Human Reproduction Update, Vol.26, No.3, pp. 356–367, 2020

Advance Access Publication on February 26, 2020 doi:10.1093/humupd/dmz048

human reproduction update

Recurrent pregnancy loss: diagnostic workup after two or three pregnancy losses? A systematic review of the literature and meta-analysis

Myrthe M. van Dijk [1,*], Astrid M. Kolte [2], Jacqueline Limpens [3], Emma Kirk [4], Siobhan Quenby [5], Madelon van Wely [1], and Mariëtte Goddijn [1]

[1]Centre for Reproductive Medicine, Department of Obstetrics and Gynaecology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands [2]Recurrent Pregnancy Loss Unit, Fertility Clinic 4071, Rigshospitalet, University Hospital Copenhagen, Copenhagen, Denmark [3]Medical Library, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands [4]Department of Obstetrics and Gynaecology, Royal Free Hospital NHS Trust, London, UK [5]University Hospital Coventry, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, UK

*Correspondence address: Centre for Reproductive Medicine, Department of Obstetrics & Gynaecology, Amsterdam UMC, University of Amsterdam, PO Box 22660, 1100 DD Amsterdam, The Netherlands. E-mail: m.m.vandijk@amsterdamumc.nl

http://orcid.org/0000-0002-9148-587X

Submitted on June 16, 2019; resubmitted on October 9, 2019; editorial decision on October 20, 2019

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BACKGROUND: Recurrent pregnancy loss (RPL) occurs in 1–3% of all couples trying to conceive. No consensus exists regarding when to perform testing for risk factors in couples with RPL. Some guidelines recommend testing if a patient has had two pregnancy losses whereas others advise to test after three losses.

OBJECTIVE AND RATIONALE: The aim of this systematic review was to evaluate the current evidence on the prevalence of abnormal test results for RPL amongst patients with two versus three or more pregnancy losses. We also aimed to contribute to the debate regarding whether the investigations for RPL should take place after two or three or more pregnancy losses.

SEARCH METHODS: Relevant studies were identified by a systematic search in OVID Medline and EMBASE from inception to March 2019. A search for RPL was combined with a broad search for terms indicative of number of pregnancy losses, screening/testing for pregnancy loss or the prevalence of known risk factors. Meta-analyses were performed in case of adequate clinical and statistical homogeneity. The quality of the studies was assessed using the Newcastle-Ottawa scale.

OUTCOMES: From a total of 1985 identified publications, 21 were included in this systematic review and 19 were suitable for meta-analyses. For uterine abnormalities (seven studies, odds ratio (OR) 1.00, 95% CI 0.79–1.27, $I^2 = 0\%$) and for antiphospholipid syndrome (three studies, OR 1.04, 95% CI 0.86–1.25, $I^2 = 0\%$) we found low quality evidence for a lack of a difference in prevalence of abnormal test results between couples with two versus three or more pregnancy losses. We found insufficient evidence of a difference in prevalence of abnormal test results between couples with two versus three or more pregnancy losses for chromosomal abnormalities (10 studies, OR 0.78, 95% CI 0.55–1.10), inherited thrombophilia (five studies) and thyroid disorders (two studies, OR 0.52, 95% CI: 0.06–4.56).

WIDER IMPLICATIONS: A difference in prevalence in uterine abnormalities and antiphospholipid syndrome is unlikely in women with two versus three pregnancy losses. We cannot exclude a difference in prevalence of chromosomal abnormalities, inherited thrombophilia and thyroid disorders following testing after two versus three pregnancy losses. The results of this systematic review may support investigations after two pregnancy losses in couples with RPL, but it should be stressed that additional studies of the prognostic value of test results used in the RPL population are urgently needed. An evidenced-based treatment is not currently available in the majority of cases when abnormal test results are present.

Key words: recurrent pregnancy loss / investigations / screening tests / diagnostic strategy

Introduction

Recurrent pregnancy loss (RPL), defined as two pregnancy losses prior to 20 weeks from the last menstrual period, occurs in 1–3% of all couples trying to conceive (ESHRE, 2017). Based on available data, there is consensus that women should not undergo extensive evaluation after a single first trimester or early second trimester pregnancy loss, given that these are relatively common and sporadic events with only a modestly increased risk of recurrence (Knudsen *et al*., 1991; Nybo Andersen *et al*., 2000; Cohain *et al*., 2017). In prospective studies, the risk of pregnancy loss increases with each loss from approximately 11% amongst nulligravidae to approximately 40% after three or more losses (Magnus *et al*., 2019).

Known risk factors for RPL are female age, previous pregnancy losses, parental structural chromosomal abnormalities, uterine anomalies, endocrine disturbances, antiphospholipid syndrome (APS) and inherited thrombophilia (Jauniaux *et al*., 2006). Even after comprehensive investigations, a cause for RPL is identified in fewer than 50% of couples (Alijotas-Reig and Garrido-Gimenez, 2013). Consequently, the majority of cases remain without a modifiable risk factor (Jaslow *et al*., 2010). Only female age and number of prior pregnancy losses have been consistently found to be prognostic factors for the majority of patients (ESHRE, 2017). The tests currently performed are often expensive, time-consuming and of uncertain prognostic value (Christiansen *et al*., 2005). Furthermore, there is no consensus about how many pregnancy losses couples should have experienced before evaluation is warranted, leading to a variety of RPL definitions.

The Royal College of Obstetricians and Gynaecologists defines RPL as three or more consecutive pregnancy losses (RCOG, 2011). The American Society for Reproductive Medicine Practice Committee defines RPL as two or more pregnancy losses confirmed by ultrasound or histology, not necessarily consecutive (ASRM Practice Committee, 2012). The most recent RPL guideline from ESHRE set the definition after a significant debate. It states that RPL could be considered after the loss of two or more pregnancies and stresses the importance of the need for further scientific research, including epidemiological studies on the effect of various RPL definitions on diagnosis, prognosis and treatment (ESHRE, 2017).

Although an evidence-based treatment is lacking for RPL, couples value a plan for the next pregnancy (Musters *et al*., 2013). Before trying to conceive, couples and clinicians attempt to find an explanation for their pregnancy losses and a treatment that will prevent a recurrence, especially in cases with modifiable risk factors, such as thyroid disorders and APS. This is why most guidelines advise investigations in RPL. However, there is no consensus on when to perform investigations for risk factors in couples with RPL.

