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RECURRENT PREGNANCY LOSS

Guideline of the European Society of Human Reproduction and Embryology

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ESHRE Early Pregnancy Guidline Development Group

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Introduction to the Guideline

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 However, the ESHRE SIG Implantation and Early Pregnancy believed that these guidelines were outdated and initiated the current guideline.

The guideline was 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) was composed of (previous) members of the coordination of the SIG, with addition of experts in the field that replied on a call for experts to the ESHRE audience. 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 will be developed.

References

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

List of all recommendations

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

2	4	Couples with RPL should be informed that smoking could have a negative impact on their chances of a live birth, and therefore cessation of smoking is recommended.	GPP		Smoking is a moderate risk factor for RPL, and associated with poor obstetric outcomes. Cessation of smoking could be recommended in couples with RPL even in the absence of studies on the effect of smoking cessation.
2	5	Couples with RPL should be informed that maternal obesity or being significantly underweight is associated with obstetric complications and could have a negative impact on their chances of a live birth and on their general health.	Strong	⊕⊕◯◯	Maternal obesity is a strong risk factor in RPL, and weight loss in overweight women has a positive impact on fertility outcomes and reduced weight is associated with reduced complications during pregnancy and birth. Striving for a normal BMI is recommended, even in the absence of studies on the impact of weight loss on a subsequent pregnancy loss.
2	6	Striving for a healthy normal range body mass index (BMI) is recommended.	GPP		
2	7	Couples with RPL should be informed that excessive alcohol consumption is a possible risk factor for pregnancy loss and proven risk factor for 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 number of preceding pregnancy losses and female age provide the best available prognostic information
3	10	The guideline development group (GDG) recommends to base prognosis on the number of preceding pregnancy losses and female age.	Strong	⊕⊕⊕◯	
4	11	Genetic analysis of pregnancy tissue is not routinely recommended but it could be performed for explanatory purposes.	Conditional	⊕⊕◯◯	As the impact of further clinical decision-making and the exact influence on prognosis for an individual patient is unclear, the GDG decided to formulate a conditional recommendation on genetic testing of the pregnancy tissue.
4	12	For genetic analysis of the pregnancy tissue, Array-based Comparative Genomic Hybridization (array-CGH) is recommended based on a reduced maternal contamination effect.	Strong	⊕⊕◯◯	
4	13	Parental karyotyping is not routinely recommended in couples with RPL. It could be carried out after individual assessment of risk.	Conditional	⊕⊕◯◯	Couples should primarily be informed that, even if a parental abnormality is found, the cumulative live birth rates are good. Furthermore, they should be informed of the limitations of karyotyping and the impact of the test result.
5	14	For women with RPL, we suggest not to screen for hereditary	Conditional	⊕⊕⊕◯	There is no, or at best a weak, association between RPL

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

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

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.	
9	38	In the male partner, it is suggested to assess life style factors (smoking, alcohol consumption, exercise pattern, and body weight).	GPP		Based on suggested association between life style and sperm quality.	
9	39	Assessing sperm DNA fragmentation in couples with RPL can be considered for explanatory purposes, based on indirect evidence.	Conditional	⊕⊕◯◯	There is a moderate body of evidence indicating associations between RPL and poor quality sperm, particularly sperm with elevated DNA fragmentation.	
TREATMENT TO INCREASE LIVE BIRTH RATE IN RPL						
10	*10*	The guideline development group (GDG) recommends to base prognosis on the number of preceding pregnancy losses and female age.	Strong	⊕⊕⊕◯	There is consistent evidence for an impact of the number of preceding pregnancy losses and female age on the prognosis in couples with RPL, while evidence for other factors is less clear.	
10	40	Prognostic tools (Lund, Brigham) can be used to provide an estimate of subsequent chance of live birth in couples with unexplained RPL.	GPP			
11	41	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	42	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	43	For women with hereditary thrombophilia and a history of RPL, we suggest not to use antithrombotic prophylaxis unless in the context of research, or if indicated for venous thromboembolism (VTE) prevention	Conditional	⊕⊕◯◯	We found no evidence of a beneficial effect of anticoagulant treatment in women with RPL and hereditary thrombophilia.	SOF table 2
12	44	For women who fulfill the laboratory criteria of APS and a history of three or more pregnancy losses, we suggest administration with low-dose aspirin (75 to 100 mg/day) starting before conception, and a prophylactic dose heparin (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
12	45	The GDG suggests offering anticoagulant treatment for women with two pregnancy losses and APS, only in the context of clinical research.	GPP			
14	46	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	SOF table 6
14	47	There is conflicting evidence regarding treatment effect of levothyroxine for women with subclinical hypothyroidism (SCH) and RPL. Treatment of women with SCH may reduce the risk of miscarriage, but the potential benefit of treatment should be balanced against the risks.	Conditional	⊕⊕◯◯	Treatment with levothyroxine is insufficiently evidence-based and the efficacy and safety should be further investigated.	
14	48	If women with subclinical hypothyroidism and RPL are pregnant again, thyroid stimulating hormone (TSH) level should be checked in early gestation (7-9 weeks AD), and hypothyroidism should be treated with levothyroxine.	GPP			
14	49	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	50	There is insufficient evidence to support treatment with levothyroxine in euthyroid women with thyroid antibodies and RPL outside a clinical trial.	Conditional	⊕⊕◯◯	There is no convincing evidence on the efficacy of levothyroxine treatment for increasing the chance of a live birth in women with a history of RPL and thyroid autoimmunity (normal TSH and TPO Ab+).	

14	51	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	52	There is insufficient evidence to recommend the use of human chorionic gonadotrophin (hCG) to improve live birth rate in women with RPL and luteal phase insufficiency.	Conditional	⊕⊕◯◯	Studies are considered too limited to recommend the use of hCG in women with RPL and luteal phase insufficiency.	
14	53	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	54	Bromocriptine treatment can be considered in women with RPL and hyperprolactinemia to increase live birth rate.	Conditional	⊕◯◯◯	In women with hyperprolactinemia, bromocriptine treatment normalizes serum prolactin levels and could improve the chance of a live birth.	
14	55	Preconception counseling 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	Whether hysteroscopic septum resection has beneficial effects (improving live birth rates, and decreasing miscarriage rates, without doing harm), should be evaluated in the context of surgical trials in women with RPL and septate uterus.	Conditional	⊕◯◯◯	Observational studies suggest a benefit of treatment in reducing the miscarriage rate, but at least one study showed that women were less likely to get pregnant after surgery. Therefore, the GDG decided to formulate a recommendation for more research on the topic.	
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	Sperm selection is not recommended as a treatment in couples with RPL	GPP		In the absence of any data in RPL, the GDG recommends against sperm selection in a GPP.	
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 8
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 9
17	69	Intravenous immunoglobulin (IvIg) is not recommended as a treatment of RPL.	Strong	⊕⊕◯◯	Based on the available evidence, IVIG cannot be recommended for clinical use in women with RPL. Further randomized controlled trials are needed.	SOF table 10
17	70	Glucocorticoids are not recommended as a treatment of	Strong	⊕⊕◯◯	The evidence points toward some beneficial effect of prednisolone in selected women with RPL. However,	SOF table 11

		unexplained RPL or RPL with selected immunological biomarkers.			based on adverse events associated with the use of prednisone, the GDG decided to recommend against treatment awaiting further studies.	
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 12
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 does not improve live birth rates in women with unexplained RPL.	Conditional	⊕⊕⊕◯	Vaginal progesterone during early pregnancy has no beneficial effect in women with unexplained RPL. 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 13
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 insufficient evidence to recommended G-CSF in women with unexplained RPL.	Conditional	⊕⊕◯◯	A single randomized controlled trial suggests a substantial benefit of G-CSF in RPL but it needs to be confirmed in other trials in different populations.	SOF table 14
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 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 observational study that there is no difference in the probability of carrier status (of a structural chromosomal abnormality) between couples that had two or three consecutive pregnancy losses, compared to two or three non-consecutive losses (van den Boogaard et al., 2010).
- There is some evidence from one observational study that whether the pregnancy losses are consecutive or not impacts on prognosis in unexplained RPL (Egerup et al., 2016).
- Only a minority of the RPL couples (estimated to be less than 10%) experience 2 or more non-consecutive pregnancy losses (van den Boogaard et al., 2010, van den Boogaard et al., 2013)

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

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

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

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

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

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

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

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

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

Recurrent "Early" Pregnancy Loss (REPL) is the loss of two or more pregnancies before 10 weeks of gestational age (Kolte *et al.*, 2015a).

Terminology

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

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

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

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

> The GDG concludes to use the term Recurrent Pregnancy Loss.

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 more recent 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 to date have focused mainly on women with RPL (Rowsell *et al.*, 2001, Craig *et al.*, 2002, Andalib *et al.*, 2006, Kagami *et al.*, 2012, Mevorach-Zussman *et al.*, 2012, Sugiura-Ogasawara *et al.*, 2013a, Toffol *et al.*, 2013, Kolte *et al.*, 2015b) and while there are several studies on (usually male) partners' reactions to sporadic pregnancy loss (Boynton, 2015) there are few if any that focus on RPL.

