernardi et al. 2013](#)). The cumulative LBR was 71% (17/24) and 67% (10/15), respectively. The authors did not find a statistically significant difference in the subsequent live-birth rate when comparing women with SCH and euthyroid women, or treated and untreated SCH.

In addition, levothyroxine therapy during pregnancy might carry the potential risk of adverse child neurodevelopment outcomes, since high maternal free thyroxine concentrations during pregnancy are recently be reported to be associated with lower child IQ and lower grey matter and cortex volume (Korevaar et al. 2016).

In conclusion, the effect of levothyroxine for women with subclinical hypothyroidism and RPL is only assessed in one observational study. There is a need for further investigation of the potential treatment effect and risks of levothyroxine supplementation by means of large RCTs.

### Thyroid autoimmunity

There are no studies evaluating the effect of treatment on the pregnancy outcomes in women with RPL and thyroid autoimmunity. Indirect evidence on pregnancy outcomes, including miscarriage rate, after levothyroxine treatment in euthyroid women with thyroid autoimmunity has been summarized in two meta-analyses (Thangaratinam et al. 2011 ; Vissenberg et al. 2012). A reduction in the risk of miscarriage with levothyroxine treatment was reported (RR 0.52; 95% CI 0.22-1.15) based on two RCTs of women with thyroid autoantibodies, TSH within the reference ranges of 0.27–4.2 mIU/L, but no history of RPL (Negro et al. 2005; Negro et al. 2006; Vissenberg et al. 2012).

In a case-control study thyroid autoimmunity, prevalence of subclinical hypothyroidism and maternal and foetal complications were assessed in 100 healthy pregnant women and 100 pregnant women with a history of RPL, of which 31% showed thyroid autoimmunity (thyroid peroxidase antibody (TPOAb+) >34 U/ml). All women with TPOAb+ received levothyroxine therapy. The authors found no difference in prevalence of miscarriage between hypothyroid and euthyroid individuals in TPOAb+ women (all receiving levothyroxine) and suggested treatment for all TPOAb+ RPL women (Lata et al. 2013).

A recent RCT, the TABLET trial, showed that levothyroxine therapy in a dose of 50 µg per day does not improve live birth rate in euthyroid women with thyroid peroxidase antibodies (Dhillon-Smith et al. 2019). The live birth rate was 37% in the levothyroxine group and 38% in the placebo group, (RR 0.97, 95%CI 0.83 to 1.14, P= 0.74). The T4life trial showed that the treatment of women with RPL and positive for TPO-Ab with levothyroxine did not result in higher live birth rates compared to placebo (50% vs 48%, RR 1.03, 95%CI 0.77 to 1.38)(Van Dijk et al. 2022).

### Recommendations (updated 2022)

Overt hypothyroidism arising before conception or during early gestation should be treated with levothyroxine in women with RPL.

Strong ⊕⊕○○

There is conflicting evidence regarding treatment effect of levothyroxine for women with subclinical hypothyroidism and RPL. Treatment of women with SCH may reduce the risk of miscarriage, but the potential benefit of treatment should be balanced against the risks.

Conditional ⊕⊕○○

If women with subclinical hypothyroidism and RPL are

GPP

pregnant again, TSH level should be checked in early gestation (7-9 weeks gestational age), and hypothyroidism should be treated with levothyroxine.

3594

If women with thyroid autoimmunity and RPL are pregnant again, TSH level should be checked in early gestation (7-9 weeks gestational age), and hypothyroidism should be treated with levothyroxine.

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Euthyroid women with thyroid antibodies and RPL should not be treated with levothyroxine.

Conditional ⊕⊕○○

3596

### 3597 Justification

3598 If hypothyroidism is identified in women with RPL, treatment with levothyroxine is recommended based  
 3599 on existing guidelines and possible maternal and foetal complications associated with untreated  
 3600 hypothyroidism during pregnancy. For women with subclinical hypothyroidism and RPL, treatment with  
 3601 levothyroxine is insufficiently evidence-based and it should be further investigated. Moreover, recent  
 3602 evidence of thyroid hormone treatment in pregnant women with SCH reported reduced miscarriage  
 3603 rates (OR 0.62; 95% CI 0.48-0.82), but higher odds of preterm delivery (OR 1.60; 95% CI 1.14-2.24),  
 3604 gestational diabetes (OR 1.37; 95% CI 1.05-1.79) and pre-eclampsia (OR 1.61; 95% CI 1.10-2.37)  
 3605 ([Maraka et al. 2017](#)).

3606 The GDG advises that women with a thyroid abnormality be treated and/or referred to a specialist in  
 3607 endocrinology or internal medicine, depending on the clinical setting and local protocols.

3608 While before 2017, there were no convincing evidence on the efficacy of levothyroxine treatment, new  
 3609 evidence from TABLET trial and the T4life trial showed that levothyroxine treatment does not increase  
 3610 the chance of a live birth in women with a history of RPL and thyroid autoimmunity ([Dhillon-Smith et al. 2019](#); [Van Dijk et al. 2022](#)), and therefore the use of levothyroxine is not recommended to treat  
 3611 euthyroid women with thyroid antibodies and RPL.  
 3612

## 3613 14.2 PROGESTERONE OR HUMAN CHORIONIC GONADOTROPHIN (HCG) (FOR LUTEAL 3614 PHASE INSUFFICIENCY)

3615 Evidence (*see also summary of evidence table 15 and 7*).

3616 Progesterone is indispensable for the establishment and maintenance of pregnancy and thus, luteal  
 3617 phase insufficiency has been suggested a causative factor in RPL. However, testing for luteal phase  
 3618 insufficiency is not routinely performed or recommended based on limited evidence on tests to use of  
 3619 the relevance thereof (*see chapter 7*).

3620 The effect of progesterone, both vaginal and oral, has been studied in women with unexplained RPL,  
 3621 and although study conclusions vary significantly, the guideline development group recommends not

to prescribe progesterone in women with unexplained RPL based on the recently published PROMISE trial ([Coomarasamy et al. 2015](#)) (see chapter 17).

The effect of vaginal progesterone treatment (100–200 mg every 12 hours starting 3 days after the LH surge) was recently evaluated in a cohort of women with RPL and abnormally elevated levels of nCyclinE ([Stephenson et al. 2017](#)). Of 116 women with RPL, 59 (51%) had abnormally elevated levels (in the luteal phase) of nCyclinE, a marker of endometrial development, on the endometrial biopsy. Vaginal progesterone administration resulted in a normalization of nCyclinE expression in 84% of 25 women undergoing a repeat endometrial biopsy. Pregnancy success in women with abnormal n-CyclinE who were treated with vaginal micronized progesterone starting in the early luteal phase was 69% (57/83) compared with 51% (19/37) in women with normal nCyclinE who were not treated with vaginal progesterone (OR = 2.1; 95% CI 1.0-4.4). The study was not randomized.

Studies on human chorionic gonadotrophin (hCG) for improving the LBR in women with RPL have been recently summarized in a Cochrane review ([Morley et al. 2013](#)). The results demonstrated a significant benefit in using hCG to prevent RPL (RR 0.51; 95% CI 0.32-0.81; five RCTs), but power of the meta-analysis was limited due to the small number of studies and methodological and clinical heterogeneity. None of the studies reported any adverse effects from the use of hCG.

## Recommendations

There is insufficient evidence to recommend the use of progesterone to improve live birth rate in women with RPL and luteal phase insufficiency.

Conditional ⊕⊕⊕○

There is insufficient evidence to recommend the use of hCG to improve live birth rate in women with RPL and luteal phase insufficiency.

Conditional ⊕⊕○○

## Justification

Based on the absence of evidence in women with RPL and luteal phase insufficiency and the recommendation that luteal phase insufficiency should not be tested in women with RPL, the GDG recommends against progesterone in women with RPL and luteal phase insufficiency, consistent with the recommendation in women with unexplained RPL.

Results on hCG as a treatment for RPL show a positive effect of treatment on miscarriage rate. However, studies are considered too limited to recommend the use of hCG in women with RPL and luteal phase insufficiency.

## 14.3 METFORMIN / INSULIN

### Evidence

Metformin is a low-risk and effective oral hypoglycemic agent for Type 2 Diabetes Mellitus, and considered safe and effective for gestational diabetes.

Several studies on metformin found that it is effective in improving pregnancy outcomes in women with PCOS or insulin resistance. In patients with PCOS, metformin was found to significantly reduce the rate of miscarriage ([Jakubowicz et al. 2002](#); [Khattab et al. 2006](#); [Wang et al. 2011](#); [Al-Biate 2015](#)).

Based on these results, it could be suggested that treatment with metformin increases the chance of a live birth in women with PCOS and a history of recurrent pregnancy loss. However, there are no studies focusing on women with RPL and PCOS.

One of the only studies on metformin treatment for women with RPL and glucose metabolism defects is the small study of Zolghadri and colleagues. Metformin or placebo was administered to women with RPL and abnormal glucose tolerance test. The miscarriage rate was significantly reduced after metformin therapy compared to placebo in women without PCOS (15% vs. 55%). The results in women with PCOS and RPL were not significant (small groups) ([Zolghadri et al. 2008](#)).