There is a clear need for an evidence-based recommendation for when to initiate investigations in RPL. As such, the goal of this study was two-fold: first, to determine whether abnormal test results for factors that are definite or probable risk factors for RPL occur with equal frequency in women with two pregnancy losses versus those who have had three or more pregnancy losses; second, to recommend if investigations for RPL should take place after two or three or more pregnancy losses.

Methods

Search strategy

This review followed the PRISMA guidelines for reporting systematic reviews and meta-analyses (Fig. 1). A medical information specialist (JL) performed a systematic search in OVID MEDLINE and OVID EMBASE from inception to March 11th 2019, using both free text and controlled terms (i.e. MeSH-terms in MEDLINE). A search for RPL was combined with search filters for primary or secondary studies and a broad search for terms indicative of screening, obstetric history, two versus three or more pregnancy losses and the relevance/prevalence of known risk factors (Supplementary Table SI). We cross-checked reference lists and citing articles of identified relevant papers (in Web of Science) and adapted the search in case of additional relevant studies. The bibliographic records retrieved were

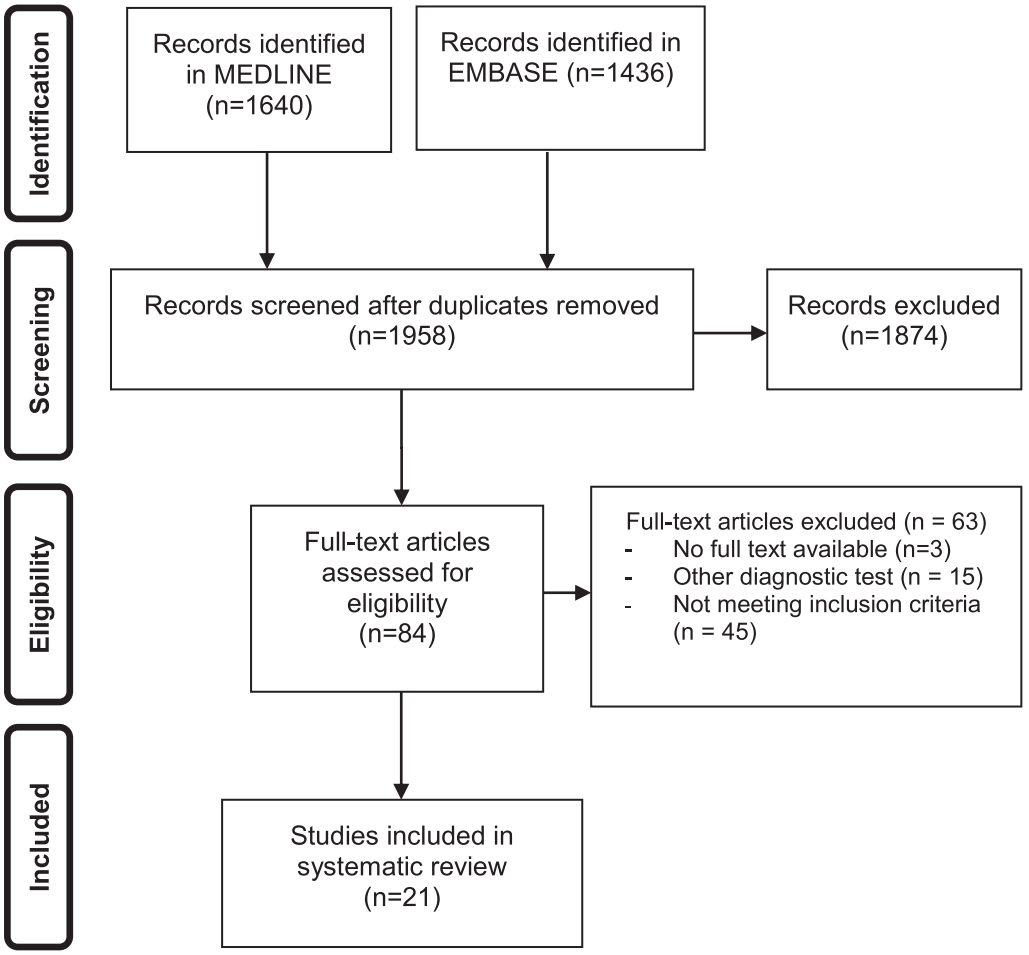

Figure 1 Study selection process for systematic review on the prevalence of abnormal evidence-based test result in women with recurrent pregnancy loss.

imported and de-duplicated in ENDNOTE X7 © (Clarivate Analytics, Boston, MA, USA). Authors were contacted for additional details when required.

Selection criteria

Studies were selected if the prevalence of the abnormal test results for RPL was reported. Only studies which compared women with two pregnancy losses to women with three or more losses were included. Based on current reviews of the literature, the following evidence-based risk-factors for RPL were considered in this review: parental structural chromosomal abnormalities, uterine anomalies, APS, inherited thrombophilia and thyroid disorders. Results of parental chromosomal analysis were considered abnormal if significant rearrangements (e.g. balanced translocations and mosaics) were present. Studies were selected when chromosome analyses were performed with parental peripheral blood lymphocyte cultures. Studies for uterine anomalies were selected if diagnostic testing was performed by hysterosalpingography, hysteroscopy or sonohysterography. Congenital abnormalities (e.g. arcuate uterus, septate uterus, bicornuate uterus and unicornuate uterus) were considered as uterine anomalies.

APS was defined as the presence of thrombosis, pregnancy loss or female morbidity and persistent circulating antiphospholipid antibodies (aPL). aPLs (lupus anticoagulant, IgM anticardiolipin antibodies, IgG anticardiolipin antibodies and beta-2 glycoprotein I antibodies) were considered to be present if a test was positive on two occasions >12 weeks apart (Miyakis *et al.*, 2006).