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

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

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

Pregnancy after RPL

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

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

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

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

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

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PART B: Organization of care

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

ACCESS TO CARE

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

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

THE RPL CLINIC

The following elements are required in a RPL clinic:

- *Staffing*

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

- *First Visit*

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

- *Equipment/ Location*

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

- *Provision of information*

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

- *Appropriate evaluation (testing)*

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

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

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

- *Care tailored to psychological needs of the couples*

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

- *Treatment plan*

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

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

- *Research*

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

TREATMENT PLAN, SUPPORTIVE CARE AND PSYCHOLOGICAL CARE

Couples coming to the RPL clinic primarily seek expertise, investigations and a treatment plan that will reduce the risk of further losses. A plan for the subsequent pregnancy involves potential treatments, life style 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 aborter, 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 behavior

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

1. Risk factors for recurrent pregnancy loss

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

1.1 Age

Advanced female age is a well-established risk factor for female subfertility, 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). 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 poor quality. A more recent study reported an increased risk of pregnancy loss in personnel exposed to anesthetic 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 anesthetic gases (Guirguis *et al.*, 1990)

Conclusion

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

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

1.4 Chronic endometritis

Evidence

Chronic endometritis is characterized by a plasma cell infiltrate in the endometrium associated with a range of pathogenic organisms. There have been a series of papers suggesting a 7-58% prevalence of chronic endometritis in women with RPL (Cicinelli *et al.*, 2014, McQueen *et al.*, 2014, McQueen *et al.*, 2015). The prevalence is dependent on the method of detection with high rates reported when hysteroscopy and /or immunohistochemistry with antibodies to CD138 are used (Kitaya, 2011, Russell *et al.*, 2013, Cicinelli *et al.*, 2014, McQueen *et al.*, 2014, McQueen *et al.*, 2015, Bouet *et al.*, 2016). However, there are no studies comparing rates of endometritis in women with RPL to control women, or discussing the predictive value of a positive test for endometritis.

Antibiotics were found to remove 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

The mechanism of endometrial changes in early pregnancy (i.e. endometrial decidualization) and during the menstrual cycle is not yet completely elucidated. Endometrial cells seem to form a checkpoint for embryo quality resulting in implantation processes for normal embryos and rapid demise of endometrium (menstruation) in case of “abnormal” embryos (Lucas *et al.*, 2016).

Recent observations have suggested that in women with RPL, abnormal decidualization may render the endometrium excessively permissive to implantation (i.e. a defect in the checkpoint) but unable to sustain the pregnancy, but further (prospective) studies are needed before any firm conclusions or recommendations can be formulated for clinical practice.

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2. Health behavior modifications

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

2.1 Smoking cessation

Evidence

Smoking is strongly associated with adverse obstetric and neonatal outcomes, including ectopic pregnancy, stillbirth, placenta praevia, preterm birth, low birth weight, and congenital anomalies. Studies have also reported associations between maternal smoking during pregnancy and problems during childhood, including sudden infant death syndrome, obesity, psychosocial problems and malignancies (Leung 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 behavior in 326 women with RPL and 400 controls who had at least one live birth, environmental exposure to tobacco smoke (passive smoking) significantly increased the risk of RPL compared with tobacco-free controls. The risk increased with the daily duration of exposure (adjusted OR 2.30; 95% CI 1.50-3.52 for short exposure of <1h/day; adjusted OR 4.75; 95% CI 3.23-6.99 for long exposure of ≥1 h/day). Female smoking, consumption of alcohol or coffee intake were not associated with the risk of RPL (Zhang *et al.*, 2010).

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

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

Recommendation

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

Justification

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

2.2 Striving for a healthy, normal range body mass index

Evidence

Weight loss

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

A normal BMI for a Caucasian population is considered 20-30 kg /m^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 (Lashen *et al.*, 2004, Boots and Stephenson, 2011). In women with RPL (n=491), there was a higher miscarriage rate in the obese versus non-obese women (OR 1.71; 95% CI 1.05-2.8) (Metwally *et al.*, 2010). The latter study also reported that an increased BMI was the second-most significant factor predicting early pregnancy loss (after advanced female age). The presence of PCOS or the number of previous losses did not predict a pregnancy loss in the next pregnancy (Metwally *et al.*, 2010)

More recent studies on obesity and RPL also found an association. Boots and colleagues assessed the frequency of a euploid miscarriage in 372 women with RPL. There were 117 subsequent miscarriages and the frequency of a euploid miscarriage among obese women was 58% compared with 37% of non-obese women (relative risk RR 1.63; 95% CI 1.08-2.47) (Boots *et al.*, 2014). In the retrospective study of Zhang, mentioned above, evaluating the impact of lifestyle factors on the risk of RPL, a BMI of 24.0 or greater was associated with an increased risk of RPL (adjusted OR 1.55; 95% CI 1.12-2.14) (Zhang *et al.*, 2010). Lo and colleagues assessed the relationship between maternal BMI and the future outcomes of pregnancy in 696 couples with unexplained RPL. They found that BMI, female age, number of previous pregnancy losses, and ethnicity were significantly associated with pregnancy outcome. Logistic regression demonstrated that maternal obesity (BMI ≥ 30 kg/m^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 recent review, also reported diverging results concerning the association between exercise during early pregnancy and miscarriage. Two case-control studies found that exercise was associated with a lower risk of miscarriage, one large cohort study reported a graded association between exercise and higher risk of miscarriage, and two studies (of which one was also included in the review of Schlussel) showed the same risk for miscarriage in exercising versus non-exercising pregnant women (Hegaard *et al.*, 2016). With regards to occupational physical activity, three studies reported no effect, while two high quality studies pointed to high-intensity occupational activity as a risk factor for miscarriage (Schlussel *et al.*, 2008).

2.5 Avoiding alcohol

Evidence

Alcohol has a clear negative impact on pregnancy and neonatal outcomes, not the least of which are 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[1] 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.

Recently, 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

[1] 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.

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 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.

Medical and family history could be helpful in deciding which investigations are relevant for the individual patient (age, fertility/sub-fertility, pregnancy history, family history, previous investigations and/or treatments). However, no studies have been performed that could advise clinicians on which diagnostic tests are relevant for a specific patient and, more importantly, which are not.

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

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

The GDG recommends to base prognosis on the number of preceding pregnancy losses and female age.	Strong	⊕⊕⊕○

Justification

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.

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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

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.

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 analyzing 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 (Kudesia *et al.*, 2014, Mathur *et al.*, 2014). New techniques such as next generation sequencing (NGS) have not yet been extensively investigated in genetic analysis of pregnancy tissue but may be useful in the near future (Shamseldin *et al.*, 2013).

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

Determining the chromosomal status of pregnancy tissue from women with recurrent pregnancy loss may provide them with a cause or reason for the particular loss being investigated, but it does not necessarily rule out other underlying conditions. No clear effect of genetic testing of the pregnancy tissue on prognosis (subsequent live birth) has been described so far and the role of genetic analysis of pregnancy tissue should be further elaborated within a prognostic model.

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

Recommendation

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

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

Justification

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

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

The preferred method of genetic analysis is array-CGH, as this is not limited by tissue culture failure or false negative results due to maternal cell contamination. However, array-CGH has some limitations with regard to not being able to detect balanced rearrangements and low-level mosaicism (<10–15%) (Sahoo *et al.*, 2017) and low sensitivity for minor copy number variants (Freeman *et al.*, 2006). A recent study suggests that array-CGH can also be used for cytogenetic analysis of spontaneously discharged pregnancy tissue, although high incidence of maternal contamination needs to be taken into account (Ozawa *et al.*, 2016). New techniques such as next generation sequencing (NGS) may be useful in the near future (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 (Franssen *et al.*, 2006, Barber *et al.*, 2010). In another retrospective study of 795 couples with two or more pregnancy losses, chromosomal abnormalities were found in 3.5% of the couples. The subsequent miscarriage rate was higher and the live birth rate was lower in carrier couples, although the cumulative live birth rate was 64%. (Flynn *et al.*, 2014). Another cohort study reported a lower live birth rate in carrier couples (63.0%) compared to women with a normal karyotype (78.7%). This study did not mention the number of carrier couples deciding not to attempt to conceive again (Sugiura-Ogasawara *et al.*, 2008).

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

Ongoing pregnancies with unbalanced translocations were detected in less than 1% in carrier couples seen for prenatal diagnosis in a large retrospective study (Barber *et al.*, 2010), and in 2.9% of 34 pregnancies in carrier couples in a smaller study (Sugiura-Ogasawara *et al.*, 2004) These numbers are in contrast with a case-control study showing that couples have a high-perceived risk of receiving an abnormal result and a suboptimal understanding of the tests carried out (Vansenne *et al.*, 2011). Deduction from two large nationwide studies reveals a negligible chance, an estimated 0,02%, of a live born handicapped child with unbalanced chromosome abnormalities in the unselected RPL population (Franssen *et al.*, 2006, Barber *et al.*, 2010).

