Thrombophilia and outcomes of assisted reproduction technologies: a systematic review and meta-analysis

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Thrombophilia has been associated with pregnancy complications and recurrent miscarriage. The aim of this systematic review was to evaluate the controversial association between thrombophilia and failures of assisted reproduction technology (ART). A systematic search of the literature for studies reporting on thrombophilia in women undergoing ART up to April 2011 yielded 33 studies (23 evaluating anti-phospholipid antibodies, 5 inherited thrombophilia, and 5 both) involving 6092 patients. Overall, methodologic quality of the studies was poor. Combined results from case-control studies showed that factor V Leiden was significantly more prevalent among women with ART failure compared with fertile parous women or those achieving pregnancy after ART (odds ratio = 3.08; 95% confidence interval, 1.77-5.36). The prothrombin mutation, methylenetetrahydrofolate reductase mutation, deficiency of protein S, protein C, or anti-thrombin were all not associated with ART failure. Women with ART failure tested more frequently positive for anti-phospholipids antibodies (odds ratio = 3.33; 95% confidence interval, 1.77-6.26) with evidence of high degree of between-study heterogeneity (I^2 = 75%; P < .00001). Prospective cohort studies did not show significant associations between thrombophilia and ART outcomes. Although case-control studies suggest that women experiencing ART failures are more frequently positive for factor V Leiden and anti-phospholipid antibodies, the evidence is inconclusive and not supported by cohort studies. (*Blood*. 2011;118(10):2670-2678)

Introduction

Assisted reproductive technologies (ARTs) offer a chance to conceive to many otherwise infertile couples; however, the average pregnancy rate after ART remains as low as 30%, despite the transfer of morphologically normal embryos.[1-3] The reasons behind the high failure rate are largely unexplained and may involve unsuccessful implantation or placentation. Both inherited and acquired thrombophilia have been associated with recurrent pregnancy loss and pregnancy complications, such as severe preeclampsia, fetal growth restriction, and stillbirth.[4-6] However, this relationship was recently called into question for factor V Leiden and the prothrombin mutation by a meta-analysis of prospective cohort studies.[7] A possible mechanism behind the development of pregnancy complications among carriers of a thrombophilic defect is the thrombosis of maternal vessels, which could cause a reduced perfusion of the intervillous space leading to placentation failure.[4,5] Similar mechanisms could hamper the implantation of the embryos and early placentation after ART. Whereas some studies have suggested a causal relationship between thrombophilia and ART failures, others have not confirmed these observations.[8,9] Many of these previous studies lacked power to detect a small, but clinically important, association. The debate over the role of thrombophilia in ART has been fired up by intervention studies that have evaluated thromboprophylaxis in women with repeated ART failures.[10-13] These preliminary findings suggested that heparin treatment may increase the chances of a live birth among carriers of a thrombophilic defect.[10-12]

Because of the conflicting results with respect to the presence, direction, and magnitude of the relationship between thrombophilia and ART outcomes, we undertook a systematic review of the literature to estimate the strength and precision of this controversial association.

Methods

Search strategy

A systematic search of the MEDLINE and EMBASE databases from inception up to April 2011 was performed to identify studies reporting on thrombophilia in women undergoing ART. The following search terms were used: thrombophilia, factor V Leiden mutation, methylenetetrahydrofolate reductase mutation (MTHFR), prothrombin gene mutation (G20210A), protein C deficiency, protein S deficiency, anti-thrombin deficiency, anti-phospholipid antibody, lupus anticoagulant, anti-cardiolipin antibodies, anti–β_2-glycoprotein antibodies, anti-phosphatidylserine antibodies, anti-phosphatidylinositol antibodies, anti-phosphatidic acid antibodies, anti-phosphatidylglycerol antibodies, and anti-phosphatidylethanolamine antibodies, assisted reproduction technology, in vitro fertilization (IVF), assisted conception, in vitro fertilization-embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, and frozen embryo transfer, intracytoplasmic sperm injection. Reference lists of all included studies and of reviews related to the topic were manually searched for additional potentially eligible studies.

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Study eligibility

Two investigators (M.D.N. and N.F.) independently evaluated titles and abstracts from the initial search for eligibility. We resolved disagreements by discussion or by involving a third reviewer (A.W.S.R.). Any report evaluating the prevalence of acquired (ie, presence of lupus anticoagulant, anti-cardiolipin, β_2-glycoprotein, anti-phosphatidylserine antibodies, anti-phosphatidylinositol, anti-phosphatidic acid, anti-phosphatidylglycerol, and anti-phosphatidylethanolamine antibodies) or inherited (factor V Leiden mutation, MTHFR mutation, prothrombin gene mutation, protein C deficiency, protein S deficiency, or antithrombin III deficiency) thrombophilia in women undergoing ART was eligible. Both case-control and cohort studies were considered. Case reports, commentaries, editorials, reviews, and meta-analyses were not eligible. We excluded cohort studies if data could not be extracted separately for women achieving a viable pregnancy or a live birth after ART and those with ART failure. In addition, we excluded case-control studies if the control groups were considered not representative, such as men or women not attempting to get pregnant. Studies in women who received oral or parenteral anticoagulation were not considered. The use of antiplatelet agents was permitted, however. In case-control studies, cases with ART failure were defined as failure of 1 or more IVF attempts, whereas controls were either women who achieved a pregnancy after ART or fertile women who attempted to get pregnant with or without a history of one miscarriage. Published or unpublished reports were eligible for inclusion, without language restrictions.

