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Recurrent Pregnancy Loss

Guideline of European Society of Human Reproduction and Embryology

Update 2022

ESHRE Recurrent Pregnancy Loss Guideline Development Group

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Contents

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 However, the ESHRE SIG Implantation and Early Pregnancy believed that these guidelines were outdated and initiated the first version of this guideline, published in 2017.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, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Human reproduction (Oxford, England)* 2006;21: 2216-2222.

List of all recommendations[1]

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	

[1] Abbreviations: GDG, guideline development group; GPP, good practice point; RCT, randomized controlled trial; RPL, Recurrent Pregnancy Loss; SOF, summary of findings table.

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 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	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 fetal problems (Fetal 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			
		INVESTIGATIONS IN RPL				
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 the complete pregnancy history and female age provide the best available prognostic information	
3	10	The GDG recommends to base prognosis on woman's age and her complete pregnancy history, including number of previous pregnancy losses, live births and their sequence.	Strong	⊕⊕⊕■		UPDATED (2022)
4	11	Genetic analysis of pregnancy tissue following pregnancy loss 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 could be carried out after individual assessment of risk for diagnostic or explanatory purposes.	Conditional	⊕⊕■■	There is no need for routine testing. 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.	
5	14	For women with RPL, we suggest not to screen for hereditary thrombophilia unless in the context of research, or in women with	Conditional	⊕⊕⊕■	There is no, or at best a weak, association between RPL and hereditary thrombophilia.	

		additional risk factors for 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 β2 glycoprotein I antibodies (aβ2GPI) can be considered after two pregnancy losses.	GPP		Based on a study showing treatment can improve LBR in women with RPL and aβ2GPI, 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.	UPDATED (2022)
6	18	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	19	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 an association to RPL has been reported in a meta-analysis 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.	
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) 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 (former AFS bicornuate uterus) with normal cervix.	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 could have 2D ultrasound to rule out adenomyosis	Conditional	⊕⊕■■		New (2022)
9	39	In couples with RPL, it is recommended to assess lifestyle in the male partner (paternal age, smoking, alcohol consumption, exercise pattern, and body weight).	Strong	⊕⊕■■	Based on suggested association between lifestyle and sperm quality.	UPDATED (2022)
9	40	Assessing sperm DNA fragmentation in couples with RPL could be considered for diagnostic purposes.	Conditional	⊕⊕⊕■	There is a growing body of evidence showing strong association between sperm DNA damage and RPL.	UPDATED (2022)
		TREATMENT TO INCREASE LIVE BIRTH RATE IN RPL				
10	*10*	The guideline development group (GDG) recommends to base prognosis on woman's age and her complete pregnancy history, including number of previous pregnancy losses, live births and their sequence.	Strong	⊕⊕⊕■	The GDG concludes that a thorough reproductive history should be taken in couples presenting with RPL and stresses that the complete pregnancy history and female age provide the best available prognostic information	
10	41	Prognostic tools (Kolte & Westergaard) can be used to provide an estimate of subsequent chance of live birth in couples with RPL.	GPP			UPDATED (2022)
11	42	All couples with results of an abnormal fetal 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 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.	SOF table 1
11	43	All couples with results of an abnormal fetal or parental karyotype may be informed about the possible treatment options available including their advantages and disadvantages.	GPP			
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	Conditional	⊕⊕■■	We found no evidence of a beneficial effect of anticoagulant treatment in women with RPL and hereditary thrombophilia.	SOF table 2

		(VTE) prevention				
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	SOF table 3-5
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 fetal 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.	SOF table 6
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 gestational age), 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 hypothyroidism should be treated with levothyroxine	GPP			
14	51	Euthyroid women with thyroid antibodies and RPL should not be treated with levothyroxine.	Strong	⊕⊕⊕■	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) SOF table 6
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.	SOF table 7

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	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 / fetal complications, prescribing vitamin D supplementation can be considered, even though evidence for the effectiveness is absent. Vitamin D supplementation can be considered safe.	
15	56	Only one small RCT showed no benefit of using hysteroscopic septum resection to reduce the rate of pregnancy loss.	Conditional	⊕■■■	The only RCT published regarding this hysteroscopic septum resection (Rikken et al 2021) showed that using this intervention in women with septate uterus and RPL does not improve the pregnancy outcomes.	UPDATED (2022)
15	57	Metroplasty is not recommended for bicorporeal uterus with normal cervix (former AFS bicornuate 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	58	Uterine reconstruction is not recommended for hemi-uterus (former AFS unicornuate uterus) and RPL.	Strong	⊕■■■		
15	59	There is insufficient evidence in favor of metroplasty in women with bicorporeal uterus and double cervix (former AFS didelphic uterus) and RPL.	Conditional	⊕■■■		
15	60	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	61	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	62	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	63	Women with a history of second-trimester pregnancy losses and suspected cervical weakness should be offered serial cervical sonographic surveillance.	Strong	⊕⊕■■	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.	
15	64	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	⊕⊕■■		
16	65	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	66	There is no evidence to support sperm selection by physiological intracytoplasmic sperm injection (PICSI) in couples with RPL.	Conditional	⊕■■■	Although sperm selection by PICSI leads to a significant decrease in pregnancy loss rates in women >35y (not RPL) as shown by a RCT, more evidence is needed to recommend this treatment for couples with RPL.	UPDATED (2022) SOF table 8
16	67	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	SOF table 9
17	68	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.	SOF table 10
17	69	The use of repeated and high doses of Intravenous immunoglobulin (IvIg) very early in pregnancy may improve live birth rate in women with 4 or more unexplained RPL.	Conditional	⊕⊕■■	Only one high-quality RCT showed a beneficial effect of the treatment of unexplained RPL with repeated and high doses of IvIg when used very early in pregnancy in women with 4 or more pregnancy losses. However, more RCTs are needed to study the effect of IvIg treatment in women with RPL	UPDATED (2022) SOF table 11
17	70	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.	SOF table 12
17	71	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.	SOF table 13
17	72	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	73	Vaginal progesterone may improve live birth rate in women with 3 or more pregnancy losses and vaginal blood loss in a subsequent	Conditional	⊕⊕⊕■	Vaginal progesterone during early pregnancy may have beneficial effects in women with unexplained RPL with	UPDATED (2022)

		pregnancy			vaginal bleeding. There is some evidence that oral dydrogesterone initiated when fetal heart action can be confirmed may be effective, but more trials are needed.	SOF table 14
17	74	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	75	There is no evidence to recommended Granulocyte colony-stimulating factor (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 and showed that there is no beneficial effect of G-CSF in unexplained RPL.	UPDATED (2022) SOF table 15
17	76	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	77	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 fetus 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 showing 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 two or more non-consecutive pregnancy losses (van den Boogaard, et al., 2013, van den Boogaard, et al., 2010)

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 two 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.

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 fetal 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 and 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 ≥3 times (Alberman, 1988). In another study, 1.4% of women with ≥2 previous pregnancies had experienced RPL (Stray-Pedersen and Lorentzen-Styr, 1979) and in a Danish questionnaire-based study, 0.8% had experienced RPL among women with ≥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 ≥2 pregnancies. In a Japanese questionnaire-based study among an unselected group of women aged 35-79 years, 0.88% reported a history of ≥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 (Andalib et al., 2006, Craig et al., 2002, Kagami et al., 2012, Kolte et al., 2015b, Mevorach-Zussman et al., 2012, Rowsell et al., 2001, Sugiura-Ogasawara et al., 2013a, Toffol et al., 2013). With regards to the emotional impact on the partner, there are studies on (usually male) partners' reactions to sporadic pregnancy loss (Boynton, 2015). A recent study in RPL specifically suggests the impact for (usually male) partners maybe less significant (Hedegaard et al., 2021). Consequently, 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 (Bardos et al., 2015, Brier, 2008, Stirtzinger and Robinson, 1989). 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, Farren et al., 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 (Clifford et al., 1997, Liddell et al., 1997, Stray-Pedersen and Stray-Pedersen, 1984) 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 (gynaecologists/ 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 those 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, fetus, 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 behaviour

For some lifestyle behaviours 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 behaviours 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 behaviour modifications that could reduce the risk of pregnancy loss in couples with recurrent pregnancy loss (RPL).

1. Risk factors for RPL

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, fetal 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 ≥ 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). 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 ≥45 age category (du Fossé et al., 2020). 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	⊕⊕■■

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 ≥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	⊕■■■

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 and 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 low 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 (Bouet et al., 2016, Cicinelli, et al., 2014, Kitaya, 2011, McQueen, et al., 2014, McQueen, et al., 2015, Russell et al., 2013). 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 (Al-Memar et al., 2020, Fan et al., 2020, Zhang et al., 2019). To date, there are no studies on the predictive value of vaginal or endometrial dysbiosis in RPL.

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 and 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-fetal interface in pregnancy (Brighton, et al., 2017, Kong et al., 2021, Lucas, et al., 2020). In parallel, bone marrow-derived decidual progenitor cells are recruited, enabling rapid expansion of the decidua (Diniz-da-Costa et al., 2021, Tal et al., 2019). 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 (Diniz-da-Costa, et al., 2021, Lucas et al., 2016, Lucas, et al., 2020). 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 associates with higher miscarriage rates (Fukui et al., 2017, Hiby et al., 2008, Katano et al., 2013, Lucas, et al., 2020). Further, the frequency abnormal cycles appear to correlate with the recurrence risk of miscarriage (Diniz-da-Costa, et al., 2021, Lucas, et al., 2016, Lucas, et al., 2020). At present, however, biomarkers of senescent decidual cells have not been validated for clinical use.

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2. Health behaviour 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 and 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 behaviour 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 meta-analysis of 8 studies, paternal smoking of >10 cigarettes per day in the preconception period was found to be associated with an increased risk of pregnancy loss, after adjustment for maternal smoking status (1–10 cigarettes per day, OR 1.01; 95%CI 0.97–1.06; 11–19 cigarettes per day, OR 1.12; 95%CI 1.08–1.16; ≥20 cigarettes per day, OR 1.23; 95%CI 1.17–1.29) (du Fossé et al., 2021).

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

Updated (2022)

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^2. As such a BMI of 25-30 kg/m^2 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^2 is recommended for people of Asian origin rather than 30kg/m^2 (Misra et al., 2009).

