
# Effects of multiple inherited and acquired thrombophilia on outcomes of in-vitro fertilization

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PII: S0049-3848(18)30338-4
DOI: doi:10.1016/j.thromres.2018.05.006
Reference: TR 7027

To appear in: *Thrombosis Research*

Received date: 26 February 2018
Revised date: 24 April 2018
Accepted date: 6 May 2018

Please cite this article as: Di Nisio Marcello, Ponzano Adalisa, Tiboni Gianmario, Guglielmi Maria Domenica, Rutjes Anne Wilhelmina Saskia, Porreca Ettore , Effects of multiple inherited and acquired thrombophilia on outcomes of in-vitro fertilization. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Tr(2017), doi:10.1016/j.thromres.2018.05.006

**Effects of multiple inherited and acquired thrombophilia on outcomes of in-vitro fertilization**

Running title: Thrombophilia and in-vitro fertilization

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**Text word count (including Tables and Legends):** 3472

**Abstract word count:** 235

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**Abstract**

**Introduction:** The effects of multiple inherited and acquired thrombophilic defects on the outcome of in-vitro fertilization (IVF) remain unexplored. The aim of this study was to evaluate the association between multiple thrombophilia and clinical outcomes in a large prospective cohort of women undergoing IVF.

**Materials and Methods:** Consecutive women scheduled for IVF were eligible. The primary study outcome was live birth. Secondary outcomes included spontaneous abortion, clinical pregnancy, and symptomatic venous thromboembolism.

**Results:** 687 women with a mean age of 34.6 (±3.2) years were included. Overall, 22 women (3.2%) had two or more thrombophilic defects. The probability of live birth was not statistically significantly different between women with ≥2 thrombophilia (odds ratio [OR] 0.62; 95% confidence interval [CI], 0.18 to 2.11) or ≥1 thrombophilia (OR 0.67;95% CI, 0.41 to 1.09) and women without any thrombophilia. None of the individual inherited thrombophilia nor positivity to antiphospholipid antibodies or lupus anticoagulant were associated with live birth. Single positivity for lupus anticoagulant carried a more than threefold higher risk of abortion (OR 3.74; 95% CI, 1.30 to 10.75). There were no statistically significant associations between individual or multiple thrombophilic defects and clinical pregnancy or pregnancy test results. No woman had a history of venous thromboembolism and none developed a thrombotic event during the study.

**Conclusions:** In women undergoing IVF, the presence of two or more thrombophilic defects was rare and showed no statistically significant associations with IVF outcomes.

**Keywords:** assisted reproductive technique; thrombophilia; live birth; spontaneous abortion; prospective studies.

**Highlights**

- The presence of two or more thrombophilic defects was infrequent in women undergoing in-vitro fertilization
- The effects of multiple thrombophilic defects on outcomes of in-vitro fertilization remain unclear.
- Live birth was lower with one or more thrombophilia, albeit differences were not significant.

**Abbreviations**

CI = Confidence Intervals

IVF = In-Vitro Fertilization

OR = Odds Ratio

## Introduction

The failure rate of assisted reproductive techniques such as in-vitro fertilization (IVF) remains as high as 60-70% causing intense emotional distress in women undergoing these procedures [1-2]. The pathophysiology behind the high failure rate is largely unexplained and likely multifactorial [3]. One of the potential mechanisms includes the abnormal coagulation activation at the maternal–fetal interface leading to thrombosis of the placental vessels and secondary implantation or placentation failures [4]. In a previous systematic review on the association between thrombophilia and outcomes of assisted reproduction techniques, we summarized the evidence from 33 studies involving 6,093 patients and found that women with IVF failures tested more frequently positive for factor V Leiden and antiphospholipid antibodies [5]. However, these associations were only observed in case-control studies with a number of methodological limitations and were not confirmed in prospective cohorts. Since that meta-analysis, few other studies evaluated the potential influence of thrombophilia on the outcomes of IVF. In a large prospective cohort of 1,717 women undergoing fresh non-donor IVF cycles, none of the eight inherited thrombophilia seemed to predict clinical pregnancy, live birth, or pregnancy loss [6]. In a retrospective analysis of 594 women with unexplained infertility initiating IVF treatment, none of the thrombophilia tested were significantly associated with the number of IVF cycles nor with lower fertility success rate [7]. Unexpectedly, carriers of factor V Leiden and lupus anticoagulant had significantly higher live birth rates (12.3% and 12.6%, respectively) in comparison to women who tested negative (9.0% and 9.7%, respectively). These and previous observations focused on inherited thrombophilia or separately evaluated individual thrombophilic defects without assessing the potential effects of multiple inherited and acquired thrombophilia that remain therefore unexplored.