A meta-analysis on the risks of metformin during pregnancy concluded that exposure to metformin during the first trimester of pregnancy does not increase the risk of birth defects ([Andrade 2016](#)).

## Recommendation

There is insufficient evidence to recommend metformin supplementation in pregnancy to prevent PL in women with RPL and glucose metabolism defects.

Conditional ⊕○○○

## Justification

Indirect evidence could support the use of metformin treatment to increase the live birth rate in women with PCOS, but in the absence of any substantial studies in women with RPL and PCOS, the GDG decided metformin is not recommended.

## 14.4 OVULATION INDUCTION

### Evidence

The efficacy of controlled ovarian stimulation to increase the chance of a live birth in women with RPL (three or more consecutive first-trimester pregnancy losses) and a luteal phase defect was shown in a small study by Li and colleagues. They studied 21 subjects with unexplained RPL and retarded (>2 days behind chronological dating) endometrial development in the mid-luteal phase, as shown by LH-timed endometrial biopsy taken around day LH + 7, and histological dating. The women underwent at least one cycle of controlled ovarian stimulation by human menopausal gonadotropins (hMG). Out of 36 treatment cycles analyzed, 13 (33%) cycles from 12 subjects resulted in a pregnancy, of which two resulted in a miscarriage. In comparison, seven of 12 pregnancies in non-treatment cycles resulted in miscarriage ([Li et al. 2001](#)).

Two other studies on ovulation induction as a treatment for RPL selected women with PCOS and RPL. In the study of Clifford and colleagues, 106 ovulatory women with a history of recurrent miscarriage, polycystic ovaries, and hypersecretion of luteinizing hormone were randomly assigned to pituitary suppression with a luteinizing hormone releasing hormone analogue followed by low dose ovulation induction and luteal phase progesterone or were allowed to ovulate spontaneously and then given

luteal phase progesterone alone or luteal phase placebo alone. There was no difference in conception rate (80% vs 82%) or live birth rate (65% vs 76%) between the groups, nor was there a difference between the women given progesterone and those given placebo pessaries (Clifford et al. 1996).

In the study by Johnson, ovulation was induced by clomiphene or pituitary suppression with buserelin followed by pure FSH in 42 women with PCOS and RPL. The miscarriage rate was 48% (11/23) for the clomiphene group compared with 9% (2/23) for the buserelin group. The authors concluded that pituitary suppression before induction of ovulation significantly reduces the risk of pregnancy loss in women with PCOS and RPL (Johnson, Pearce 1990).

## Conclusion

Based on the study of Li, controlled ovarian stimulation by human menopausal gonadotropins could be beneficial for decreasing the chance of a next pregnancy loss in women with RPL diagnosed with luteal phase insufficiency (Li et al. 2001), however the GDG decided that the evidence was too limited to support recommending controlled ovarian stimulation in women with RPL but without PCOS.

Based on a small study of women with RPL and PCOS, pituitary suppression with buserelin before induction of ovulation in women with RPL and PCOS could be an option to reduce the risk of PL (Johnson, Pearce 1990).

## 14.5 BROMOCRIPTINE FOR RPL ASSOCIATED WITH HYPERPROLACTINEMIA

Prolactin testing is only recommended in women with RPL if they have clinical symptoms (oligo-amenorrhea) indicative of hyperprolactinemia. Patients with hyperprolactinemia who require medical therapy are typically treated with dopamine agonist therapy (bromocriptine or cabergoline).

## Evidence

In a study by Hirahara, it was confirmed that also in women with RPL, bromocriptine effectively normalizes serum prolactin levels. Women with RPL and (occult) hyperprolactinemia were assigned to bromocriptine (2.5–5.0 mg/d, depending on individual response) from before conception until the end of the 9th week of gestation or no treatment. Twenty-one of the 24 women treated with bromocriptine conceived: 18 had a live birth (85.7%) and three miscarried (14.3%), while in the non-treated group 21 of 22 women conceived, 11 had a live birth (52.4%) and 10 miscarried (47.6%). In addition, serum prolactin levels during early pregnancy (5–10 weeks of gestation) were significantly higher in women who miscarried (31.8–55.3 ng/mL) than in women with successful pregnancies (4.6–15.5 ng/mL) (Hirahara et al. 1998).

## Recommendation

**Bromocriptine treatment can be considered in women with RPL and hyperprolactinemia to increase live birth rate.**

Conditional ⊕○○○

## Justification

In women with RPL and hyperprolactinemia, bromocriptine treatment normalizes serum prolactin levels and it could be effective for increasing the chance of a live birth. However, this conclusion is based on a single small study, and hence should be confirmed.

## 14.6 VITAMIN D

### Evidence

Vitamin D deficiency has been studied extensively in relation to obstetrical complications and was described as a risk factor for gestational diabetes, small for gestational age infants and preeclampsia in systematic reviews ([Aghajafari et al. 2013](#)). Furthermore, vitamin D deficiency during pregnancy adversely affects health, growth and development of the child ([McAree et al. 2013](#)). Even though vitamin D deficiency seems prevalent in women with RPL (47.4%, <30 ng/ml) ([Ota et al. 2014](#)), testing of vitamin D levels is not recommended with the aim of identifying cause or providing treatment options in women with RPL.

There are no studies evaluating the effect of vitamin D supplementation on the chance of a live birth in the next pregnancy in women with RPL. One recent study concluded that vitamin D supplementation in women with RPL and vitamin D deficiency or insufficiency (n=64) could reduce abnormalities of cellular immune responses observed in women with low vitamin D levels ([Chen et al. 2016](#)).

Independent of RPL, concerns have been raised on the prevalence of vitamin D deficiency and insufficiency among pregnant women. Vitamin D status is affected by factors that regulate its production in the skin, including skin pigmentation, latitude, season, dressing codes, aging, sunscreen use and air pollution ([De-Regil et al. 2016](#)).

A recent review combining trials on vitamin D supplementation in pregnancy, which cumulatively involved more than 2000 pregnant women, reported that there were no adverse events observed attributable to vitamin D supplementation ([De-Regil et al. 2016](#); [Wagner et al. 2017](#)). All trials started vitamin D supplementation after 20 weeks of gestation, and daily doses ranged from 200 to 2000 IU. Regarding the benefit of vitamin D supplementation on pregnancy related outcomes, evidence is scarce and inconsistent. Vitamin D supplementation during pregnancy seems to reduce the risk of preterm birth (three trials) and low birth weight (four trials). Miscarriage was not discussed ([De-Regil et al. 2016](#)).

### Recommendation

Preconception counselling in women with RPL could include the general advice to consider prophylactic vitamin D supplementation.

GPP

### Justification

Based on the significant prevalence of vitamin D deficiency in women with RPL and the possibly associated obstetrical and foetal complications, prescribing vitamin D supplementation can be considered, even though evidence for the effectiveness is absent. With regard to harm, most experts agree that supplemental vitamin D is safe in dosages up to 4,000 IU per day during pregnancy or lactation, even though data on the safety of higher doses are lacking ([ACOG Committee Opinion No. 495: Vitamin D: Screening and supplementation during pregnancy 2011](#); [Del Valle et al. 2011](#)).

## 14.7 TREATMENT FOR HYPERHOMOCYSTEINEMIA

There is inconsistent evidence for an association of elevated homocysteine (Hcy) levels with RPL and assessment of Hcy levels is not recommended in women with RPL. However, studies have evaluated the effects of different treatments on Hcy levels and pregnancy outcomes in women with RPL and HHcy.

## Evidence

A first study showed that daily supplementation of 0.5 mg folic acid (for 2 months) in 49 women with a history of unexplained RPL substantially reduced homocysteine concentrations. The greatest decline in median fasting total plasma Hcy concentration (-41%) was detected in women with the homozygous (T/T) MTHFR genotype (Nelen et al. 1998).

The second study, a non-controlled pilot study, reported improved live birth rates (20 live births in 22 pregnancies) in 25 women with RPL, HHcy and homozygous for the C677T mutation of the MTHFR gene after treatment with high-dose folic acid (15 mg daily, reduced to 5 mg after 3 months) and vitamin B6 (750 mg daily for 3 months). (Quere et al. 2001).

Another study reported benefit of treatment with LMWH (prophylactic dose of 2500 IU sc everyday) in concomitant with aspirin (5 mg/day) since foetal cardiac activity was observed by US and continuing up to 12 weeks of gestation with regard to pregnancy salvage in women with RPL and HHcy (Chakraborty et al. 2013). Pregnancy salvage was significantly higher after combined treatment in 76 women with HHcy as compared to 111 women with normal Hcy levels (84.2% versus 54.9%; OR 1.55; 95% CI 129-1.88).

## Conclusion

In the absence of consistent evidence for an association between HHcy and RPL, assessing Hcy levels is not routinely recommended. However, if HHcy is detected in women with RPL, treatments are available that can lower Hcy levels and possibly improve the chance of a live birth rate in the next pregnancy.

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# 15. Treatment for uterine abnormalities in RPL

## KEY QUESTION: WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO WOMEN WITH RPL AND UTERINE ABNORMALITIES TO INCREASE LIVE BIRTH RATES?