Obesity (BMI >30 kg/m^2 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 (Boots and Stephenson, 2011, Lashen et al., 2004). 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 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^2) 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/m²), 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^2 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 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 fetal 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[2] 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 fetal problems (Fetal 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 advice 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.

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

[2] 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.

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 fetus, including congenital malformations, mental retardation and fetal 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.

Recommendation (updated in 2022)

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

The GDG recommends to base prognosis on woman's age and her complete pregnancy history, including number of previous pregnancy losses, live births and their sequence.	Strong	⊕⊕⊕■

Updated (2022)

Justification

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

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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 following pregnancy loss

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 fetus 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 following pregnancy loss.

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 fetus (aneuploidy) by analysing the pregnancy or fetal 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 fetus (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 following pregnancy loss but may be useful in the near future (Colley et al., 2019, Quintero-Ronderos and Laissue, 2020, Rajcan-Separovic, 2020, Shamseldin et al., 2013, Wang et al., 2017).

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 (Bernardi et al., 2012, Foyouzi et al., 2012, Hogge et al., 2003, 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 following pregnancy loss should be further elaborated within a prognostic model.

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

Recommendation

Genetic analysis of pregnancy tissue following pregnancy loss is not routinely recommended but it could be performed for explanatory purposes.	Conditional	⊕⊕■■

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

Justification

	Association	Contributing factor	Prognosis	Treatment
Karyotyping of the pregnancy tissue following pregnancy loss	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 following pregnancy loss 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 following pregnancy loss.

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). Another 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 (Colley, et al., 2019, Quintero-Ronderos and Laissue, 2020, Rajcan-Separovic, 2020, Shamseldin, et al., 2013).

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 (Barber et al., 2010, Franssen et al., 2006). 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 (Franssen, et al., 2006, Stephenson and Sierra, 2006, Sugiura-Ogasawara et al., 2004). For example, in one case-control study 85 of 157 (54%) with reciprocal translocations had one or more pregnancy losses 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 (Barber, et al., 2010, Franssen, et al., 2006).

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 (≥ 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 (Flynn, et al., 2014, Franssen, et al., 2006). 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 could be carried out after individual assessment of risk for diagnostic purposes.	Conditional ⊕⊕■■

Justification

	Association	Contributing factor	Prognosis	Treatment
Parental genetic testing	Yes	Yes[1]	Yes[2]	PGT, adoption, gamete donation or other alternatives

[1] 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.
[2] 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 for diagnostic purposes. 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

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 fetal loss (>10 weeks) (rather than embryonic loss i.e. <10 weeks) (Gao and 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; 4 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 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 and 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 (Chen et al., 2016, Govindaiah et al., 2009, Nelen et al., 2000), 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 (Chen, et al., 2016, Hickey et al., 2013)

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 β2 glycoprotein I antibodies (aβ2GPI, 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 an another 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).

β2 glycoprotein I antibodies

Based on five studies included in a meta-analysis from 2006, no statistically significant association was found between aβ2GPI 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 aβ2GPI 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 aβ2GPI 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 aβ2GPI antibodies is limited and inconsistent, and should be confirmed before applied in clinical practice (Aoki et al., 1993, Sater et al., 2012, Subrt et al., 2008, Subrt et al., 2013, Tebo et al., 2008).

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

Updated (2022)

Recommendations

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

Updated (2022)

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6. Immunological screening

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[3] 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 fetal 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 and 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 ≥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 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 frequency of HLA-DRB*O7 did not differ significantly between patients with primary RPL and patients 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 or other HLA genes in patients

[3] 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.

with primary RPL whereas several studies of the impact of carrying 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.[4] 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 and 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 (Vargas et al., 2009, Varla-Leftherioti et al., 2003). 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 an 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) (Fan et al., 2014, Wang et al., 2013). Since the HLA-G14 bp insertion is in strong positive linkage disequilibrium with the HLA-DRB1*03 allele (Hviid and 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-DRB*03 allele.

[4] 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
Anti-HY immunity	Moderate (Only shown in Scandinavian women)	YES, especially for secondary 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-α, a marker for the degree of systemic inflammation. High plasma TNF-α levels are reported to increase the risk of miscarriage in women with RPL (Mueller-Eckhardt et al., 1994) and high TNF-α and TNF-α/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-α 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-α in peripheral blood lymphocytes and a significantly increased TNF-α/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-α 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 TGFB1 or TNF-α 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 and Kwak-Kim, 2008, Medica et al., 2009).

Recommendations

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

Cytokine polymorphisms should not be tested in women with RPL.	Strong	⊕⊕⊕■

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-α 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

A meta-analyses concluded that there is an association between RPL and ANA, when the latter is determined by immunofluorescence (Chen et al., 2020). The presence of ANA was associated with a significantly increased ORs for RPL (OR 2.97; 95%CI 1.91-4.64). 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 a new pregnancy loss (Ogasawara et al., 1996).

A direct pathophysiological link between the presence of autoantibodies such as ANA in women with RPL and fetal 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
ANA antibodies	Yes	Probably not – no documentation	Unclear	NA

Measurement of ANA in women with RPL could be considered. One meta-analysis documents an association to RPL (Chen, et al., 2020) and there is some evidence (from small prospective studies) that ANA presence affects the prognosis negatively (Cavalcante, et al., 2014, Harger, et al., 1983). 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 (Karami et al., 2012, King et al., 2010, Kwak et al., 1995, Lee, et al., 2013, Perricone et al., 2007, Prado-Drayer et al., 2008, Shakhar et al., 2003, Yoo et al., 2012) , or had predictive value for subsequent pregnancy outcome (Emmer et al., 2000, Emmer et al., 1999) whereas other studies did not find NK cell numbers or percentages associated to RPL (Carbone et al., 2009, Chao et al., 1995, Wang et al., 2008) or predictive for outcome (Liang et al., 2012, Morikawa et al., 2001, Yamada et al., 2003). 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 (Hadinedoushan et al., 2007, Karami, et al., 2012, Lee, et al., 2013, Shakhar et al., 2006) 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, Katano et al., 2013, Liang, et al., 2012).

NK cells in endometrial biopsies or decidual tissue

One small case-control study reported that the CD56^{bright} 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 (Bao et al., 2012, Ozcimen et al., 2009, Vassiliadou and Bulmer, 1996). However, since the tissue in the former cases is necrotic and often inflamed and the latter cases is fresh and vital, this 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 (Faridi and Agrawal, 2011, Hiby, et al., 2008, Vargas, et al., 2009, Varla-Leftherioti, et al., 2003, Witt, et al., 2004). 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	⊕■■■

Update 2021

Justification

	Association	Contributing factor	Prognosis	Treatment
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 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-waited 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 and 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 and 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.	Strong ⊕⊕⊕■

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

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 fetal development. A review on the thyroid function and reproduction concluded that thyroid hormone disorders and increased Thyroid peroxidase (TPO) antibodies (TPOAb) 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 (≥ 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 and Boelaert, 2015).

Thyroid autoantibodies

In women with RPL, thyroid peroxidase autoantibodies (TPOAb) are mostly studied, and shown to be more relevant than other 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 were 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 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.	Strong	⊕⊕⊕■

Abnormal TSH levels should be followed up by T4 testing in women with RPL.	Strong	⊕⊕⊕■

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

**** No association has been found based on the evidence included in this guideline.***

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.

7.2 PCOS AND DISTURBANCES OF THE INSULIN METABOLISM

Evidence

Polycystic ovary syndrome (PCOS)

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

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

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

Insulin metabolism

Several markers for insulin metabolism have been assessed in women with RPL and controls, including fasting insulin (FI), fasting glucose (FG), the fasting glucose to insulin ratio (FG/FI), and insulin resistance (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 µU/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 β-cell function to the fasting hyperglycaemia, 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 (FI)+log (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 case-control study found higher levels of maternal serum fructosamine (a marker of glycaemic 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

** IR calculated based on fasting insulin and fasting glucose*

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±6.7 ng/ml versus 10.5±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 ≥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
Prolactin	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 low 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 (DOR) could be a causative or prognostic factor in RPL.

Ovarian reserve can be assessed with measurements of FSH, oestrogen (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, oestradiol or FSH:LH ratio - and RPL were summarised (Bunnewell et al., 2020). The reviewers found that more women with RPL seemed to have DOR 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 DOR 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. However, their results were not adjusted for the effect of age in the original studies.

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).

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 ⊕⊕■■

Justification

	Association	Contributing factor	Prognosis	Treatment
Ovarian reserve	Unclear	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. Furthermore, there is no gold standard test for diminished ovarian reserve and due to the lack of standardized reporting thresholds, conclusions are difficult to make.

Several studies have attempted to evaluate the association between DOR with RPL, however, these studies seem to be inadequately adjusting for age and report conflicting results.

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 ≤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 and 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 and 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).

Recommendation

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

Justification

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

** Midluteal progesterone or endometrial biopsy*

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

7.6 Androgens

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

Evidence

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

Two prognostic studies found no association between testosterone levels and the pregnancy outcome (LBR) in the next pregnancy (Nardo et al., 2002, Rai, et al., 2000).

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

Recommendation

Androgen testing is not recommended in women with RPL.	Strong	⊕⊕■■

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 for vitamin D in the complex immunoregulation at the fetal-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_3-1α-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_3-1α-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 fetal 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 14.6for more details).

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

7.8 Luteinizing hormone (LH)

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

Evidence

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

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

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

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

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

Recommendation

LH testing is not routinely recommended in women with RPL	Strong	⊕■■■

Justification

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

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

7.9 Hyperhomocysteinemia

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

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

Evidence

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

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

In a large case-control study of postpartum patients who had a history of vascular-related pregnancy complications, 569 patients experienced recurrent early pregnancy loss. Associations were detected of Hcy levels with pregnancy-induced hypertension, abruption placentae and Intrauterine growth retardation, but these associations were no longer significant after correction for time interval (between delivery and testing) and maternal age (Steegers-Theunissen et al., 2004). In another case-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 ±7.94 versus 8.72 ± 6.86 µmol/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 µmol/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 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 ± 1.6; n=60) compared to women with PCOS and without RPL (7.3 ± 1.1; n=60), women with RPL and without PCOS (9.65 ± 0.9; n=60), and controls (6.7 ± 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 ± 9.5 µmol/l in 140 men of couples with RPL and 14.2 ± 7.4 µmol/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
Hyperhomo-cysteinemia	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 (Binia et al., 2014, Wilcken et al., 2003).