The aim of this study was to evaluate the association between multiple inherited and acquired thrombophilia and clinical outcomes in a large prospective cohort of women undergoing IVF.

## Materials and Methods

### *Study population*

Consecutive women scheduled for IVF were eligible for this study. Exclusion criteria were an ongoing or indication for anticoagulant treatment, thrombophilia screening not available before IVF, age ≥ 40 years, embryo transfer not performed, lack of informed consent. The study was approved by the local institutional review board and all women signed a written informed consent before study procedures. The study is registered in clinicaltrial.gov with accession number NCT02407730.

### *Study outcomes*

The primary study outcome was live birth. Secondary outcomes were spontaneous abortion, clinical pregnancy and symptomatic venous thromboembolic events occurring during the IVF procedure and, in case of pregnancy, during the gestation period up to 6 weeks post-partum. For descriptive purposes, we recorded positive pregnancy tests and pregnancy-related complications which included preeclampsia, placental abruption, and intrauterine growth retardation. Pregnancy test was performed by measuring β-human chorionic gonadotropin 14 days after the embryo transfer. A viable (clinical) pregnancy was defined as a pregnancy diagnosed initially by serum β-human chorionic gonadotropin levels with evidence of one or more gestational sacs at six weeks gestation. The clinical pregnancy rate was defined as the proportion of women achieving a clinical pregnancy out of the total number of women undergoing embryo transfer. The live birth rate was the number of deliveries divided by the total of women undergoing the procedure. The risk of abortion was defined as the risk of pregnancy loss out of all women who underwent the procedure. The study considered only the outcomes of fresh non-donor embryo transfers.

### *Study procedures*

All patients underwent controlled ovarian stimulation, follicle growth monitoring, and ovum pick-up as previously described [8]. All IVF procedures were performed by intracytoplasmic sperm injections. The

embryo transfer took place under ultrasound guidance about 72-76 hours after ovum pick-up. Up to three embryos were transferred in uterus and the number of embryos transferred reflected national guidelines, with some variation according to individual patient needs. The luteal phase was supported with daily intramuscular injections of 100 mg of progesterone (Prontogest, IBSA, Italy).

All women attending our University Center of Reproductive Medicine to undergo IVF have regular clinical visits to closely monitor controlled ovarian stimulation, need for gonadotropin dosage adjustments, and to identify any ensuing complications. As part of the clinical risk assessment, all women undergo thrombophilia testing before IVF, and consult an expert in thrombosis and haemostasis to decide on the potential indications for low-molecular-weight heparin according to the guidelines of the American College of Chest Physicians, which suggest antepartum thromboprophylaxis for women with a history of unprovoked, pregnancy- or estrogen-associated venous thromboembolic events or women with a family history of venous thromboembolism who are homozygous or compound heterozygote factor V Leiden and prothrombin gene mutation [9]. The panel of thrombophilia routinely tested included factor V Leiden, factor II mutation (G20210A), deficiency of protein C, protein S or antithrombin, hyperhomocysteneimia, lupus anticoagulant, anti-cardiolipin and anti-beta2 glycoprotein antibodies. To avoid any potential effect of hormonal stimulation on antiphospolipid antibody levels, blood for thrombophilia measurement was withdrawn and analyzed before IVF procedures [10-11]. All patients with positive lupus anticoagulant or antiphospolipid antibodies repeated testing after 12 weeks. Blood samples were collected in 3.8% trisodium citrate and centrifuged at 4000 g for 15 min to obtain platelet-poor plasma. We measured lupus anticoagulant, anticardiolipin and anti-beta2 glycoprotein antibodies (QUANTA Lite™, INOVA Diagnostics, San Diego, CA), antithrombin and protein C (Berichrom® Antithrombin and Berichrom® Protein C, SIEMENS, Germany), and free protein S antigen (INNOVANCE FREE PS Ag assay, SIEMENS, CT). DNA was extracted from peripheral blood leukocytes according to standard protocols. Factor V Leiden and prothrombin mutation genotyping were performed by a TaqMan® (Applied Biosystems, Foster City, CA) probe-based real time PCR technique.