Data extraction

Two reviewers (M.D.N. and N.F.) independently extracted study characteristics using standardized forms. Any disagreements concerning the extracted data were resolved by consensus and, if necessary, by involving a third reviewer (A.W.S.R.). No attempts to mask for authorship, journal name, or institution were made here or in any other step of the review process. The information extracted from each study consisted of: age, race (whites only or mixed), number of failures, type of ART, ART outcome (clinical/viable pregnancy, live birth, or pregnancy test result) in cohort studies, type of thrombophilia (inherited or acquired), subtypes of thrombophilia, and the evaluation for other causes of ART failure. The pregnancy test is a widely used biochemical evaluation that ascertains the presence of a pregnancy measuring the serum or urinary β-human chorionic gonadotrophin levels. For the purpose of this study, a viable (clinical) pregnancy was defined as a pregnancy diagnosed initially by serum β-human chorionic gonadotrophin levels with consequent evidence of a gestational sac with or without a fetal heart observed on transvaginal ultrasound. The number of women with and without thrombophilia experiencing an ART failure or who achieved a live birth after ART was extracted to calculate 2×2 tables. If available, we extracted data on both recurrent and first ART failure, and on both combined and individual thrombophilia. If odds ratios (ORs) or relative risks (RRs) could not be calculated, we contacted the authors for additional data.

The methodologic quality assessment included type of design (cohort vs case-control), type of control (women achieving a live birth after ART vs fertile parous women vs mixed controls including both), type of sampling method (consecutive vs nonconsecutive or unclear), type of data collection (prospective vs retrospective vs unclear), and number of dropouts or withdrawals, with the latter 2 items assessed in prospective studies only.

Statistical analysis

Our main interest was the association between thrombophilia and ART failure in case-control and the development of viable pregnancy or live birth in cohort studies. In addition, we evaluated the proportion of women with a negative pregnancy test evaluation.

Data from cohort studies and case-control designs were analyzed separately. Individual RRs or ORs, as appropriate and associated 95% confidence intervals (CIs) were calculated. We used inverse variance random-effects meta-analyses to combine the trials. We assessed heterogeneity between studies using the χ^2 test and the I^2 statistic,[14] which describes the percentage of variation across studies that is attributable to heterogeneity rather than to chance. I^2 values of 25%, 50%, and 75% may be interpreted as low, moderate, and high, respectively, between-study heterogeneity, although its interpretation depends on the size and number of studies included.[15]

Publication bias and other biases related to small study size were evaluated using funnel plots, plotting ORs on the vertical axis against their SEs on the horizontal axis and assessing asymmetry by the asymmetry coefficient: the difference in OR per unit increase in SE.[16] Funnel plots were only considered in the presence of moderate to high between-study heterogeneity. We planned stratified analyses to evaluate the impact of type of controls (fertile parous women or mixed controls vs women achieving a live birth after ART) used on the association of interest, using meta-regression.

Meta-analysis was done with RevMan Version 5 software (Nordic Cochrane Center, Cochrane Collaboration). Funnel plot evaluation and the stratified analysis were performed in Stata Version 10.1 (Stata Corp). Two-sided P values $< .05$ were considered to indicate statistical significance. This study had no external funding source.

Results

The initial search strategy yielded 694 studies. Of these, 622 articles were excluded for the following reasons: case reports, editorials, reviews, and meta-analyses (n = 39), inadequate outcomes (n = 20), population (ie, women were not treated with ART or nonparous women; n = 30) or controls (n = 2), absence of thrombophilia/anti-phospholipid antibodies (n = 9), and more than one of the above (n = 522). Of the remaining 72 studies eligible based on the title or abstract, 39 were excluded (Figure 1), leaving 33 studies (6092 patients) for the final analysis.[8-10,17-46] All studies used IVF as ART, with 6 studies reporting intracytoplasmic sperm injection in adjunction.[9,23,29-31,36] In 2 studies, patients received antiplatelet agents during ART.[22,46]

Overall, the methodologic quality in case-control studies was poor (Tables 1-2). Of the 23 case-control comparisons included, only 6 used a representative control group consisting of women achieving a live birth after ART. The remainder included either healthy women with a history of successful delivery (n = 11) or mixed controls (n = 6). In the 13 cohort studies, women were sampled consecutively in 6 (46%) studies, data collection was prospective in 10, and the percentage of drop-outs ranged from 0%-55% (median, 0.006%).

Inherited thrombophilia

Ten studies (1763 patients) evaluated the relationship between inherited thrombophilia and ART[8-9,17-24]; and in 5 of them, anti-phospholipid antibodies were also assessed.[9,20-22,26] Seven studies were case-control, 2 were cohorts, and in 1 case-control study patients undergoing IVF (cases) were followed to assess the pregnancy outcomes[9] (Table 1).

Factor V Leiden

Pooled data on ART failure associated with factor V Leiden from 8 case-control studies showed an overall 3-fold increased risk (OR = 3.08; 95% CI, 1.77-5.36) with no evidence of statistical heterogeneity ($I^2 = 0\%$, $P = .44$; Figure 2).[8,9,17-20,22,24] The risk of ART failure was still significantly higher among the heterozygotes (5 studies, 914 patients; OR = 2.74; 95% CI, 1.43-5.25), with a trend for the homozygotes (2 studies, 381 patients; OR = 8.43; 95% CI, 0.96-73.80).