Inherited thrombophilia was defined in four different sub-categories: Factor V Leiden mutation, prothrombin gene mutation, protein S deficiency and protein C deficiency. Factor V Leiden mutation was considered abnormal if there was a heterozygous or homozygous factor V Leiden G1691A mutation found. Prothrombin gene mutation was defined as heterozygous or homozygous mutations for the G20210A prothrombin (factor II) gene. Functional protein C activity less than 70% and functional protein S activity less than 70% were considered abnormal.

Thyroid disorders were defined as serum levels of thyroid-stimulating hormone (TSH) <0.45 mU/L or TSH >4.5 mU/L with an abnormal free thyroxine level with or without the presence of thyroid peroxidase antibodies.

Studies were excluded when the population examined or the diagnostic methods used were not accurately defined. Only publications in English were considered in our selection.

Study selection

Studies were selected in a two-stage process using Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia). First, the titles and abstracts from the electronic searches were examined independently by two reviewers (M.D. and A.M.K.), and full manuscripts of all citations that met the predefined selection criteria were obtained. Secondly, examinations of the full manuscripts were carried out to decide on inclusion or exclusion (M.D. and M.W.). In cases of duplicates, the most recent or the most complete publication was used. Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer (M.G.).

All selected papers were assessed for the following: study design, adequate sampling, adequate description of population characteristics, completeness of information in the data sets, and use of a validated diagnostic method.

Data collection and extraction

Data collection was performed by two reviewers (M.D. and M.W.) independently. Data were extracted based on patients' characteristics, study quality, inclusion and exclusion criteria, diagnostic tools used and abnormal diagnostic test occurrence rates. Articles were judged on scientific quality according to the Strengthening the Reporting of Observational Studies in Epidemiology statement (White *et al*., 2015). Levels of evidence were attributed according to the Oxford Centre for evidence-based medicine (Oxford Centre for Evidence-based Medicine, 2009). The quality of each study was assessed with the Newcastle-Ottawa Scale.

Statistical analysis

In order to reach a consistent presentation of the data, all individual study results were translated into an odds ratio (OR) and 95% CI. In case of adequate clinical and statistical homogeneity with the same outcome measure, we performed meta-analyses using a random effect model. Heterogeneity was assessed using the I^2 statistic. We took an I^2 measurement greater than 50% to indicate substantial heterogeneity. To evaluate the possible presence of publication bias, a funnel plot was made for outcomes with data of at least 10 studies (Cochrane handbook). Review Manager 5 (RevMan version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used to perform the meta-analyses.

Results

Of the 1958 publications identified, 21 publications met the inclusion criteria, entailing 8301 couples with RPL. Reference checking of the cited and citing articles of the included articles yielded no additional relevant articles (Fig. 1 shows the PRISMA flowchart of the selection process). Of the 21 articles included in this systematic review, 10 studies reported on chromosomal abnormalities (Michels *et al*., 1982; Diedrich *et al*., 1983; FitzSimmons *et al*., 1983; Schwartz and Palmer, 1983; Sachs *et al*., 1985; Sider *et al*., 1988; Goddijn *et al*., 2004; Jaslow *et al*., 2010; Bashiri *et al*., 2012; Asgari *et al*., 2013), 7 studies reported on testing for uterine anomalies (Weiss *et al*., 2005; Bohlmann *et al*., 2010; Jaslow *et al*., 2010; Souza *et al*., 2011; Bashiri *et al*., 2012; Seckin *et al*., 2012; Jaslow and Kutteh, 2013), 4 studies reported on testing for antiphospholipid syndrome (Jaslow *et al*., 2010; Bashiri *et al*., 2012; van den Boogaard *et al*., 2013; Guzel *et al*., 2015), 7 studies reported on testing for inherited thrombophilia (Sotiriadis *et al*., 2007; Jaslow *et al*., 2010; Bashiri *et al*., 2012; Karadeniz *et al*., 2012; Baumann *et al*., 2013; Ali *et al*., 2014; Guzel *et al*., 2015) and 2 studies reported on testing for thyroid disorders (Jaslow *et al*., 2010; Bashiri *et al*., 2012).

Quality of the studies

The characteristics of the included articles and quality assessment are reported in Table I and Supplementary Table SII. The studies were evidence-level IIb studies, i.e. cohort studies. Nineteen studies presented appropriate data and could be included in meta-analyses.

Chromosomal abnormalities

A total of 10 studies ($n = 2498$) reported on the difference in prevalence of parental structural chromosomal abnormalities in women with two versus three or more pregnancy losses (Table I). When pooling the studies, we found insufficient evidence for a difference in the frequency of abnormal test results for parental structural chromosomal abnormalities between women with two pregnancy losses and three or more pregnancy losses (10 studies, OR 0.78, 95% CI: 0.55–1.10) (Fig. 2).

When summarizing the individual proportions in the studies using meta-analysis, we found a chromosomal abnormality prevalence of 5.3% (95% CI 2.8–7.8) after two pregnancy losses and 6.6% (95% CI 3.8–9.3) after three pregnancy losses. These results indicate that differences in prevalence of chromosomal abnormalities after two or three pregnancy losses might be small, but that larger differences cannot be fully excluded.

The funnel plot did not show an indication of publication bias (Supplementary Fig. SI).

Uterine anomalies

Seven studies described the prevalence of uterine anomalies in women with two pregnancy losses compared to three or more pregnancy losses. Seven cohort studies ($n = 2343$) were eligible for meta-analysis and no significant difference in frequency of abnormal test results for uterine anomalies could be detected between women with two pregnancy losses and three or more pregnancy losses (seven studies, OR 1.00, 95% CI 0.79–1.27) (Fig. 3). When summarizing the individual proportions in the studies using meta-analysis, we found a prevalence of 18% (95% CI 11–25) after two pregnancies and 17% (95% CI 11–23) after three pregnancies. These results suggest that a clinically relevant difference in prevalence is unlikely.

