The influence of the thyroid on pregnancy outcomes

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Chapter 2

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

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ABSTRACT

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.

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.[1] 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.[2-4] In prospective studies, the risk of pregnancy loss increases with each loss from approximately 11% among nulligravidae to approximately 40% after three or more losses.[5]

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.[6] Even after comprehensive investigations a cause for RPL is identified in fewer than 50% of couples.[7] Consequently, the majority of cases remain without a modifiable risk factor.[8] Only female age and number of prior pregnancy losses have been consistently found to be prognostic factors for the majority of patients.[1] The tests currently performed are often expensive, time-consuming and of uncertain prognostic value.[9] 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.[10] The American Society for Reproductive Medicine Practice Committee defines RPL as two or more miscarriages confirmed by ultrasound or histology, not necessarily consecutive.[11] 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.[1]

Although evidence-based treatment is lacking for RPL, couples value a plan for the next pregnancy.[12] 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 (Figure 1). A medical information specialist (J.L.) 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 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 result 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, 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, beta-2 glycoprotein 1 antibodies) were considered to be present if a test was positive on two occasions >12 weeks apart.[13]

Inherited thrombophilia was defined in four different sub-categories: Factor V Leiden mutation, prothrombin gene mutation, protein S deficiency, 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 >4.5mU/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 were 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 The Strengthening the Reporting of

Observational Studies in Epidemiology statement.[14] Levels of evidence were attributed according to the Oxford Centre for evidence-based medicine. 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 8,301 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 or the selection process). Of the 21 articles included in this systematic review, 10 studies reported on chromosomal abnormalities[15-24], seven studies reported on testing for uterine anomalies [20,22,25-30], four studies reported on testing for antiphospholipid syndrome [20,22,31,32], seven studies reported on testing for inherited thrombophilia[20,22,32-36] and two studies reported on testing for thyroid disorders.[20,22]

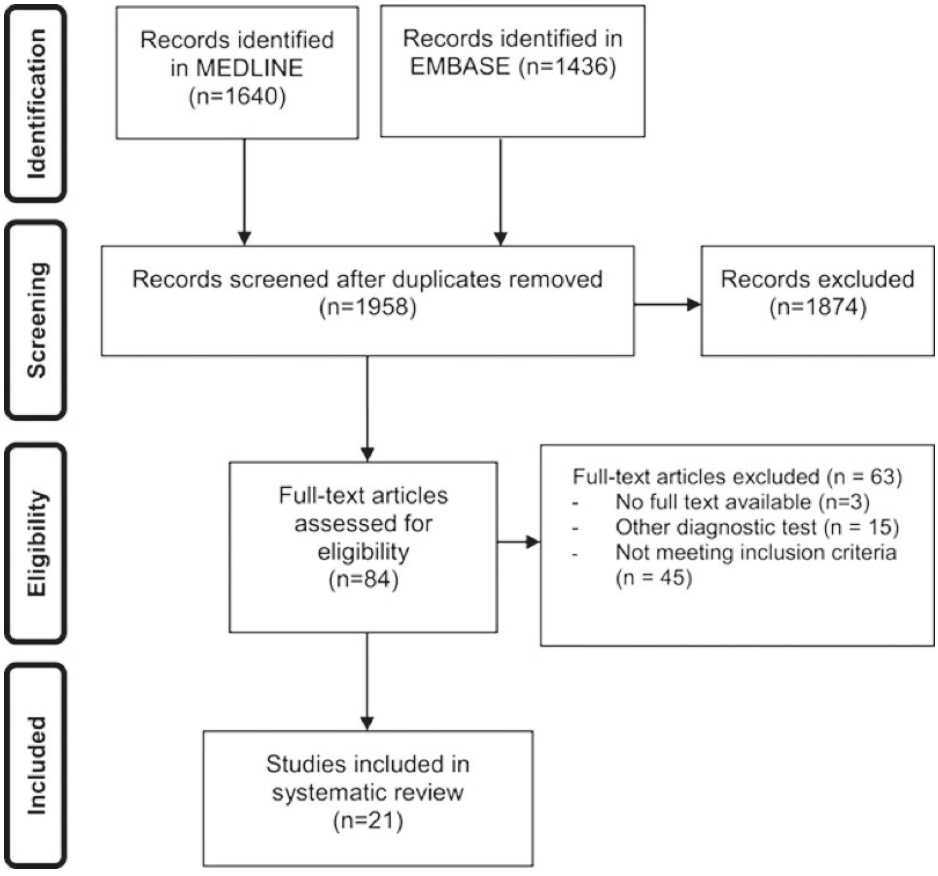


Figure 1. Study selection process for systematic review on the prevalence of abnormal evidence-based test results in women with recurrent pregnancy loss.