3D ultrasound is recommended for the detection of Müllerian uterine malformations that are associated with RPL. With 3D ultrasound, several other uterine abnormalities can be seen. This chapter will explore treatment options for Müllerian uterine malformations that can improve the chances of a live birth in women with RPL, but we will also elaborate briefly on treatment options for other abnormalities.

### 15.1 CONGENITAL UTERINE MALFORMATIONS

#### Evidence

Reconstructive surgery is a treatment option for congenital uterine malformations, but it depends on the type and the severity of the malformation.

#### Septate uterus

For a septate uterus, hysteroscopic metroplasty has become the indicated treatment of choice (Valle, Ekpo 2013). Older studies have discussed abdominal metroplasty, but based on lower morbidity, ease of the procedure and the reduced risk of intrauterine adhesions, hysteroscopic metroplasty is the preferred option, and widely applied (Grimbizis et al. 2001; Valli et al. 2004; Mollo et al. 2011).

A meta-analysis (not specific for RPL) reported a significantly decreased risk of pregnancy loss in women who underwent hysteroscopic septostomy as compared to women who did not undergo treatment (RR 0.37; 95% CI 0.25-0.55; I<sup>2</sup> = 0%; five studies) (Venetis et al. 2014). One meta-analysis of 7 comparative studies involving women with uterine septum and a history of subfertility and/or poor reproductive outcomes showed that hysteroscopic septum resection reduced the rate of pregnancy loss (Krishnan et al. 2021). The women treated by hysteroscopic septum resection had a lower rate of pregnancy loss compared with women with conservative management (OR .25, 95%CI 0.07-0.88). However, no significant effect was seen on live birth, clinical pregnancy rate or preterm delivery.

A more recent meta-analysis showed also that hysteroscopic metroplasty reduced the risk of pregnancy loss in patients with complete uterine septum (OR 0.16, 95%CI 0.03-0.78) or partial uterine septum (OR 0.36 (95% CI 0.19-0.71) (Carrera et al. 2021). The clinical pregnancy rates, the live birth rates and the risk of caesarean delivery were not significantly different between the treated and untreated group.

A prospective study reported pregnancy outcomes in women with RPL ( $\geq 2$  PLs) and uterine malformations. Of the 124 women with a septate uterus, 109 underwent surgery. In women that achieved pregnancy, 78 of 96 (81.3%) women treated with surgery and 8 of 13 (61.5%) women without surgery delivered a live born at the first pregnancy after examination (Sugiura-Ogasawara et al. 2015). There were no significant differences in preterm birth, low birth weight or caesarean section.

Non-controlled and observational studies have suggested a beneficial effect of surgery ([Homer et al. 2000](#)) but are biased by comparing miscarriage rates before and after treatment. Furthermore, most of them describe women with RPL as a small subgroup. One of the largest study on 63 women with RPL and septate uterus reported a decrease in the miscarriage rate from 90% to 10-20% after surgery ([Porcu et al. 2000](#)).

Recent data from the TRUST trial, comparing 79 women with a septate uterus, showed no evidence for benefit from septum resection in term of pregnancy loss (RR 2.3, 95% CI 0.86–5.9), clinical pregnancy (RR 1.2, 95%CI 0.77–1.2), ongoing pregnancy (RR 0.95, 95%CI 0.52–1.8), live birth (RR 0.88, 95% CI 0.47–1.7) or preterm birth (RR 1.3, 95% CI 0.37–4.4) rates ([Rikken et al. 2021](#)). Similar results were obtained in an earlier large cohort study and showed that septum resection does not lead to improved reproductive outcomes compared to expectant management for women with a septate uterus ([Rikken et al. 2020](#)).

Although a reduction in the miscarriage rate in 72 women with RPL and septate uterus was reported in the study of Venturoli, they also reported on pregnancies and deliveries. They found that surgery had a negative impact on fertility, with only 52% becoming pregnant in the first year after surgery. For those becoming pregnant, they found a reduction in the miscarriage rate ([Venturoli et al. 2002](#)).

Hysteroscopic treatment of a symptomatic septate uterus can be accomplished via various methods including hysteroscopic scissors, and electrosurgical electrodes fitted through the hysteroscope (or resectoscope), which are the most common used methods. There is no evidence to elect one method over the others ([Colacurci et al. 2007](#); [Valle, Ekpo 2013](#)). However, the efficiency of such treatment for septum resection has not been defined as most studies do not have a follow up hysteroscopy to compare before and after treatment, and there are no objective morphometric parameters.

#### Other uterine malformations

For hemi-uterus (former AFS unicornuate uterus), uterine reconstruction is not feasible ([Jaslow 2014](#)). However, in cases of hemi-uterus with rudimentary horn and cavity, laparoscopic removal of the rudimentary horn should be considered to avoid “ectopic” pregnancy in this cavity and, in some cases, hematocavity (obstructive symptoms).

Metroplasty (transabdominal or laparoscopically) is the only option for a bicornuate uterus ([Papp et al. 2006](#); [Alborzi et al. 2015](#)). Surgery however showed no benefit for having a live born in women with a bicornuate uterus, but tended to decrease the preterm birth rate and the low birth weight in women with RPL ([Sugiura-Ogasawara et al. 2015](#)). Overall, there is no strong evidence in favor of metroplasty in women having RPL and a bicornuate uterus ([Bailey et al. 2015](#)).

In women with RPL and bicorporeal uterus and double cervix (former AFS didelphic uterus), laparoscopic unification of the uterus has been described, but the efficacy for improving live birth rate, is unclear as the data are based on few studies and few patients ([Alborzi et al. 2009](#); [Jaslow 2014](#); [Alborzi et al. 2015](#)).

We found no evidence supporting any recommendations on treatment of T-shaped uterus or bicorporal septate uterus in women with RPL.

## 3972 Recommendations

There is low quality evidence in favor of hysteroscopic septum resection to reduce the rate of pregnancy loss.

Conditional ⊕○○○

3973

Metroplasty is not recommended for bicorporeal uterus with normal cervix (former AFS bicornuate uterus) and RPL.

Strong ⊕○○○

3974

Uterine reconstruction is not recommended for hemi-uterus (former AFS unicornuate uterus) and RPL.

Strong ⊕○○○

3975

There is insufficient evidence in favor of metroplasty in women with bicorporeal uterus and double cervix (former AFS didelphic uterus) and RPL.

Conditional ⊕○○○

3976

## 3977 Justification

3978 Women with (untreated) congenital uterine malformations have significantly impaired pregnancy  
3979 outcome (*see also chapter 8*) ([Grimbizis et al. 2001](#)).

3980 For women with RPL and septate uterus, several meta-analysis of low quality studies showed a benefit  
3981 of treatment in reducing the miscarriage rate: women who underwent hysteroscopic septum resection  
3982 had a significantly decreased probability of pregnancy loss compared with women who did not undergo  
3983 treatment. There was no effect on the clinical pregnancy rate and live birth rate. However, an  
3984 international, multicentre, open label, randomized controlled trial showed that septum resection does  
3985 not improve the reproductive outcomes in treated women ([Rikken et al. 2021](#)). Larger randomized  
3986 controlled trials are still needed to demonstrate a clear benefit of hysteroscopic septum resection.  
3987 Therefore, the GDG decided to formulate a conditional recommendation base on low quality evidence  
3988 in favor of hysteroscopic septum resection to reduce the rate of pregnancy loss.

3989 For Müllerian malformations other than septate uterus, there are currently no high quality studies to  
3990 support surgery for improving the live birth rate or decreasing the miscarriage rate. Existing studies are  
3991 difficult to summarize as they use different diagnostic criteria, various techniques, different endpoints,  
3992 and a wide range of therapeutic options (transabdominal, hysteroscopic metroplasty by using  
3993 monopolar, bipolar, loop, or scissors).

3994 To establish the value of metroplasty for bicorporeal uterus with normal cervix (former AFS bicornuate  
3995 uterus) conclusively, controlled trials comparing women after surgery with matched controls  
3996 undergoing expectant management are needed. Furthermore, the risk of subfertility after surgery  
3997 should be clarified. For other Müllerian malformations, good quality randomized trials with carefully  
3998 classified patients are urgently needed ([Sugiura-Ogasawara et al. 2013](#)).

### Additional information

In the event of irreparable anatomic uterine abnormalities and RPL, IVF with transfer of embryos to an appropriately selected gestational carrier (surrogacy) can be an option.

More information on the ESHRE/ESGE classification system of female genital tract congenital malformations ([Grimbizis et al. 2013](#)) is available on the ESHRE website ([www.eshre.eu/guidelines](http://www.eshre.eu/guidelines))

## 15.2 ACQUIRED INTRAUTERINE MALFORMATIONS

Although not clearly associated with RPL, acquired intrauterine malformations are detected in women with RPL when performing recommended pelvic ultrasound for the detection of congenital malformations, and studies have evaluated whether treatment of the acquired intrauterine malformations affects the miscarriage rate and the chance of a live birth.

In a recent RCT in women with normal transvaginal ultrasound and subfertility, there was no evidence for improved pregnancy outcomes when performing routine hysteroscopy (including surgical correction of acquired intrauterine malformations) before IVF treatment as compared to immediate IVF (RR 1.06; 95% CI 0.93-1.20) ([Smit et al. 2016](#)).