*Data collection*

We collected information on demographics (maternal age, body mass index), comorbidities (e.g. prior venous thromboembolism or a family history of venous thromboembolism, cardiovascular disease), personal obstetric history, causes of infertility, prior IVF attempts, concomitant treatments, thrombophilia, results of pregnancy test. Venous thromboembolic events had to be objectively confirmed by standard diagnostic methods which included compression ultrasonography for deep vein thrombosis and computed tomography pulmonary angiography or lung scan for pulmonary embolism [12].

*Statistical considerations*

Data are reported as frequencies, mean (± standard deviations), and/or median (range). Categorical variables were analyzed with the chi-square test, and continuous variables with a Student t test or Mann–Whitney U test as appropriate. We assessed the association between study outcomes and multiple or individual inherited and acquired thrombophilia. Positivity for lupus anticoagulant or antiphospholipid antibodies that was not confirmed at repeat testing were considered in the analysis as thrombophilic defects because of their potential effects on IVF outcomes [5]. The effect of thrombophilia on primary and secondary outcomes was first evaluated in univariable analysis calculating odds ratio (OR) and the relative 95% confidence intervals (CIs). Other variables that were considered for their potential effect on study outcomes were age, body mass index ($kg/m^2$), smoking, cardiovascular disease, IVF indication, number of previous cycles, number of previous abortions, previous pregnancies either spontaneous or following intracytoplasmic sperm injections. All explaining variables significantly associated with the outcome at the 0.05 level in univariable models were included in multivariable logistic regression analyses. Explorative subgroup analysis was conducted for the primary study outcome in women with idiopathic infertility and women < 35 years. P-values of 0.05 (two tailed) were considered significant. The sample size was calculated based on a reported success rate of live birth using fresh embryo transfers of 35.5% [13]. We assumed that the prevalence of multiple inherited and acquired thrombophilia defined as two or more thrombophilic defects was about 10%. Assuming 40% live birth in women without any thrombophilia and a relative risk of at least 0.55, 715 women would need to be

included to reach 80% power at two-sided alpha level of 5%. Descriptive and analytical analyses were conducted using IBM SPSS version 19 (SSPS Inc., Chicago, IL, USA). Sample size calculations were done in STATA (StataCorp. 2013. Stata Statistical Software: Release 13. Texas, USA).

**Results**

From March 2015 to July 2017, a total of 1,008 eligible women were evaluated, of whom 321 were excluded because of an ongoing anticoagulant treatment with low-molecular-weight heparin for ovarian hyperstimulation syndrome (n = 22), IVF cancelled or treatment discontinued for any reason (n = 75), no thrombophilia available before IVF or patients refused measuring any thrombophilia (n = 93), age ≥ 40 years (n = 125), or more than one of above reasons (n = 1, Figure). Five additional patients moved to another IVF center after initial evaluation and were not accessible to follow-up. The final study population consisted of 687 women with a mean age of 34.6 (± 3.2) years. The most frequent indications for IVF were infertility due to tubaric (n = 153, 22%) or male (n = 197, 28.7%) factors, and idiopathic infertility (145, 21.1%). Four women had at least one first-degree family member with a history of venous thromboembolism while none had experienced past venous thrombotic events. Baseline demographic characteristics of study population are reported in Table 1.