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

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

Recommendations

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

Justification

	Association	Contributing factor	Prognosis	Treatment
Parental genetic testing	Yes	Yes[1]	Yes[2]	No

[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 other 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. 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 currently 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 mutation

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

Studies on the Factor V Leiden mutation and RPL were summarized and analyzed 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 mutation), 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 mutation

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 mutation 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 analyzed 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 mutation and the risk of a next pregnancy loss was not significant (OR 3.29; 95%CI 0.594-18.19, 1 study), nor was the occurrence rate (OR 1.77; 95%CI 0.87-3.61; four cohort studies). Similar to Factor V Leiden, the clinical utility was judged as minimal and the harms of testing outweigh the benefits (Bradley *et al.*, 2012).

Protein C, Protein S and Antithrombin deficiency

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

Methylenetetrahydrofolate reductase (MTHFR) mutation

MTHFR gene polymorphisms have historically been classified as a hereditary thrombophilia factor but the mutations are no longer considered for routine assessment of thrombosis risk (Levin 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 (Nelen *et al.*, 2000, Govindaiah *et al.*, 2009, Chen *et al.*, 2016), while others did not find evidence of an association (Rey *et al.*, 2003). Although less well studies, no significant associations were found between other mutations of the MTHFR gene and RPL (Hickey *et al.*, 2013, Chen *et al.*, 2016)

Recommendation

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

Justification

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

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

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

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

5.2 Acquired thrombophilia

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

Three clinically relevant and well-characterized antiphospholipid antibodies (i.e. antibodies associated with thrombosis) are lupus anticoagulant (LA), anticardiolipin antibodies (ACA, IgG and IgM), and 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 nonconsecutive, 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).

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).

β2 glycoprotein I antibodies

Based on five studies, 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).

Other Antibodies

Recently, 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, Subrt *et al.*, 2008, Tebo *et al.*, 2008).

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

Recommendations

For women with RPL we recommend screening for antiphospholipid antibodies (LA and ACA [IgG and IgM]), after two pregnancy losses.	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 a recent prospective study, although needing confirmation, suggests that a decrease in aβ2GPI antibodies (IgM) with anticoagulant treatment was correlated with better pregnancy outcomes (Song *et al.*, 2017).

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

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

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6. Immunological screening

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[2] 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). 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 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).

[2] 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.

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.[3] 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 (Varla-Leftherioti *et al.*, 2003, Vargas *et al.*, 2009). Due to the contradictive findings concerning KIR genotyping in couples with RPL, KIR and HLA-C typing is not suitable for diagnostic and therapeutic purposes at present.

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

Recommendation

HLA determination in women with RPL is not recommended in clinical practice. Only HLA class II determination (HLA-DRB1*15:01 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 ⊕⊕◯◯

[3] 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.

Justification

	Association	Contributing factor	Prognosis	Treatment
HLA-compatibility	Controversial evidence	NA	No prognostic potential	NA
HLA class II: HLA-DR (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.

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 sec RPL after first born boy	Negative impact on future live birth*	None available

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

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

6.3 Cytokines

Evidence

In general, investigation of the cytokine levels in peripheral blood is not informative except for TNF-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 flushings 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

In an older review, 10 out of 12 case-control studies found an increased prevalence of ANA in women with RPL (Christiansen, 1996). In the nine relevant studies subsequently published, six found a significantly increased prevalence of ANA in women with RPL compared with controls (Ogasawara *et al.*, 1996, Stern *et al.*, 1998, Kaider *et al.*, 1999, Matsubayashi *et al.*, 2001, Ticconi *et al.*, 2010, Molazadeh *et al.*, 2014) whereas three did not (Bustos *et al.*, 2006, Giasuddin *et al.*, 2010, Hefler-Frischmuth *et al.*, 2017).

Some studies reported that ANA positivity was more prevalent in women with RPL with a new miscarriage (n=24) as compared to those who gave birth (n=82) (Cavalcante *et al.*, 2014). Similarly, a higher miscarriage rate was reported in ANA-positive as compared to ANA-negative women with RPL in a small prospective study (Harger *et al.*, 1983). However, the study by Ogasawara did not find that the presence of ANA could predict new pregnancy loss (Ogasawara *et al.*, 1996).

A direct pathophysiological link between the presence of autoantibodies such as ANA in women with RPL and 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	Association found in most studies	Probably not – no documentation	Unclear	NA

Measurement of ANA in women with RPL could be considered since the majority of case-control studies document an association to RPL and there is some evidence (from smaller prospective studies) that ANA presence affects the prognosis negatively (Harger *et al.*, 1983, Cavalcante *et al.*, 2014). Whether ANA positivity can identify a subset of women with RPL that responds beneficially to various forms of immunotherapy is unknown and can only be shown in randomized controlled trials.

6.5 Natural killer cells (NK cells)

Evidence

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

NK cells in peripheral blood

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

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

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

NK cells in endometrial biopsies or decidual tissue

One small case-control study reported that the CD56^{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 (Vassiliadou and Bulmer, 1996, Ozcimen *et al.*, 2009, Bao *et al.*, 2012). However, since the tissue in the former cases is necrotic and often inflamed and the latter cases is fresh and vital, these kind of studies provide limited valid information.

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

Recommendation

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

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

Furthermore, there are significant technical challenges; the frequencies of NK cell subsets between the endometrium and peripheral blood are extremely different. NK cells can be measured in endometrial biopsies taken in non-pregnant cycles by immunohistochemistry or flow cytometry of homogenized tissue. The former technique is prone to subjective evaluation and using the latter can change surface

marker expression since the tissue undergoes enzymatic digestion. Furthermore, endometrial and peripheral blood NK cell numbers fluctuate hugely in the menstrual cycle so exact timing of samples is crucial but has rarely been done.

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.

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

Evidence

Hyperthyroidism

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

Hypothyroidism

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

Three studies investigated a possible association between subclinical hypothyroidism (SCH) and RPL. In the cohort study of Bernardi and colleagues, 19% of 286 women with RPL (≥ 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 recent 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 antibodies against the thyroid gland (Marai *et al.*, 2004).

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

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

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

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

Recommendations

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

Abnormal TSH and TPO-antibody 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

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 hyperglycemia, calculated by comparing the patient's fasting values with the model's predictions.

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

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

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

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

PCOS and insulin metabolism

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

Another case-control study by Kazerooni compared several parameters in four groups of 60 women: PCOS with RPL, RPL without PCOS, PCOS without RPL, and women without RPL or PCOS. They found the highest levels for fasting insulin in women with PCOS and RPL; and significantly lower levels in all other groups. For the Quantitative Insulin Sensitivity Check Index (calculated 1/log(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 recent case-control study found higher levels of maternal serum fructosamine (a marker of glycemic control) in women with RPL (n=117) as compared to controls, which could indicate an association between subclinical glucose intolerance and RPL, although this needs confirmation (Romero *et al.*, 2016).

Recommendation

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

Justification

	Association	Contributing factor	Prognosis	Treatment
PCOS	YES	YES	NO	Metformin for sporadic PL no studies for RPL
Insulin resistance*	YES (OR 3.6)	Unclear	No studies	No studies
Fasting insulin	Inconsistent (2 YES, 1 NO)	Unclear	No studies	No studies
Fasting glucose	NO	NO	No studies	No studies

** 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 Prolactin Deficiency

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 recent 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 poor quality and many did not include a control group. It was concluded that in the absence of consistent evidence on an association between prolactin and RPL, prolactin testing is not routinely recommended.

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

7.4 Ovarian reserve testing

Evidence

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

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

In a recent cross-sectional study, ovarian reserve was assessed in 71 women with a history of unexplained RPL and compared with 70 age-matched fertile controls. FSH levels were significantly higher in women with RPL as compared to controls (8.6±3.7 U/l versus 7.1±3.9 U/l). The levels of AMH were significantly lower in the RPL group (2.9±1.7 ng/ml versus 3.6±1.7 ng/ml). The percentage of

women with diminished ovarian reserve (defined as levels of FSH ≥11 U/l) was significantly higher in the RPL group (18.3% versus 4.3%), as was the percentage of women with levels of AMH ≤1 ng/ml (19.7% versus 5.7%). The levels of LH, FSH/LH ratios, and E2, the mean ovarian volume and AFC were similar between the groups (Atasever et al., 2016).

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

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

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

Ovarian reserve in women with unexplained RPL has also been compared to ovarian reserve markers in women with explained RPL. The percentage of women with elevated FSH and/or estradiol levels significantly higher in the unexplained RPL as compared to explained RPL (Trout and Seifer, 2000, Gurbuz *et al.*, 2004). Another study found lower levels of AMH and estradiol on Day 3-5 in women with unexplained RPL, with no difference in FSH and LH (Pils *et al.*, 2016). The relevance of these findings is unclear.