### Endometrial polyps

Endometrial polyps are found in women with RPL, but there is no clear evidence of an association with pregnancy loss. Although there are no adequate studies showing benefit for polypectomy in RPL, hysteroscopic removal can be considered for larger polyps (>1 cm) in women with RPL without any other known cause ([Lieng et al. 2010](#); [Salim et al. 2011](#); [Jaslow 2014](#)). The size-limit is derived from the observation that a significant proportion (27%) of endometrial polyps regressed spontaneously within one year, and that this was specifically seen in smaller polyps (<1 cm) ([Lieng et al. 2009](#)).

### Fibroids

There are no studies on the effect of treatment of fibroids on the miscarriage rate in women with RPL. In subfertile women with submucosal fibroids, myomectomy did not significantly improve live birth rate or miscarriage rate, as compared to controls with fibroids that did not have myomectomy (based on two observational studies) ([Pritts et al. 2009](#)). Pregnancy rates, live birth rates and miscarriage rates after myomectomy were similar to those in infertile patients without fibroids, indicating a benefit for surgery (based on three studies) ([Pritts et al. 2009](#)). A more recent study reported a benefit of myomectomy with regard to miscarriage rate in women with infertility or RPL and submucosal fibroids ([Roy et al. 2010](#)). The AAGL practice guidelines concluded that at least in selected patients, submucous myomectomy may reduce the risk of spontaneous abortion ([AAGL practice report: practice guidelines for the diagnosis and management of submucous leiomyomas ; Jaslow 2014](#)).

With regard to subserosal and intramural fibroids, these are not considered likely factors contributing to RPL ([Jaslow 2014](#)). For intramural fibroids (i.e. fibroids that do not distort the uterine cavity), myomectomy did not significantly improve live birth rate or miscarriage rate, as compared to controls with fibroids that did not have myomectomy ([Pritts et al. 2009](#)). Furthermore, women with fibroids not distorting the uterine cavity can achieve high live birth rates without intervention ([Saravolos et al. 2011](#)).

## 4037 Recommendations

There is insufficient evidence supporting hysteroscopic removal of submucosal fibroids or endometrial polyps in women with RPL.

Conditional ⊕○○○

4038

Surgical removal of intramural fibroids is not recommended in women with RPL. There is insufficient evidence to recommend removing fibroids that distort the uterine cavity.

Conditional ⊕○○○

## 4039 Justification

4040 Clinical management of RPL in patients with endometrial polyps, submucosal or intramural fibroids is  
4041 controversial, and there is no conclusive evidence that polyps or fibroids are associated with RPL and  
4042 no conclusive evidence that surgical treatment reduces the risk of pregnancy loss.

4043 Hysteroscopic myomectomy for fibroids may be associated with postoperative complications that can  
4044 affect future pregnancies, including the formation of intrauterine adhesions and the risk of uterine  
4045 rupture during pregnancy ([Di Spiezo Sardo et al. 2008](#)). Hence, myomectomy is not recommended.

4046 *Intrauterine adhesions (IUA) (Asherman's syndrome)*

4047 Intrauterine adhesions (IUA) are frequently detected in women with RPL, but the relationship and  
4048 impact of IUAs on long-term reproductive outcomes remain undetermined ([Hooker et al. 2014](#)).  
4049 Furthermore, women with RPL may be predisposed to developing intrauterine adhesions because of a  
4050 previous dilatation and curettage ([Hooker et al. 2014](#); [Jaslow 2014](#)). In reviews on the topic, surgical  
4051 removal for adhesions is recommended for women having RPL ([Kodaman, Arici 2007](#); [Jaslow 2014](#)). In  
4052 the absence of controlled trials, this conclusion is based on small observational studies comparing  
4053 miscarriage rates before and after adhesiolysis.

## 4054 Recommendation

There is insufficient evidence of benefit for surgical removal of intrauterine adhesions for pregnancy outcome. After hysteroscopic removal of intrauterine adhesions in women with RPL, precautions have to be taken to prevent recurrence of adhesions.

Conditional ⊕○○○

## 4055 Justification

4056 The treatment of adhesions is surgical removal. Although small observational studies have shown that  
4057 surgery may decrease miscarriage rates in women with RPL, the GDG decided to formulate a conditional  
4058 recommendation based on the absence of conclusive data on benefit and harm. For severe adhesions,  
4059 benefits with regard to pregnancy and pain symptoms may outweigh the potential harms of surgery. In  
4060 any case, uterine surgery is a known cause for adhesions, and treatment should attempt to prevent  
4061 recurrence of adhesions.

## Additional information

Non-surgical techniques for the removal of intrauterine adhesions (f.i. stem cell therapy) are being explored but need confirmation before being applied in routine practice ([Santamaria et al. 2016](#)).

## 15.3 CERVICAL INSUFFICIENCY

Cervical weakness is believed to be a causing factor for pregnancy loss in women experiencing recurrent second trimester pregnancy loss, but this association is complicated by the absence of a consistent definition, or diagnostic criteria ([Drakeley et al. 1998](#)). Cervical cerclage has been used in the prevention of preterm birth in women with previous second trimester pregnancy loss or risk factors such as short cervix revealed at ultrasound examination.

## Evidence

A Cochrane review on cervical stitch (cerclage) for preventing pregnancy loss found no conclusive evidence that prophylactic cerclage reduces the risk of pregnancy loss or preterm delivery in women at risk of preterm birth or mid-trimester loss due to cervical weakness (based on 4 RCTs). Similarly, there was no evidence of benefit for cerclage in women with evidence for short cervix on ultrasound (2 RCTs with limited number of patients) ([Drakeley et al. 2003](#)).

Another recent review on cerclage (not specifically on pregnancy loss) concluded that the actual groups that benefit of cerclage are limited, but include women with three prior adverse events, and those with a short cervix (<25 mm) who have had a prior preterm birth ([Story, Shennan 2014](#)).

With regard to the technical aspects, a review reported no difference in the reproductive outcomes when the cerclage was performed before or during pregnancy. There was also no difference between laparotomy and laparoscopy, except that most complications, in particular excessive intraoperative blood loss, were reported with laparotomy ([Tulandi et al. 2014](#)). In a recent trial, there was no difference in pregnancy or preterm delivery rates after single (n=14) or double cervical cerclage (n=19) in women with RPL assigned to cervical weakness, but the gestational duration was significantly longer after double cerclage ([Zolghadri et al. 2014](#)).

In a retrospective study of 55 women with prior ultrasound-indicated cerclage (not necessarily RPL), 23 underwent cervical surveillance in the next pregnancy and 57% did not require intervention for a short cervix. Of 23 women that received a history-indicated vaginal cerclage, six delivered preterm (<34 weeks), which was significantly more than the women under surveillance. Eight women receiving an abdominal elective cerclage had good outcomes ([Hall et al. 2015](#)).

## Recommendations

Women with a history of second-trimester PLs and suspected cervical weakness should be offered serial cervical sonographic surveillance.

Strong ⊕⊕○○

In women with a singleton pregnancy and a history of recurrent second-trimester PL attributable to cervical weakness, a cerclage could be considered. There is no

Conditional ⊕⊕○○

evidence that this treatment increases perinatal survival.

4094

## 4095 Justification

4096 Based on inconclusive evidence on the benefit, and taking into consideration the absence of a  
4097 consistent definition or a standardized diagnosis, and the possible harms associated with any surgery,  
4098 the GDG is cautious in the recommendations on cerclage for RPL, but strong in recommending  
4099 ultrasound surveillance.

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# 16. Treatment for RPL with Male factor

## KEY QUESTION: WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO COUPLES WITH RPL DUE TO MALE FACTOR TO INCREASE LIVE BIRTH RATE?

There is moderate evidence of associations between sperm DNA quality and miscarriage. Since there is also clear evidence that sperm DNA damage is caused by unhealthy lifestyles and disease, male partners should be advised of these risks.

### Evidence

#### Smoking cessation

Cigarette smoke contains over 4,000 chemicals, many of which are oxidative; impairing sperm quality and function (Li et al. 2011) and inducing strand breaks in sperm DNA (Arabi 2004; Hsu et al. 2009). Associations between smoking and reduced male fertility, heritable genomic damage and incidence of childhood cancer and impaired mental health of offspring has been well documented (Aitken et al. 2009; Aitken, Bakos 2021). There is no evidence on whether paternal smoking cessation has a beneficial effect on LBRs.

#### Obesity

Obesity is associated with impaired semen parameters and sperm DNA damage (Du Plessis et al. 2010; Keszthelyi et al. 2020). In one study of 520 men, a positive correlation between body mass index and sperm DNA fragmentation was reported, with a 20% increase in sperm DNA damage in obese men (Chavarro et al. 2010). Again, there is no evidence that paternal weight loss has an impact on LBR in RPL.

#### Medications

A range of prescribed drugs has deleterious effects on sperm quality (reviewed by (Sharma et al. 2013; Semet et al. 2017; Montagnoli et al. 2021)). For example, selective serotonin reuptake inhibitors, corticosteroids, antibiotics, anti-inflammatories and even codeine can harm sperm function. Many of these effects are reversible so male partners of couples being investigated for RPL should have a full history taken so that these male risks may be identified, and potentially deleterious medication avoided as part of a holistic approach for the couple.