Antiphospholipid syndrome

Four included studies described the prevalence of APS in women with two pregnancy losses compared to three or more pregnancy losses. In a retrospective cohort study of 252 women with RPL, the levels of anticardiolipin antibodies IgG and IgM were compared between women with two versus three or more pregnancy losses. The test

Table I Characteristics of the studies for chromosomal abnormalities, uterine anomalies, antiphospholipid syndrome, thrombophilia and thyroid disorders identified in a systematic review of RPL

Author	Year	Study type	Study population	Prevalence 2 pregnancy losses	Prevalence ≥ 3 pregnancy losses	Outcome measures
Chromosomal abnormalities						
Michels *et al.*	1982	Cohort	122 couples 2 PL *n* = 48 ≥ 3 PL *n* = 74	Balanced translocations 8.4% (4/48)	Balanced translocations 5.4% (4/74)	Cytogenetic analysis from peripheral blood lymphocyte cultures showed no significant difference between 2 versus 3 or more pregnancy losses.
Diedrich *et al.*	1983	Cohort	136 couples 2 PL *n* = 59 ≥ 3 PL *n* = 77	Abnormal karyotype 10.2% (6/59)	Abnormal karyotype 11.9% (9/77)	Chromosomal analysis from peripheral blood lymphocyte cultures showed no significant difference between 2 versus 3 or more pregnancy losses.
FitzSimmons *et al.*	1983	Cohort	645 couples 2 PL *n* = 340 ≥ 3PL *n* = 305	Abnormal karyotype 1.8% (6/340)	Abnormal karyotype 2.3% (7/305)	Chromosomal analysis from peripheral blood lymphocyte cultures showed no significant difference between 2 versus 3 or more pregnancy losses.
Schwartz *et al.*	1983	Cohort	164 couples 2 PL *n* = 71 ≥ 3 PL *n* = 93	Abnormal karyotype 5.6% (4/71)	Abnormal karyotype 5.4% (5/93)	Chromosomal analysis from peripheral blood lymphocyte cultures showed no significant difference between 2 versus 3 or more pregnancy losses.
Sachs *et al.*	1985	Cohort	371 couples 2 PL *n* = 182 ≥ 3PL *n* = 189	Abnormal karyotype 9.3% (17/182)	Abnormal karyotype 9.5% (18/189)	Chromosomal analysis from peripheral blood lymphocyte cultures showed no significant difference between 2 versus 3 or more pregnancy losses.
Sider *et al.*	1988	Cohort	187 couples 2 PL *n* = 99 ≥ 3PL *n* = 88	Abnormal karyotype 3.0% (3/99)	Abnormal karyotype 6.8% (6/88)	Chromosomal analysis from peripheral blood lymphocyte cultures showed no significant difference between 2 versus 3 or more pregnancy losses.
Goddijn *et al.*	2004	Cohort	95 couples 2 PL *n* = 55 ≥ 3PL *n* = 40	Abnormal karyotype 32.7% (18/55)	Abnormal karyotype 37.5% (15/40)	Chromosomal analysis from peripheral blood lymphocyte cultures showed no significant difference between 2 versus 3 or more pregnancy losses.
Jaslow *et al.*	2010	Cohort	561 women 2 PL *n* = 281 ≥ 3PL *n* = 280	Abnormal karyotype 2.8% (8/281)	Abnormal karyotype 5.4% (15/280)	Parental karyotypes showed no significant difference between 2 versus 3 or more pregnancy losses.
Bashiri *et al.*	2012	Cohort	114 couples 2 PL *n* = 34 ≥ 3 PL *n* = 80	Abnormal karyotype (0/34)	Abnormal karyotype 4.0% (4/80)	Parental genetics (significant rearrangements (balanced translocations) showed no significant difference between 2 versus 3 or more pregnancy losses.
Asgari *et al.*	2013	Cohort	140 couples 2 PL *n* = 65 ≥ 3PL *n* = 75	Abnormal karyotype 3.1% (2/65)	Abnormal karyotype 5.3% (4/75)	Chromosomal analysis from peripheral blood lymphocyte cultures showed no significant difference between 2 versus 3 or more pregnancy losses.
Uterine anomalies						
Weiss *et al.*	2005	Cohort	165 women 2 PL *n* = 67 ≥ 3 PL *n* = 98	22.4% (15/67)	17.3% (17/98)	Identified by hysteroscopy. Considered abnormal were congenital anomalies. No difference in prevalence was found between 2 versus 3 or more pregnancy losses.
Bohlmann *et al.*	2010	Cohort	206 women 2 PL *n* = 78 ≥ 3 PL *n* = 119	9.2% (8/78)	16.8% (20/119)	Identified by hysteroscopy. Considered abnormal were congenital abnormalities. No difference in prevalence was found between 2 versus 3 or more pregnancy losses.

Continued.

Table I Continued.