Table 1. Characteristics of the studies for chromosomal abnormalities, uterine anomalies, antiphospholipid syndrome, thrombophilia and thyroid disorders identified in a systematic review of recurrent pregnancy loss.

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.

Table 1. Continued.

Author	Year	Study type	Study population	Prevalence 2 pregnancy losses	Prevalence ≥ 3 pregnancy losses	Outcome measures
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.
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.

Table 1. Continued.

Author	Year	Study type	Study population	Prevalence 2 pregnancy losses	Prevalence ≥ 3 pregnancy losses	Outcome measures
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.
Antiphospolipid 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 pregnancy losses.

Table 1. Continued.

Author	Year	Study type	Study population	Prevalence 2 pregnancy losses	Prevalence ≥ 3 pregnancy losses	Outcome measures
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.
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.

Table 1. Continued.

Author	Year	Study type	Study population	Prevalence 2 pregnancy losses	Prevalence ≥ 3 pregnancy losses	Outcome measures
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/l or > 4.5 mU/l
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/l or > 4.5 mU/l.

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

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).[15-24] 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).

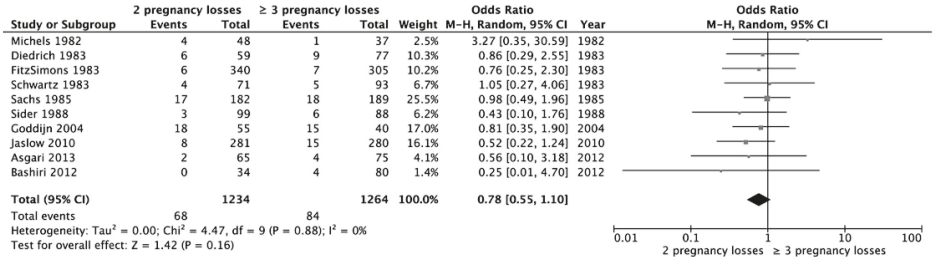


Figure 2. Forest plot of odds ratios of abnormal test results for parental chromosomal abnormalities in women with two pregnancy losses or three or more pregnancy losses.

When summarizing the individual proportions in the studies using meta-analysis, we found a chromosomal abnormalities 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.[20,22,25-29,37] 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.

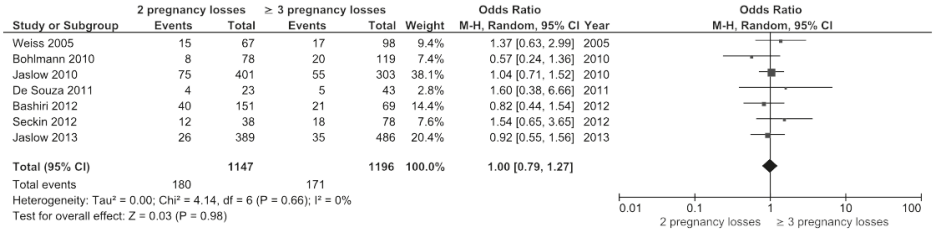


Figure 3. Forest plot of odds ratios of abnormal test results for uterine anomalies in women with two pregnancy losses or three or more pregnancy losses.

Antiphospholipid syndrome

Four included studies described the prevalence of APS in women with two pregnancy losses compared to three or more pregnancy losses.[20,22,31,32] 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 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±4.16GPLU/ml and IgM 4.76±0.69 versus 4.22±0.29MPLU/ml).[32]

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 for (three studies, OR 1.04, 95%CI 0.86 – 1.25) (Fig. 4).

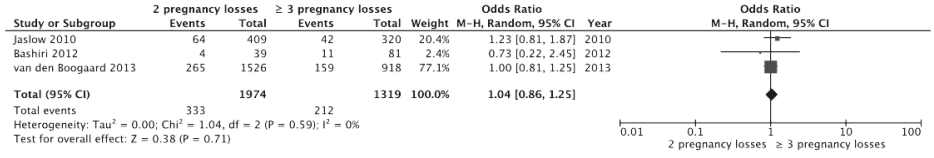


Figure 4. Forest plot of odds ratios of abnormal test results for antiphospholipid syndrome in women with two pregnancy losses or three or more pregnancy losses.