*Thrombophilia*

The number of thrombophilic defects ranged from 0 to 4. A total of 537 (78.2%) women had no inherited or acquired thrombophilia, while the remaining patients had either one (n = 128, 18.6%), two (n = 17, 2.5%), three or more (5, 0.7%) thrombophilic defects. Overall, there were 149 women (22%) with at least one thrombophilia and 22 women (3.2%) with two or more thrombophilic defects. Excluding women with positivity for lupus anticoagulant or antiphospholipid antibodies that was not confirmed at repeat testing, only 16 women (2.3%) had two or more thrombophilia. The most prevalent inherited thrombophilia was factor V Leiden, which was detected in 34 of 624 women tested (5.5%). Deficiencies of protein S, protein C and antithrombin were less common (Table 2). The mean protein S, protein C and antithrombin levels were

89.4% (15.1), 96.5% (14.5), and 101.1% (21.1), respectively. The proportion of women with missing data on individual inherited thrombophilia ranged from 8.4% for protein S to 27% for antithrombin.

Lupus anticoagulant was detected in 20 of 597 (3.3%) patients of whom only two tested again positive at the second measurement performed 12 weeks later. Anticardiolipin antibodies were positive in 31 of 622 (5.0%) patients of whom four tested positive for both IgM and IgG antibodies, 12 for IgG and 15 for IgM antibodies alone. Only two patients (0.3%) had anticardiolipin levels that fulfilled antiphospholipid syndrome criteria (>40 GPL or MPL) and both were of IgM subtype [14]. The median titers of anticardiolipin IgG and IgM antibodies were 1.0 GPL/mL (0 - 32.0) and 1.0 MPL/mL (0 - 70), respectively. Anti-beta2 glycoprotein antibodies were positive in 12 of 467 (4.7%) patients of whom four were positive for both IgM and IgG, four for either IgM or IgG alone. Only three patients - all with IgM subtype - had levels >40 GPL or MPL (0.6%). The median titer of anti-beta2 glycoprotein antibodies was 1.0 U/mL (0 - 28.5) for IgG and 1.0 U/mL (0 - 96.2) for IgM. Only one patient with both anticardiolipin and anti-beta2 glycoprotein antibodies was again positive at the control 12-weeks later. Overall, fifty-seven women tested positive to either anticardiolipin antibodies, anti-beta2 glycoprotein antibodies or lupus anticoagulant and three of them had positivity confirmed at 12 weeks. The proportion of women with missing data ranged from 9.5% for anticardiolipin antibodies to 32% for anti-beta2 glycoprotein antibodies.

Of the four women with a family history of venous thromboembolism, three tested positive for one thrombophilic defect while the fourth had no thrombophilia.

*IVF outcomes*

Overall, 231 women (33.6%) had a positive pregnancy test and 208 (90%) obtained a clinical pregnancy for a calculated clinical pregnancy rate of 30% (Supplementary Table 1). Eventually, 138 women (20.1%) delivered 178 live children leading to a live birth rate of 26% (178/684). Sixty-four women (9.3%) had spontaneous abortion, two required a therapeutic abortion after the 12th week because of a severe genetic anomaly (trisomy 13 and 18), and a third patient voluntary interrupted gestation. Twenty-two patients had at least one pregnancy complication, which included preeclampsia (n = 5), placental abruption (n = 2), and

intrauterine growth retardation (n = 8). Seven women had an ectopic pregnancy. Three patients moved to another center after the IVF procedures and follow-up was not available.

None of the participating women received antepartum thromboprophylaxis, whereas post-partum low-molecular-weight heparin prophylaxis was administered to 89 patients (13%) who underwent caesarean section. There were no venous thromboembolic events during IVF procedures, gestation or the peripartum period. One patient developed superficial vein thrombosis of the forearm following parenteral iron infusion during gestation.

*Thrombophilia and IVF outcomes*

The odds of a live birth was not significantly different between women with ≥2 thrombophilia and women with no thrombophilic defects (OR 0.62; 95% CI, 0.18 to 2.11; Table 3). Similarly, there were no statistically significant associations of multiple thrombophilia with abortion (OR 1.56; 95% CI 0.45 to 5.41), clinical pregnancy (OR 0.86; 95% CI, 0.33 to 2.23), or positive pregnancy tests (OR 0.73; 95%CI, 0.28 to 1.90) (Supplementary Tables 2 to 4).