Author	Year	Study type	Study population	Prevalence 2 pregnancy losses	Prevalence ≥ 3 pregnancy losses	Outcome measures
Jaslow *et al.*	2010	Cohort	875 women 2 PL *n* = 401 ≥ 3PL *n* = 303	18.7% (75/401)	18.2% (55/303)	Identified by hysterosalpingogram, hysteroscopy, sonohysterography. Considered abnormal were congenital anomalies, fibroids, polyps and septa, Asherman's syndrome adhesions. No difference in prevalence was found between 2 versus 3 or more pregnancy losses.
De Souza *et al.*	2011	Cohort	66 women 2 PL *n* = 23 ≥ 3 PL *n* = 43	17.3% (4/23)	11.6% (5/43)	Identified by hysteroscopy. Considered abnormal was congenital anomalies. No difference in prevalence was found between 2 versus 3 or more pregnancy losses.
Seckin *et al.*	2012	Cohort	220 women 2 PL *n* = 151 ≥ 3 PL *n* = 69	26.5% (40/151)	30.4% (21/69)	Diagnostic hysteroscopy. Considered abnormal was congenital anomaly. No difference in prevalence was found between 2 versus 3 or more pregnancy losses.
Bashiri *et al.*	2012	Cohort	114 women 2 PL *n* = 38 ≥ 3 PL *n* = 78	31.6% (12/38)	23.1% (18/78)	Hysteroscopy or 3D ultrasound. Considered abnormal were septate uterus, unicornuate, bicornuate, fibroids, polyps and Asherman's syndrome. No difference in prevalence was found between 2 versus 3 or more pregnancy losses.
Jaslow *et al.*	2013	Cohort	875 women 2 PL *n* = 389 ≥ 3 PL *n* = 486	6.7% (26/389)	7.2% (35/486)	Three dimensional sonohysterography. Considered abnormal were congenital and acquired abnormalities. No difference in prevalence was found between 2 versus 3 or more pregnancy losses.
Antiphospholipid syndrome						
Jaslow *et al.*	2010	Cohort	729 women 2 PL *n* = 409 ≥ 3 PL *n* = 320	15.6% (64/409)	13.1% (42/320)	Lupus anticoagulant levels, Anticardiolipin IgG and IgM were measured. No difference was found between 2 versus 3 or more pregnancy losses.
Bashiri *et al.*	2012	Cohort	120 women 2 PL *n* = 39 ≥ 3 PL *n* = 81	10.3% (4/39)	13.6 (11/81)	Lupus anticoagulant. No difference in prevalence was found between 2 versus 3 or more pregnancy losses.
Van den Boogaard *et al.*	2013	Cohort	2444 women 2 PL *n* = 1526 ≥ 3 PL *n* = 918	17.4% (265/1526)	17.3% (159/918)	Lupus anticoagulant levels, Anticardiolipin IgG and IgM were measured. No difference was found between 2 versus 3 or more pregnancy losses.
Thrombophilia						
Sotiriadis *et al.*	2007	Cohort	99 women 2 PL *n* = 56 ≥ 3 PL *n* = 43	2 PL = 56	3 PL = 43	There was no difference in the distribution of Factor V Leiden, FII G20210A and MTHFR between patients with 2 and 3 or more PLs.
Jaslow *et al.*	2010	Cohort	243 women	Factor V Leiden 4.2% (6/144) Prothrombin gene mutation 2.6% (3/115) Protein S 3.5% (4/115) Protein S 0.9% (1/115)	Factor V Leiden 8.1% (8/99) Prothrombin gene mutation (0/85) Protein S 2.4% (2/85) Protein C (0/85)	Factor V Leiden mutation, prothrombin gene mutation, protein C activity, protein S activity. No difference was found between 2 versus 3 or more pregnancy losses.
Bashiri *et al.*	2012	Cohort	120 women	Factor V Leiden 4.8% (1/21) Prothrombin gene mutation 13.6% (3/22) Protein S 3.8% (1/26) Protein C 7.7% (2/26)	Factor V Leiden 17.0% (8/47) Prothrombin gene mutation 4.5% (2/44) Protein S 13.6% (8/59) Protein C 8.2% (5/61)	Factor V Leiden mutation, prothrombin gene mutation, Protein S activity, Protein C activity. No difference was found between 2 versus 3 or more pregnancy losses.

Continued.

Table I Continued.

Author	Year	Study type	Study population	Prevalence 2 pregnancy losses	Prevalence ≥ 3 pregnancy losses	Outcome measures
Karadeniz et al.	2012	Cohort	108 women 2 PL $n = 42$ ≥ 3 PL $n = 66$	Factor V Leiden 9.5% (4/42) Prothrombin gene mutation (0/42) Protein S 16.6% (7/42) Protein C 16.6% (7/42)	Factor V Leiden 7.5% (5/66) Prothrombin gene mutation 1.5% (1/66) Protein S 12.2% (8/66) Protein C 18.2% (12/66)	Factor V Leiden mutation, prothrombin gene mutation, Protein S activity, Protein C activity. No difference was found between 2 versus 3 or more pregnancy losses.
Baumann et al.	2013	Cohort	641 women 2 PL $n = 240$ ≥ 3 PL $n = 401$	Factor V Leiden 8.3% (20/240) Prothrombin gene mutation 2.9% (7/240)	Factor V Leiden 7.2% (29/401) Prothrombin gene mutation 3.5% (14/401)	Factor V Leiden, prothrombin gene mutation. No difference was found between 2 versus 3 or more pregnancy losses.
Ali et al.	2014	Cohort	250 patients 2 PL $n = 125$ ≥ 3 PL $n = 125$	Factor V Leiden (0/23) Prothrombin gene mutation (0/175) Protein S 1.1% (2/175) Protein C 1.1% (2/175)	Factor V Leiden 11.5% (3/26) Prothrombin gene mutation 1.4% (2/140) Protein S 4.3% (6/140) Protein C 4.3% (6/140)	Factor V Leiden mutation, prothrombin gene mutation, protein C activity, protein S activity. No difference was found between 2 versus 3 or more pregnancy losses.
Guzel et al.	2015	Cohort	252 women 2 PL $n = 72$ ≥ 3 PL $n = 180$	Protein S deficiency (84.18 ± 11.69) Protein C deficiency (90.91 ± 23.35)	Protein S deficiency (89.02 ± 22.47) Protein C deficiency (106.57 ± 68.79)	Protein S deficiency and protein C deficiency. No difference was found between 2 versus 3 or more pregnancy losses.
Thyroid disorders						
Jaslow et al.	2010	Cohort	687 women 2 PL $n = 396$ ≥ 3 PL $n = 291$	Abnormal TSH 8.0% (32/396)	Abnormal TSH 6.5% (19/291)	Serum levels of TSH < 0.45 mU/ml or > 4.5 mU/ml
Bashiri et al.	2012	Cohort	118 women 2 PL $n = 38$ ≥ 3 PL $n = 80$	Abnormal TSH 2.6% (1/38)	Abnormal TSH 16.3%(13/80)	Serum levels of TSH < 0.45 mU/ml or > 4.5 mU/ml.

PL, pregnancy loss; TSH: thyroid-stimulating hormone, MTHFR: methylenetetrahydrofolate reductase.

results of women with two pregnancy losses ($n = 72$) and three or more ($n = 180$) were not statistically significant different (anticardiolipin IgG 7.62 ± 2.45 versus 10.01 ± 4.16 GPLU/ml and IgM 4.76 ± 0.69 versus 4.22 ± 0.29 MPLU/ml) (Guzel et al., 2015).

Three studies were appropriate to be included for meta-analysis. No significant difference in frequency of abnormal results for APS was found between women with two pregnancy losses and three or more pregnancy losses (three studies, OR 1.04, 95% CI 0.86–1.25) (Fig. 4).

When summarizing the individual proportions in the studies using meta-analysis, we found a prevalence of 16% (95% CI 14–18) after two pregnancy losses and 15% (95% CI 12–18) after three pregnancy losses. These results suggest that a clinically relevant difference in prevalence is unlikely.