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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 (Saravelos et al., 2008). Potentially relevant congenital Müllerian tract malformations include septate uterus, bicorporeal uterus with normal cervix (AFS bicornuate uterus), bicorporeal 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) (Chan et al., 2011b, Saravelos, et al., 2008).

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) (Elsokkary et al., 2018, Jaslow and Kutteh, 2013).

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 Saravelos, 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 (Saravelos, 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

(Valenzano et al., 2006, Ventolini et al., 2004). 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 (Caliskan et al., 2010, Saravelos, et al., 2008). 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 (Saravelos, 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 (Saravelos, 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, Saravelos, 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 (Hall-Craggs et al., 2013, Oppelt, et al., 2007).

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 (Ferreira et al., 2007, Frates et al., 1996, 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 (Harger, 2002, Kassanos et al., 2001, Liddell and 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 and Kutteh, 2013). Saravelos and colleagues reported fibroids in 8.2% (79/966) of women with RPL (Saravelos 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).One meta-analysis including 11 comparative studies showed that the pregnancy loss rate in women with adenomyosis was higher than in those without adenomyosis (OR 2.20; 95%CI 1.53-3.15) (Younes and Tulandi, 2017). Similarly, a small retrospective study using exclusively PGT euploid embryo transfer showed that adenomyosis was associated with higher rates of pregnancy loss independently of maternal age and BMI (44.1% vs 15.3%, for patients with and without adenomyosis, respectively) (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	⊕⊕■■
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 bicorporeal uterus (former AFS bicornuate uterus) with normal cervix.	Conditional	⊕⊕■■
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	⊕⊕■■

Update 2022

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

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 could 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 adenomyosis is associated with pregnancy loss and need to be ruled out in all women with RPL.

	Association	Contributing factor	Prognosis	Treatment
Congenital uterine malformations	Yes	Suggested some malformations	/	Surgical trials in case of a septate uterus
Acquired uterine malformations	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 ≥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 physiological ICSI (PICSI) has been observed in previous studies (Mokánszki et al., 2014, Worrilow et al., 2013). 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 PICSI (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, de Ligny et al., 2022, du Fossé, et al., 2022, du

Fossé, et al., 2020, Jensen et al., 2014, Miller, et al., 2019, Montagnoli et al., 2021, Pacey et al., 2014, 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 to –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 1.04-2.17), when compared with the reference group. The 2 groups had no significant difference of semen volume (SMD= – 0.03; 95%CI – 0.14 to 0.08) and semen pH value (SMD= –0.23; 95%CI – 0.50 to 0.05) (Li et al., 2021).

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 to 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). A meta-analysis of 29 studies including 3992 ART patients showed a higher miscarriage rate in high -DNA fragmentation index (DFI) group compared to the low-DFI group (RR=1.57; 95%CI 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 reporting a strong relationship between sperm DNA damage and pregnancy loss (Dhawan et al., 2019, Esquerré-Lamare et al., 2018, Haddock et al., 2021, Le et al., 2021, Ribas-Maynou et al., 2020, Rogenhofer et al., 2017, Zhu et al., 2020). A major cause of DNA damage is oxidative stress and this seems to be exacerbated by smoking, obesity and excessive exercise (Aitken and Bakos, 2021, Aitken et al., 2009, Du Plessis et al., 2010, Hsu et al., 2009).

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 SDF compared with those of controls. Furthermore, the risk of RPL was significantly increased when smoking, drinking and occupational exposure to environmental factors were superimposed (OR 11.97; 95%CI 1.49-95.62).

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 ≥45 age category (du Fossé, et al., 2020). In addition, there is a less favourable pro-inflammatory cytokine expression profile in seminal

plasma of men in couples with RPL and this is associated with advanced male age and lifestyle risk factors (du Fossé, et al., 2022). A more accurate prediction of future pregnancy outcome in couples with unexplained RPL is possible by adding additional male predictors (du Fossé, et al., 2022).

Recommendations (updated 2022)

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

Assessing sperm DNA fragmentation in couples with RPL could be considered for diagnostic purposes.	Conditional	⊕⊕⊕■

Justification

	Association	Contributing factor	Prognosis	Treatment
Sperm DNA damage	Yes	Yes	requires further clarification	Changing lifestyle and 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. It has not been established which test is most informative and most reliable. Therefore, the GDG recommends assessing sperm DNA fragmentation for diagnostic purposes using a validated test 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 advise male partners of couples with RPL of these connections and suggest ways to prevent sperm DNA damage caused by unhealthy lifestyles (de Ligny, et al., 2022, Sharma et al., 2013, Wright et al., 2014). Evidence shows that lifestyle modifications of the male partner (cessation of smoking, a normal body weight, limited alcohol consumption, physical activity) 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 (Bhattacharya et al., 2010, Brigham et al., 1999, Greenberg et al., 2015, Kling et al., 2016, Knudsen et al., 1991, Kolte et al., 2014, Lund, et al., 2012, Parazzini et al., 1988, Quenby and Farquharson, 1993).

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 (≥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 (Kolte, 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 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 predictive factor for the 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, Christiansen et al., 1990, Ho et al., 1991, Johnson et al., 1988, Kolte et al., 2011, Zhang et al., 2010). 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 centre 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 fetal 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 retrospective single centre cohort study including 675 women found that the women the study group having a live birth were younger compared to the women in the study group experiencing pregnancy loss (30.15±5.68 vs. 32.30±6.05,$p<0.001$, respectively) and three and below pregnancy losses were a significant predictor for a live birth (51 (26.7%) in women with ≥3 PLs vs 140 (73.3%) in women with ≤3 PLs) (Bashiri et al., 2020). However, the study has a small number of patients included to predict the chance of a live birth and showed moderate discrimination between explained and unexplained RPL.

Du Fossé and colleagues 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, they showed that showed that predicting the chance of a subsequent ongoing pregnancy beyond 24 weeks of gestation in couples with RPL becomes more accurate when more variables are incorporated into the model. These variables included both male and female characteristics, supporting a couple-focused approach in RPL. However, the predictive ability of the current model remains limited, and 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 poor predictive performance (Youssef et al., 2022). The model has too extreme predictions and poor discriminative ability.

Recommendation (updated 2022)

The GDG recommends to base prognosis on the woman's age and her complete pregnancy history, including number of previous pregnancy losses, live births, and their sequence.	Strong	⊕⊕⊕■

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

Update 2021

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 the chances of a successful pregnancy (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. None of the models cited in the text above is externally validated in an independent study and all three models showed overestimation and too extreme predictions due to small sample size. Optionally, the new prognostic tool Kolte & Westergaard validated internally using the large Denmark national database, could be used to predict a live birth in the next pregnancy (Kolte, et al., 2021) and is currently the preferred choice, even if this tool has also not yet been externally validated.

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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 *(see also summary of findings table 1).*

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 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. Women with RPL (n=41) undergoing PGT-A and women with RPL but no PGT-A (n=38) were compared with women with RIF undergoing PGT-A (n=42) or not (n=50) (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 undergoing or not undergoing 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 PGT-A for those couples with no known chromosomal abnormality concluded that there is no improvement in live birth rate with 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 PGT-A and expectant management (EM). Clinical outcomes improved in RPL couples undergoing IVF and PGT-A compared with couples who received expectant management. Among all attempts at 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 PGT-A group and 3.0 months in the EM group. However those couples whose intended PGT-A was cancelled had a lower live birth rate and higher clinical miscarriage rate as opposed to those who underwent PGT-A despite similar maternal age (Murugappan et al., 2016). In addition, IVF/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 RPL (Takeda et al., 2020). The majority had either no opinion or poor knowledge of PGT-A and tend to want PGT-A a to ensure 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 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 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.

Data on PGT-SR versus expectant management for couples with translocations reports a live birth rate of 37.8% on the first pregnancy after 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). 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 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 RPL service found that they were twice as likely to pursue natural conception than PGT-SR (Maithripala et al., 2018).

Some studies have suggested that miscarriage rates may be lower using 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 fetal or parental karyotype should receive genetic counselling.	GPP

All couples with results of an abnormal fetal 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 *(see also summary of findings table 1).* Therefore, the GDG strongly recommends that all couples with abnormal genetic results from pregnancy tissue testing following pregnancy loss or from 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 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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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 *(see also summary of findings table 2).*

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 (≥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.

Folic acid and vitamins

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

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	⊕⊕■■

Justification

We found no evidence of a beneficial effect of anticoagulant treatment in women with hereditary thrombophilia *(see also summary of findings table 2)*. An international RCT in women with RPL and hereditary thrombophilia has completed recruiting in 2021 and results are expected in 2022 (ALIFE2 trial/ trial reg nr NTR 3361).

12.2 Treatment for women with RPL and antiphospholipid syndrome (APS)

Evidence *(see also summary of findings table 3-5).*

Anticoagulants

Antithrombotic therapy (aspirin, UFH or LMWH) was summarized in a recent Cochrane review summarizing eleven RCTs of 1672 women with RPL and APS (Hamulyák et al., 2020). A benefit of heparin (UFH or LMWH) and aspirin, as compared to aspirin alone, with regard to live birth was reported (RR 1.27; 95%CI 1.09-1.49, 5 studies, n= 1295). Heparin plus aspirin may reduce the risk of pregnancy loss (RR 0.48; 95%CI 0.32 to 0.71, 5 studies, n=1295). The reviewers noted significant risk of bias in the included studies, and remarked that adverse effects were frequently not, or not uniformly, reported.

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

For thrombosis prophylaxis, LMWH is preferred over UFH, because of a lower risk of osteoporosis and 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 1.04-1.38, 3 trials, n=1155). In the comparison of UFH plus aspirin versus aspirin alone, the RR for live birth was 1.74 (95%CI 1.28-2.35, 2 trials, n=140). 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 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 (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 *(see also summary of findings table 3)*. 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) (Hamulyák, et al., 2020). 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, Bates, et al., 2018).

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 fetal 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

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 and 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 and Winger, 2005) or NK cell/cytokine aberrations (Moraru et al., 2012, Winger and Reed, 2008) but these trials are only of moderate/low quality, primarily because they were not placebo-controlled and thus not blinded. Two good-quality 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 *(see also summary of findings table 6)*

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 fetal 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 fetus 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 10.0mU/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 (≥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 mIU/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 ≤2.5 mIU/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 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

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., 2006, Negro et al., 2005, Vissenberg, et al., 2012).