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

Inherited thrombophilia

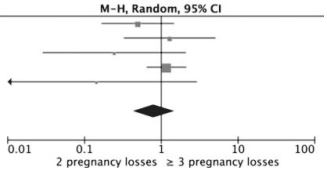
Seven studies were identified which described the prevalence of inherited thrombophilia in women with two pregnancy losses compared to three or more pregnancy losses.[20,22,32-35] A cohort study compared the prevalence of prothrombin gene mutation and Factor V Leiden mutation in 99 women with two or 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 miscarriages.[35] 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 (*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).[32]

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 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).

a)

Study or Subgroup	2 pregnancy losses Events	Total	≥ 3 pregnancy losses Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Jaslow 2010	6	144	8	99	23.4%	0.49 [0.17, 1.47]	2010
Karadeniz 2012	4	42	5	66	16.3%	1.28 [0.32, 5.08]	2012
Bashiri 2012	1	21	8	47	7.5%	0.24 [0.03, 2.09]	2012
Baumann 2013	20	240	29	401	48.9%	1.17 [0.64, 2.11]	2013
Ali 2014	0	23	3	26	3.9%	0.14 [0.01, 2.92]	2014
Total (95% CI)		**470**		**639**	**100.0%**	**0.79 [0.43, 1.47]**	
Total events	31		53				

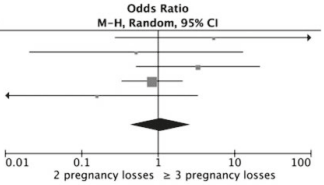
Heterogeneity: $Tau^2 = 0.11$; $Chi^2 = 5.05$, df = 4 (P = 0.28); $I^2 = 21\%$
Test for overall effect: Z = 0.74 (P = 0.46)



b)

Study or Subgroup	2 pregnancy losses Events	Total	≥ 3 pregnancy losses Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Year
Jaslow 2010	3	115	0	85	8.4%	5.32 [0.27, 104.37]	2010
Karadeniz 2012	0	42	1	66	7.3%	0.51 [0.02, 12.91]	2012
Bashiri 2012	3	22	2	44	19.6%	3.32 [0.51, 21.50]	2012
Baumann 2013	7	240	14	401	56.6%	0.83 [0.33, 2.09]	2013
Ali 2014	0	175	2	140	8.1%	0.16 [0.01, 3.31]	2014
Total (95% CI)		**594**		**736**	**100.0%**	**1.08 [0.44, 2.62]**	
Total events	13		19				

Heterogeneity: $Tau^2 = 0.14$; $Chi^2 = 4.52$, df = 4 (P = 0.34); $I^2 = 11\%$
Test for overall effect: Z = 0.16 (P = 0.87)



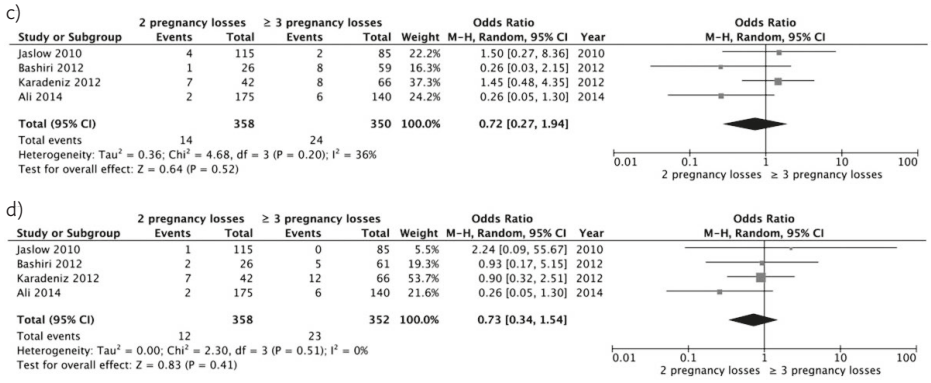


Figure 5. Forest plot of odds ratio of abnormal test result for thrombophilia women with two pregnancy losses or three or more pregnancy losses. (a) Factor V Leiden mutation (b) Prothrombin gene mutation (c) Protein S deficiency (d) Protein C deficiency.

Thyroid disorders

Two studies (*n* = 805) described the prevalence of thyroid disorders in women with two pregnancy losses versus three or more.[20,22] 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.

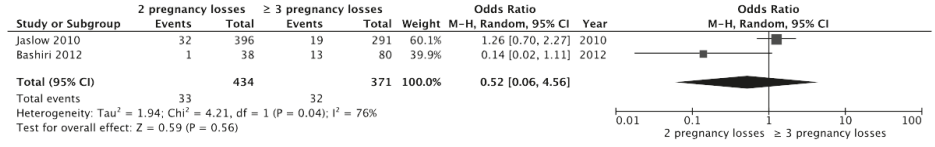


Figure 6. Forest plot of odds ratios of abnormal test results for thyroid disorders in women with two pregnancy losses or three or more pregnancy losses.