Recommendation

Ovarian reserve testing is not routinely recommended in women with RPL.	Strong	⊕⊕◯◯

Justification

	Association	Contributing factor	Prognosis	Treatment
Ovarian reserve	No evidence	No data	Abnormal CCCT = poor LBR	No studies

Several studies have attempted to evaluate ovarian reserve, but overall, there is insufficient evidence to claim an association between low ovarian reserve and RPL. Based on a single study, low ovarian reserve could be indicative of a less favorable prognosis in women with infertility and RPL. However, this study used the clomiphene citrate challenge test (CCCT) which is not very sensitive and no longer commonly used.

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

7.5 Luteal phase insufficiency

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

Evidence

The assessment of a possible association between luteal phase insufficiency and RPL is hampered by the diagnostic criteria for luteal phase insufficiency. The sensitivity and specificity of common clinical tests used for the diagnosis of luteal phase insufficiency were compared in 19 women with infertility or RPL and 15 normal controls. The recommended test for the determination of luteal phase insufficiency is a midluteal phase single serum Progesterone (P) level <10 ng/mL or the sum of three serum P levels that is <30 ng/ml. Timed endometrial biopsy (performed at late luteal phase) was found to have marginally acceptable sensitivity and specificity. Low sensitivity and/or specificity were found for the appearance of basal body temperature charts, luteal phase length ≤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 (Rai *et al.*, 2000, Nardo *et al.*, 2002).

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 recent 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, recent 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 13.6 for 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).

Recent 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 RPLand 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 recent studies reported that HHcy was associated with RPL in patients with PCOS. The incidence of HHcy was significantly higher in RPL-affected PCOS (70.63%, n=126) patients, compared to in women with RPL without PCOS (57.26%, n=117; p<0.04) (Chakraborty *et al.*, 2013). In the study of Kazerooni, mentioned before, women with RPL and PCOS had significantly higher levels of Hcy (12.4 ± 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 (Wilcken *et al.*, 2003, Binia *et al.*, 2014).

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8. Anatomical investigations

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

8.1 CONGENITAL UTERINE MALFORMATIONS

Evidence

An association between congenital uterine malformations and recurrent pregnancy loss (RPL) has been well documented, but the exact prevalence in this population has not been clearly defined (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) (Saravelos *et al.*, 2008, Chan *et al.*, 2011b).

Recent 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-trimested 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 can be considered in women with RPL (primary or secondary) (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 recently published (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 anesthetics.

Sonohysterography (or hysterosonography) (SHG) appears a safe procedure which provides more information about uterine abnormalities than hysterosalpingography (HSG) or ultrasound (US) alone (Tur-Kaspa *et al.*, 2006). SHG is accurate in diagnosing and classifying congenital uterine malformations (Ventolini *et al.*, 2004, Valenzano *et al.*, 2006). In addition, SHG has a higher sensitivity and specificity

than HSG or diagnostic hysteroscopy to diagnose uterine malformations in general (Ludwin *et al.*, 2011). SHG uses the introduction of fluid (saline or contrast) into the uterine cavity to enhance US imaging studies, which could be uncomfortable for women. The diagnosis of septate uterus by SHG eliminates the need to perform laparoscopy prior to hysteroscopic metroplasty (Ludwin *et al.*, 2011).

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

Two-dimensional US and hysterosalpingography (HSG) are non-invasive and widely available. Two-dimensional US has a low sensitivity, but a high specificity for diagnosis of malformations. HSG has a good sensitivity for diagnosing more pronounced uterine malformations, but it is limited in differentiating between the types of malformations (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 in isolation (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 (Oppelt *et al.*, 2007, Hall-Craggs *et al.*, 2013).

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

Cervical weakness is a recognized cause of second-trimester pregnancy loss, but the true incidence is unknown, since the diagnosis is essentially a clinical one (Kassanos *et al.*, 2001, Harger, 2002, Liddell 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).

Diagnosis of acquired uterine malformations

Although the relevance of acquired uterine malformations 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).

Recommendations

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 with normal cervix (former AFS bicornuate uterus).	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	⊕⊕◯◯
If a Müllerian uterine malformation is diagnosed, further investigation (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	⊕⊕◯◯

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. For acquired uterine malformations, there is no convincing evidence that these malformations are associated with or contribute to RPL.

	Association	Contributing factor	Prognosis	Treatment
Congenital uterine malformations	Yes	Suggested some malformations	/	Surgical trials in case of a septate uterus
Acquired uterine malformations	Unclear	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 recent study suggest 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 been considered an issue stemming exclusively from female causes until very recently. 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

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. 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 sperm quality, occupational exposure, and lifestyle (smoking, alcohol consumption and soft drugs) should be assessed in addition to female factors (Anifandis *et al.*, 2014, Jensen *et al.*, 2014, Pacey *et al.*, 2014, Showell *et al.*, 2014).

Several smaller studies have compared sperm parameters of couples with RPL to healthy (fertile) controls. Overall, these studies found no differences in sperm volume (7 studies) or sperm count (2 studies) (Sbracia *et al.*, 1996, Gopalkrishnan *et al.*, 2000, Bhattacharya, 2008, Brahem *et al.*, 2011, Imam *et al.*, 2011, Talebi *et al.*, 2012, Zhang *et al.*, 2012, Khadem *et al.*, 2014). The percentage of motile sperm and percentage of sperm with normal morphology were reported to be lower in RPL men in some studies, while others found no difference. Three studies consistently reported higher DNA fragmentation in RPL men. Some studies have suggested a difference in sperm parameters between RPL couples that achieve a successful pregnancy and live birth rate, and couples that experienced an additional pregnancy loss, or failed to achieve pregnancy. One study reported differences in sperm concentration and motility between successful and unsuccessful couples (Sbracia *et al.*, 1996), while another study reported differences in abnormal sperm chromatin integrity. A lower sperm concentration was only reported in infertile RPL couples, while a lower percentage of normal morphology was detected in RPL couples who experienced pregnancy loss (Zhang *et al.*, 2012)

Following the lack of a consistent association between conventional semen parameters and RPL, the majority of recent studies addressing male factors and RPL have focused on male genetic defects. These range from markers of Y chromosomal deletions, chromatin integrity and DNA damage. The few studies on chromosomal anomalies were poorly powered and overall indicated no relationship with miscarriage (Bernardini *et al.*, 2004, Carp *et al.*, 2006, Bronet *et al.*, 2012). Similarly, Y chromosome microdeletions were not associated with increased miscarriage rates in RPL couples (Kaare *et al.*, 2008, Wettasinghe *et al.*, 2010, Pereza *et al.*, 2013). Sperm DNA shows more promise. Of the systematic

reviews with meta-analysis, Robinson and colleagues interrogated 16 cohort studies (2969 couples) of which 14 were prospective (Robinson *et al.*, 2012). In 15 out of 16 included studies, sperm DNA damage was assessed in couples undergoing IVF or ICSI, while one study focused on spontaneous conception. The meta-analysis showed a significant increase in miscarriage rates in men with high sperm DNA damage compared with those with low sperm DNA damage (RR 2.16; 95% CI 1.54-3.03). A subgroup analysis showed that the miscarriage association is strongest for the TUNEL assay (RR 3.94; 95% CI 2.45-6.32) (Robinson *et al.*, 2012). Similarly, Zhao and colleagues performed a systematic review in 2014 also including 16 cohort studies (3106 couples) showing that sperm DNA fragmentation had a detrimental effect on clinical outcomes (pregnancy and miscarriage) after IVF/ICSI (Zhao *et al.*, 2014). The studies used different sperm DNA damage test assays, endpoints were for different treatment types (IVF/ICSI/IUI), different aspects of DNA damage were measured and different thresholds for DNA damage were used. Further, female inclusion and exclusion criteria were imposed and the definitions of miscarriage were not always coherent. Given a significantly increased RR despite these numerous confounding factors, both Robinson and Jing Zhao concluded that sperm DNA damage testing should be offered to couples following even a single miscarriage after fertility treatment (Robinson *et al.*, 2012, Zhao *et al.*, 2014). Two recent studies have also reported significantly increased sperm DNA fragmentation with couples who have experienced RPL after natural conception (Zidi-Jrah *et al.*, 2016, Carlini *et al.*, 2017).

The main cause of DNA damage is oxidative stress and this seems to be exacerbated by smoking, obesity and excessive exercise (Aitken *et al.*, 2009, Hsu *et al.*, 2009, Du Plessis *et al.*, 2010). Clinicians could advise male partners of couples presenting with RPL of these connections and suggest ways to prevent sperm DNA damage caused by unhealthy lifestyles (Sharma *et al.*, 2013, Showell *et al.*, 2014, Wright *et al.*, 2014).

Another possible male cause of pregnancy loss is the introduction of surgically retrieved, immature sperm to be used with intracytoplasmic sperm injection (ICSI). There are only two well-powered studies comparing pregnancy loss following ICSI with testicular sperm compared with epididymal sperm. In a chart review of 1121 men with obstructive azoospermia who underwent ICSI with surgically retrieved sperm, miscarriage rates did not differ between epididymal and testicular sperm (17.6% versus18.4%) (Kamal *et al.*, 2010). This supported a previous study by Nicopoullos who had reported no difference in miscarriage rates between similar groups (Nicopoullos *et al.*, 2004). However, the cause of azoospermia rather than the source of sperm led to differences as in a study of 108 consecutive couples where the miscarriage rate was 28% for obstructive azoospermia, and 40% for non-obstructive azoospermia (Pasqualotto *et al.*, 2002).