In a case-control study thyroid autoimmunity, prevalence of subclinical hypothyroidism and maternal and fetal 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 0.83-1.14, P= 0.74). The T4life trial showed that the treatment of women with RPL and positive for TPOAb with levothyroxine did not result in higher live birth rates compared to placebo (50% vs 48%, RR 1.03; 95%CI 0.77-1.38) (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 pregnant again, TSH level should be checked in early	GPP	

Update 2022

gestation (7-9 weeks gestational age), and hypothyroidism should be treated with levothyroxine.	
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.	GPP
Euthyroid women with thyroid antibodies and RPL should not be treated with levothyroxine.	Strong ⊕⊕⊕■

Justification

If overt hypothyroidism is identified in women with RPL, treatment with levothyroxine is recommended based on existing guidelines and possible maternal and fetal complications associated with untreated hypothyroidism during pregnancy. For women with subclinical hypothyroidism and RPL, treatment with levothyroxine is insufficiently evidence-based and should be further investigated. Moreover, evidence of thyroid hormone treatment in pregnant women with SCH reported reduced miscarriage 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), gestational diabetes (OR 1.37; 95%CI 1.05-1.79) and pre-eclampsia (OR 1.61; 95%CI 1.10-2.37) (Maraka et al., 2017).

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

While before 2017, there were no convincing evidence on the efficacy of levothyroxine treatment, new evidence from TABLET trial and the T4life trial showed that levothyroxine treatment does not increase 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 euthyroid women with thyroid antibodies and RPL.

14.2 Progesterone or human chorionic gonadotrophin (hCG) (for luteal phase insufficiency)

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

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

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

to prescribe progesterone in women with unexplained RPL based on the 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 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 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 when a sensitivity analysis restricted to good-quality trials was performed and two studies of weaker methodological quality were removed, there was no longer a statistically significant benefit (RR 0.74; 95%CI 0.44 - 1.23, three RCTs). 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.

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 hypoglycaemic 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 (Al-Biate, 2015, Jakubowicz et al., 2002, Khattab et al., 2006, Wang et al., 2011).

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 analysed, 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).

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.

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).

Conclusion

In women with RPL and hyperprolactinemia, bromocriptine treatment normalizes serum prolactin levels one single small study showed this treatment to be effective for increasing the chance of a live birth. However, this evidence is not sufficient to recommend the use of bromocriptine in women with RPL and hyperprolactinemia.

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 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 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 fetal 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 fetal 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 and 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, Mollo et al., 2011, Valli et al., 2004).

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^2 = 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 0.25; 95%CI 0.07-0.88). However, no significant effect was seen on live birth, clinical pregnancy rate or preterm delivery.

Another meta-analysis showed also that hysteroscopic metroplasty reduced the risk of pregnancy loss in patients with a complete uterine septum (OR 0.16; 95%CI 0.03-0.78) or a 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 (≥ 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 randomly assigned to septum resection (n=39) or expectant management (n=40), 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 and 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, hemato-cavity (obstructive symptoms).

Metroplasty (transabdominal or laparoscopically) is the only option for a bicornuate uterus (Alborzi et al., 2015, Papp et al., 2006). 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, Alborzi, et al., 2015, Jaslow, 2014).

In women with RPL and T-shaped uterus, low-quality evidence from one meta-analysis of 11 cohort studies showed that hysteroscopic metroplasty seems to be effective to improve reproductive outcomes including a higher pooled proportion of live birth (56.9%; 95%CI 46.4-66.9, I^2= 36.3, 6 studies) and a lower pooled proportion of pregnancy loss (21,5%; 95%CI 15.1-28.6, I^2= 30.18, 8 studies) after the metroplasty (Garzon et al., 2020).

Recommendations

Only one small RCT showed no benefit of using hysteroscopic septum resection to reduce the rate of pregnancy loss.	Conditional	⊕■■■
Metroplasty is not recommended for bicorporeal uterus with normal cervix (former AFS bicornuate uterus) and RPL.	Strong	⊕■■■
Uterine reconstruction is not recommended for hemi-uterus (former AFS unicornuate uterus) and RPL.	Strong	⊕■■■
There is insufficient evidence in favor of metroplasty in women with bicorporeal uterus and double cervix (former AFS didelphic uterus) and RPL.	Conditional	⊕■■■

Justification

Women with (untreated) congenital uterine malformations have significantly impaired pregnancy outcome *(see also chapter 8)* (Grimbizis, et al., 2001).

An international, multicentre, open label, randomized controlled trial showed that septum resection does not improve the reproductive outcomes in women with RPL (Rikken, et al., 2021). However, several meta-analyses of low-quality studies showed a benefit of treatment in reducing the miscarriage rate: women who underwent hysteroscopic septum resection had a significantly decreased probability of pregnancy loss compared with women who did not undergo treatment. There was no effect on the clinical pregnancy rate and live birth rate. Therefore, the GDG decided to formulate a conditional recommendation based on the only RCT with limited sample size showing no benefit of the use of hysteroscopic septum resection to reduce the rate of pregnancy loss. Larger randomized controlled trials are still needed to demonstrate a clear benefit of hysteroscopic septum resection.

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

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

Low level of evidence found in the literature are in favor of using hysteroscopic metroplasty in women with reproductive failure and T-shaped uterus, but the GDG did not recommend the treatment of RPL in women with T-shaped uterus to avoid unnecessary procedures for many patients.

Additional information

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

More information on the ESHRE/ESGE classification system of female genital tract congenital malformations (Grimbizis et al., 2016) is available on the ESHRE website (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 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 (Jaslow, 2014, Lieng et al., 2010, Salim et al., 2011). 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 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 (Saravelos et al., 2011).

Recommendations

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

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	⊕■■■

Justification

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

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

Intrauterine adhesions (IUA) (Asherman's syndrome)

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

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	⊕■■■

Justification

The treatment of adhesions is surgical removal. Although small observational studies have shown that surgery may decrease miscarriage rates in women with RPL, the GDG decided to formulate a conditional recommendation based on the absence of conclusive data on benefit and harm. For severe adhesions,

benefits with regard to pregnancy and pain symptoms may outweigh the potential harms of surgery. In any case, uterine surgery is a known cause for adhesions, and treatment should attempt to prevent 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 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 and 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 clinical 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 evidence that this treatment increases perinatal survival.	Conditional ⊕⊕■■

Justification

Based on inconclusive evidence on the benefit and taking into consideration the absence of a consistent definition or a standardized diagnosis, 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.

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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 *(see also summary of findings tables 8 and 9)*

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 and Bakos, 2021, Aitken et al., 2009). 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 (Montagnoli et al., 2021, Semet et al., 2017, Sharma et al., 2013)). 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 (Dieamant et al., 2017, Nagler et al., 1997) and it leads to impaired semen quality and increased sperm DNA damage in comparison to healthy donors (Dieamant, et al., 2017, Wright et al., 2014). Evidence suggests that varicocelectomy could improve sperm DNA integrity in infertile patients, but there have not been any studies in RPL (Birowo et al., 2020, Wang et al., 2012). 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 RCT involving 136 women with RPL, 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). In the varicocelectomy group, the semen parameters including mean sperm concentration, sperm progressive motility and sperm with normal morphology were significantly improved. The chance of pregnancy increased with an increase in the number of sperms/million/ml (OR 3.7; 95%CI 2.7-6.4, P=0.001); The pregnancy rate increased the >6-month varicocelectomy compared to the ≤6-month varicocelectomy (OR=3,4; 95%CI 2.4-5.8, P=0.001). The pregnancy loss rate was higher in the untreated group (13.3% vs 68.2%, P=0.001, group 1 vs group 2). The results of this RCTs showed that varicocelectomy improves semen quality, increases pregnancy rate and decreases pregnancy loss rate but further studies with a larger sample size are needed to confirm the results.

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 and Majumdar, 2013, Worrilow 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.

A Cochrane systematic review involving patients (not RPL) undergoing PICSI decreased pregnancy loss rates compared with ICSI with sperm selected using standard methods (Lepine et al., 2019). In this systematic review, two RCTs reported live birth and there may be little or no difference between PICSI and ICSI (RR 1.09; 95%CI 0.97-1.23, 2 RCTs, n=2903, I^2=0%, low-quality evidence). In contrast, three RCTs showed a significant decrease in pregnancy loss rates among couples in the PICSI group (RR 0.61; 95%CI 0.45-0.83, 3 RCTs, 3005 women, I^2=0%, although low-quality evidence). This impact of HA-ICSI sperm selection was also observed when the pregnancy loss was calculated per clinical pregnancy (RR 0.62; 95%CI 0.46-0.82, 3 RCTs, n=1065, I^2=0%, low-quality evidence). In a mechanistic study further examining the data of the HABSelect trial included in the former meta-analysis, 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 (Miller, et al., 2019, West et al., 2022). This reduction in miscarriage by selecting sperm with less DNA damage by PICSI has been observed in previous studies (Mokánszki et al., 2014, Worrilow, et al., 2013).

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 and Drevet, 2020) and indeed may be deleterious.

A Cochrane review of 90 studies has reported that men with poor semen quality showed improvement in sperm parameters following antioxidant therapy. In six studies reporting miscarriage, no significant difference was found in miscarriage rate between couples randomized to antioxidant therapy compared to placebo (OR 1.46; 95%CI 0.75-2.83). Live birth rate was higher in couples randomized to treatment (OR 1.43; 95%CI 1.07-1.91, 12 RCTs, n=1283, I^2= 44%, very low-quality evidence). When studies at high risk of bias were removed from the analysis, there was no evidence of increased live birth (Peto OR 1.22, 95%CI 0.85-1.75, 8 RCTs, n= 827, I^2= 32%) (de Ligny et al., 2022) . 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

There is no evidence to recommend the sperm selection by PICSI in couples with RPL	Conditional ⊕■■■

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 in subfertile couples (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, comparable data are lacking for couples with RPL and therefore, further randomized studies including RPL women are needed to assess whether sperm selection by PICSI could improve ART outcomes. Since there is little evidence for male factors linked to recurrent pregnancy loss, the GDG believe that the area should be extended in this instance to include papers on sporadic pregnancy loss.