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 recommends screening for antiphospholipid antibodies after two pregnancy losses.[1] 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.[3,8] 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.[1]

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 should be on tailor-made supportive care in women with RPL. Couples suffering 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.[12]

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

A methodological limitation of this study is the definitions 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 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.[20,22] 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 miscarriages, which was not significantly different (p = 0.572).[37]

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.[39,40] These considerations have resulted in the recommendation not to perform routine karyotyping of all couples with RPL, but rather after an individual risk assessment.[1] 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.[1]

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.[1]

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.[3,41-48] 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.[49,50] 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.[51] It should also be the result of shared decision-making by the doctor and couple while being compliant with available resources.[1] 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.[52] 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.

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SUPPLEMENTARY DATA

Supplementary Table SI. Search strategy for articles on recurrent pregnancy loss and testing after two or three pregnancy losses.

Database(s): **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily** 1946 to March 08, 2019. Search Strategy: **2019-03-11**

#	Searches	Results
1	abortion, habitual/	6608
2	((habitual* or recurr* or multiple or repeat* or repetit*) adj4 (abortion* or miscarriage* or (pregnanc* adj2 loss*))).tw,kf.	8726
3	((habitual* or recurr* or repeat* or repetit*) adj2 f?etal loss*).tw,ot,kf.	585
4	(frequent adj2 (abortion* or miscarriage* or ((pregnanc* or f?etal) adj2 loss*))).tw,kf.	74
5	(("more than two" or "more than 2" or "more than three" or "more than 3" or "two or more" or "2 or more" or "three or more" or "3 or more") adj9 (miscarriag* or ((pregnanc* or f?etal) adj2 loss*) or abort*)).tw.	1003
6	(RPL or REPL or ERFL or RFL).tw,kf. and (pregnan* or abortion* or miscarriag*).mp.	746
7	**or/1-6 [RPL]**	**11317**
8	exp abortion, induced/ not habitual abortion/	38638
9	((terminat* adj2 pregnanc*) or ((surgic* or medic* or induced) adj2 abortion*)).ti.	5563
10	**8 or 9**	**39397**
11	**7 not 10 [RPL]**	**10671**
12	animals/ not humans/	4521762
13	**11 not 12 [human-RPL]**	**10419**
14	(current or cochrane or clinical evidence or EBM).jw. or (meta analy* or metaanaly* or meta?analy* or (systematic* adj3 (review or literature or evidence)) or ((summari* or review) adj3 evidence)).ti,ot. or ((systematic or PubMed or MEDLINE or EMBASE) adj5 search*).tw. or ((review or editorial or letter or comment).pt. not (Comparative Study.pt. or exp Cohort Studies/ or Cross-Sectional Studies/ or case-control studies/))	4145494
15	**13 not 14 [RPL - exclusion non primary studies]**	**8033**
16	(meta-analysis or systematic review).pt. or (meta analy* or metaanaly* or meta?analy*).ti,ot. or ((systematic* adj3 (review or literature or evidence or search*)) or ((summari* or review) adj3 evidence) or ((search* or evidence) adj12 (literature* or ((electronic or medical or biomedical) adj3 database*) or exhaustive)) or medline or pubmed or cochrane).tw,ot,kf. or (cochrane or clinical evidence or EBM).jw.	365284
17	**13 and 16 [RPL - secondary studies]**	**442**
18	("more than" adj3 ("two" or "2" or three or "3" or four or "4")).ti.	865
19	((exactly or precisel* or exceed*) adj3 ("two" or "2" or three or "3")).tw,kf.	9118
20	(("two" or "2") adj2 (versus or vs or compared) adj2 ("3" or "three" or more or many)).tw.	8012
21	(((two or "2") adj3 loss*) and ((three or "3" or four or "4" or more) adj3 loss*)).tw.	1390