The effects of factor V Leiden on ART failure were evaluated in 3 cohort studies, which found a comparable chance of a positive

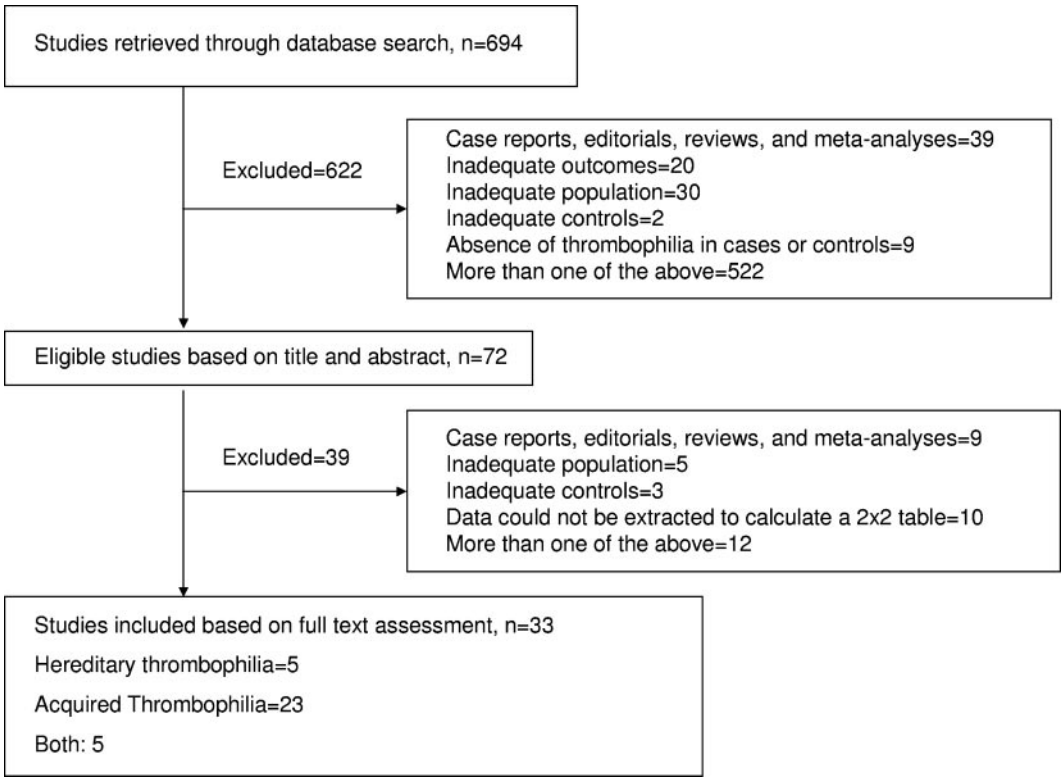

Figure 1. Flow chart.

pregnancy test result[21] (182 patients; RR = 0.16; 95% CI, 0.01-3.11) or viable pregnancy[9,23] (435 patients; RR = 0.62; 95% CI, 0.35-1.08) between women with and without the mutation.

Other inherited thrombophilia

Eight case-control studies examined the association between the prothrombin gene mutation and ART failure (Figure 2).[8,9,17-20,22,24] The overall association was not significant (OR = 1.48; 95% CI, 0.71-3.06) with an intermediate degree of statistical heterogeneity (I^2 = 26%, P = .23). The risk was comparable between heterozygotes (3 studies, n = 734; OR = 0.88; 95% CI, 0.39-2.00) or homozygotes (1 study, n = 280; OR = 2.12;95% CI, 0.13-34.34) relative to noncarriers. The funnel plot did not show asymmetry (P for asymmetry < .53) and the corresponding asymmetry coefficient was 0.79 (95% CI, −2.19-3.77).

The MTHFR mutation was evaluated in 7 studies.[9,17,18,20,22,24] The percentage of women with ART failure testing positive for

Table 1. Characteristics of studies that evaluated inherited thrombophilia in women undergoing assisted reproductive technology

Study	Design	Thrombophilia	Participants
Azem (2004)[17]	Case-control	FVL, prothrombin mutation, MTHFR, protein C and S, AT	Cases: ≥ 4 IVF failures (n = 45); controls: healthy women, (n = 44); live birth after the first IVF attempt (n = 15)
Bellver (2008)[18]	Case-control	FVL, prothrombin mutation, MTHFR, protein C and S, AT, APCR	Cases: repeated unexplained IVF failure (n = 26); controls: healthy women (n = 32)
Grandone (2001)[8]	Case-control	FVL, prothrombin mutation, MTHFR, protein C and S, AT	Cases: at least 3 IVF failures (n = 18); controls: healthy women (n = 216)
Martinelli (2003)[9]	Case-control	FVL, protrombin mutation, MTHFR	Cases: ≥ 1 IVF failures (n = 162);* controls: healthy women (n = 234)
Martinuzzo (2005)[19]	Case-control	FVL, prothrombin mutation	Cases: ≥ 2 IVF failures (n = 48); controls: healthy women (n = 80)
Qublam (2006)[20]	Case-control	FVL, prothrombin mutation, MTHFR, proteins C and S, AT	Cases: ≥ 3 IVF failures (n = 90); controls: live birth after the first IVF attempt (n = 90); healthy women (n = 100)
Rudick (2009)[21]	Prospective cohort	FVL	Women undergoing IVF (n = 182)
Simur (2009)[22]	Case control	FVL, prothrombin mutation, MTHFR	Cases: ≥ 3 IVF failures (n = 51); controls: healthy women (n = 50)
Tormene (2011)[23]	Prospective cohort	FVL, prothrombin mutation, MTHFR	Women undergoing IVF (n = 201)
Vaquero (2006)[24]	Case-control	FVL, prothrombin mutation, MTHFR	Cases: ≥ IVF failures (n = 59); controls: healthy women (n = 20)

FVL indicates factor V Leiden; AT, anti-thrombin; and APCR indicates activated protein C resistance.
*Cases followed prospectively to assess pregnancy outcomes.