#### Varicocele repair

Varicocele has an incidence of 40% in men presenting with infertility (Nagler et al. 1997; Dieamant et al. 2017) and it leads to impaired semen quality and increased sperm DNA damage in comparison to healthy donors (Wright et al. 2014; Dieamant et al. 2017). Evidence suggests that varicocelectomy could improve sperm DNA integrity in infertile patients, but there have not been any studies in RPL (Wang et al. 2012; Birowo et al. 2020). In a retrospective study, no significant difference in miscarriage rates were observed after ICSI in 169 men who had undergone varicocele repair when compared with 79 men with clinical varicocele (Pasqualotto et al. 2012). Surgical intervention of varicocele, although it could improve sperm DNA quality, does not translate to a reduction in miscarriages (Cho et al. 2016; Pathak et al. 2016).

In a randomized clinical trial, couples were assigned randomly into two groups: group one (n = 68), in which male partners underwent varicocele repair, and group two (n = 68), which underwent expectant therapy ([Mansour Ghanaie et al. 2012](#)). All of the couples were followed up monthly up to 12 months. All of the women who conceived were followed up until delivery. In each 3-month follow-up visits, two semen analyses were performed. Mean sperm concentration, sperm progressive motility, and sperm with normal morphology improved significantly after elapsing 6 months from varicocelectomy by 75.0%, 15.9%, and 14.3%, respectively, versus the expectant group ( $P < .01$ ). The overall pregnancy rate was 44.1% and 19.1% within a 12-month period in groups 1 and 2, respectively ( $P = .003$ ). Of women who conceived in groups 1 and 2, 13.3% and 69.2% developed miscarriage ( $P = .001$ ). Sperm density/mL ( $r = 0.072$ ;  $P = .001$ ), time elapsed from varicocelectomy ( $r = 0.068$ ;  $P = .001$ ), and female age ( $r = -0.062$ ;  $P = .002$ ) were three most significantly related independent factors to pregnancy rate by multiple regression analysis. Varicocelectomy improves semen quality, increases pregnancy rate, and decreases miscarriage rate significantly. Further controlled studies to confirm our results seem warranted.

#### Sperm selection

Two randomised trials involving patients (not RPL) undergoing an IVF-ICSI cycle reported that ICSI with hyaluronan-selected sperm (so-called physiological ICSI or PICSI) decreased pregnancy loss rates compared with ICSI with sperm selected using standard methods ([Majumdar, Majumdar 2013](#); [Worilow et al. 2013](#)).

A more recent parallel, two-group, randomised trial ([Miller et al. 2019](#)) included couples undergoing an ICSI procedure with fresh embryo transfer at 16 assisted conception units in the UK. Couples were randomly assigned (1:1) with an online system to receive either PICSI or a standard ICSI procedure. This study called HABSelect is the largest randomised trial of PICSI to date and supports the earlier studies. A significant decrease was observed in pregnancy loss rates among couples in the PICSI group. There were no differences between groups in any other outcome. In a mechanistic study further examining this data, the authors concluded that among older women (>35 years) in particular, avoiding sperm with poor DNA quality was the most likely reason for the decrease in pregnancy loss reported in the trial's PICSI arm. It must be noted that these miscarriages were not in women who had a history of recurrent miscarriage.

#### Nutrition and antioxidants

A balanced diet, rich in carbohydrates, fiber, vegetable protein and water, is associated with healthy sperm (i.e. good motility, morphology and DNA quality). Restricting intake of fats, especially trans-fats and sugars is also associated with good sperm quality. Natural antioxidants in the form of vitamins C and E and minerals like Selenium, Iron and Zinc decrease levels of reactive oxygen species (ROS). However, as a small physiological level of ROS is necessary for normal sperm function ([Aitken et al. 2012](#); [Doshi et al. 2012](#)), men would be advised to test for seminal oxidative stress prior to embarking on additional dietary antioxidant supplementation. A major reason for the conflicting evidence is that some men have ROS and need antioxidants whilst others do not have ROS and so antioxidants are of no benefits ([Aitken 2020](#)) and indeed may be deleterious.

A Cochrane review of 61 studies has reported that men with poor semen quality showed improvement in sperm parameters following antioxidant therapy. In three studies reporting miscarriage, no significant difference was found in miscarriage rate between couples randomized to antioxidant therapy compared to placebo (OR 1.74; 95% CI 0.40-7.60). Live birth rate was higher in couples randomized to treatment (OR 1.79, 95% CI 1.20 to 2.67,  $P = 0.005$ , 7 RCTs, 750 men,  $I(2) = 40\%$ , low-

quality evidence) (Smits et al. 2019). However, these numbers are too small to be definitive and further research is needed. We found no studies assessing antioxidant therapy in couples with RPL.

#### Recommendations (updated 2022)

Couples with RPL should be informed that smoking, alcohol consumption, obesity and excessive exercise could have a negative impact on their chances of a live birth, and therefore cessation of smoking, a normal body weight, limited alcohol consumption and a normal exercise pattern is recommended.

GPP

Sperm selection by PICSi may be considered for older women (>35 y) who have experienced RPL

GPP

Antioxidants for men have not been shown to improve the chance of a live birth.

Conditional ⊕○○○

#### Justification

Sperm selection by PICSi leads to a significant decrease in pregnancy loss rates in woman >35y (not RPL) as shown by a RCT (Miller et al. 2019; West et al. 2022) and may be considered as a treatment for couples experiencing RPL. However, further randomized studies including RPL women are needed to assess whether sperm selection by PICSi could improve ART outcomes. Antioxidants for men are often used, but there is no evidence that antioxidants could be helpful in couples with RPL. In a Cochrane review, antioxidants did improve live birth rate after ART in subfertile men, but it did not significantly decrease the chance of a pregnancy loss. Therefore, a conditional recommendation was formulated.

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DRAFT for REVIEW

# 17. Treatment for unexplained RPL

**KEY QUESTION: WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO COUPLES WITH UNEXPLAINED RPL TO INCREASE LIVE BIRTH RATE?**

## 17.1 LYMPHOCYTE IMMUNIZATION THERAPY

In the 1980s deliberate immunization of women with RPL with allogeneic lymphocytes (lymphocyte immunization therapy or LIT) became increasingly used after a randomized controlled trial suggested a beneficial effect of immunization with partner lymphocytes ([Mowbray et al. 1985](#)). The theory for using LIT was that women with RPL lack anti-paternal antibodies or blocking antibodies that protect the foetus against rejection, and the subsequent production of these antibodies after LIT was suggested to be beneficial ([Beer et al. 1981](#)). In most of the randomized trials of LIT, patients were selected due to absence of anti-paternal cytotoxic or blocking antibodies in the blood; however, the clinical impact of such antibodies is unclear ([Lashley et al. 2013](#)), which weakens the scientific rationale for the therapy.

### Evidence

A Cochrane systematic review on the efficacy of LIT found an OR for live birth in treated patients to be 1.23 (95% CI 0.89-1.70) based on 12 randomized trials using paternal lymphocytes and 1.39 (95% CI 0.68-2.82) based on three trials using third-party lymphocytes compared with placebo ([Wong et al. 2014](#)). There was no significant benefit for LIT treatment on live birth rate neither with paternal, nor with third-party donor lymphocytes in women with RPL.

Several of the included randomized controlled trials did not meet current criteria for methodological quality (uncertain/high risk of bias) and potential adverse effects were not adequately described. Treatment with allogeneic cells raises serious safety concerns and in transfusion practice great efforts are made to lymphocyte-deplete blood before used for transfusion. There is a substantial risk of neonatal alloimmune thrombocytopenia and production of red blood cell antibodies, which can result in erythroblastosis fetalis ([Christiansen et al. 1994](#)), some risks of transferring infectious agents such as hepatitis and HIV and maybe an increased long term risk of hematological malignancies.

However, injections with paternal lymphocytes before conception seems to be associated with a low risk of serious adverse events as reported in a long-term follow-up study of immunized women with RPL or implantation failure ([Kling et al. 2006](#)).

### Recommendation

Lymphocyte immunization therapy should not be used as treatment for unexplained RPL as it has no significant effect and there may be serious adverse effects.

Strong ⊕⊕○○

### Justification

LIT should not be used in clinical practice since its scientific foundation is weak, its effect to prevent miscarriage is not established and proven and potential adverse effects have been described. If further

4412 randomized controlled trials on LIT are carried out they should be conducted using strict  
4413 methodological rigor and include long-term follow-up of mothers and babies.

## 4414 17.2 INTRAVENOUS IMMUNOGLOBULIN (IVIG)

4415 Intravenous immunoglobulin (IVlg) is known to reduce symptoms in many autoimmune and  
4416 inflammatory diseases through a multitude of mechanisms including elimination of immune complexes,  
4417 interactions with Fc-receptors, elimination of activated complement factors, interference with antigen  
4418 presentation and neutralization of inflammatory cytokines.