Inherited thrombophilia

Seven studies were identified which described the prevalence of inherited thrombophilia in women with two pregnancy losses compared to three or more pregnancy losses. A cohort study compared the prevalence of prothrombin gene mutation and Factor V Leiden mutation in 99 women with two or more pregnancy losses with 102 healthy controls. There was no difference in the distribution of Factor V Leiden and prothrombin gene mutation between patients with two and three or more pregnancy losses (Sotiriadis et al., 2007). In a retrospective cohort study of 252 women with RPL, different diagnostic tests were investigated. The results of cases with two pregnancy losses ($n = 72$) and more than two pregnancy losses($n = 180$) were not significantly different for Protein S deficiency (84.18 ± 11.69 versus 89.02 ± 22.47) and Protein C deficiency (90.91 ± 23.35 versus 106.57 ± 68.79) (Guzel et al., 2015).

Five studies eligible for meta-analysis described the difference in prevalence of factor V Leiden mutation ($n = 1109$). Meta-analysis showed no significant difference in the prevalence of factor V Leiden mutation between women with two pregnancy losses and three or more pregnancy losses (five studies, OR 0.79, 95% CI 0.43–1.47) (Fig. 5a). Five studies described the difference in prevalence of

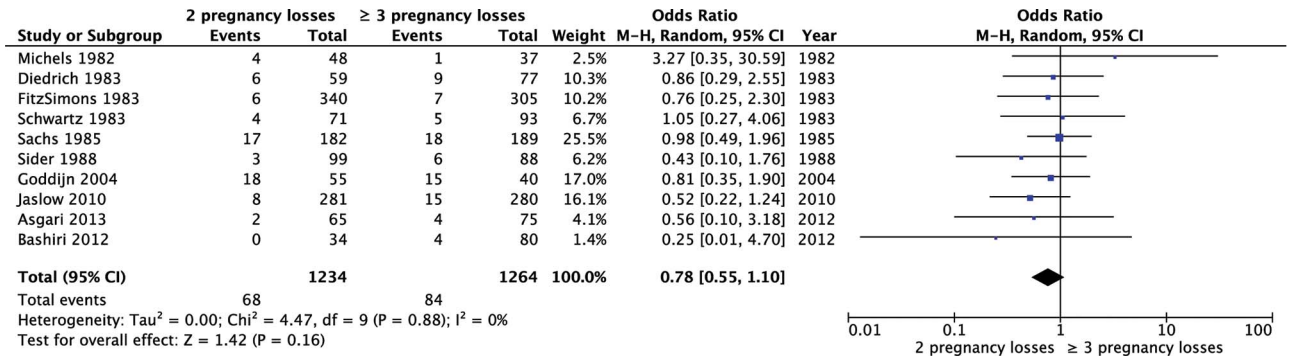

Figure 2 **Forest plot of odds ratios of abnormal test results for parental chromosomal abnormalities in women with two pregnancy losses or three or more pregnancy losses.**

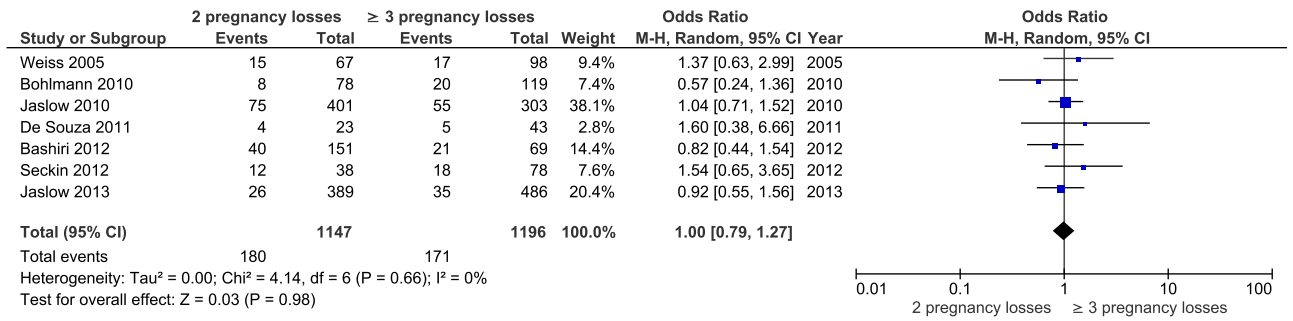

Figure 3 **Forest plot of odds ratios of abnormal test results for uterine anomalies in women with two pregnancy losses or three or more pregnancy losses.**

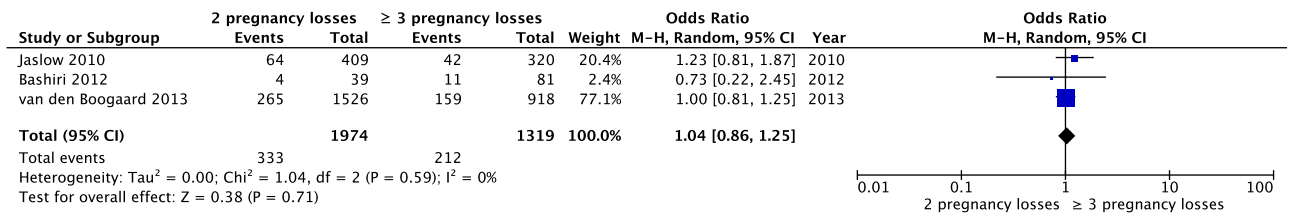

Figure 4 **Forest plot of odds ratios of abnormal test results for antiphospholipid syndrome in women with two pregnancy losses or three or more pregnancy losses.**

prothrombin gene mutation ($n = 1330$). A meta-analysis showed no significant difference in frequency of prothrombin gene mutation between women with two pregnancy losses and three or more pregnancy losses (five studies, OR 1.08, 95% CI 0.44–2.62) (Fig. 5b). Four studies described the difference in prevalence of protein S deficiency ($n = 708$). A meta-analysis showed no significant difference in frequency between women with two pregnancy losses and three or more pregnancy losses (four studies, OR 0.72, 95% CI 0.27–1.94) (Fig. 5c). Four studies described the difference in prevalence of protein C deficiency ($n = 710$). A meta-analysis showed no significant difference in frequency between women with two pregnancy losses and three or more pregnancy losses (four studies, OR 0.73, 95% CI 0.34–1.54) (Fig. 5d).