Table 2. Characteristics of studies that assessed the prevalence of anti-phospholipid antibodies in women undergoing assisted reproductive technology

Study	Design	Anti-phospholipid antibodies	Participants
Balasch (1996)[25]	Case-control	LA, ACA	Cases: repeated unexplained IVF failure (n = 40); controls: healthy women (n = 125); women in labor after normal pregnancies at term (n = 52); live birth after the first IVF attempt (n = 49)
Balasch (1999)[26]	Case-control	LA, ACA, β_2-GPI	Cases: repeated unexplained IVF failure (n = 75); controls: healthy women (n = 100); live birth after the first IVF attempt (n = 60)
Bellver (2008)[18]	Case-control	LA, ACA	Cases: repeated unexplained IVF failure (n = 26); controls: healthy women (n = 32)
Birdsall (1996)[27]	Retrospective cohort	ACA, APS	Less than 3 previous IVF failures (n = 240)
Birkenfeld (1994)[28]	Case-control	LA, ACA	Cases: ≥ 1 IVF failures (n = 56); controls: healthy women or live birth after IVF attempt (n = 14)
Buckingham (2006)[29]	Prospective cohort	ACA, β_2-GPI, APS	Women undergoing IVF treatment (n = 99)
Caccavo (2007)[30]	Prospective cohort	ACA	Women undergoing their first IVF (n = 50)
Chilcott (2000)[31]	Prospective cohort	LA, ACA, β_2-GPI, APS	Women undergoing IVF (n = 380)
Coulam (1997)[32]	Case-control	ACA, APA, APC, APE, API, APG, APS	Cases: ≥ 1 IVF failures (n = 312); controls: healthy women (n = 100)
Coulam (2002)[33]	Case-control	ACA	Cases: IVF failure (n = 122); controls: pregnancy after IVF (n = 20)
Geva (1994)[34]	Case-control	LA, ACA	Cases: chemical pregnancies and no deliveries (n = 21); controls: patients who had conceived and delivered after IVF (n = 21)
Geva (1995)[35]	Case-control	LA, ACA	Cases: ≥ 3 IVF failures (n = 50); controls: live birth after ≤ 3 IVF attempts (n = 40)
Eldar-Geva (1999)[36]	Case-control	LA, ACA, APA, APE, API, APG, APS	Cases: ≥ 2 IVF failures (n = 96); controls: live birth after IVF (n = 45)
El-Roeiy (1987)[37]	Prospective cohort	ACA, APS	Women undergoing IVF (n = 26)
Gleicher (1994)[38]	Retrospective cohort	APL	Randomly chosen women who had undergone IVF (n = 105)
Kaider (1996)[39]	Case-control	ACA, APA, APC, APE, APG, API, APS	Cases: repeated IVF failures (n = 42); controls: live birth after IVF (n = 42)
Kaider (1999)[40]	Case-control	APL, LA	Cases: repeated IVF failures (n = 122); controls: healthy women (n = 20)
Kowalik (1997)[41]	Retrospective cohort	ACA, APS	Women undergoing IVF (n = 570)
Kutteh (1997)[42]	Case-control	APL	Cases: women undergoing their first cycle of IVF (n = 79);* controls: healthy women (n = 200)
Lucena (1999)[43]	Case-control	LA, ACA, APE, APS, APG, APA, API	Cases: women undergoing IVF (n = 162); controls: healthy women (n = 35)
Martinelli (2003)[9]	Case-control	LA, ACA	Cases: ≥ 1 IVF failures (n = 162); controls: healthy women (n = 234).
Martinuzzo (2005)[19]	Case-control	LA, ACA, β_2-GPI	Cases: ≥ 2 IVF failures (n = 48); controls: healthy women (n = 80)
Putowski (2004)[44]	Case-control	LA, ACA	Cases: repeated IVF failure (n = 17); controls: live birth after IVF (n = 10)
Qublan (2006)[20]	Case-control	LA, ACA	Cases: ≥ 3 IVF failures (n = 90); controls: live birth after first IVF attempt (n = 90); healthy women (n = 100)
Sanmarco (2007)[45]	Case-control	LA, ACA, β_2-GPI, APE	Cases: ≥ 3 IVF failures (n = 101);* controls: healthy women (n = 160)
Sher (1994)[10]	Prospective cohort	APL	Women undergoing IVF (n = 260)
Stern (1998)[46]	Case-control	LA; ACA, β_2-GPI, APE, API, APS	Cases: repeated IVF failures (n = 105); controls: healthy women (n = 106)
Vaquero (2006)[24]	Case-control	APL, LA, ACA, β_2-GPI	Cases: ≥ 2 IVF failures (n = 59); controls: healthy women (n = 20)

LA indicates lupus anticoagulant; ACA, anti-cardiolipin; β_2-GPI, β_2-glycoprotein; APS, anti-phosphatidylserine; APA, anti-phosphatidic acid; APC, anti-phosphatidylcholine; APE, anti-phosphatidylethanolamine; API, anti-phosphatidylinositol; APG, anti-phosphatidylglycerol; and APL, anti-phospholipid antibodies (antibody subtype not specified).

*Cases followed prospectively to assess pregnancy outcomes.

MTHFR was similar to controls overall (Figure 2) and when MTHFR homozygotes (5 studies, 622 patients; OR = 1.66; 95% CI, 0.74-3.75) and heterozygotes (2 studies, 381 patients; OR = 1.16; 95% CI, 0.64-2.13) were evaluated separately.