### 4419 Evidence

4420 A recent systematic review and meta-analysis of IVlg in RPL ([Egerup et al. 2015](#)) included 11 RCTs and  
4421 found in 531 patients a RR of 0.92 (95% CI 0.75-1.12) for *no* live birth (= miscarriage) after IVlg. In women  
4422 with secondary RPL, a subset that in previous randomized controlled trials seemed to benefit from IVlg  
4423 ([Hutton et al. 2007](#)), the RR for *no* live birth after IVlg was 0.77 (95% CI 0.58-1.02), which can be  
4424 translated into a borderline non-significant benefit of IVlg in secondary RPL. In women with primary  
4425 RPL, the RR for *no* live birth after IVlg was 1.32 (95% CI 0.88-1.98). A more recent meta-analysis reported  
4426 a similar conclusion but also suggests that live birth rate was significantly improved in women with RPL  
4427 if treatment was started before conception (RR 1.67; 95% CI 1.30-2.14), but not if started after  
4428 implantation ([Wang et al. 2016](#)).

4429 A trial sequential analysis in the review concluded that even with meta-analysis, studies are  
4430 underpowered for definitive conclusions about the efficacy of IVlg in RPL. Furthermore, the protocols  
4431 used in the randomized trials were very heterogeneous with substantial variations between IVlg  
4432 dosages used and start of treatment before or during pregnancy.

4433 Moderate adverse events such as headache and skin rash were significantly more frequent in IVlg-  
4434 treated compared to placebo-treated patients but there was no difference in the incidence of serious  
4435 adverse events.

### 4436 Recommendation

Intravenous immunoglobulin (IVlg) is not recommended as  
a treatment of RPL.

Strong

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### 4437 Justification

4438 IVlg cannot be recommended for clinical use in women with either primary RPL or secondary RPL.  
4439 Further randomized controlled trials should be carried out and given the results of meta-analysis, the  
4440 focus should be on women with secondary RPL and on treatment started before conception.

## 4441 17.3 PREDNISOLONE

4442 Glucocorticoids exhibit a beneficial clinical effect in most autoimmune inflammatory diseases and are  
4443 therefore a potential useful therapy in women with RPL with a suspected immune etiology. They have  
4444 only been tested in two randomized placebo-controlled trials in women with RPL positive for specific  
4445 immunological biomarkers.

## Evidence

An RCT including 150 women with unexplained RPL; showed that the rate of ongoing pregnancy beyond 20 weeks was higher in 74 women receiving prednisolone (5 mg/day) treatment compared to 76 women receiving placebo (RR 7.63; 95% CI 3.70-15.70). Both the intervention and placebo group received empiric treatment with low dose aspirin and heparin (Gomaa et al. 2014). In a feasibility study, women with unexplained RPL and high uNK cell density were randomized when pregnant to prednisolone treatment (20 mg for 6 weeks, 10 mg for 1 week, 5 mg for 1 week) (n=20) or placebo (n=20). The live birth rate was 60% in the prednisolone group and 40% in the placebo group (RR 1.5; 95% CI 0.8-209.0).

Laskin and colleagues carried out a placebo-controlled trial of prednisolone and low-dose aspirin to women with RPL and positivity for antiphospholipid, antinuclear, anti-DNA or anti-lymphocyte antibodies (Laskin et al. 1997). A very high prednisolone dose (40-50 mg/day) was administered for the whole duration of pregnancy. In the treatment group, a 9% higher live birth rate was found, which was not significantly different from controls (OR 1.5; 95% CI 0.8-2.6). However, the treated patients had a significantly higher risk of preterm birth (62% versus 12%,  $p < 0.001$ ) and higher risks for diabetes and hypertension, which is well known to be associated with high and prolonged administration of prednisolone.

## Recommendation

Glucocorticoids are not recommended as a treatment of unexplained RPL or RPL with selected immunological biomarkers.

Strong

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## Justification

The evidence points toward some beneficial effect of prednisolone in women with RPL selected due to positivity for selected biomarkers. However, based on adverse events associated with the use of prednisone, the GDG decided to recommend against treatment awaiting further studies.

New randomized trials administering lower doses of prednisone (in order to reduce side effects) to RPL patients before pregnancy and in the first trimester should be carried out. Patients could be selected for such trials due to presence of biomarkers suggesting immune activation. Trials may also be conducted in women with unexplained RPL realizing that we still have no biomarkers that can identify patients with an immune etiology with sufficient specificity.

## 17.4 ANTICOAGULANTS

Due to the evidence from randomized controlled trials that heparin and low-dose aspirin seem to be beneficial in the treatment of women with RPL and antiphospholipid antibodies, heparin and low-dose aspirin have been increasingly administered to RPL women without antiphospholipid antibodies.

## Evidence

In a Cochrane review, live birth rate after anticoagulant (aspirin, heparin, or combination of aspirin and heparin) or placebo/no treatment or another anticoagulant in women with RPL with or without

hereditary thrombophilia. There were no significant benefits for any of the anticoagulants in comparison to placebo or no treatment ([de Jong et al. 2014](#)).

For the comparison of heparin versus placebo, three RCTs were published after the inclusion deadline of the review ([Pasquier et al. 2015](#); [Schleussner et al. 2015](#)). There was no benefit of heparin compared to placebo/multivitamins with regard to live birth rate. Two of these RCTs showed no benefit ([Pasquier et al. 2015](#); [Schleussner et al. 2015](#)), while the third study reported a decrease in miscarriage rate and an increase in LBR ([Shaaban et al. 2016](#)).

#### Recommendation

Heparin or low dose aspirin are not recommended, as there is evidence that they do not improve live birth rate in women with unexplained RPL.

Strong

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#### Justification

Based on a meta-analysis and results of two subsequent large randomized controlled trials there is no evidence that heparin alone, aspirin alone, or heparin in combination with low-dose aspirin improves the live birth rate in unexplained RPL.

### 17.5 FOLIC ACID

#### Evidence

Folic acid in pregnancy is recommended for the prevention of neural tube defects and high-dose supplementation can reduce high plasma homocysteine levels that may be harmful in pregnancy. However, there has been performed no randomized controlled trials testing folic acid supplementation versus no folic acid supplementation in the prevention of pregnancy loss in women with RPL with or without hyperhomocysteinemia. One randomized controlled trial found similar live birth rates in women with RPL and specific polymorphisms in the MTHFR gene supplemented with either folic acid or methyltetrahydrofolate during pregnancy ([Hekmatdoost et al. 2015](#)).

High folic acid intake may have negative effects especially in elderly people with low B12 vitamin levels and a study also suggested a higher frequency of insulin resistance in children born to mothers taking high dose folic acid ([Selhub, Rosenberg 2016](#)). Therefore, high-dose folic acid supplementation is only recommended for selected groups of women trying to conceive ([Yajnik et al. 2008](#)).

#### Recommendation

Low dose folic acid is routinely started preconceptionally to prevent neural tube defects, but it has not been shown to prevent pregnancy loss in women with unexplained RPL.

Strong

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## Justification

Based on the absence of evidence for a benefit, and possible harms, high-dose folic acid supplementation should not be used for women with RPL without hyperhomocysteinemia or underlying conditions (diabetes, epilepsy) associated with increased risk of neural tube defects.

## 17.6 PROGESTERONE

### Evidence

A double blind, placebo-controlled, randomized trial of oral dydrogesterone (given from the time that a live foetus was confirmed by ultrasound until 20 weeks of gestation) among 360 women with a RPL also showed a benefit of progesterone in reducing a subsequent risk of miscarriage compared with placebo (RR 2.4; 95% CI 1.3-5.9) ([Kumar et al. 2014](#)). The main problem with this study is that treatment was initiated at a late stage of first trimester (mean gestational age  $6.5 \pm 1.1$  weeks and  $6.5 \pm 1.2$  weeks, for treatment and placebo group respectively), which is also reflected in very high live birth rates in both the treatment (93%) and the placebo (83%) groups.

A multicenter, double blind, placebo-controlled, randomized trial (PROMISE trial) investigated vaginal progesterone as a treatment to improve live births in women with unexplained RPL ([Coomarasamy et al. 2015](#)). Women were randomized to twice daily vaginal suppositories containing either 400 mg of micronized progesterone (n=398) or matched placebo (n=428) from a time soon after a positive urinary pregnancy test (up until 6 weeks) up through 12 weeks of gestation. There was no difference in the rate of live births in the progesterone group (65.8%) compared to the placebo group (63.3%) (RR 1.04; 95% CI 0.94-1.15).

A meta-analysis combined 10 trials, including the trials of Kumar and Coomarasamy, to a total of 802 women receiving progesterone and 784 receiving placebo. Women with RPL who were randomized to the intervention group had a lower risk of subsequent pregnancy loss (RR 0.72; 95% CI 0.53-0.97) and higher live birth rate (RR 1.07; 95% CI 1.02-1.15) compared with those who did not. Discrepancies in the conclusion of this meta-analysis with the largest included trial were explained by the differences in progesterone supplement, and the inclusion of seven trials published before 1990 when the quality standards for RCTs were lower ([Saccone et al. 2017](#)). In the most recent Cochrane systematic review on progesterone administration in unexplained RPL including 10 trials with 1684 patients (probably almost the same patients as in the Saccone et al. analysis ([Saccone et al. 2017](#))), the risk of subsequent pregnancy loss in the intervention group was the same as in this meta-analysis (RR 0.73; 95% CI 0.54-1.00) ([Haas et al. 2019](#)).