Compared to women with no thrombophilic defects, the odds of a live birth was not statistically significantly different in women with one or more thrombophilia (OR 0.67; 95% CI, 0.41 to 1.09). The presence of one or more thrombophilic defect seemed associated with a higher risk of abortion, whereas no association was observed with clinical pregnancy or positive pregnancy test (Supplementary Table 2 to 4).

The direction of the association estimates of individual thrombophilic defects were typically towards lower rates of live birth and higher risk of abortion compared to no thrombophilia, but these associations were not statistically significant (Table 3 and Supplementary Table 2). Women with lupus anticoagulant had a more than threefold higher risk of abortion compared to women without lupus anticoagulant (9.6% versus 2.8%, OR 3.74;95% CI, 1.30 to 10.75). There were no statistically significant associations between individual thrombophilia and clinical pregnancy or pregnancy test results, and direction of these associations varied (Supplementary Tables 3 and 4).

Age, body mass index, smoking, cardiovascular disease, indication for IVF, number of previous IVF cycles, history of abortion, or previous spontaneous or assisted pregnancies had no significant effect on live birth nor abortion. The odds of a positive pregnancy test was lower in older women with a 6% reduction for each additional year of age (OR 0.94; 95% CI, 0.90 to 0.99). Previous pregnancy after intracytoplasmic sperm injections carried higher chances of a positive pregnancy test result (OR 1.89; 95% CI, 1.10 to 3.23) and clinical pregnancy (OR 1.76; 95% CI, 1.02 to 3.03).

**Discussion**

In women undergoing IVF, the presence of two or more thrombophilic defects is rare. Our study did not detect statistically significant associations between multiple thrombophilia and live birth, abortion, clinical pregnancy, or positive pregnancy test.

A previous meta-analysis found an inconsistent association between thrombophilia and IVF outcomes [5]. While case-control studies suggested that infertile women tested more frequently positive for anti-phospholipid antibodies than fertile controls, cohort studies did not confirm a significant association between anti-phospholipid antibodies and live birth. Recent studies have also reported conflicting results. In a large prospective cohort of 1,717 women undergoing IVF, Patounakis and colleagues found that none of the eight inherited thrombophilia predicted IVF outcomes [6]. Antiphospholipid antibodies and lupus anticoagulant were not evaluated and about 16% of women received antithrombotic treatment. In a retrospective analysis of 594 women with unexplained infertility initiating IVF, positivity for factor V Leiden and lupus anticoagulant were unexpectedly associated with higher live birth rates [7]. Interestingly, three other studies showed a potential benefit of factor V Leiden mutation [15-17]. Along the same line, recent data suggested that the association between thrombophilia and recurrent pregnancy loss or pregnancy complications is weak and does not translate into large absolute increased risk of recurrent complications [18-23].

With the exception of lupus anticoagulant, which seemed to increase the odds of spontaneous abortion by more than threefold, none of the individual thrombophilia was statistically significantly

associated with IVF outcomes, although the direction of the associations typically suggested lower live birth and higher risk of abortion. The relatively low prevalence and the difficulty in obtaining adequate measurements of lupus anticoagulant in every laboratory remain major obstacles. There were no venous thromboembolic events during IVF or gestation, consistent with data from previous studies, which indicated no or very modest thrombotic risk with IVF [24-28].