Two studies (n = 435) prospectively assessed the impact of the prothrombin and the MTHFR mutations on the chance of a viable pregnancy after IVF.[9,23] None of these thrombophilic defects increased the risk of failure (RR = 0.85; 95% CI, 0.36-1.97, and RR = 1.04; 95% CI, 0.60-1.83, respectively).

Deficiencies of protein C, protein S, and anti-thrombin were evaluated in 3 case-control studies (n = 442),[17,18,20] whereas only one study[18] of 58 participants assessed the relationship between protein C resistance and ART failure. As shown in Figure 2, the presence of any of these thrombophilia was similar between women with and those without ART failure (Figure 2).

Anti-phospholipid antibodies

Twenty-nine studies (5270 patients) assessed anti-phospholipid antibodies in women treated with ART.[9,10,18-20,24-46] Eighteen were case-control, 8 cohorts, and 2 case-control followed cases for pregnancy outcomes (Table 2). In 23 studies reporting on the

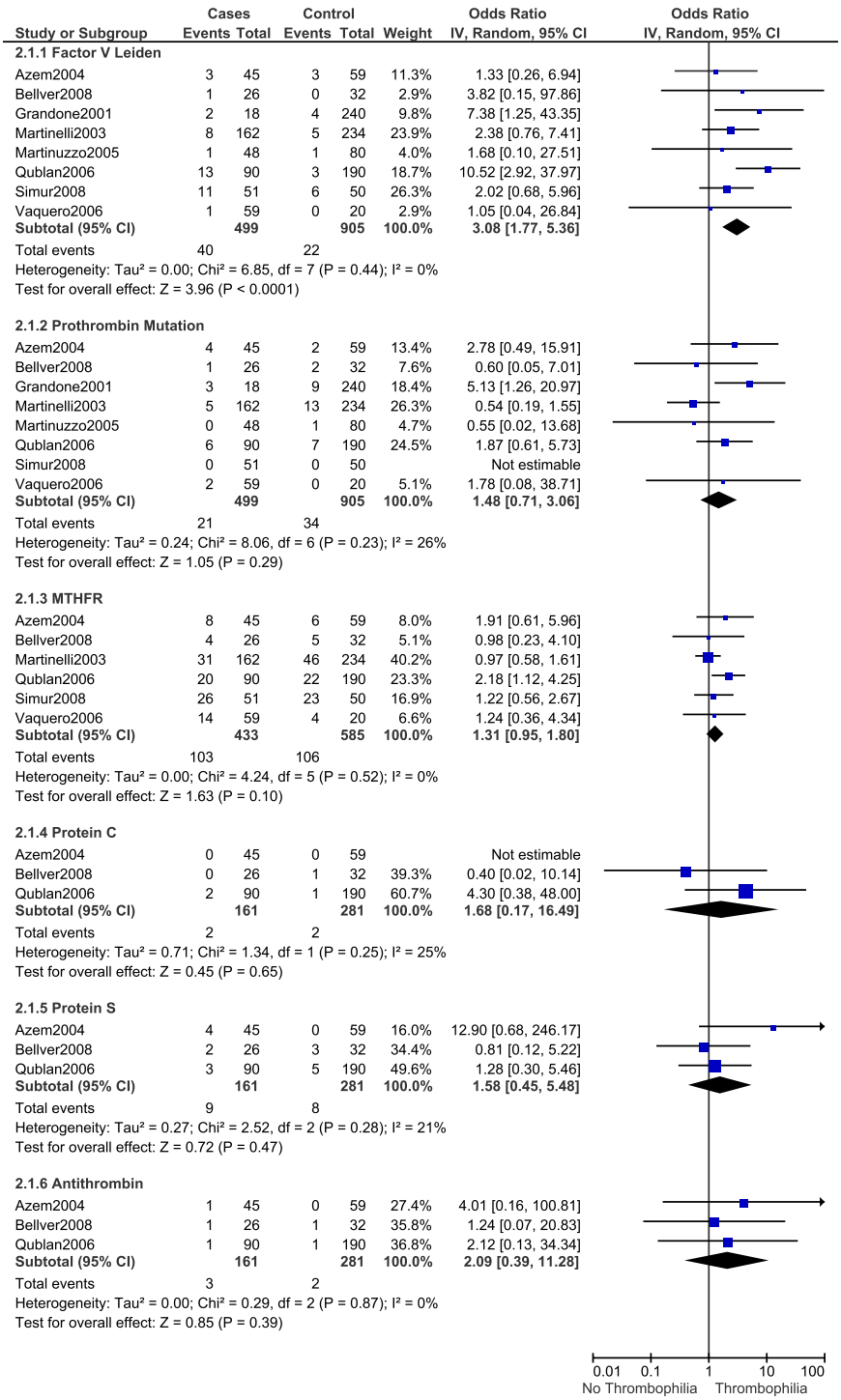

Figure 2. Forest plot of case-control studies on inherited thrombophilia and assisted reproductive technology.

number of antibodies tested, this latter varied between ≤ 2 (n = 14) and ≥ 3 antibodies (n = 8). The antibody cut-off for positivity was reported in 23 of 29 studies. The prevalence of antibodies in infertile patients varied from 0%-45%.

Overall, the presence of one or more anti-phospholipid antibodies was associated with a > 3-fold higher risk of ART failure (20 studies, 3542 patients; OR = 3.33; 95% CI, 1.77-6.26; Figure 3). There was a significant high-degree heterogeneity across the studies (I^2 = 75%, P < .00001). We explored heterogeneity in stratified analysis based on the type of controls. Studies using fertile parous control women alone or mixed controls showed a similar association between the presence of antiphospholipid antibodies and ART failure (OR = 3.79; 95% CI, 2.27-6.36) compared with studies using controls who achieved a live birth after ART (OR = 3.18; 95% CI, 0.91-11.1; P value for interaction from meta-regression = .55). The funnel plot did not show asymmetry (Figure 4, P for asymmetry < .23), and the corresponding asymmetry coefficient was 1.38 (95% CI, −0.94-3.70).