Antioxidants for men are often used, but there is no evidence that antioxidants could be helpful in couples with RPL. In a Cochrane review, very low-quality evidence showed that antioxidants may improve live birth rate after ART in subfertile men, but it did not significantly decrease the chance of a

pregnancy loss (de Ligny, et al., 2022) *(see also summary of findings table 8)*. Therefore, a conditional recommendation was formulated.

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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 fetus 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 *(see also Summary of findings table 10)*

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 haematological 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

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

17.2 Intravenous immunoglobulin (IvIg)

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

Evidence *(see also Summary of findings table 11)*

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

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

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

Recently, a high-quality RCT found that IvIg given in repeated doses (400 mg/kg) for five consecutive days very early in pregnancy to women with 4 or more unexplained PLs increased the LBR significantly (OR 2.60; 95%CI 1.15-5.86) (Yamada et al., 2022).

Recommendation

The use of repeated and high doses of IvIg very early in pregnancy may improve live birth rate in women with 4 or more unexplained RPL	Conditional ⊕⊕■■

Justification

Only one high-quality RCT showed a beneficial effect of the treatment of unexplained RPL with repeated and high doses of IvIg when used very early in pregnancy in women with 4 or more pregnancy losses. However, more RCTs are needed to study the effect of IvIg treatment in women with RPL and future update of meta-analysis on the topic with this study is likely to change the conclusions positively towards the use of IvIg in the treatment of unexplained RPL.

17.3 Prednisolone

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

Evidence *(see also Summary of findings table 12)*

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.

Another small size RCT showed that the live birth rate was higher when RPL women with high µNK cell density (≥5%) were treated with prednisolone (20mg for 6 weeks, 10mg for 1 week, 5 mg for 1 week) compared to placebo treatment (60% vs 40%, RR 1.5; 95%CI 0.8-2.9), but this difference was not significant (Tang et al., 2013).

Recommendation

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

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 increasing administered to RPL women without antiphospholipid antibodies.

Evidence *(see also Summary of findings table 13)*

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	⊕⊕⊕■

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 and 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	⊕⊕■■

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 Progestogen

Evidence *(see also Summary of findings table 14)*

A double blind, placebo-controlled, randomized trial of oral dydrogesterone (given from the time that a live fetus was confirmed by ultrasound until 20 weeks of gestation) among 360 women with a RPL showed a benefit of dydrogesterone 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 not initiated immediately after confirmation of pregnancy (mean gestational age 6.5 ± 1.1 weeks and 6.5 ± 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 multicentre, 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) through 12 weeks of gestation. There was no difference in the live birth rate 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 Cochrane systematic review on progesterone administration in unexplained RPL including 10 trials with 1684 patients (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 progestogen or placebo. The finding of importance for this guideline is that the risk ratio for

subsequent live birth in progestogen-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).

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.	Conditional ⊕⊕⊕■

Justification

A meta-analysis and a Cochrane review showed a benefit of progestogen on miscarriage rate and live birth rate (Haas, et al., 2019, Saccone, et al., 2017).. However, the 2 papers are flawed by the quality of the older included studies, and hence, we decided to base the updated recommendation on the combination of recent high- quality trials (Coomarasamy, et al., 2019, Coomarasamy, et al., 2015). Vaginal progesterone during early pregnancy (started <12 weeks) may have a beneficial effect in women with unexplained RPL (16 to 39-years old) with vaginal bleeding and it is therefore recommended to use vaginal progesterone (twice-daily 400 mg progesterone from presentation to 16 completed weeks of gestation) to improve live birth rate in a subsequent pregnancy.

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

17.7 Intralipid therapy

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

Evidence

Clark and colleagues reported that infusions of Intralipid reduced the fetal resorption rate in specific mice matings (Clark, 1994). Roussev and colleagues reported that NK cell cytotoxicity declined after Intralipid infusions to recurrent implantation failure patients (to the same level as after IvIg infusions) and they therefore extrapolated that Intralipid had a beneficial effect in RPL (Roussev et al., 2008).

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

No serious adverse effects has been reported after the use of low dose intralipid treatment in women with RPL (Meng, et al., 2015, Roussev, et al., 2008). However, a series of serious adverse effects has been reported after the use of higher doses of intravenous lipid emulsions: acute kidney injury, cardiac

arrest, acute lung injury, venous thromboembolism, fat embolism, fat overload syndrome, pancreatitis, allergic reactions and increased susceptibility to infection (Hayes et al., 2016).

Recommendation

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

Justification

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

17.8 Granulocyte colony-stimulating factor (G-CSF)

Evidence *(see also Summary of findings table 15)*

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-abortive effects based on animal studies. Although the mechanism is unknown, studies have been conducted in women with RPL and recurrent implantation failure (RIF).

A review found two studies on G-CSF as treatment for RPL. Both studies (Santjohanser et al., 2013, Scarpellini and Sbracia, 2009) 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 IvIg, 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 and 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 RIF. 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 recommended G-CSF in women with unexplained RPL.	Strong	⊕⊕⊕■

Justification

The results from a recent trial (Eapen, et al., 2019) overrule those from the Scarpellini and Sbracia trial (Scarpellini and Sbracia, 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 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

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 *(see also Summary of findings table 16)*

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 an 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 PGT-A 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 and 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. Bioresonans therapy and naprotechnology have been suggested as treatment options for pregnancy loss, but there are no data available supporting their use in clinical practice.

Recommendation

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

Justification

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

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Annexes

Annex 1: Guideline development group

This guideline was developed and updated by the ESHRE Early Pregnancy Guideline Development Group (GDG). The GDG included gynaecologists 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.

Chair of the GDG

Mariëtte Goddijn	Centre for Reproductive Medicine, Amsterdam University Medical Centre, Amsterdam (The Netherlands)

GDG members

Ole Bjarne Christiansen	Aalborg University Hospital, Aalborg, (Denmark)
Janine Elson	Liverpool Women's Hospital, Crown Street, Liverpool (UK)
Astrid Marie Kolte	University Hospital Copenhagen, Rigshospitalet, Copenhagen (Denmark) Amager and Hvidvore Hospitals, University Hospital Copenhagen, Hvidvore (Denmark)
Sheena Lewis	Queen's University Belfast (UK)
Saskia Middeldorp (Thrombophilia)	Radboud university medical centre, Nijmegen, Gelderland (The Netherlands)
Henriette Svarre Nielsen	University Hospital Copenhagen Hvidovre and University of Copenhagen, Hvidovre (Denmark)
Braulio Peramo	Al Ain Fertility Clinic, Al Ain (United Arab Emirates)
Siobhan Quenby	University of Warwick, Warwick (UK)
Marie-Louise van der Hoorn	Leiden University Medical Centre (The Netherlands)

Patient representative

Ruth Bender Atik	Miscarriage Association (UK)

Invited experts

Peter Bisschop *(Thyroid abnormalities)*	Amsterdam University Medical Centre, Amsterdam (The Netherlands)

Methodological support

Nathalie Vermeulen	European Society of Human Reproduction and Embryology (Belgium)
Saria Mcheik	European Society of Human Reproduction and Embryology (Belgium)

DECLARATIONS OF INTEREST

All members of the guideline development group were asked to declare possible conflicts of interest by means of the disclosure forms (see *ESHRE Manual for Guideline Development*).

	Conflicts of Interest
Mariette Goddijn	*Research and educational grant received by the Centre for Reproductive Medicine, Amsterdam UMC (location VUMC) from Guerbet, Merck and Ferring, not related to the presented work.*
Ole Bjarne Christiansen	*Salary as specialty editor at European Journal of Obstetrics & Gynaecology and Reproductive Biology*
Janine Elson	*None declared*
Astrid Marie Kolte	*None declared*
Sheena Lewis	*Salary or position funding from EXAMENLAB Ltd* *Ownership interest by stock or partnership of a healthcare company, from EXAMENLAB LTD*
Henriette Nielsen	*Grants with payment to institution: Freya Biosciences A/S, Ferring Pharmaceuticals, Merck* *Speakers fee for lectures from Astra Zeneca, Cook Medical*
Braulio Peramo	*None declared*
Siobhan Quenby	*Speaker's fees from Ferring*
Marie-Louise van der Hoorn	*None declared*
Ruth Bender Atik	*None declared*
Nathalie Vermeulen	*None declared*
Saria Mcheik	*None declared*

Annex 2: Summary of findings tables

EXPLANATIONS

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio

SUMMARY OF FINDINGS TABLES 1 – 16

1 PGT compared to no treatment for RPL

Patient or population: Unexplained RPL (PGT-A) and RPL with known genetic abnormality (PGT-SR)
Intervention: Preimplantation genetic testing (PGT)
Comparison: No treatment (expectant management)

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no treatment	Risk with PGT				
Live birth rate (PGT-A) *(Musters et al., 2011)*	421 per 1.000	*not estimable* *354 per 1000***	not estimable	442 (12 observational studies) [a]	⊕■■■ VERY LOW [b,c,d,e]	
Cumulative live birth rate (PGT-SR) *(Ikuma et al., 2015)*	654 per 1.000	**675 per 1.000** (505 to 836)	**OR 1.10** (0.54 to 2.70)	89 (1 observational study)	⊕■■■ VERY LOW [f]	Single study
Live birth rate (PGT-SR) *(Franssen et al., 2011)*	531 per 1.000	*not estimable* *349 per 1000***	not estimable	595 (25 observational studies)	⊕■■■ VERY LOW [a,b,g]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. cohort studies, as no RCTs comparing PGT with NC are available
b. low-quality studies
c. unclear from review
d. no direct comparison available
e. combination of very small studies
f. one small study
g. no meta-analysis due to high heterogeneity
** observed event rate as anticipated absolute effect is not estimable

2 Anticoagulant therapy compared to no treatment for RPL + hereditary thrombophilia

Patient or population: RPL + hereditary thrombophilia
Intervention: anticoagulant therapy
Comparison: no treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no treatment/placebo	Risk with anticoagulant therapy				
Live birth rate *(LMWH vs no treatment)* (Skeith et al., 2016)	862 per 1.000	**836 per 1.000** (690 to 1.000)	**RR 0.97** (0.80 to 1.19)	66 (2 RCTs)	⊕⊕■■ LOW [a]	*early RPL + hereditary thrombophilia*
Live birth rate *(LMWH vs no treatment)* (Skeith, et al., 2016)	590 per 1.000	**478 per 1.000** (224 to 1000)	**RR 0.81** (0.38 to 1.72)	308 (5 RCTs)	⊕⊕■■ LOW [a,b,c]	*late loss + hereditary thrombophilia*