Recommendations

In the male partner, it is suggested to assess life style factors (smoking, alcohol consumption, exercise pattern, and body weight).	GPP	

Assessing sperm DNA fragmentation in couples with RPL can be considered for explanatory purposes, based on indirect evidence.	Conditional	⊕⊕◯◯

Justification

	Association	Contributing factor	Prognosis	Treatment
Sperm DNA damage	Moderate	Probably, but no prospective studies to confirm	Unclear	No treatment has been studied

There is a moderate body of evidence indicating associations between RPL and poor quality sperm; particularly sperm with elevated DNA fragmentation. These associations are independent of female factors.

Since there is also clear evidence that sperm DNA damage is caused by unhealthy lifestyles (such as smoking, obesity and excessive exercise), clinicians could make couples aware of these risks.

Prospective studies with appropriate controls (matched for age, fertility status and lifestyle) are needed to elucidate these trends further.

From the literature searched on male factors and RPL few studies were retrieved, therefore, the search was extended to include studies on single miscarriage.

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Part E: Prognosis and treatment

10. Assessing prognosis of a couple with RPL

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

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

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

10.1 Factors affecting prognosis

Evidence

Reproductive history

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

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

One retrospective cohort study of 587 women with unexplained RPL (≥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 recent cohort study suggested that only consecutive pregnancy losses after the birth influenced the subsequent prognosis, while the number of losses prior to the birth did not affect the prognosis in the next pregnancy (Egerup *et al.*, 2016).

In a multicenter 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

A number of studies have reported that sporadic or recurrent (≥2) pregnancy loss is more common among RPL patients' first-degree relatives than controls, approximately a doubled incidence or per pregnancy loss rate (Alexander *et al.*, 1988, Johnson *et al.*, 1988, Christiansen *et al.*, 1990, Ho *et al.*, 1991, Zhang *et al.*, 2010, Kolte *et al.*, 2011). While this may suggest a familial or hereditary component to RPL, none of the abovementioned studies investigated whether affected family members are important for the prognosis of an individual patient. Furthermore, it should be remembered that studies evaluating risk of pregnancy loss among patients' relatives may be subject to information bias, especially if information on relatives' pregnancy losses is derived from the patients. In families where one person suffers from RPL, there may be more openness about reproductive history than in other families.

10.2 Prognostic tools

Evidence

In a descriptive cohort study, prognosis was evaluated in 987 women with primary or secondary RPL referred to a tertiary center in Denmark (Lund *et al.*, 2012). Five years after the first consultation, 66.7% (95% CI 63.7-69.7) had achieved a live birth, increasing to 71.1% (95% CI 68.0-74.2) after 15 years. There was a significantly decreased chance of at least one subsequent live birth with increasing maternal age; of women aged 40 years or older, 41.7% (95% CI 29.8-56.1) achieved a live birth within 5 years compared to 81.3% (95% CI 69.2-90.7) of women aged 20–24 years. There was also a significant decrease in chance of a live birth by increasing number of miscarriages before first consultation ranging

from 71.9% (95% CI 67.5-76.1) in women with 3 miscarriages to 50.2% (95% CI 40.5-60.8) in women with 6 or more previous miscarriages. There was no evidence of an interaction between maternal age and the number of previous miscarriages.

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.

Recommendation

The GDG recommends to base prognosis on the number of preceding pregnancy losses and female age.	Strong	⊕⊕⊕○

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

Justification

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

The studies of Lund and Brigham show that RPL couples have a good prognosis for a next live birth, especially if female age and the number of previous miscarriages are low. Information from the studies *(summarized in the graphs and tables below)* can be used by clinicians to estimate the chance of a live birth in a next pregnancy in couples with RPL.

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

Additional information:

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

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

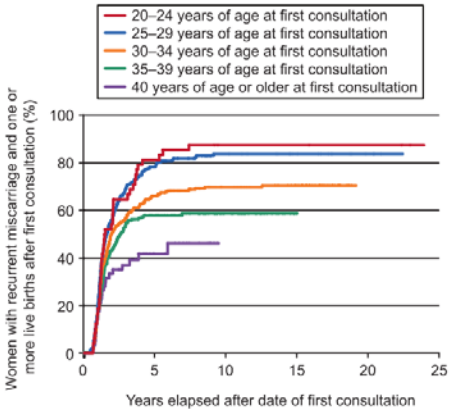

Figure 2: Kaplan-Meier plot showing percentage of women in the recurrent miscarriage cohort who have had at least one live birth after first consultation by number of miscarriages before first consultation (Lund *et al.*, 2012) (reproduced with permission).

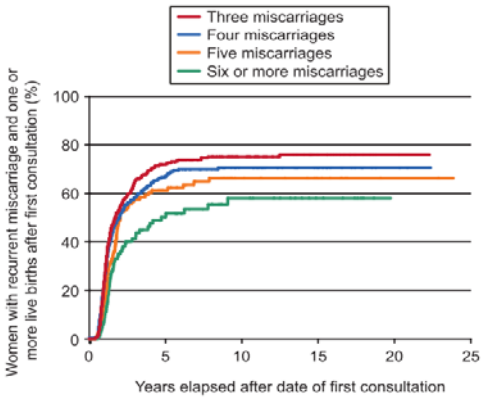

Table 1: Hazard Ratio (95% CI) of Achieving a Live Birth After Referral According to Age at First Consultation and Number of Previous Miscarriages (Lund *et al.*, 2012) (reproduced with permission).

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

NE, not estimable.

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

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

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

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11. Treatment for RPL with genetic background

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

Evidence *(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 PGS (PGT-A) technique used, as the vast majority have employed FISH with an embryo biopsy at Day 3, which only looks at a specific number of chromosomes at an early stage of embryo development where mosaicism is higher. Whole genome techniques such as array-CGH or Next Generation Sequencing (NGS) with a biopsy taken at blastocyst stage, looking at all chromosomes, are recognized to be more accurate screening techniques. To date only one study has explored the use of the array-CGH technique, but it only included 40 women with RPL and focused on the value of morphokinetic analysis (Basile *et al.*, 2014). A systematic reviews looking at PGS (PGT-A) for those couples with no known chromosomal abnormality concluded that there is no improvement in live birth rate with PGS (PGT-A), however FISH was used, the numbers were relatively small and the end points different (Musters *et al.*, 2011). Two recent studies of the same group compared PGS (PGT-A) and expectant management (EM).Clinical outcomes improved in RPL couples undergoing IVF and PGS (PGT-A) compared with couples who received expectant management. Among all attempts at PGS (PGT-A) or EM among couples with RPL, clinical outcomes (pregnancy rate, live birth rate, clinical miscarriage rate) were similar. Median time to pregnancy was 6.5 months in the PGS (PGT-A) group and 3.0 months in the EM group. However those couples whose intended PGS (PGT-A) was cancelled had a lower live birth rate and higher clinical miscarriage rate as opposed to those who underwent PGS (PGT-A) despite similar maternal age (Murugappan *et al.*, 2016). In addition, IVF/PGS (PGT-A) was not a cost-effective strategy for increasing live birth (Murugappan *et al.*, 2015).

11.2 Preimplantation genetic testing for RPL with genetic background

Preimplantation genetic testing for monogenic/single gene defects (PGT-M) or chromosomal structural rearrangements (PGT-SR), previously PGD, is an established alternative to invasive prenatal diagnosis and as such may avoid termination of pregnancy in couples with a high risk of transmitting genetic

disorders such as various monogenic diseases and for structural chromosome abnormalities, the latter being found in the RPL population.

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

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

In a cohort study, it was found that 76.9% (206/268) of couples with a translocation opted for PGD (PGT-SR) following genetic counselling (De Krom *et al.*, 2015).

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

Recommendations

All couples with results of an abnormal 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 or parental karyotypes should be offered genetic counselling to discuss likely prognosis and further diagnostic options. Couples may also receive information on the treatment options so they can make an informed decision on treatment. Clinicians are encouraged to elaborate on the advantages and disadvantages of PGT, depending on the techniques used (Brezina *et al.*, 2016). In addition, couples should be informed that PGT-SR could reduce the miscarriage rate, but will not improve live birth rate or time to pregnancy. Finally, PGT is not permitted in some countries.

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

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

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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.*, 2012).

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 recent 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 is currently recruiting which will provide much needed data on the topic (ALIFE2 trial/ trialreg nr NTR 3361).