In cohort studies, anti-phospholipid antibodies were not associated with a lower rate of viable pregnancy, live birth, or a higher incidence of negative pregnancy tests (Figure 5).

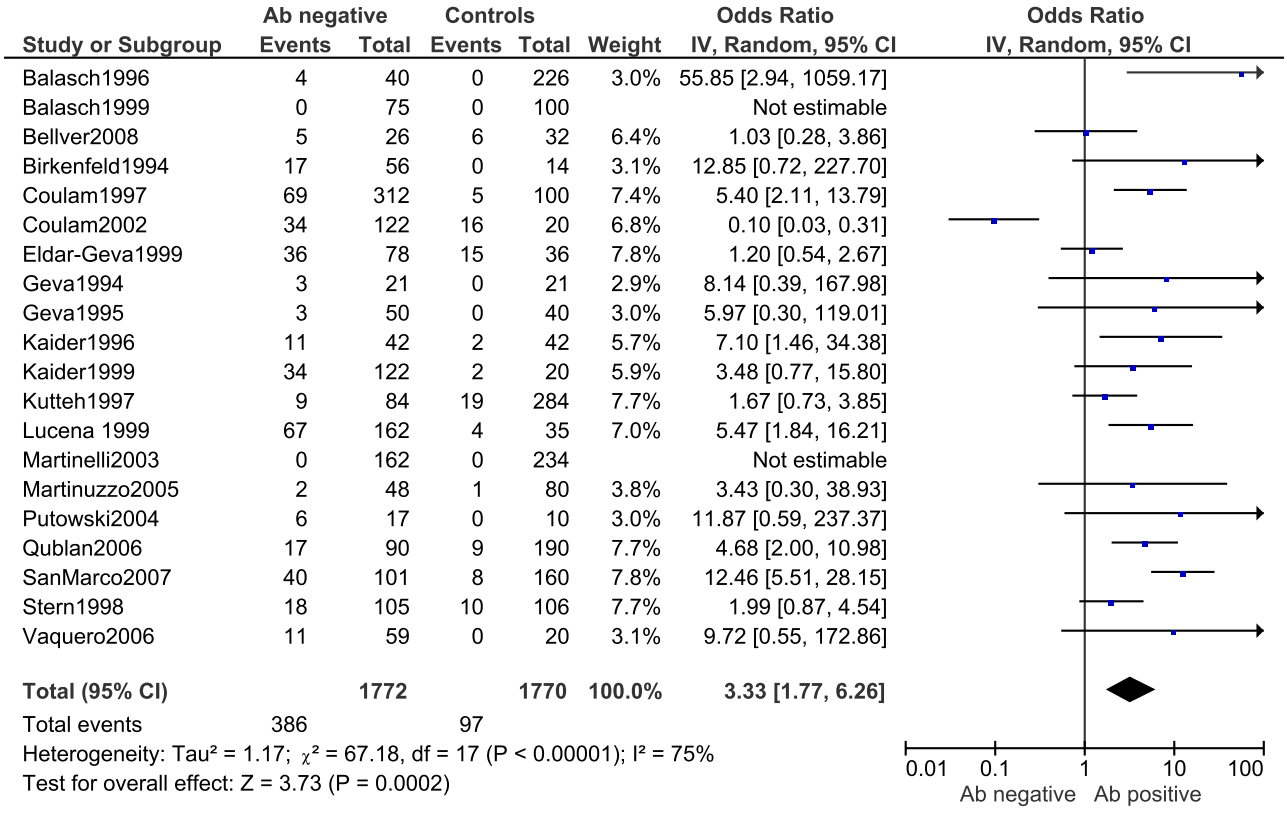

Figure 3. Forest plot of case-control studies on anti-phospholipid antibodies and assisted reproductive technology.

Anti-phospholipid antibody subtypes

Compared with controls, women experiencing ART failure were more often positive for lupus anticoagulant, antibodies anti-phosphatidylserine, anti-phosphatidylinositol, anti-phosphatidic acid, and anti-phosphatidylglycerol (Table 3). No significant associations were observed for anticardiolipin antibodies, anti–β_2-glycoprotein-I antibodies, and anti-phosphatidylethanolamine (Table 3). One cohort study prospectively assessed the ART outcome in 50 patients with and without anti-cardiolipin antibodies and found no association with viable pregnancy (RR = 0.59; 95% CI, 0.05-7.08) or pregnancy test results (RR = 0.49; 95% CI, 0.19-1.28).[30] None of the other individual antibodies was evaluated in cohort studies.

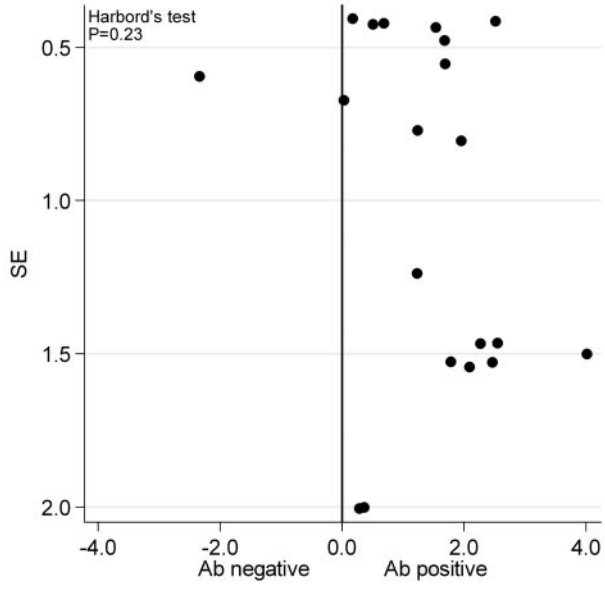

Figure 4. Funnel plot of studies on anti-phospholipid antibodies.