#	Searches	Results
22	(((two or "2") adj3 (consecutive or RPL or REPL or RM or RSA or IRM or ERFL or RFL or abortion* or ((pregnancy or f?et*) adj2 loss*) or losses or miscarriag* or recurrent)) and ((three or "3" or four or "4") adj3 (consecutive or RPL or REPL or RM or RSA or IRM or ERFL or RFL or abortion* or ((pregnancy or f?et*) adj2 loss*) or losses or miscarriag* or recurrent))).tw.	3401
23	((RM>2 or RPL>2 or REPL>2 or RSA>2 or IRM>2 or ERFL>2 or RFL>2 or RM-2 or RPL-2 or REPL-2 or RSA-2 or IRM-2 or ERFL-2 or RFL-2) and (RM>3 or RPL>3 or REPL>3 or RSA>3 or IRM>3 or ERFL>3 or RFL>3 or RM>4 or RPL>4 or RSA>4 or IRM>4 or ERFL>4 or RFL>4 or RM-3 or RPL-3 or REPL-3 or RSA-3 or IRM-3 or ERFL-3 or RFL-3 or RM-4 or RPL-4 or RSA-4 or IRM-4 or ERFL-4 or RFL-4)).tw.	35
24	((number* or frequenc*) adj2 (RPL or REPL or RM or RSA or IRM or ERFL or RFL or loss* or abortion* or miscarr* or pregnanc* or birth* or live or liveborn* or childbirth*)).tw.	13509
25	((number* or sequence or frequenc*) adj3 (preceding or prior or previous or past or former or subsequent or consecutive)).tw.	17481
26	((preceding or prior or previous or past or former or antecedent* or subsequent or consecutive) adj3 (birth* or childbirth* or live or liveborn* or viable or obstetric histor* or obstetric record* or reproductive histor*)).tw.	7519
27	(history adj3 (consecutive or number)).tw.	1002
28	((obstetric* or etiologic* or aetiologic*) adj2 characteristic*).tw.	1025
29	index pregnan*.tw.	1015
30	(nonconsecutiv* or non-consecutiv*).tw.	1930
31	((risk or odds) adj3 (further or next or subsequent) adj3 (birth* or childbirth* or RPL or REPL or RM or RSA or IRM or ERFL or RFL or abortion* or ((pregnancy or f?etal) adj2 loss*) or miscarriag*)).tw.	254
32	carrier status.tw.	2766
33	Mass Screening/	96550
34	Diagnostic Tests, Routine/	10590
35	Patient Selection/	60805
36	(diagnostic adj3 (work-up* or workup* or protocol or strategy or yield* or factor* or marker* or investigative or screen* or evaluation or value or significance or relevance or abnormal)).tw,kf.	99540
37	((routin* or uniform* or universal* or selective* or history-based or evidence-based) adj6 (test* or screen* or diagnos* or work-up or workup or counseling or karyotyp* or cytogenetic* or chromosomal)).tw,kf.	88009
38	((strategy or specific or targeted or unique* or selected or limited or restricted or confined) adj3 (screening or counseling or karyotyping)).tw.	15092
39	(need adj3 (test* or screen*)).tw.	9170
40	(additional adj3 test*).tw.	10448
41	((subgroup* or sub-group* or different group*) and (regression or multivariate or logistic)).tw.	31710
42	((subgroup* or sub-group* or different group*) adj3 (RPL or REPL or RM or RSA or IRM or ERFL or RFL or abortion* or ((pregnancy or f?et*) adj2 loss*) or miscarriag*)).tw.	93

#	Searches	Results
43	(((clinical adj (significanc* or utility or relevan* or value)) or prevalence or incidence or frequencies or occurrenc* or likelihood or impact) adj6 (subgroup* or test result* or laboratory test* or aberration* or abnormal* or anomal* or malformat* or congenital or defect* or mutation* or chromosomal or Mullerian* or uterine or anatomic or uterus or arcuat* or subseptat* or septa* or septum or septus or subseptus or sub-sept* or hysteroscop* or hyperhomocystein* or homocystein* or karyotyp* or cytogenetic or carrier* or translocat* or aneuplod* or hereditary or inherited or familial or thrombophila* or HTP or carrier* or autoimmun* or auto-immun* or autoantibod* or auto-antibod* or auto-Ab* or t?yroid* or hypert?yr* or hypot?yr* or TFT or TSH or TRH or antit?yroid* or t?yroglobulin* or TG or TGs or antiTG* or TPO or TPOAb or thyroperoxidas* or iodide peroxidase* or ATA or antit?yroglobulin* or etiologic* factor* or aetiologic* factor* or factor V or FV or FVL or factor VI or FVI or F-VI or factor VII or FVII or F-VI or factor XII or FXII or f-XII or factor XIII or FXIII or F-XIII or G1691A or G20210* or C677T or A1298C or prothrombin* or anti-PT or thrombophil* or MTHFR or methylene tetrahydrofolate reductase* or methylenetetrahydrofolat* or prothrombin* or antiphospho* or phospho* or aPL or aPLs or annexin* or antiphosphatidyl* or phosphatidyl* or APA or APAs or APSA or APSAs or APS or anti-PS or aPE or aPEs or cardiolip* or anticardiolipin* or ACA or ACAs or aCL or aCLs or CLAb or CLAbs or CL-Ab or CL-Abs or antinuclear* or ANA or ANAs or lupus or anticoag* or coagulat* or thrombogen* or beta-2-glycoprotein* or beta2-glycoprotein* or abeta2GPI* or beta-2-GPI* or beta2GPI* or beta2-GPI* or aAnAV or plasminogen* or PLG or t-PA or PAPS)).tw,kf.	110037
44	or/18-43	567159
45	15 and 44 [I primary studies]	1437
46	remove duplicates from 45 [primary studies -deduplicated]	1436
47	17 and 44 [II SR]	96
48	remove duplicates from 47 [II secondary studies -deduplicated]	93