In the study by Coomarasamy and colleagues, data were combined from the PROMISE trial ([Coomarasamy et al. 2015](#)) of 836 women with RPL and from the PRISM study ([Coomarasamy et al. 2019](#)) of 4153 women (some of them with RPL) with bleeding in early pregnancy who were randomized to vaginal progesterone or placebo. The finding of importance for this guideline is that the risk ratio for subsequent live birth in progesterone-treated women with a minimum of 3 previous pregnancy losses and current bleeding was significantly increased (RR = 1.28, 95% CI 1.08-1.51; rate difference 15%) ([Coomarasamy et al. 2020](#)).

## 4546 Recommendation (updated 2022)

Vaginal progesterone may improve live birth rate in women with 3 or more pregnancy losses and vaginal blood loss in a subsequent pregnancy.

Strong

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## 4547 Justification

4548 Recent meta-analysis showed a benefit of progesterone on miscarriage rate and live birth rate ([Saccone et al. 2017](#)). However, the meta-analysis is flawed by the quality of the older included studies, and  
4549 hence, we decided to base the updated recommendation on the combination of recent high quality  
4550 trials ([Coomarasamy et al. 2015](#); [Coomarasamy et al. 2019](#)). Vaginal progesterone during early  
4551 pregnancy (started <12 weeks) may have a beneficial effect in women with unexplained RPL (16–39-  
4552 year-old) with vaginal bleeding and it is therefore recommended to use vaginal progesterone (twice-  
4553 daily 400 mg progesterone from presentation to 16 weeks of gestation) to improve live birth rate in a  
4554 subsequent pregnancy.  
4555

4556 There is some evidence that oral dydrogesterone initiated when foetal heart action can be confirmed  
4557 may be effective. Furthermore, as progesterone is important during implantation of the embryo,  
4558 benefit from supplementation may be realized if progesterone is administered from the luteal phase,  
4559 rather than after a positive pregnancy test. More trials are needed to evaluate oral progesterone and  
4560 administration of progesterone from the luteal phase.

## 4561 17.7 INTRALIPID THERAPY

4562 Intravenous lipid emulsions (such as Intralipid) were initially developed to boost nutrition after surgery  
4563 and in premature babies. In recent years, Intralipid has emerged as a treatment for poisoning by local  
4564 anesthetics and various other drugs.

## 4565 Evidence

4566 Clark and colleagues reported that infusions of Intralipid reduced the foetal resorption rate in specific  
4567 mice matings ([Clark 1994](#)). Roussev and colleagues reported that NK cell cytotoxicity declined after  
4568 Intralipid infusions to recurrent implantation failure patients (to the same level as after Ivlg infusions)  
4569 and they therefore extrapolated that Intralipid had a beneficial effect in RPL ([Roussev et al. 2008](#)).

4570 No randomized controlled trial has so far tested Intralipid versus no treatment or placebo, but one trial  
4571 found that the live birth rate in women with RPL after Intralipid treatment was similar (92%) to that  
4572 after Ivlg (88%) ( $p=0.415$ ) ([Meng et al. 2015](#)).

4573 No serious adverse effects has been reported after the use of low dose intralipid treatment in women  
4574 with RPL ([Roussev et al. 2008](#); [Meng et al. 2015](#)). However, a series of serious adverse effects has been  
4575 reported after the use of higher doses of intravenous lipid emulsions: acute kidney injury, cardiac arrest,  
4576 acute lung injury, venous thromboembolism, fat embolism, fat overload syndrome, pancreatitis, allergic  
4577 reactions and increased susceptibility to infection ([Hayes et al. 2016](#)).

## 4578 Recommendation

There is insufficient evidence to recommend intralipid therapy for improving live birth rate in women with unexplained RPL.

Strong ⊕○○○

## 4579 Justification

4580 There is no clinical evidence at all to support the use of Intralipid therapy in the treatment of RPL.

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DRAFT for REVIEW

## 17.8 GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF)

### Evidence

Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) are growth factors that may promote trophoblast growth and have been proposed to have anti-abortion effects based on animal studies. Although the mechanism is unknown, studies have been conducted in women with RPL and recurrent implantation failure.

A review found two studies on G-CSF as treatment for RPL. Both studies ([Scarpellini, Sbracia 2009](#); [Santjohanser et al. 2013](#)) found a beneficial effect of G-CSF on the outcome of subsequent pregnancy ([Cavalcante et al. 2015](#)). In the first included RCT, 68 women with a history of unexplained RPL who had previously been unsuccessfully treated with IVF, were randomized to placebo (n=33) (saline) or recombinant G-CSF treatment (n=35) (a dose of 1 µg [100,000 IU]/kg/day of Filgrastim subcutaneously from the sixth day after ovulation until the onset of menstruation or the end of the ninth week of pregnancy) ([Scarpellini, Sbracia 2009](#)). All women in the study became pregnant spontaneously within 3 months. The success rate was 82.8% in the treated group (29 live births in 35 pregnancies) and 48.5% in the placebo group (16 in 33 pregnancies). The difference between the groups was statistically significant (OR 5.1; 95% CI 1.5-18.4; p=0.0061). The second study, a retrospective cohort study, evaluated the effect of G-CSF in women with a history of RPL and infertility who underwent IVF/ICSI by comparing a group treated with G-CSF (49 women), a group not treated with any medication (33 patients) and a group treated with other medications (45 women). For the G-CSF group a pregnancy rate of 47% and a live-birth rate of 32% was reported ([Santjohanser et al. 2013](#)). The group who received other medications had a pregnancy rate of 27% (p=0.016) and a live birth rate of 14% (p=0.006), and the subgroup who received no medications had a pregnancy rate of 24% (p=0.016) and a live birth rate of 13% (p=0.016). There were several methodological problems in this study: it was retrospective, many women were treated in several IVF/ICSI cycles and there is no information about whether the pregnancy and live birth rates were calculated per cycle or per patient. Furthermore, prognostic variables were not equally distributed in the three groups.

A high-quality multicentre RCT included 150 patients with RPL who were randomized to recombinant G-CSF or placebo ([Eapen et al. 2019](#)). The live birth rate was 59.2% in the G-CSF group and 64.9% in the placebo group (RR 0.9 95% CI 0.7-1.2) suggesting no beneficial effect of G-CSF in unexplained RPL. Due to its high quality, the results of this trial should overrule those of previous reports.

There are several ongoing randomized trials of G-CSF in women with recurrent implantation failure. One of these is completed but found no beneficial effect of uterine instillations with G-CSF on implantation and pregnancy rates after IVF ([Barad et al. 2014](#)).

### Recommendation (updated 2022)

There is no evidence to recommend G-CSF in women with unexplained RPL.

Strong

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## Justification

The results from a recent trial ([Eapen et al. 2019](#)) overrule those from the Scarpellini and Sbraca trial ([Scarpellini, Sbraca 2009](#)) due to the much larger size of the former study and its high quality, and showed that there is no beneficial effect of G-CSF in unexplained RPL.

## 17.9 ENDOMETRIAL SCRATCHING

### Evidence

Scratching of the endometrium in the luteal phase prior to an IVF/ICSI cycle has gained widespread use in women with recurrent implantation failure; the theory is that the procedure will liberate cytokines and chemo-attractants of importance for subsequent embryo implantation. In an editorial comment the editor-in-chief of Human Reproduction has recently challenged the evidence for using this procedure in any patient before awaiting results from more controlled trials ([Evers 2016](#)). So far, no trial has been performed in women with RPL.

### Recommendation

There is no evidence to recommended endometrial scratching in women with unexplained RPL

GPP

### Justification

There is no evidence that endometrial scratching improves subsequent pregnancy outcome in women with RPL. Based on clinical expertise, the GDG decided to formulate this in a recommendation.

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## 18. Non-conventional treatments for RPL

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### KEY QUESTION: WHICH THERAPEUTIC INTERVENTIONS COULD BE OFFERED TO ALL COUPLES, IRRESPECTIVE OF A CAUSE, TO INCREASE LIVE BIRTH RATES?

A range of treatments has been proposed for women with RPL, especially women with unexplained RPL, with the aim of increasing live birth rates.

#### Evidence

##### Chinese Herbal treatment

A Cochrane review included nine RCTs (involving 861 women) on Traditional Chinese Medicine for improving live birth or pregnancy rate in couples with RPL. The reviewers concluded that the methodological quality was too poor to comment on the efficacy of Traditional Chinese Medicine for RPL, based on small sample sizes and unclear risk of bias (Li et al. 2016). Another older review came to a similar conclusion based on 41 studies (involving 3660 participants) comparing Chinese herbal medicine alone or in combination with conventional medicine, with placebo or conventional medicine (Yang et al. 2013). Overall, it is unclear, based on the available studies – all conducted in China and with different compositions of herbs – whether Chinese Herbal treatment is effective, and in addition, data on safety are scarcely reported, which may evoke serious concerns.

##### Acupuncture

The effectiveness of acupuncture for improving the chance of a live birth in couples with RPL has been described in case reports (Hullender Rubin et al. 2013). However, we did not find any studies systematically evaluating acupuncture as a treatment for RPL.

##### IVF/ICSI

A detailed description on IVF/ ICSI combined with PGS can be found in chapter 10: treatment of RPL due to genetic/ chromosomal causes. To our knowledge there are no studies evaluating IVF/ICSI (without PGT) in couples with RPL.