Discussion

Data from case-control studies suggest that women with ART failure test more frequently positive for factor V Leiden and some subtypes of anti-phospholipid antibodies compared with fertile parous women or women who achieve a live birth after ART. These findings need, however, to be considered with caution because of the substantial between-study heterogeneity and the lack of a significant association between thrombophilia and failures to achieve a pregnancy in cohort studies.

ART failure in women with thrombophilia could be explained by excessive thrombosis of the placental vessels causing hypoperfusion of the intervillous space with secondary placentation failure,[4,5] although other mechanisms, such as the damage of decidual or chorionic vessels, or reduction of trophoblast invasiveness, could prevent the conceptus implantation.[47,48] Factor V Leiden and other inherited thrombophilias have been associated with an unfavorable pregnancy outcome and could cause embryos implantation failure after ART.[4-6] The risk of pregnancy complications in carriers of the factor V Leiden and the prothrombin mutation was, however, recently called into question by a meta-analysis of prospective cohort studies.[7] In the current analysis, pooled estimates from case-control studies seemed to support the hypothesis that factor V Leiden carriership is more prevalent among women with repetitive ART failures. Although the risk

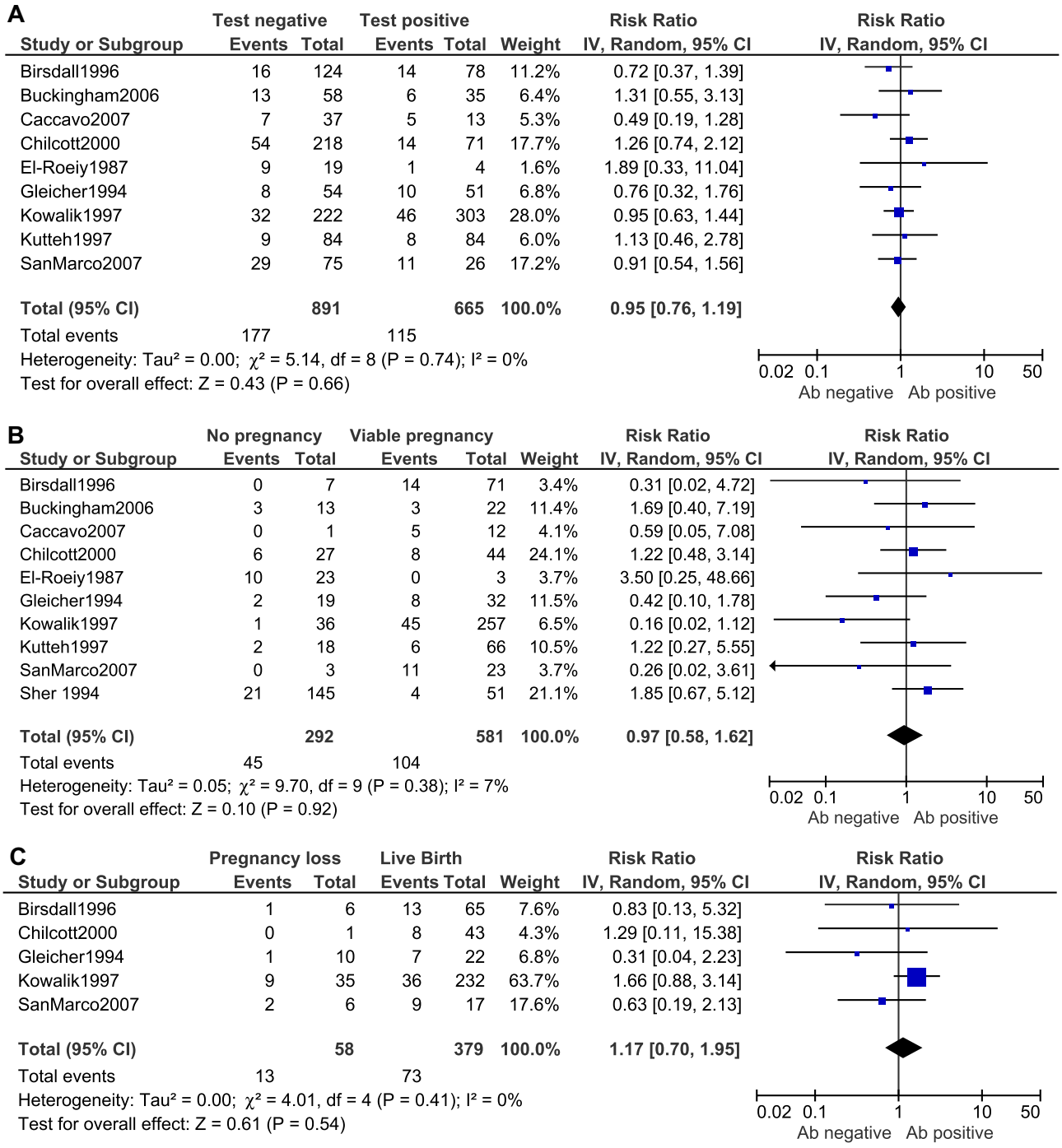

Figure 5. Forest plot of cohort studies on anti-phospholipid antibodies and outcomes-assisted reproductive technology. Studies on anti-phospholipid antibodies and (A) pregnancy test results, (B) viable pregnancy, and (C) live birth after assisted reproductive technology.