Database(s): **Embase Classic+Embase** 1947 to 2019 March 08

Search Strategy: **2019-03-11**

#	Searches	Results
1	recurrent abortion/	7296
2	((habitual* or recurr* or multiple or repeat* or repetit*) adj4 (abortion* or miscarriage* or (pregnanc* adj2 loss*))).tw,kw.	13706
3	((habitual* or recurr* or repeat* or repetit*) adj2 f?etal loss*).tw,ot,kw.	875
4	(frequent adj2 (abortion* or miscarriage* or ((pregnanc* or f?etal) adj2 loss*))).tw,kw.	149
5	(("more than two" or "more than 2" or "more than three" or "more than 3" or "two or more" or "2 or more" or "three or more" or "3 or more") adj9 (miscarriag* or ((pregnanc* or f?etal) adj2 loss*) or abort*)).tw.	1581
6	(RPL or REPL or ERFL or RFL).tw,kw. and (pregnan* or abortion* or miscarriag*).mp.	1385
7	**or/1-6 [RPL]**	**16447**
8	exp induced abortion/ not recurrent abortion/	36346
9	((terminat* adj2 pregnanc*) or ((surgic* or medic* or induced) adj2 abortion*)).ti.	6992
10	**8 or 9**	**38675**
11	**7 not 10**	**15856**
12	(animal/ or animal experiment/ or animal model/ or nonhuman/ or exp female animal/) not human/	6106070
13	**11 not 12 [human-RPL]**	**15431**
14	(book or editorial).pt. or (current or cochrane or clinical evidence or EBM).jw. or (meta analy* or metaanaly* or meta?analy* or (systematic* adj3 (review or literature or evidence)) or ((summari* or review) adj3 evidence)).ti,ot. or ((systematic or PubMed or MEDLINE or EMBASE) adj5 search*).tw. or book/ or editorial/ or case report/ or case study/ or (conference abstract or conference review or note).pt. or ((("review" or letter).pt. or "review"/ or letter/ or meta analysis/ or "systematic review"/) not (exp case control study/ or exp controlled clinical trial/ or controlled study/ or longitudinal study/ or major clinical study/ or observational study/ or prospective study/ or retrospective study/ or cohort analysis/ or cross-sectional study/)) [filter to exclude aggregated evidence, books and editorials]	10254185
15	**13 not 14 [RPL - primary studies]**	**8739**
16	meta analysis/ or "systematic review"/ or (meta analy* or metaanaly* or meta?analy*).ti,ot. or ((systematic* adj3 (review or literature or evidence or search*)) or ((summari* or review) adj3 evidence) or ((search* or evidence) adj12 (literature* or ((electronic or medical or biomedical) adj3 database*) or exhaustive)) or medline or pubmed or cochrane).tw,ot,kw. or (cochrane or clinical evidence or EBM).jw.	507599
17	**13 and 16 [RPL - secondary studies]**	**668**
18	("more than" adj3 ("two" or "2" or three or "3" or four or "4")).ti.	1143
19	((exactly or precisel* or exceed*) adj3 ("two" or "2" or three or "3")).tw,kw.	12028
20	(("two" or "2") adj2 (versus or vs or compared) adj2 ("3" or "three" or more or many)).tw.	15297
21	(((two or "2") adj3 loss*) and ((three or "3" or four or "4" or more) adj3 loss*)).tw.	2229