##### Diet – antioxidants

A narrative review summarized the basic science and clinical case reports for antioxidants to improve pregnancy outcome by reducing oxidative stress in the placenta based on a literature search (Hovdenak, Haram 2012). The authors concluded that whilst vitamin C may confer some benefit to pregnancy outcomes, vitamin E could be harmful. In the absence of well-designed and controlled studies, vitamin supplements or antioxidants cannot be recommended to improve pregnancy outcome in women with RPL, except where a specific deficiency has been detected.

##### Other treatments

We found no studies on other therapies for couples with RPL, including homeopathy. Recently, bioresonance therapy and nanotechnology have been suggested as treatment options for pregnancy loss, but there are no data available supporting their use in clinical practice.

## 4773 Recommendation

If women with RPL ask about using multivitamin supplements, they should be advised on multivitamin supplements that are safe in pregnancy.	GPP
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## 4774 Justification

4775 Based on frequent questions from couples, it was decided to add a recommendation on vitamin  
 4776 supplements. As there is no conclusive evidence supporting the use of vitamin supplements, they are  
 4777 not recommended as treatment. However, based on the possible harms associated with some vitamin  
 4778 supplements (vitamin E, A), the GDG recommends advice on safe options.

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## Annexes

Annex 1: Guideline development group

Annex 2: Recommendations for research

Annex 3: Methodology

DRAFT for REVIEW

# Annex 1: Guideline Development Group

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This guideline was developed and updated by the ESHRE Early Pregnancy Guideline Development Group (GDG). The GDG included gynecologists with expertise in reproductive medicine, miscarriage and recurrent miscarriage, thrombophilia, and male infertility. A representative of the Miscarriage Association (UK) was added to the GDG to represent the patient perspective. We aimed for an equal distribution in gender, region and expertise.

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## Annex 2: Recommendations for research in RPL

From the literature and discussion of the available evidence, several topics were identified for which evidence is inconsistent, insufficient or non-existing. For the benefit of couples with RPL, the GDG recommends that future research, where possible in well-designed RCTs, should focus on these research gaps.

### Definition of RPL

- Perform epidemiological studies on the effect of various RPL definitions on diagnosis, prognosis, or treatment.

### Organization of Care

- Develop a prognostic model to provide an individually based live birth prognosis.
- Develop E-health tools for support to couples with RPL and staff.

### Genetics

- Establish the value of using NGS for PGD-A in couples with RPL.
- The role of genetic analysis of pregnancy tissue needs to be clarified (prognostic modelling).

### Thrombophilia

- Study the effect of anticoagulant treatment for RPL women with hereditary thrombophilia
- With regard to RPL and APS:
  - Study clinical criteria for diagnosis and treatment of APS (e.g. female age, number of pregnancy losses, consecutive or non-consecutive losses).
  - Assess the effectiveness of heparin treatment from comparison with placebo/no treatment
  - Compare the efficacy and safety of LMWH versus UFH.
  - How should heparin be administered; start before conception (ante partum), start after foetal heartbeat, throughout whole pregnancy from positive pregnancy test, up to 36 weeks or later?
  - Evaluate the effect of hydroxychloroquine in couples with RPL. Hydroxychloroquine has been safely used in APS pregnancies and lupus pregnancies for preventing obstetric complications.

### Immunology

- Study the association between subsequent pregnancy outcome and HLA polymorphism in non-Scandinavian women with RPL
- Study the effect of moderate dosages of prednisolone in RPL (preferably in large, controlled trials).
- Study the effect of IVIg treatment in women with secondary RPL.
- Study the effect of immunotherapy in subsets of women with RPL with specific HLA class II alleles (in RCTs)

Metabolic factors

- Study the effect of Levothyroxine treatment in women with RPL and identified subclinical hypothyroidism.

Uterine malformations

- Clarify the role of congenital uterine malformations in RPL and the associated live birth rates per type of congenital uterine abnormality (preferably in well-controlled prospective trials).
- Evaluate whether hysteroscopic septum resection has beneficial effects in women with RPL (increasing live birth rates, and decreasing miscarriage rates, without doing harm).

Male factor

- Study the mechanisms of sperm DNA damage.
- Study the effect of male lifestyle alterations with outcomes of both sperm DNA per se and RPL (in randomized controlled trials).
- Study the effect of antioxidant therapy for men on RPL; specifically to determine the best combinations and extent of dietary vitamin supplementation in the protection of sperm DNA from fragmentation.

Female factor

- Study the effect of pre-conceptual weight loss on live birth rate using diet, exercise of therapeutic interventions.
- Define optimal endometrial characteristics for pregnancy; develop tests that detect women with sub-optimal endometrium and treatments to improve it.
- Further research is needed on the role of (chronic) endometritis in RPL, including prospective observational studies and randomized controlled trials.

## Annex 3: Methodology

### GUIDELINE DEVELOPMENT

European Society of Human Reproduction and Embryology (ESHRE) guidelines are developed based on the Manual for ESHRE guideline development (N. Vermeulen, A. D'Angelo, P. de Sutter, W.L.D.M. Nelen, Manual for ESHRE guideline development, version 2013), which can be consulted at the ESHRE website ([www.eshre.eu/guidelines](http://www.eshre.eu/guidelines)). The principal aim of this manual is to provide stepwise advice on ESHRE guideline development for members of ESHRE guideline development groups. The manual describes a 12-step procedure for writing clinical management guidelines by the guideline development group, supported by the ESHRE methodological expert.

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The current guideline was developed and funded by ESHRE, which covered expenses associated with the guideline meetings (travel, hotel and catering expenses) associated with the literature searches (library costs, costs associated with the retrieval of papers) and with the implementation of the guideline (printing, publication costs). Except for reimbursement of their travel expenses, GDG members did not receive any payment for their participation in the guideline development process.

The scope of the guideline and first version of the key questions were drafted by the coordinator and deputies of the ESHRE Special Interest Group Implantation and Early Pregnancy. A call was launched for experts in the field interested in joining the guideline development group. All applications were reviewed, and experts were selected based on expertise and geographical location. We strived towards a balance in gender and location within Europe. A meeting of the guideline development group was organized to discuss the key questions and redefine them through the PICO process (patients – interventions – comparison – outcome). This resulted in a final list of 18 key questions. Based on the defined key words, literature searches were performed by the methodological expert. Key words were sorted to importance and used for searches in PUBMED/MEDLINE and the Cochrane library. We searched the databases from inception up to 31 March 2017. The literature search was repeated in November 2021 and February 2022.

Literature searches were performed as an iterative process. In a first step, systematic reviews and meta-analyses were collected. If no results were found, the search was extended to randomized controlled trials, and further to cohort studies and case reports, following the hierarchy of the levels of evidence. Reference were selected or excluded by the methodological expert and expert GDG member based on title and abstract and knowledge of the existing literature. If necessary, additional searches were performed in order to get the final list of papers. The quality of the selected papers was assessed by means of the quality assessment checklist, defined in the ESHRE guideline manual. Furthermore, the evidence was collected and summarized in an evidence table according to format suggested by the

Guidelines International network (GIN) (<http://www.g-i-n.net/activities/etwg>). The quality assessment and evidence tables were constructed by the expert GDG members. Summary of findings tables (Annex 2) were prepared according to the GRADE approach for all interventions with at least two studies per outcome. Where available, summary of findings tables were based on existing up-to-date well-executed systematic reviews, if necessary supplemented with additional recent RCTs. When there was no recent valid systematic review available, we systematically searched for relevant studies, as described above. Cumulative live birth rate, live birth rate and pregnancy loss rate (or miscarriage rate) were considered the critical outcomes.

GDG meetings were organized to discuss the draft recommendations and the supporting evidence and to reach consensus on the final formulation of the recommendations. In a final step, all evidence and recommendations were combined in the ESHRE guideline: "Recurrent Pregnancy Loss".

The studies retrieved from the update of the literature searches (i.e. studies published between March 2017 and February 2022) were evaluated with regards to their relevance for the existing guideline and impact on the recommendations. Some references were added in the text without further implications for the recommendations. In specific sections, new studies or reviews retrieved from the literature were added and recommendations reformulated. All modified sections were labelled as "updated (2022)" and adaptations to the recommendations explained in the justification sections. For all other sections and recommendations, no new publications were found in the literature search, and the evidence and recommendations are considered up-to-date and are reconfirmed in the current update.

## FORMULATION OF RECOMMENDATIONS

We labelled the recommendations as either "strong" or "conditional" according to the GRADE approach. We used the words "we recommend" for strong recommendations and "we suggest" for conditional recommendations. Suggested interpretation of strong and conditional recommendations by patients, clinicians and health care policy makers is as follows:



4925 For each recommendation, it is mentioned whether it is strong or conditional and what the quality of  
4926 the supporting evidence was. In the justification section, more data are provided on the considerations  
4927 taken into account when formulating the recommendations: balance between desirable and  
4928 undesirable effects, certainty of the evidence of effects, certainty in how people value the outcome,  
4929 acceptability and feasibility of the intervention. Impact on health equity and resource impact were only  
4930 discussed where relevant.

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