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. optimal information size not met
b. Every trial included had adequate random sequence generation, good allocation concealment and no selective reporting, and most trials clearly addressed incomplete outcome data.
c. difference in direction of effect

3 Anticoagulant therapy compared to no treatment/placebo for RPL + APS

Patient or population: RPL + APS
Intervention: Anticoagulant therapy
Comparison: No treatment/placebo/other treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no treatment/placebo	Risk with Anticoagulant therapy				
Pregnancy loss (Heparin* + aspirin versus aspirin only) (Hamulyák et al., 2020)	325 per 1000	**156 per 1000** (104 to 231)	**RR 0.48** (0.32 to 0.71)	1295 (5 RCTs)	⊕⊕■■ LOW [ab]	
Live birth rate (Heparin*+ aspirin versus aspirin) (Hamulyák, et al., 2020)	675 per 1000	**857 per 1000** (736 to 1000)	**RR 1.27** (1.09 to 1.49)	1295 (5 RCTs)	⊕⊕■■ LOW [ac]	Subgroup analysis: UFH+ aspirin vs aspirin: RR 1.74 (1.28to 2.35) LMWH + aspirin vs aspirin: RR 1.20 (1.04 to 1.38)
Pregnancy loss (aspirin versus placebo) (Hamulyák, et al., 2020)	150 per 1000	**200 per 1000** (51 to 782)	**RR 1.33** (0.34 to 5.21)	40 (1 RCT)	⊕■■■ VERY LOW [d e]	
Live birth rate (Aspirin versus placebo) (Hamulyák, et al., 2020)	850 per 1000	**799 per 1000** (603 to 1000)	**RR 0.94** (0.71 to 1.25)	40 (1 RCT)	⊕■■■ VERY LOW [d e]	

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

* Unfractionated or LMWH
a Downgraded one level due to serious inconsistency: heterogeneity in interventions ($I^2 > 45\%$)
b Downgraded one level due to serious risk of bias for limitations (selection, attrition, and reporting bias)
c Downgraded one level due to serious risk of bias for limitations (selection and attrition bias)
d Downgraded one level due to serious risk of selection and attrition bias
e Downgraded two levels due to very serious imprecision: few participants and wide confidence intervals crossing the line of no effect

4 Prednisolone compared to placebo/other treatment for RPL + APS

Patient or population: RPL + APS
Intervention: Prednisolone (+ aspirin)
Comparison: Placebo/other treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo/no treatment	**Risk with Prednisolone**				
Miscarriage rate *(Prednisone and aspirin versus aspirin or placebo)* *(Empson et al., 2005)*	324 per 1.000	**275 per 1.000** (171 to 440)	**RR 0.85** (0.53 to 1.36)	122 (2 RCTs)	⊕■■■ VERY LOW [a,b,c,d]	
Miscarriage rate *(Prednisone and aspirin versus heparin and aspirin)* *(Empson, et al., 2005)*	269 per 1.000	**315 per 1.000** (127 to 789)	**RR 1.17** (0.47 to 2.93)	45 (1 RCT)	⊕■■■ VERY LOW [a,c,d,e]	Single study

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. performance bias suspected
b. no miscarriages in one of the 2 studies
c. no direct comparison of prednisolone with placebo
d. optimal information size not met
e. single RCT

5 IvIg compared to other treatment for RPL and antiphospholipid antibodies

Patient or population: RPL and antiphospholipid antibodies
Intervention: IvIg *(± heparin + aspirin)*
Comparison: other treatment: *heparin* + aspirin or prednisone + aspirin.*

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo/no treatment	**Risk with IVIG**				
Miscarriage rate *(Empson, et al., 2005)*	175 per 1.000	**258 per 1.000** (91 to 726)	**RR 1.47** (0.52 to 4.14)	138 (3 RCTs)	⊕■■■ VERY LOW [a,b,c,d]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

* Unfractionated or LMW
a. suspected bias in randomization and allocation concealment
b. difference in direction of effects, borderline heterogeneity
c. no direct comparison of IVIG with placebo
d. optimal information size not met

6 Levothyroxine compared to placebo/no treatment for RPL

Patient or population: RPL with hormonal/metabolic background
Intervention: Levothyroxine
Comparison: Placebo/no treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo/no treatment	**Risk with Levothyroxine**				
Subclinical hypothyroidism						
Cumulative live birth rate (*Bernardi et al., 2013*)	667 per 1.000	**708 per 1.000** (375 to 907)	**OR 1.21** (0.30 to 4.87)	39 (1 observational study)	⊕■■■ VERY LOW [a, b, c]	Single observational study
Miscarriage rate (*Negro et al., 2010*)	206 per 1.000	**47 per 1.000** (10 to 210)	**RR 0.23** (0.05 to 1.02)	77 (1 observational study)	⊕⊕■■ LOW [a, b]	Single observational study
Thyroid autoantibodies with normal thyroid function						
Live birth rate (Van Dijk et al., 2022)	484 per 1000	**498 per 1000** (373 to 668)	**RR 1.03** (0.77 to 1.38)	187 (1 RCT)	⊕⊕⊕⊕ HIGH [b]	1 RCT
Live birth rate (Dhillon-Smith et al., 2019)	379 per 1000	**367 per 1000** (314 to 432)	**RR 0.97** (0.83 to 1.14)	940 (1 RCT)	⊕⊕⊕■ MODERATE [d]	
Miscarriage rate (Van Dijk, et al., 2022)	329 per 1000	**233 per 1000** (135 to 398)	**RR 0.71** (0.41 to 1.21)	142 (1 RCT)	⊕⊕⊕⊕ HIGH [b]	1 RCT
Miscarriage rate (Dhillon-Smith, et al., 2019)	296 per 1000	**281 per 1000 (216 to 364)**	**RR 0.95 (0.73 to 1.23)**	540 (1 RCT)	⊕⊕⊕■ MODERATE [d]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. Optimal information size not met
b. Single study
c. About 70% of the women were treated for concomitant factors associated with RPL
d. RPL women were a subpopulation of the RCT

7 HCG compared to no treatment for RPL

Patient or population: Couples with RPL
Intervention: HCG
Comparison: Placebo/ no treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo/no treatment	**Risk with HCG**				
Miscarriage rate (1st trimester) *(Morley et al., 2013)*	291 per 1.000	**149 per 1.000** (93 to 236)	**RR 0.51** (0.32 to 0.81)	302 (5 RCTs)	⊕⊕■■ LOW [a,b,c]	

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. for most studies selection, performance and reporting bias was detected
b. exclusion of 2 studies significantly changed results (OR 0.74; 95%CI 0.44 - 1.23)
c. optimal information size not met

8 Sperm selection by PICSI compared to other treatment

Patient or population: Couples with DNA damage (not RPL)
Intervention: Sperm selection by PICSI
Comparison: other treatment: *ICSI*.

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo/no treatment	**Risk with IVIG**				
Live birth per woman randomly assigned (Lepine et al., 2019)	253 per 1000	**276 per 1000** (245 to 311)	**RR 1.09** (0.97 to 1.23)	2903 (2 RCTs)	⊕⊕■■ VERY LOW [a,b,c,d]	
Miscarriage rate per woman randomly assigned (Lepine, et al., 2019)	70 per 1000	**43 per 1000** (31 to 58)	**OR 0.61** (0.45 to 0.83)	3005 (3 RCTs)	⊕⊕■■ VERY LOW [a,b,c]	
Miscarriage rate per clinical pregnancy (Lepine, et al., 2019)	197 per 1000	**122 per 1000** (90 to 161)	**OR 0.62** (0.46 to 0.82)	1065 (3 RCTs)	⊕⊕■■ VERY LOW [a,,c,d]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. performance bias was detected
b. imprecision, direction of effect inconsistent
c. not RPL patients
d. optimal information size not met

Update 2022

9 Antioxidants compared to placebo

Patient or population: Couples with male subfertility (not RPL)
Intervention: Antioxidants (Vitamin E, Zinc, combined antioxidants)
Comparison: Placebo/no treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo/no treatment	**Risk with Antioxidants**				
Live birth rate (subfertile men) (de Ligny et al., 2022)	162 per 1.000	**216 per 1.000** (171 to 269)	**OR 1.43** (1.07 to1.91)	1283 (12 RCTs)	⊕⊕■■ very low [a,b,c]	
Miscarriage rate (subfertile men) (de Ligny, et al., 2022)	48 per 1.000	**68 per 1.000** (36 to 125)	**OR 1.46** (0.75 to2.83)	664 (6 RCTs)	⊕■■■ VERY LOW [a,b,d]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. performance and selection bias suspected
b. analysis in subfertile men, not RPL
c. optimal information size not met

Update 2022

10 Immunotherapy (paternal - donor) compared to placebo for unexplained RPL

Patient or population: Unexplained RPL
Intervention: Immunotherapy (paternal - donor)
Comparison: Placebo

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo	Risk with Immunotherapy (paternal - donor)				
Live birth rate *(paternal lymphocytes)* (*Wong et al., 2014*)	600 per 1.000	**649 per 1.000** (572 to 718)	**OR 1.23** (0.89 to 1.70)	641 (12 RCTs)	⊕⊕⊕■ MODERATE [a,b,c]	
Live birth rate *(donor lymphocytes)* (*Wong, et al., 2014*)	596 per 1.000	**672 per 1.000** (500 to 806)	**OR 1.39** (0.68 to 2.82)	156 (3 RCTs)	⊕⊕⊕■ MODERATE [d,e]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. No explanation was provided
b. Significant inconsistency across studies, with different effects
c. Sample size should be sufficient
d. Concerns on performance bias in one of the included studies (Illeni 1994)
e. Optimal information size not met

11 Immunotherapy IvIg compared to usual treatment/placebo for unexplained RPL

Patient or population: unexplained RPL
Intervention: Immunotherapy IvIg
Comparison: Usual treatment/placebo