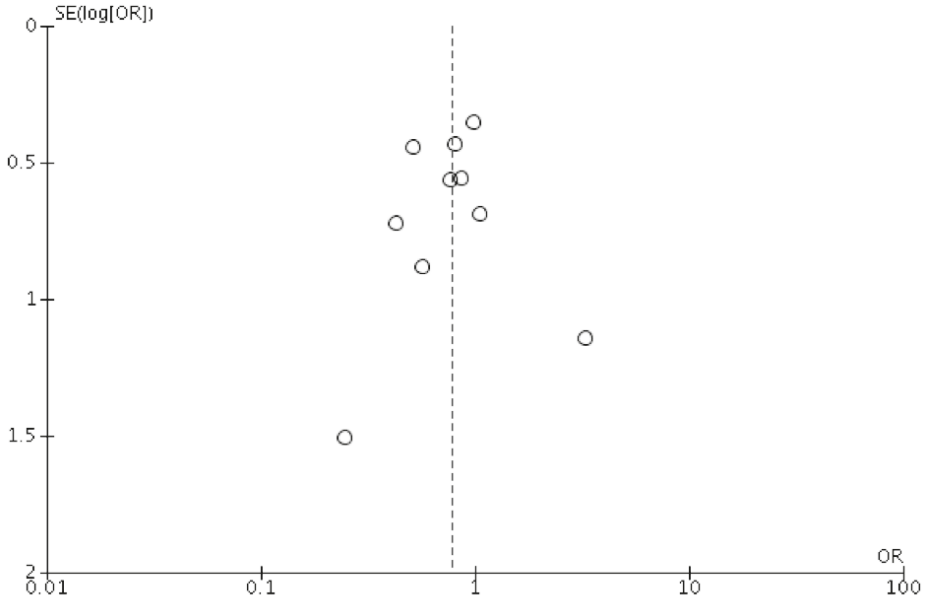
#	Searches	Results
22	(((two or "2") adj3 (consecutive or RPL or REPL or RM or RSA or IRM or ERFL or RFL or abortion* or ((pregnancy or f?et*) adj2 loss*) or losses or miscarriag* or recurrent)) and ((three or "3" or four or "4") adj3 (consecutive or RPL or REPL or RM or RSA or IRM or ERFL or RFL or abortion* or ((pregnancy or f?et*) adj2 loss*) or losses or miscarriag* or recurrent))).tw.	5307
23	((RM>2 or RPL>2 or REPL>2 or RSA>2 or IRM>2 or ERFL>2 or RFL>2 or RM-2 or RPL-2 or REPL-2 or RSA-2 or IRM-2 or ERFL-2 or RFL-2) and (RM>3 or RPL>3 or REPL>3 or RSA>3 or IRM>3 or ERFL>3 or RFL>3 or RM>4 or RPL>4 or RSA>4 or IRM>4 or ERFL>4 or RFL>4 or RM-3 or RPL-3 or REPL-3 or RSA-3 or IRM-3 or ERFL-3 or RFL-3 or RM-4 or RPL-4 or RSA-4 or IRM-4 or ERFL-4 or RFL-4)).tw.	39
24	((number* or frequenc*) adj2 (RPL or REPL or RM or RSA or IRM or ERFL or RFL or loss* or abortion* or miscarr* or pregnanc* or birth* or live or liveborn* or childbirth*)).tw.	18621
25	((number* or sequence or frequenc*) adj3 (preceding or prior or previous or past or former or subsequent or consecutive)).tw.	28668
26	((preceding or prior or previous or past or former or antecedent* or subsequent or consecutive) adj3 (birth* or childbirth* or live or liveborn* or viable or obstetric histor* or obstetric record* or reproductive histor*)).tw.	10479
27	(history adj3 (consecutive or number)).tw.	1698
28	((obstetric* or etiologic* or aetiologic*) adj2 characteristic*).tw.	1551
29	index pregnan*.tw.	1463
30	(nonconsecutiv* or non-consecutiv*).tw.	2743
31	((risk or odds) adj3 (further or next or subsequent) adj3 (birth* or childbirth* or RPL or REPL or RM or RSA or IRM or ERFL or RFL or abortion* or ((pregnancy or f?etal) adj2 loss*) or miscarriag*)).tw.	352
32	carrier status.tw.	4012
33	mass screening/	56818
34	abnormal laboratory result/	4132
35	(diagnostic adj3 (work-up* or workup* or protocol or strategy or yield* or factor* or marker* or investigative or screen* or evaluation or value or significance or relevance or abnormal)).tw,kw.	149900
36	((routin* or uniform* or universal* or selective* or history-based or evidence-based) adj6 (test* or screen* or diagnos* or work-up or workup or counseling or karyotyp* or cytogenetic* or chromosomal)).tw,kw.	131332
37	((strategy or specific or targeted or unique* or selected or limited or restricted or confined) adj3 (screening or counseling or karyotyping)).tw.	21277
38	(need adj3 (test* or screen*)).tw.	13785
39	(additional adj3 test*).tw.	16101
40	((subgroup* or sub-group* or different group*) and (regression or multivariate or logistic)).tw.	50027
41	((subgroup* or sub-group* or different group*) adj3 (RPL or REPL or RM or RSA or IRM or ERFL or RFL or abortion* or ((pregnancy or f?et*) adj2 loss*) or miscarriag*)).tw.	143