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

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

Anticoagulants

A benefit of heparin (UFH or LMWH) and aspirin, as compared to aspirin alone, with regard to first trimester losses was reported in the review of Ziakas summarizing five RCTs of 398 women with RPL and APS (OR 0.39; 95% CI 0.24-0.65; NNT 4) (Ziakas *et al.*, 2010). Based on similar studies, Mak reported overall live birth rates of 74.27% (127/171) and 55.83% (91/163) in women who received the heparin (UFH or LMWH)/aspirin combination and aspirin alone, respectively (based on 5 RCTs; RR 1.301; 95% CI 1.040-1.62; NNT=5.6) (Mak *et al.*, 2010). The observed benefit of LMWH and aspirin as compared to aspirin alone did not reach statistical significance for RPL (OR 0.70; 95% CI 0.34-1.45; n=186; 2 RCTs), and was absent when the analysis was limited to studies that included late pregnancy losses (OR 2.28; 95% CI 0.43-12.13; n=150; 2 RCTs) (Ziakas *et al.*, 2010). It should be noted that there is significant risk of bias in the included studies.

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).

A Bayesian network analysis (i.e. indirect evidence from the theoretical comparisons of results of various small studies) showed no statistically significant effect of any treatment (aspirin, LMWH, LMWH

+ aspirin, UFH + aspirin) compared to placebo in 543 women with RPL and APS based on the results of six studies (Zhang *et al.*, 2015).

These studies overrule the Cochrane review on the topic, which has not been updated since 2005 (Empson *et al.*, 2005).

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, but it should be realized that the evidence for efficacy of LMWH in RPL is absent.

Steroids

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

Intravenous immunoglobulin

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

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

Recommendations

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

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

Justification

Although several reviews have been published, the overall quality of evidence for live birth rate and miscarriage rate is low to very low *(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). 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).

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

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

Additional information

In most of the included studies, UFH/LMWH combined with low-dose aspirin treatment was started as soon as pregnancy was confirmed (6 weeks' gestation), except for Kutteh and colleagues who started aspirin before conception, and added heparin treatment after 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 recommended.

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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. There 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 (Winger and Reed, 2008, Moraru *et al.*, 2012) but these trials are only of moderate/low quality, primarily because they were not placebo-controlled and thus not blinded. Two good placebo-controlled trials have tested prednisone in patients selected due to presence of auto- or alloantibodies (Laskin *et al.*, 1997) or endometrial NK cell abnormalities (Tang *et al.*, 2013). However, since the importance of these immune biomarkers is uncertain, we have chosen to include these trials in chapter 17 where they can be put into the best context

Conclusion

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

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14. Treatment of RPL with metabolic or endocrinologic abnormalities

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

14.1 Treatment for thyroid abnormalities associated with RPL

Evidence *(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 recently be reported to be associated with lower child IQ and lower grey matter and cortex volume (Korevaar *et al.*, 2016).

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

Thyroid auto-immunity

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

In a case-control study thyroid autoimmunity, prevalence of subclinical hypothyroidism and maternal and 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).

The published studies so far did not have an adequate sample size and overall, the studies were too small to draw robust conclusions. A potential treatment effect of levothyroxine needs further study by means of large RCTs.

Recommendations

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 gestation (7-9 weeks AD), and hypothyroidism should be treated with levothyroxine.	GPP	

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

There is insufficient evidence to support treatment with levothyroxine in euthyroid women with thyroid antibodies and RPL outside a clinical trial.	Conditional ⊕⊕◯◯

Justification

If 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 it should be further investigated. Moreover, recent 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.

There is no convincing evidence on the efficacy of levothyroxine treatment for increasing the chance of a live birth in women with a history of RPL and thyroid autoimmunity (normal TSH and TPOAb+). Results of ongoing trials should be awaited (TABLET trial, T4 life trial).

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 recently published PROMISE trial (Coomarasamy *et al.*, 2015) *(see chapter 17).*

The effect of vaginal progesterone treatment (100–200 mg every 12 hours starting 3 days after the LH surge) was recently evaluated in a cohort of women with RPL and abnormally elevated levels of nCyclinE (Stephenson *et al.*, 2017). Of 116 women with RPL, 59 (51%) had abnormally elevated levels (in the

luteal phase) of nCyclinE, a marker of endometrial development, on the endometrial biopsy. Vaginal progesterone administration resulted in a normalization of nCyclinE expression in 84% of 25 women undergoing a repeat endometrial biopsy. Pregnancy success in women with abnormal n-CyclinE who were treated with vaginal micronized progesterone starting in the early luteal phase was 69% (57/83) compared with 51% (19/37) in women with normal nCyclinE who were not treated with vaginal progesterone (OR = 2.1; 95% CI 1.0-4.4). The study was not randomized.

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

Recommendations

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

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

Justification

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

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

14.3 Metformin / insulin

Evidence

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

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

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

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

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

Recommendation

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

Justification

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

14.4 Ovulation induction

Evidence

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

Two other studies on ovulation induction as a treatment for RPL selected women with PCOS and RPL. In the study of Clifford and colleagues, 106 ovulatory women with a history of recurrent miscarriage, polycystic ovaries, and hypersecretion of luteinizing hormone were randomly assigned to pituitary suppression with a luteinizing hormone releasing hormone analogue followed by low dose ovulation induction and luteal phase progesterone, or were allowed to ovulate spontaneously and then given luteal phase progesterone alone or luteal phase placebo alone. There was no difference in conception

rate (80% vs 82%) or live birth rate (65% vs 76%) between the groups, nor was there a difference between the women given progesterone and those given placebo pessaries (Clifford *et al.*, 1996).

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

Conclusion

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

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

14.5 Bromocriptine for RPL associated with hyperprolactinemia

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

Evidence

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

Recommendation

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

Justification

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

14.6 Vitamin D

Evidence

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

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

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

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

Recommendation

Preconception counseling 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 (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, Valli *et al.*, 2004, Mollo *et al.*, 2011).

A Cochrane review on the topic found no RCTs evaluating hysteroscopic metroplasty with expectant management in women with RPL (Kowalik *et al.*, 2011, Rikken *et al.*, 2017). Another meta-analysis (not specific for RPL) reported a significantly decreased risk of pregnancy loss in women who underwent hysteroscopic septotomy as compared to women who did not undergo treatment (RR 0.37; 95% CI 0.25-0.55; I2 = 0%; five studies) (Venetis *et al.*, 2014).

One recent 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. Another prospective study reported a higher rate of pregnancies in women with RPL and septate uterus undergoing hysteroscopic resection (n=46) as compared to those that elected expectant management (n=32) (80.4% vs 56.3%; p<0.005). The miscarriage rate was 21.6% and 50.0%, respectively for surgery and EM (p<0.005) (Pang *et al.*, 2011).

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). A few studies have also reported on the live birth rate, and found an increase after surgery (Choe and Baggish, 1992, Valli *et al.*, 2004, Giacomucci *et al.*, 2011, Ghahiry *et al.*, 2014).

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)

Other uterine malformations

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

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

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

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

Recommendations

Whether hysteroscopic septum resection has beneficial effects (improving live birth rates, and decreasing miscarriage rates, without doing harm), should be evaluated in the context of surgical trials in women with RPL and septate uterus.	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).

For women with RPL and septate uterus, observational studies suggest a benefit of treatment in reducing the miscarriage rate. This was also the conclusion of a recent meta-analysis of uterine malformations (not specific for RPL): women who underwent hysteroscopic septum resection had a significantly decreased probability of spontaneous miscarriage compared with women who did not undergo treatment (RR 0.37; 95% CI 0.25 - 0.55; heterogeneity I^2 0%; 6 datasets) (Venetis *et al.*, 2014). However, the effect on fertility (i.e. the chance of becoming pregnant after surgery) is unclear. Therefore, the GDG decided to formulate a recommendation for more research on the topic.

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).

Additional information

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

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

15.2 Acquired Intrauterine Malformations

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

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

Endometrial polyps

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

Fibroids

There are no studies on the effect of treatment of fibroids on the miscarriage rate in women with RPL. In subfertile women with submucosal fibroids, myomectomy did not significantly improve live birth rate or miscarriage rate, as compared to controls with fibroids that did not have myomectomy (based on two observational studies) (Pritts *et al.*, 2009). Pregnancy rates, live birth rates and miscarriage rates after myomectomy were similar to those in infertile patients without fibroids, indicating a benefit for surgery (based on three studies). A more recent study reported a benefit of myomectomy with regard to miscarriage rate in women with infertility or RPL and submucosal fibroids (Roy *et al.*, 2010). The AAGL practice guidelines concluded that at least in selected patients, submucous myomectomy may reduce the risk of spontaneous abortion (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 (Kodaman and Arici, 2007, Jaslow, 2014). 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 recent review on cerclage (not specifically on pregnancy loss) concluded that the actual groups that benefit of cerclage are limited, but include women with three prior adverse events, and those with a short cervix (<25 mm) who have had a prior preterm birth (Story 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 recent trial, there was no difference in pregnancy or preterm delivery rates after single (n=14) or double cervical cerclage (n=19) in women with RPL assigned to cervical weakness, but the gestational duration was significantly longer after double cerclage (Zolghadri *et al.*, 2014).