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with usual treatment/placebo	Risk with Immunotherapy IVIG				
No live birth (*Egerup et al., 2015*)	425 per 1.000	**391 per 1.000** (319 to 476)	**RR 0.92** (0.75 to 1.12)	531 (11 RCTs)	⊕⊕■■ LOW [a,b,c]	
No live birth *Primary RPL only* (*Egerup, et al., 2015*)	278 per 1.000	**367 per 1.000** (244 to 550)	**RR 1.32** (0.88 to 1.98)	181 (6 RCTs)	⊕⊕■■ LOW [b,c,d]	
No live birth *Secondary RPL only* (*Egerup, et al., 2015*)	527 per 1.000	**406 per 1.000** (306 to 538)	**RR 0.77** (0.58 to 1.02)	221 (6 RCTs)	⊕⊕■■ LOW [b,c,d]	
Live birth rate (*Yamada et al., 2022*)	480 per 1000	**706 per 1000** (515 to 844)	**OR 2.60** (1.15 to 5.86)	204 (1 RCT)	⊕⊕⊕■ MODERATE[e,f]	Single study ≥4 pregnancy losses

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. 10 out of 11 trials classified as 'high risk of bias'
b. differences in direction of effect
c. optimal effect size not met
d. most trials classified as 'high risk of bias'
e. Single study
f. Small sample size

12 Prednisolone compared to placebo/other treatment for unexplained RPL

Patient or population: Unexplained RPL
Intervention: Prednisolone
Comparison: Placebo/other treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo/other treatment	**Risk with prednisolone**				
Ongoing Pregnancy rate *(Gomaa et al., 2014)*	92 per 1.000	**703 per 1.000** (341 to 1000)	**RR 7.63** (3.70 to 15.70)	150 (1 RCT)	⊕■■■ VERY LOW [a,b,c]	Single study
Live birth rate *(Tang et al., 2013)*	400 per 1.000	**600 per 1.000** (320 to 1000)	**RR 1.5** (0.8 to 2.9)	40 (1 RCT)	⊕■■■ VERY LOW [b,c,d]	Single study

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. The control group used low dose aspirin and heparin, while the experimental group was treated with prednisolone combined with low dose aspirin and heparin
b. Optimal information size not met
c. Single study
d. RPL patients selected due to NK cell density ≥ 5%

13 Anticoagulant compared to placebo/no treatment for unexplained RPL

Patient or population: unexplained RPL
Intervention: anticoagulant
Comparison: placebo/no treatment/other treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no/ other treatment	Risk with anticoagulant				
Anticoagulant versus no treatment						
Live birth rate *(aspirin)* *(de Jong et al., 2014)*	700 per 1.000	**658 per 1.000** (560 to 777)	**RR 0.94** (0.80 to 1.11)	256 (2 RCTs)	⊕⊕⊕■ MODERATE [a]	
Live birth rate *(LMWH)* *(de Jong, et al., 2014)*	784 per 1.000	**964 per 1.000** (658 to 1.000)	**RR 1.23** (0.84 to 1.81)	453 (3 RCTs)	⊕⊕■■ LOW [e,f]	
Live birth rate *(LMWH + aspirin)* *(de Jong, et al., 2014)*	702 per 1.000	**709 per 1.000** (611 to 814)	**RR 1.01** (0.87 to 1.16)	322 (2 RCTs)	⊕⊕⊕■ MODERATE [a]	
Live birth rate *(LMWH ± aspirin)* *(de Jong, et al., 2014)*	749 per 1.000	**802 per 1.000** (742 to 862)	**RR 1.07** (0.99 to 1.15)	793 (5 RCTs)	⊕⊕⊕■ MODERATE [b]	
Anticoagulant versus other treatment						
Live birth rate *(LMWH vs aspirin)* *(de Jong, et al., 2014)*	681 per 1.000	**790 per 1.000** (633 to 987)	**RR 1.16** (0.93 to 1.45)	325 (3 RCTs)	⊕■■■ VERY LOW [a,b,c,d]	
Live birth rate *(LMWH + aspirin vs aspirin)* *(de Jong, et al., 2014)*	609 per 1.000	**677 per 1.000** (573 to 792)	**RR 1.11** (0.94 to 1.30)	327 (2 RCTs)	⊕⊕⊕■ MODERATE [a]	
Live birth rate *(LMWH + aspirin vs LMWH)* *(de Jong, et al., 2014)*	723 per 1.000	**658 per 1.000** (521 to 832)	**RR 0.91** (0.72 to 1.15)	126 (1 RCT)	⊕■■■ VERY LOW [a,g]	*Single RCT*

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. Optimal information size not met
b. Analysis includes studies assessed as high risk of bias
c. Direction of effects, heterogeneity
d. One study includes women with hereditary thrombophilia and RPL
e. Two studies assessed as high risk of bias
f. Heterogeneity
g. Single RCT

14 Progesterone compared to no treatment/placebo for unexplained RPL

Patient or population: Unexplained RPL
Intervention: Progesterone
Comparison: No treatment/placebo

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no treatment/placebo	**Risk with Progesterone**				
Miscarriage rate (Haas et al., 2019)	376 per 1.000	**191 per 1.000** (112 to 303)	**OR 0.39** (0.21 to 0.72)	225 (4 RCTs)	⊕■■■ VERY LOW [a,b]	*(Women with 3 previous pregnancy losses only)*
Miscarriage rate (Coomarasamy et al., 2020)	358 per 1000	**326 per 1000** (290 to 362)	**RR 0.91** (0.81 to 1.01)	285 (2 RCTs)	⊕⊕⊕■ MODERATE [c]	*≥3 pregnancy losses*
Live birth rate (Coomarasamy, et al., 2020)	709 per 1000	**730 per 1000** (709 to 758)	**RR 1.03** (1.00 to 1.07)	4864 (2 RCTs)	⊕⊕⊕■ MODERATE [c]	*Review including Coomarasamy 2019 (women with spontaneous pregnancy loss) and Coomarasamy 2015 (women with recurrent pregnancy loss)*
Miscarriage rate *(Saccone et al., 2017)*	282 per 1.000	**203 per 1.000** (149 to 273)	**RR 0.72** (0.53 to 0.97)	1586 (10 RCTs)	⊕⊕⊕■ MODERATE [d]	*Review including (Coomarasamy et al., 2015)*

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. 4 RCTs of very low quality (as assessed by reviewers)
d. differences in direction of effect
c. RPL women with ≥3 pregnancy losses

Update 2022

15 G-CSF compared to placebo for unexplained RPL

Patient or population: unexplained RPL
Setting:
Intervention: G-CSF
Comparison: placebo

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo	Risk with G-CSF				
Live birth rate (Eapen et al., 2019)	649 per 1000	**584 per 1000** (454 to 778)	**RR 0.9** (0.7 to 1.2)	150 (1 RCT)	⊕⊕⊕■ MODERATE[a]	*Single RCT*
Miscarriage rate (Eapen, et al., 2019)	338 per 1000	**372 per 1000** (236 to 574)	**RR 1.1** (0.7 to 1.7)	150 (1RCT)	⊕⊕⊕■ MODERATE[a]	*Single RCT*

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. single study

Update 2022

16 Chinese Herbal medicine compared to placebo/no treatment for RPL

Patient or population: RPL
Intervention: Chinese Herbal medicine
Comparison: Placebo/no treatment

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with placebo/no treatment	Risk with Chinese Herbal medicine				
Live birth rate *(Chinese herbal medicines versus other pharmaceuticals)* *(Li et al., 2016)*	475 per 1.000	**499 per 1.000** (318 to 784)	**RR 1.05** (0.67 to 1.65)	80 (1 RCT)	⊕■■■ VERY LOW [b,c,d,e]	
Live birth rate *(Combined medicines versus other pharmaceuticals)* *(Li, et al., 2016)*	442 per 1.000	**685 per 1.000** (504 to 928)	**RR 1.55** (1.14 to 2.10)	601 (6 RCTs)	⊕■■■ VERY LOW [a,c,f]	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

a. High or unclear risk of bias in most studies (selection, performance, reporting)
b. Single study
c. Comparison with conventional medicine, instead of placebo
d. Optimal information size not met
e. No explanation was provided
f. Significant heterogeneity

Annex 3: 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 more dynamic and validated prognostic model including more risk factors 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 PGT-A in couples with RPL.
- The role of genetic analysis of pregnancy tissue following pregnancy loss needs to be clarified (prognostic modelling).
- Study the cost-effectiveness of the parental karyotyping.

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 administrated; start before conception (antepartum), start after fetal 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 RPL.

- Study the effect of immunotherapy in subsets of women with RPL with specific HLA class II alleles (in RCTs)
- Study the endometrial decidualization and senescence
  Study the prognostic value of ANA antibodies and potential treatment

Metabolic factors

- Study the effect of Levothyroxine treatment in women with RPL and identified subclinical hypothyroidism.
- Study the potential involvement of insulin resistance in RPL
- Study the effectiveness and safety of metformin for RPL and glucose metabolism defects

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 which DNA fragmentation test is most informative and most reliable
- 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.
- Study the use of PICSI as a treatment to improve pregnancy outcomes for couples with RPL and DNA 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 4: Abbreviations

AAGL	American Association of Gynecologic Laparoscopists
ACA	Anticardiolipin antibodies
AFC	Antral follicle count
AMH	Anti-Müllerian hormone
ANA	Antinuclear antibody
APS	Antiphospholipid syndrome
Array-CGH	Array-based Comparative Genomic Hybridization
ART	Assisted reproductive technology
aβ2GPI	β2 glycoprotein I antibodies
BMI	Body mass index
bp	Base pair
CCCT	clomiphene citrate challenge test
CI	Confidence Interval
CT	Computed tomography
DFI	DNA fragmentation index
E2	Estrogen
EM	Expectant management
EPL	Early pregnancy loss
ESGE	European Society for Gynaecological Endoscopy
FAI	Free androgen index
FG	Fasting glucose
FI	Fasting insulin
FISH	Fluorescent in situ Hybridization
FSH	Follicle Stimulating Hormone
G-CSF	Granulocyte colony-stimulating factor
GDG	Guideline Development Group
hCG	Human Chorionic Gonadotrophin
Hcy	Homocysteine
HHcy	Hyperhomocysteinemia
HLA	Human Leukocyte Antigen
hMG	Human Menopausal Gonadotropins
HOMA-IR	Homeostatic Model Assessment Insulin Resistance
HSG	Hysterosalpingography
HY	male-specific minor histocompatibility
ICSI	Intracytoplasmic sperm injection
IL	Interleukin
IR	Insulin Resistance
IU	International units
IUA	Intrauterine adhesions
IUI	Intrauterine insemination
IVF	In vitro fertilisation
Ivlg	Intravenous Immunoglobulin