Thyroid disorders

Two studies ($n = 805$) described the prevalence of thyroid disorders in women with two pregnancy losses versus three or more. We found insufficient evidence of a difference in frequency of abnormal results for thyroid disorders (two studies, OR 0.52, 95% CI 0.06–4.56, very low quality of evidence) (Fig. 6). There was substantial statistical heterogeneity (I^2 of 76%) between the studies; therefore, this finding should be considered with care.

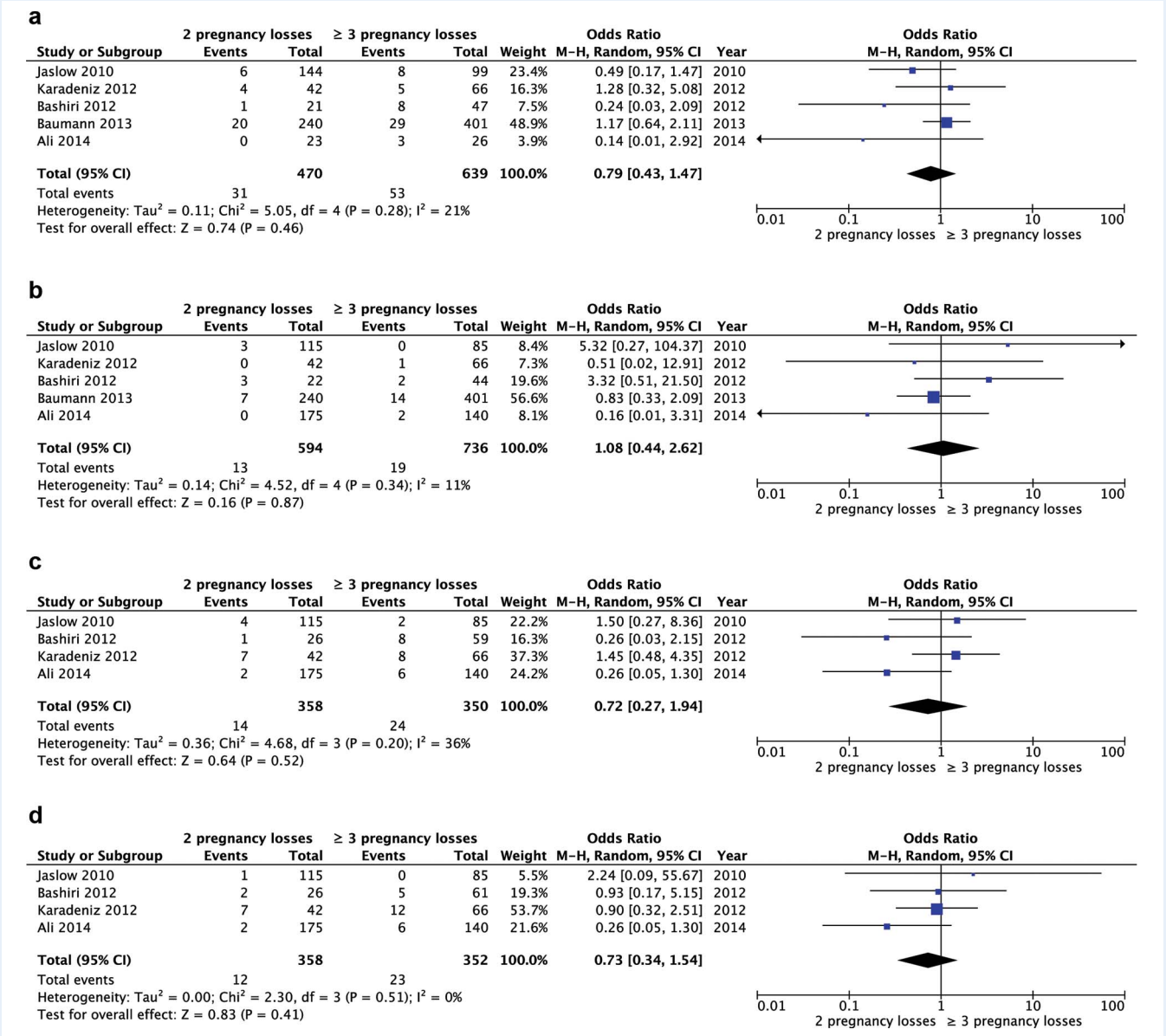

Figure 5 Forest plot of odds ratio of abnormal test results for thrombophilia in women with two pregnancy losses or three or more pregnancy losses. (**a**) Factor V Leiden mutation. (**b**) Prothrombin gene mutation. (**c**) Protein S deficiency. (**d**) Protein C deficiency.

Discussion

This systematic review investigated the available literature on the prevalence of abnormal test results in women with RPL with different numbers of previous pregnancy losses. Overall, we found no difference in prevalence of abnormal test results for parental structural chromosome abnormalities, uterine anomalies, APS, inherited thrombophilia and thyroid disorders in women with two pregnancy losses compared with three or more pregnancy losses.

The most recent RPL guideline (ESHRE, 2017) recommends screening for antiphospholipid antibodies after two pregnancy losses. Thyroid screening and assessment of uterine anatomy is recommended for RPL, but no recommendation is given after how many pregnancy losses. Parental karyotyping is not routinely recommended. As the chance of finding an abnormality is very low, it should only be considered after an individual risk assessment (Franssen *et al.*, 2005). As there is a weak association between RPL and hereditary thrombophilia and no available evidenced-based treatment, screening for hereditary thrombophilia is not routinely recommended in couples with RPL (ESHRE, 2017).