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

Recommendations

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

In women with a singleton pregnancy and a history of recurrent second-trimester PL attributable to cervical weakness, a cerclage could be considered. There is no 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 table 8)*

Smoking cessation

Cigarette smoke contains over 4,000 chemicals, many of which are oxidative; impairing sperm quality and function (Li *et al.*, 2011) and inducing strand breaks in sperm DNA (Arabi, 2004, Hsu *et al.*, 2009). Associations between smoking and reduced male fertility, heritable genomic damage and incidence of childhood cancer and impaired mental health of offspring has been well documented (Aitken *et al.*, 2009). 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). In one study of 520 men, a positive correlation between body mass index and sperm DNA fragmentation was reported, with a 20% increase in sperm DNA damage in obese men (Chavarro *et al.*, 2010). Again, there is no evidence that paternal weight loss has an impact on LBR in RPL.

Medications

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

Varicocele repair

Varicocele has an incidence of 40% in men presenting with infertility (Nagler *et al.*, 1997) and it leads to impaired semen quality and increased sperm DNA damage in comparison to healthy donors (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 (Wang *et al.*, 2012). In a recent 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).

Sperm selection

Some studies have shown that there is less sperm DNA fragmentation in sperm separated by post density centrifugation as compared to semen (Donnelly *et al.*, 2000, Sakkas *et al.*, 2000). However, no

studies have been performed investigating whether sperm selection/preparation could improve LBR in couples with RPL. Therefore, this option should not be recommended to male partners.

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 Cochrane review of 34 studies has reported that men with poor semen quality showed improvement in sperm parameters following antioxidant therapy. In three studies reporting miscarriage, no significant difference was found in miscarriage rate between couples randomized to antioxidant therapy compared to placebo (OR 1.74; 95% CI 0.40-7.60). Live birth rate was higher in couples randomized to treatment (OR 4.21; 95% CI 2.08-8.51; 4 RCTs). However, these numbers are too small to be definitive and further research is needed (Showell *et al.*, 2014). We found no studies assessing antioxidant therapy in couples with RPL.

Recommendations

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

Sperm selection is not recommended as a treatment in couples with RPL.	GPP

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

Justification

No relevant papers on treatment for miscarriage, or recurrent pregnancy loss associated with a male factor problem have been found. In the absence of any data in RPL, the GDG recommends against sperm selection in a GPP.

Antioxidants for men are often used, but there is no evidence that antioxidants could be helpful in couples with RPL. In a Cochrane review, antioxidants did improve live birth rate after ART in subfertile

men, but it did not significantly decrease the chance of a pregnancy loss *(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 9)*

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

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

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

Recommendation

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

Justification

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

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 10)*

A recent 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 more recent 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.

Recommendation

Intravenous immunoglobulin (IvIg) is not recommended as a treatment of RPL.	Strong	⊕⊕◯◯

Justification

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

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 two randomized placebo-controlled trials in women with RPL positive for specific immunological biomarkers.

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

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

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

Recommendation

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

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 12)*

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 PROGESTERONE

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

A Cochrane review summarized progesterone for treatment of miscarriage in all women and in women with previous miscarriage (RPL) (Haas and Ramsey, 2013). The Cochrane analysis pooled the results from four small trials that had substantive methodologic limitations none of the trials specified the method of concealment of study-group assignments, and only two trials used a placebo for comparison. The miscarriage rate was lower in women with RPL receiving progesterone treatment, compared to placebo (OR 0.39; 95% CI 021-0.72).

A more recent 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 also showed a benefit of progesterone in reducing a subsequent risk of miscarriage compared with placebo (RR 2.4; 95% CI 1.3-5.9) (Kumar *et al.*, 2014). The main problem with this study is that treatment was initiated at a late stage of first trimester (mean gestational age 6.5 ± 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.

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

A recent 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).

Recommendation

Vaginal progesterone does not improve live birth rates in women with unexplained RPL	Conditional ⊕⊕⊕◯

Justification

The Cochrane meta-analysis combined 4 small studies with high risk of bias and the results are overruled by the recent well performed RCTs on oral and vaginal progesterone (Kumar *et al.*, 2014, Coomarasamy *et al.*, 2015). A recent meta-analysis, including the recent trials showed a benefit of progesterone on miscarriage rate and live birth rate (Saccone *et al.*, 2017). However, the meta-analysis is flawed by the quality of the older included studies, and hence, we decided to base the recommendation on the included and recent high quality trials. Vaginal progesterone during early pregnancy has no beneficial effect in women with unexplained RPL. 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 progesterone and administration of progesterone 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 anesthetics 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 (Roussev *et al.*, 2008, Meng *et al.*, 2015). 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 14)*

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.

A recent review found two studies on G-CSF as treatment for RPL. Both studies (Scarpellini and Sbracia, 2009, Santjohanser *et al.*, 2013) found a beneficial effect of G-CSF on the outcome of subsequent pregnancy (Cavalcante *et al.*, 2015). In the first included RCT, 68 women with a history of unexplained RPL who had previously been unsuccessfully treated with 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 no equally distributed in the three groups.

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

Although promising, the use of G-CSF as a treatment for RPL needs to be confirmed in more trials of good quality in different populations before it can be recommended for use in clinical practice. A new phase II study is underway.

Recommendation

There is insufficient evidence to recommended G-CSF in women with unexplained RPL.	Strong	⊕○○○

Justification

A single randomized controlled trial of good quality suggests a substantial benefit of G-CSF in RPL but it needs to be confirmed in other trials in different populations.

17.9 Endometrial scratching

Evidence

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

Recommendation

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

Justification

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

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18. Non-conventional treatments for RPL

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 15)*

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

Diet – antioxidants

A narrative review summarized the basic science and clinical case reports for antioxidants to improve pregnancy outcome by reducing oxidative stress in the placenta based on a literature search (Hovdenak 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. Recently, 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

Annex 2: Summary of findings tables

Annex 3: Recommendations for research

Annex 4: Abbreviations

Annex 5: Methodology

Annex 6: Stakeholder consultation

Annex 7: Literature study: flowcharts, list of excluded studies

Annex 8: Evidence tables

Annex 7 and 8 are available on the ESHRE website as seperate documents

Annex 1: Guideline development group

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

Chair of the GDG

Mariëtte Goddijn	Center for Reproductive Medicine, Academic Medical Center, Amsterdam (The Netherlands)

GDG members

Ole Bjarne Christiansen	Department of Obstetrics and Gynaecology, Aalborg University Hospital, Aalborg, (Denmark) Recurrent Pregnancy Loss Clinic, Rigshospitalet, Copenhagen University Hospital (Denmark)
Janine Elson	CARE Fertility Group , Nottingham (UK)
Astrid Marie Kolte	Copenhagen University Hospital Rigshospitalet, Copenhagen (Denmark)
Sheena Lewis	Queen's University Belfast (UK)
Saskia Middeldorp	Department of Vascular Medicine, Academic Medical Center, Amsterdam (The Netherlands)
Willianne Nelen	Department of Obstetrics and Gynaecology, Radboudumc, Nijmegen (The Netherlands)
Braulio Peramo	Al Ain Fertility Clinic, Al Ain (United Arab Emirates)
Siobhan Quenby	University of Warwick, Warwick (UK)

Patient representative

Ruth Bender Atik	Miscarriage Association (UK)

Invited experts

Peter Bisschop ***(Thyroid abnormalities)***	Academic Medical Center, Amsterdam (The Netherlands)

Methodological support

Nathalie Vermeulen	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	*None declared*
Ole Bjarne Christiansen	*Salary as specialty editor at European Journal of Obstetrics & Gynecology and Reproductive Biology*
Janine Elson	*Salary or position funding from CARE Fertility*
Astrid Marie Kolte	*None declared*
Sheena Lewis	*Salary or position funding from SpermComet Ltd*
Saskia Middeldorp	*Research grants from GSK - BMS/Pfizer – Sanquin -Aspen - Daiichi Sankyo. Consulting fees from GSK - Bayer - BMS/Pfizer - Boehringer Ingelheim – Daiichi Sankyo. Speaker's fees from GSK - Bayer - BMS/Pfizer - Boehringer Ingelheim - Aspen - Daiichi Sankyo.*
Willianne Nelen	*None declared*
Braulio Peramo	*None declared*
Siobhan Quenby	*Speaker's fees from Ferring*
Ruth Bender Atik	*None declared*
Nathalie Vermeulen	*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 – 15

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. poor 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 + heriditary 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				
Miscarriage rate (Heparin + aspirin versus aspirin only)* (Ziakas *et al.*, 2010)	357 per 1.000	**178 per 1.000** (117 to 265)	**OR 0.39** (0.24 to 0.65)	398 (5 RCTs)	⨁◯◯◯ VERY LOW [a,b,c]	
Live birth rate *(Heparin* + aspirin versus aspirin only)* (*Mak et al., 2010*)	558 per 1.000	**726 per 1.000** (581 to 909)	**RR 1.301** (1.040 to 1.629)	334 (5 RCTs)	⨁⨁◯◯ LOW [c,d]	
Miscarriage rate *(aspirin versus placebo/usual care)* (*Empson et al., 2005*)	235 per 1.000	**244 per 1.000** (169 to 341)	**OR 1.05** (0.66 to 1.68)	71 (3 RCTs)	⨁⨁◯◯ LOW [a,b]	