KIR	Killer immunoglobulin-like receptor
LA	Lupus Anticoagulant
LAI-P	Lupus activity index-pregnancy
LBR	Live Birth Rate
LH	Luteinizing Hormone
LIT	lymphocyte immunization therapy
LMWH	Low molecular weight heparin
MRI	Magnetic resonance imaging
MTHFR	Methylenetetrahydrofolate reductase
NGS	Next Generation Sequencing
NK	Natural Killer
OR	Odd's ratio
P	Progesterone
PCOS	Polycystic ovary syndrome
PGD	Preimplantation Genetic Diagnosis
PGD-A	Preimplantation Genetic Diagnosis of aneuploidy
PGS	Preimplantation Genetic Screening
PGT	Preimplantation Genetic Testing
PGT-A	PGT for aneuploidies
PGT-M	PGT for monogenic/single gene defects
PGT-SR	PGT for chromosomal structural rearrangements
PICO	Patients – interventions – comparison – outcome
PICSI	Physiological Intracytoplasmic Sperm Injection
PL	Pregnancy loss
POI	Premature Ovarian Insufficiency
PSS	Perceived stress scale
RCT	Randomized controlled trial
ROS	reactive oxygen species
RPL	Recurrent pregnancy loss
RR	Relative risk
SCH	Subclinical hypothyroidism
SDF	Sperm DNA fragmentation
SHBG	sex hormone-binding globulin
SHG	Sonohysterography (or hysterosonography)
SMD	Standard mean deviation
T3	Triiodothyronine
T4	Thyroxine
TPO	Thyroid peroxidase
TPOAb	Thyroid peroxidase antibodies
TSH	Thyroid stimulating hormone
tTG	Tissue transglutaminase antibodies
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL).
TV-US	Transvaginal ultrasound
UFH	Unfractionated heparin
Vs	Versus
VTE	venous thromboembolism

Annex 5: Methodology

GUIDELINE DEVELOPMENT

European Society of Human Reproduction and Embryology (ESHRE) guidelines are developed based on the Manual for ESHRE guideline development (N. Vermeulen, N. Le Clef, S. Mcheik, A. D'Angelo, K. Tilleman, Z. Veleva, W. Nelen, version 4.0 2019), which can be consulted at the ESHRE website (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.

The two versions of this guideline (2017 and 2022) were 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. For the original version of this guideline (2017).

To update the original guideline, the literature search in PUBMED/MEDLINE and the Cochrane library was updated to include studies published between March 2017 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. References 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" (2017).

To update the guideline, the studies retrieved from the update of the literature searches (i.e. studies published between March 2017 and February 2022) were evaluated by the experts 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'' or "should" for strong recommendations and ''we suggest'' or "could" for conditional or weak recommendations. Suggested interpretation of strong and conditional recommendations by patients, clinicians and health care policy makers is as follows:

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

Strategy for review of the Guideline draft

After finalization of the guideline draft, the review process was initiated. The draft guideline was published on the ESHRE website, accompanied by the reviewers' comments form and a short explanation of the review process. The guideline was open for review between 28March and 9 May 2022. All reviewers are listed in annex 6. The Reviewer comments processing report, including further information on the review and a list of all comments per reviewer with the response formulated by the GDG will be published on the ESHRE website.

Guideline Implementation strategy

The standard dissemination procedure for all ESHRE guidelines comprises publishing and announcement.

Each guideline is published on the ESHRE Website and in Human Reproduction. The announcement procedure includes a newsflash on the ESHRE website homepage. All participants in the annual ESHRE meeting and all related national societies and patient organizations are informed about the update of the RPL guideline. The latter are asked to encourage local implementations by, for instance, translations or condensed versions, but they are also offered a website link to the original document.

The patient version of the guideline was updated in line with the update of this document. The patient version is a translation of the recommendations in everyday language, with emphasis on questions important to patients. It aims to help patients understand the guideline's recommendations and facilitates clinical decision-making. The patient version of the guideline is available on the ESHRE website.

Schedule for updating the guideline

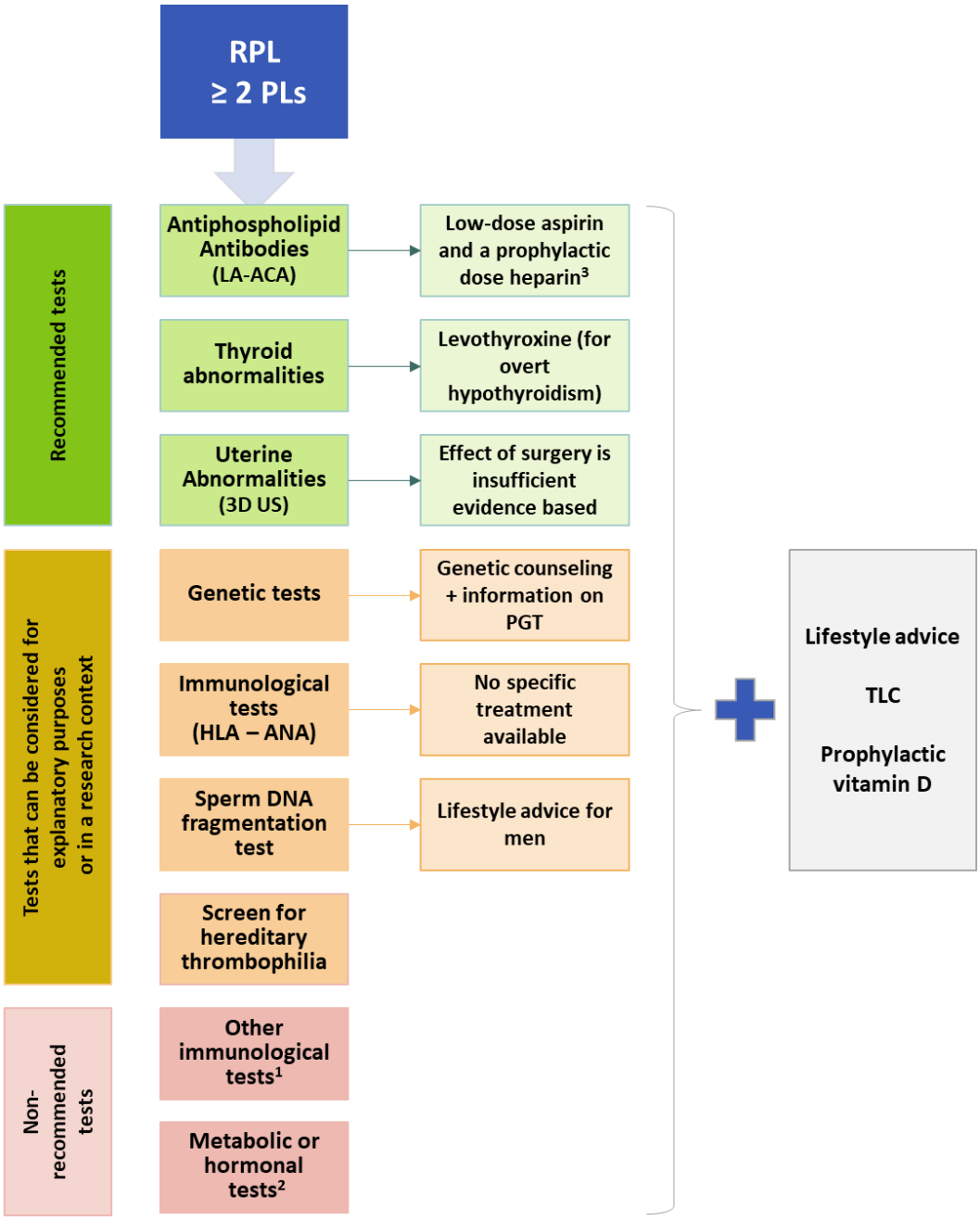
The current guideline is an update of the guideline in 2017, a revision performed as initially scheduled (four years after publication). The current guideline will be again revised (and updated) in 2026. An intermediate search for new evidence will be performed two years after publication, which will inform the GDG of the necessity of an update.

Every care is taken to ensure that this publication is correct in every detail at the time of publication. However, in the event of errors or omissions, corrections will be published in the web version of this document, which is the definitive version at all times. This version can be found at www.eshre.eu/guidelines.

For more details on the methodology of ESHRE guidelines, visit www.eshre.eu/guidelines

Annex 6: Flowchart

Figure 1 – Pictorial summary of the diagnosis test and treatments of RPL



1 *Including anti-HY antibodies, Natural Killer (NK) cell testing, anti-HLA antibodies*
2 *Including cytokine testing/polymorphisms, assessment of Polycystic ovary syndrome (PCOS), fasting insulin and fasting glucose, prolactin testing, ovarian reserve testing, luteal phase insufficiency testing, androgen testing, luteinizing hormone (LH) testing, homocysteine plasma levels*
3 *Low-dose aspirin and heparin are recommended after 3 or more pregnancy losses, or in the context of a clinical trial*

LA: Lupus anticoagulant; ACA: anticardiolipin antibodies; 3D US: three-dimensional ultrasound; HLA: Human Leukocyte Antigen; ANA: Antinuclear Antibodies

Annex 7: List of reviewers

As mentioned in the methodology, the updated guideline was open for review for 6 weeks, between 28 March and 9 May 2022. All reviewers of the 2017 version and the updated version, their comments and the reply of the guideline development group are summarized in the review report, which is published on the ESHRE website as supporting documentation to the guideline. The list of representatives of professional organizations, and of individual experts that provided comments to the updated version of the guideline are summarized below.

Reviewers of the RPL guideline (Update 2022)

Representative	Participation on behalf of
Michael Massoud Kamrava	West Coast IVF Clinic, Inc. - USA

Reviewer of the updated guideline	Country
Catherine Rongiers	France
Asher Bashiri	Israel
Zeev Shoham	Israel
Elena Kostova	The Netherlands
Lisa Lashley	The Netherlands
Mitranovici Melinda Ildiko	Romania
Abeer Issa	Saudi Arabia
Nicolas Garrido	Spain
Tansu Kucuk	Turkey
Shehnaaz Jivraj	UK
Roy Farquharson	UK
Peter Bisschop	The Netherlands

The list of reviewers of the 2017 version can be found in the full RPL guideline, version 2017 (annex 6: list of reviewers) on the ESHRE website (https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Recurrent-pregnancy-loss).