appeared to increase in homozygotes relative to heterozygotes suggesting a dose effect, the estimate for homozygotes was not significant and the confidence interval was wide. In addition, the 3 cohort studies addressing 2 different outcomes (ie, viable pregnancy and pregnancy test result) failed to show a relationship between the factor V Leiden and ART failures.[9,21,23] These studies were, however, highly underpowered and the observed and apparent protective effect of factor V Leiden may even be considered as a chance finding. Other inherited thrombophilia were not predictive of ART failure. Because not all studies tested for all major types of thrombophilia, it cannot be excluded that some women with ART failure were indeed carriers of one or more of the untested thrombophilia. This partial verification could have underestimated the risk associated with other inherited thrombophilia.

It has been suggested that anti-phospholipid antibodies are implicated in the development of pregnancy complications and that thromboprophylaxis may improve the pregnancy outcome in antibody-positive patients.[49] The association between anti-phospholipid antibodies and ART remains controversial, and the American Society for Reproductive Medicine advices against the routine assessment of anti-phospholipid antibodies among couples undergoing ART.[50] In a sample of > 5000 patients, we found a significant higher risk of ART failure among women positive for anti-phospholipid antibodies with the strength of the association

Table 3. Risk of assisted reproductive technology failure with individual anti-phospholipid antibodies

Antibody	No. of studies (no. of patients)	OR (95% CI)	I^2, % (*P*)
Lupus anticoagulant	12 (2013)	5.60(2.16-14.55)	0 (.80)
Anti-cardiolipin	15 (2685)	3.27(0.92-11.63)	76 (< .001)
Anti-phosphatidylserine	3 (820)	4.51(1.37-14.80)	0 (.46)
Anti-phosphatidylinositol	2 (408)	5.03(1.13-22.47)	0 (.82)
Anti-phosphatidic acid	1 (394)	4.65(1.10-19.74)	—
Anti-phosphatidylglycerol	1 (394)	2.77(1.06-7.20)	—
Anti–β_2-glycoprotein I	3 (574)	1.19(0.39-3.67)	—
Antiphosphatidylethanolamine	2 (408)	1.31(0.46-3.71)	70 (.27)

— indicates not applicable.

varying based on the type of antibody tested. These findings, although intriguing, need to be taken with caution for several reasons. A lower prevalence of thrombophilia might be expected in control groups composed only of women with previous uncomplicated pregnancies; thus, an overestimation of the association between thrombophilia and ART failure could have occurred in studies including such controls. However, we did not observe any differences based on the type of controls included. Additional possible sources of heterogeneity could be the lack of standardization of the assays, the variable panels of autoantibodies tested, and the inclusion of women with transiently positive results. Some authors have suggested that levels of anti-phospholipid antibodies may increase during IVF cycles,[29] although this has not been confirmed by others.[51] The time of blood withdrawing for antibody measurement was reported in only half of the studies; and in 41% of cases, it appeared performed away from the hormonal stimulation. Finally, but not lastly, anti-phospholipid antibodies were not associated with ART failure in cohort studies, which could, however, depend on a type II error. In a future cohort or database study, we estimated that 1300 women with anti-phospholipid antibodies and 8200 women without anti-phospholipid antibodies would have to be enrolled to demonstrate an increase of RR = of ~ 20% with 80% power, assuming a prevalence of anti-phospholipid antibodies of 16% and a background risk of pregnancy loss in ~ 0.2% (Figure 5C).

Meta-analyses have general limitations, including publication bias and variability of methodologic quality of the original studies. Although publication bias might threaten results, funnel plot evaluation did not show any asymmetry. Although our study included all women undergoing ART, it may be that the relevance of thrombophilia differs for those at the first versus third or more attempts. Moreover, a comprehensive risk assessment should consider other risk factors recognized to affect either success or failure rate of ART, including, for instance, age, parity, and previously successful pregnancy.[52-54] Poor reporting did not allow sensitivity analyses on the basis of these characteristics. Finally, the lack of reporting of coexisting thrombophilic defects did not allow an analysis of possible cumulative effects of thrombophilias.

For testing to be truly beneficial, effective treatment of carriers is needed. In an early pilot investigation of women undergoing ART and testing positive for anti-phospholipid antibodies, Sher et al suggested that combined treatment with aspirin and heparin increases the implantation and viable pregnancy rates.[10] These findings were confirmed by the same authors a few years later in a larger sample.[11] Both latter studies included only women positive for anti-phospholipid antibodies and lacked an adequate control group. More recently, in a randomized placebo-controlled clinical trial, the use of prophylactic-dose low-molecular-weight heparin was associated with higher implantation, pregnancy, and live birth rates in women with a history of 3 or more previous ART failures and at least one thrombophilic defect.[12] In another open-label randomized pilot trial, low-molecular-weight heparin thromboprophylaxis did not improve the live birth rates after ART in women with 2 or more ART failures without known thrombophilia.[12] The main implication of our results, if confirmed, is that antithrombotic therapy should not be currently administered in patients with ART failure and thrombophilia until further evidence becomes available.

In conclusion, the relationship between ART failure and thrombophilia remains largely inconclusive. Large prospective investigations are eagerly warranted to confirm this association before embarking in screening or intervention studies.

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Authorship

Contribution: M.D.N., A.W.S.R., and E.P. designed the research; M.D.N., N.F., and A.W.S.R. performed the search and collected data; and all authors analyzed and interpreted data and wrote the paper.

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