***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. selection and performance bias suspected
b. Optimal information size not met
c. studies compared to aspirin only (not placebo)
d. mean Jadad score 1.6 (range 0-3)

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 Ivlg compared to other treatment for RPL and antiphospholipid antibodies

Patient or population: RPL and antiphospholipid antibodies
Intervention: Ivlg *(± 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 [b,c,d]	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 [b,c]	Single observational study
Thyroid autoantibodies with normal thyroid function (not RPL)						
Miscarriage rate (*Vissenberg et al., 2012*)	241 per 1.000	**115 per 1.000** (60 to 221)	**RR 0.48** (0.25 to 0.92)	160 (2 RCTs)	⨁⨁◯◯ LOW [a,b]	

*__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. Not RPL
b. Optimal information size not met
c. Single study
d. About 70% of the women were treated for concomitant factors associated with REPL

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 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) *(Showell et al., 2014)*	50 per 1.000	**181 per 1.000** (99 to 309)	**OR 4.21** (2.08 to 8.51)	277 (4 RCTs)	⨁◯◯◯ VERY LOW a,b,c	
Miscarriage rate (subfertile men) *(Showell et al., 2014)*	19 per 1.000	**33 per 1.000** (8 to 129)	**OR 1.74** (0.40 to 7.60)	247 (3 RCTs)	⨁◯◯◯ VERY 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. performance and selection bias suspected
b. analysis in subfertile men, not RPL
c. optimal information size not met

9 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

10 Immunotherapy Ivlg compared to usual treatment/placebo for unexplained RPL

Patient or population: unexplained RPL
Intervention: Immunotherapy Ivlg
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]	

***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'

11 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 209.0)	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%

12 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

13 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 and Ramsey, 2013)	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 previous miscarriage only)*
Miscarriage rate *(Coomarasamy et al., 2015)*	334 per 1.000	**321 per 1.000** (264 to 391)	**RR 0.96** (0.79 to 1.17)	826 (1 RCT)	⨁⨁◯◯ LOW [b,c]	*Single RCT*
Live birth rate *(Coomarasamy et al., 2015)*	633 per 1.000	**659 per 1.000** (595 to 728)	**RR 1.04** (0.94 to 1.15)	826 (1 RCT)	⨁⨁⨁◯ MODERATE [b,c]	*Single RCT*
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 poor quality (as assessed by reviewers)
b. Optimal information size not met
c. Single RCT
d. differences in direction of effect

14 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 *(Scarpellini and Sbracia, 2009)*	485 per 1.000	**828 per 1.000** (585 to 945)	**OR 5.1** (1.5 to 18.4)	68 (1 RCT)	⨁◯◯◯ VERY LOW [a,b]	*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. women with unexplained primary RM, all with at least 4 consecutive miscarriages and negative for all clinical investigations
b. No explanation was provided

15 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

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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

- Study the (emotional) impact of RPL on men.
- Develop a prognostic model to provide an individually based live birth prognosis.
- Develop E-health tools for support to couples with RPL and staff.

Genetics

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

Thrombophilia

- Study the effect of anticoagulant treatment for RPL women with hereditary thrombophilia
- With regard to RPL and APS:
    - Study clinical criteria for diagnosis and treatment of APS (e.g. female age, number of pregnancy losses, consecutive or non-consecutive losses).
    - Assess the effectiveness of heparin treatment from comparison with placebo/no treatment
    - Compare the efficacy and safety of LMWH versus UFH.
    - How should heparin be 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 effect of moderate dosages of prednisolone in RPL (preferably in large controlled trials).
- Study the effect of IvIg treatment in women with secondary RPL.
- Study the effect of immunotherapy in subsets of women with RPL with specific HLA class II alleles (in RCTs)

Metabolic factors

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

Uterine malformations

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

Male factor

- In general, there is very little evidence on the role of the man in RPL couples.
- Study the impact of unhealthy lifestyle (such as obesity, poor diet and smoking) on RPL through sperm DNA damage (preferably in prospective studies with appropriate controls, matched for age, fertility status and lifestyle).
- Study the mechanisms of sperm DNA damage.
- Study the effect of male lifestyle alterations with outcomes of both sperm DNA per se and RPL (in randomized controlled trials).
- Study the effect of antioxidant therapy for men on RPL; specifically to determine the best combinations and extent of dietary vitamin supplementation in the protection of sperm DNA from fragmentation.

Female factor

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

Annex 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
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
Ivig	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
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
SHBG	sex hormone-binding globulin
SHG	Sonohysterography (or hysterosonography)
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, A. D'Angelo, P. de Sutter, W.L.D.M. Nelen, Manual for ESHRE guideline development, version 2013), which can be consulted at the ESHRE website (www.eshre.eu/guidelines). 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 current guideline was developed and funded by ESHRE, which covered expenses associated with the guideline meetings (travel, hotel and catering expenses) associated with the literature searches (library costs, costs associated with the retrieval of papers) and with the implementation of the guideline (printing, publication costs). Except for reimbursement of their travel expenses, GDG members did not receive any payment for their participation in the guideline development process.

The scope of the guideline and first version of the key questions were drafted by the coordinator and deputies of the ESHRE Special Interest Group Implantation and Early Pregnancy. A call was launched for experts in the field interested in joining the guideline development group. All applications were reviewed and experts were selected based on expertise and geographical location. We strived towards a balance in gender and location within Europe. A meeting of the guideline development group was organized to discuss the key questions and redefine them through the PICO process (patients – interventions – comparison – outcome). This resulted in a final list of 18 key questions. Based on the defined key words, literature searches were performed by the methodological expert (Dr. N. Vermeulen). 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.

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

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

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

FORMULATION OF RECOMMENDATIONS

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

Implications for	Strong recommendation	Conditional recommendation
Patients	Most individuals in this situation would want the recommended course of action, and only a small proportion would not	The majority of individuals in this situation would want the suggested course of action, but many would not
Clinicians	Most individuals should receive the intervention Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences	Recognize that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences Decision aids may be useful in helping individuals to make decisions consistent with their values and preferences
Policy makers	The recommendation can be adopted as policy in most situations	Policy making will require substantial debate and involvement of various stakeholders

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 30 June and 15 August 2017.

To notify interested clinicians, we sent out an invitation to review the guideline by email to all members of the ESHRE SIG of Implantation and Early Pregnancy. Selected reviewers were invited personally by email. These reviewers included:

- *Coordinators and deputies of the ESHRE SIG Implantation and Early Pregnancy and the ESHRE SIG Quality and Safety in ART.*
- *Contact persons of patient organizations across Europe.*
- *Contact persons of international and national societies focused on miscarriage across Europe.*

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 an announcement in "Focus on Reproduction", a newsflash on the ESHRE website homepage and a news item in the next digital ESHRE newsletter. All participants in the annual ESHRE meeting will be informed about the development and release of new guidelines; all related national societies and patient organizations are informed about the guideline release. They are asked to encourage local implementation by, for instance, translations or condensed versions, but they are also offered a website link to the original document.

Patient versions of the guideline will be developed by a subgroup of the GDG together with patient representatives. 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.

To further enhance implementation of the guideline, the members of the GDG, as experts in the field, will be asked to make suggestions for tailor-made implementation interventions (e.g. option grids, flow-charts, additional recommendations, addition of graphic/visual material to the guideline).

Schedule for updating the guideline

The current guideline will be considered for revision in 2021 (four years after publication). 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: Stakeholder consultation

As mentioned in the methodology, the guideline draft was open for review for 6 weeks, between 30 June and 15 August 2017. All reviewers, 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 organization, and of individual experts that provided comments to the guideline are summarized below.

Representative	Organization
ESHRE/ESGE CONUTA Group Grigoris Grimbizis	ESHRE/ESGE CONUTA Group
RCOG Guidelines Committee Bethany King	Royal College of Obstetricians and Gynaecologists
Giovanni Scambia SIGO President Elsa Viora AOGOI President Nicola Colacurci AGUI President	SIGO - AOGOI – AGUI (Italy)

Reviewer	Country
Stephan Gordts	Belgium
T C Li	China
Fang Ma	China
Henriette Svarre Nielsen	Denmark
Aboubakr Elnashar	Egypt
Thomas Strowitzki	Germany
Christiane Kling	Germany
Pratip Chakraborty	India
Mayumi Sugiura-Ogasawara	Japan
Raminta Bausyte	Lithuania
Michal Kunicki	Poland
Kersti Lundin	Sweden
Recurrent Pregnancy Loss group LUMC Harjo Verburg, Sandra Dieben, Lisa Lashley, Marie-Louise van der Hoorn	The Netherlands
H.D.L. Ockhuijsen	The Netherlands
Ahmet Berkiz Turp	Turkey
Alessandra Pipan	UAE
Hena Zaheer	UAE
Mahmoud Moussa	UK
Shehnaaz Jivraj	UK
Arianna D'Angelo	UK
Ying Cheong	UK
Joe Leigh Simpson	USA
Channing Burks, Mary D. Stephenson, Theresa S. Falcon-Cullinan	USA