#	Searches	Results
42	(((clinical adj (significanc* or utility or relevan* or value)) or prevalence or incidence or frequencies or occurrenc* or likelihood or impact) adj6 (subgroup* or test result* or laboratory test* or aberration* or abnormal* or anomal* or malformat* or congenital or defect* or mutation* or chromosomal or Mullerian* or uterine or anatomic or uterus or arcuat* or subseptat* or septa* or septum or septus or subseptus or sub-sept* or hysteroscop* or hyperhomocystein* or homocystein* or karyotyp* or cytogenetic or carrier* or translocat* or aneuplod* or hereditary or inherited or familial or thrombophila* or HTP or carrier* or autoimmun* or auto-immun* or autoantibod* or auto-antibod* or auto-Ab* or t?yroid* or hypert?yr* or hypot?yr* or TFT or TSH or TRH or antit?yroid* or t?yroglobulin* or TG or TGs or antiTG* or TPO or TPOAb or thyroperoxidas* or iodide peroxidase* or ATA or antit?yroglobulin* or etiologic* factor* or aetiologic* factor* or factor V or FV or FVL or factor VI or FVI or F-VI or factor VII or FVII or F-VI or factor XII or FXII or f-XII or factor XIII or FXIII or F-XIII or G1691A or G20210* or C677T or A1298C or prothrombin* or anti-PT or thrombophil* or MTHFR or methylene tetrahydrofolate reductase* or methylenetetrahydrofolat* or prothrombin* or antiphospho* or phospho* or aPL or aPLs or annexin* or antiphosphatidyl* or phosphatidyl* or APA or APAs or APSA or APSAs or APS or anti-PS or aPE or aPEs or cardiolip* or anticardiolipin* or ACA or ACAs or aCL or aCLs or CLAb or CLAbs or CL-Ab or CL-Abs or antinuclear* or ANA or ANAs or lupus or anticoag* or coagulat* or thrombogen* or beta-2-glycoprotein* or beta2-glycoprotein* or abeta2GPI* or beta-2-GPI* or beta2GPI* or beta2-GPI* or aAnAV or plasminogen* or PLG or t-PA or PAPS)).tw,kw.	166334
43	**or/18-42**	**682010**
44	**43 and 15 [I primary studies]**	**1653**
45	**remove duplicates from 44 [I primary studies - deduplicated]**	**1640**
46	**43 and 17 [II secondary studies]**	**157**
47	**remove duplicates from 46 [II secondary studies - deduplicated]**	**153**

Supplementary Table SII. Quality assessment of cohort studies included in the meta-analysis using the Newcastle-Ottawa Scale.

Article	NOS star rating	Quality assessment
Ali 2014	8	Good
Asgari 2012	7	Good
Bashiri 2012	8	Good
Bauman 2013	8	Good
Bohlmann 2010	8	Good
van den Boogaard 2013	8	Good
Diedrich et al. 1983	6	Fair
FitzSimons et al. 1983	5	Poor
Goddijn 2004	7	Good
Guzel 2015	7	Good
Jaslow 2010	8	Good
Jaslow 2013	8	Good
Karadeniz 2012	7	Good
Michels 1982	5	Poor
Sachs 1985	6	Poor
Schwartz 1983	6	Poor
Seckin 2012	7	Good
Sider 1988	6	Fair
de Souza 2011	8	Good
Sotiriadis 2007	6	Poor
Weiss 2005	7	Good

Thresholds for converting the Newcastle-Ottawa scales (NOS) to standards (good, fair, and poor): Good quality: 3 or 4 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain. Fair quality: 2 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain. Poor quality: 0 or 1 star in selection domain OR 0 stars in comparability domain OR 0 or 1 stars in outcome/exposure domain.



Supplementary Figure SI. Funnel plot for the studies reporting on abnormal test results for parental structural chromosomal abnormalities in women with two pregnancy losses or three or more pregnancy losses.
OR: odds ratio