The results of this systematic review may support investigations after two pregnancy losses in couples with RPL, but it should be stressed that additional studies of the prognostic value of test results used in the RPL population are urgently needed. There is a paucity of effective evidenced-based treatments for the majority of abnormal tests for possible contributing factors for RPL. This is because many factors have been associated with RPL but few meet accepted criteria for causation. Therefore, testing should not be overvalued and the focus

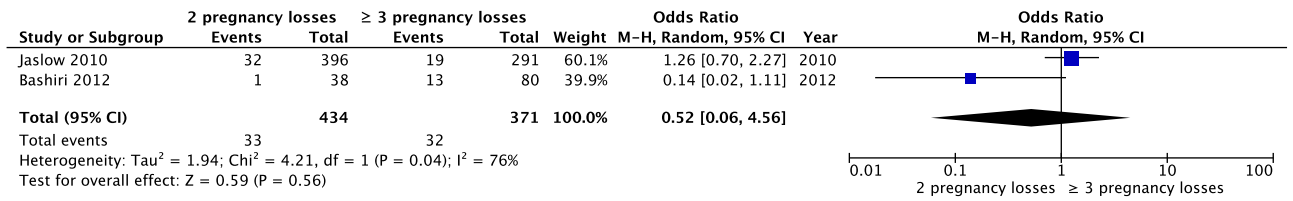

Figure 6 Forest plot of odds ratios of abnormal test results for thyroid disorders in women with two pregnancy losses or three or more pregnancy losses.

should be on tailor-made supportive care in women with RPL. Couples suffering from RPL need individualized management plans that include appropriate support and, in this context, testing for associated factors may help to reduce anxiety and manage expectations (Musters *et al.*, 2013).

In this systematic review, the quantity and quality of the evidence on the comparison between the prevalence of abnormal test results between groups were low. It follows that any conclusions and recommendations should be drawn with care.

A methodological limitation of this study is the definition of the study groups. As, on average, 15–20% of women with two losses will experience a loss in the next pregnancy, some of the women in the group with two pregnancy losses would be in the other group if evaluated at a different time point. Comparing these groups at a certain moment in time is a fictitious reality, and large studies of the prognostic importance of test results would provide significant new insights into the clinical relevance of diverse clinical tests.

There was no statistical heterogeneity across studies; this suggests that the relative chance of pregnancy loss might be similar in different countries, which could imply that our results are highly generalizable. Two large cohort studies were present in all the meta-analyses and had an important weight factor in the analysis (Jaslow *et al.*, 2010; Bashiri *et al.*, 2012). The results of this systematic review were in line with these two studies. A large systematic review on uterine anomalies in women with RPL reported a prevalence of 10.9% (95% CI 3.6–33.3) uterine anomalies in women with two and 15.4% (95% CI 10.3–23) in women with three or more pregnancy losses, which was not significantly different ($P = 0.572$) (Chan *et al.*, 2011).

In this systematic review, parental karyotyping was included, although in the last few years, less karyotyping is performed in some countries. In the work-up for couples with RPL, parental karyotyping of both parents is expensive, and there is a very low chance of a live born handicapped child with unbalanced chromosome abnormalities in the unselected RPL population (Franssen *et al.*, 2006; Barber *et al.*, 2010). These considerations have resulted in the recommendation not to perform routine karyotyping of all couples with RPL, but rather after an individual risk assessment (ESHRE, 2017). The treatment option for chromosome abnormalities in couples with RPL consists of PGD. However, limited evidence for PGD in couples with RPL shows no clear benefit of treatment. Couples should be offered genetic counselling and information on the treatment options (ESHRE, 2017).

We did not address genetic analysis of miscarriage tissue in this systematic review. Since genetic analysis is not routinely recommended, finding a fetal chromosomal abnormality does not necessarily rule out an underlying condition. However, it could be performed for explanatory purposes (ESHRE, 2017).

It is important to note that the presence of a particular abnormal test result in women with RPL does not prove causality for the pregnancy losses. Female age and number of prior pregnancy losses have been consistently found to be negative prognostic factors in numerous cohort studies (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).

Female age at first live birth is almost 30 years in European populations, and with an increasing female age, the risk of embryonic aneuploidy increases. Therefore, embryonic aneuploidy will often be the etiology behind RPL, especially in women older than 36 years (Stephenson *et al.*, 2002; Marquard *et al.*, 2010). The decision on when to start investigations should depend on female age and previous pregnancy losses as well as other maternal conditions such as manifest autoimmune or coagulative disease, family history and the results from miscarriage tissue karyotyping, if performed (Bernardi *et al.*, 2012). It should also be the result of shared decision-making by the doctor and couple whilst being compliant with available resources (ESHRE, 2017). Customized diagnostic testing should be considered, where some test can be performed and others omitted.

It should be noted that performing diagnostic testing after two pregnancy losses means that a higher number of couples will have to be investigated. Further studies are needed to assess the economic implications of such a change in policy.

We propose that future research should focus on the design of a dynamic prediction model for couples experiencing RPL. A dynamic model has the advantage that it allows for adaptations to changes in the underlying data over time (van Eekelen *et al.*, 2017). In this model, age, previous pregnancy losses and other risk factors for RPL, such as APS, can be incorporated. If treatment possibilities are present for risk factors (i.e. APS), correction should be applied. With this prediction model, the chance of a live birth could be estimated more precisely. A prediction model can also be used to give positive message to couples suffering anxiety and depression following their pregnancy losses.

Conclusion

The prevalence of abnormal test results for RPL is low after two and three or more pregnancy losses. A difference in prevalence in uterine abnormalities and APS is unlikely in women with two versus three

pregnancy losses. We cannot exclude a lower prevalence of chromosomal abnormalities, inherited thrombophilia and thyroid disorders following testing after two versus three pregnancy losses. The results of this systematic review may support testing after two pregnancy losses in couples with RPL, but it should be stressed that additional studies of the prognostic value of test results used in the RPL population are urgently needed. An evidenced-based treatment is not currently available in the majority of cases when abnormal test results are present.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Authors' roles

M.D., A.M.K., J.L., S.Q., E.K., M.W. and M.G. all contributed substantially to the design of this review. The literature search was performed by J.L. M.D. A.M.K. and M.W. participated in the selection of studies and data-extraction. M.G. was reviewer in case consensus could not be reached directly. M.D. drafted the article; all other authors critically revised multiple versions of the manuscript. All authors gave their final approval of the version to be published.

Funding

No external funding was either sought or obtained for this study.

Conflict of interest

None declared.

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