Strengths of the current study include the prospective evaluation of multiple thrombophilia in a relatively large population undergoing standard IVF procedures. There are, however, some limitations that need to be acknowledged. Since not every woman got tested for all thrombophilia, reflecting a real-world scenario, we may have underestimated the risk associated with some thrombophilia. Partial testing of thrombophilia in the same individual was often related to the patient's decision to proceed to IVF avoiding further delays caused by laboratory testing. Our study lacked statistical power, because the prevalence of women with two or more thrombophilia and observed effects were smaller than anticipated. Although some of the observed associations may be due to chance, the odds of lower live birth and higher abortion rates are tenable. To avoid the confounding effect of age, we included women younger than 40 years and the current findings may not apply to older women in whom the role of thrombophilia, if any, remains to be elucidated. Early reports on the association between thrombophilia and outcomes of assisted reproductive techniques fostered the adoption of antepartum aspirin or low-molecular-weight heparin to increase pregnancy rates, despite limited and conflicting evidence to support their use [29-34]. Currently available evidence on the association between thrombophilia and IVF outcomes is very weak and questions the use of antithrombotic agents in women undergoing IVF.



**Conclusion**

The presence of two or more thrombophilic defects is uncommon in women undergoing IVF. Because of the imprecision in the estimates, the current study provides very weak evidence for an association between multiple thrombophilia and lower odds of live birth and higher risk of abortion. While larger studies may inform the debate about the effects of thrombophilia on IVF outcomes, the rarity of multiple thrombophilic defects questions their clinical relevance. Furthermore, the rare occurrence of multiple thrombophilia poses a major obstacle to perform an adequately powered study.

**Authors' roles**

Concept and design: MDN, GMT, EP. Interpretation of data, critical writing or revising the intellectual content, and final approval of the version to be published: MDN, AP, GMT, MDG, AWSR, EP.

**Acknowledgments**

None.

**Conflict of interest**

None of the authors have potential conflicts of interest to declare in relation to the current work.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

**References**


1. de Mouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, Korsak V, et al; European IVF-Monitoring (EIM); Consortium for the European Society on Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2007: results generated from European registers by ESHRE. Hum Reprod. 27 (2012) 954-966.
2. Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine. Assisted reproductive technology in the United States: 2000 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril. 81 (2004) 1207-1220.
3. Norwitz ER, Schust DJ, Fischer SJ. Implantation and survival of early pregnancy. N Engl J Med. 345 (2001) 1400-1408.
4. Hossain N, Paidas MJ. Adverse pregnancy outcomes, the uteroplacental interface, and preventative strategies. Seminars in Perinatology. 31 (2007) 209–212.
5. Di Nisio M, Rutjes AW, Ferrante N, Tiboni GM, Cuccurullo F, Porreca E. Thrombophilia and outcomes of assisted reproduction technologies: a systematic review and meta-analysis. Blood. 118 (2011) 2670-2678.
6. Patounakis G, Bergh E, Forman EJ, Tao X, Lonczak A, Franasiak JM, et al. Multiple thrombophilic single nucleotide polymorphisms lack a significant effect on outcomes in fresh IVF cycles: an analysis of 1717 patients. J Assist Reprod Genet. 33 (2016) 67-73.
7. Steinvil A, Raz R, Berliner S, Steinberg DM, Zeltser D, Levran D, et al. Association of common thrombophilias and antiphospholipid antibodies with success rate of in vitro fertilisation. Thromb Haemost. 108 (2012) 1192-1197.
8. Di Nisio M, Porreca E, Di Donato V, Tiboni GM. Plasma concentrations of D-dimer and outcome of in vitro fertilization. J Ovarian Res. 22 (2014) 7:58.
9. Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos A-M, Vandvik PO. VTE, thrombophilia, antithrombotic therapy, and pregnancy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 141 (2012) e691S-e736S.

10. Buckingham KL, Stone PR, Smith JF, Chamley LW. Antiphospholipid antibodies in serum and follicular fluid: is there a correlation with IVF implantation failure? Hum Reprod. 21 (2006) 728-734.
11. Fisch B, Rikover Y, Shohat L, Zurgil N, Tadir Y, Ovadia J, et al. The relationship between in vitro fertilization and naturally occurring antibodies: evidence for increased production of antiphospholipid antibodies. Fertil Steril. 56 (1991) 718-724.
12. Di Nisio M, van Es N, Büller HR. Deep vein thrombosis and pulmonary embolism. Lancet. 388 (2016) 3060-3073.
13. Dieamant FC, Petersen CG, Mauri AL, Comar V, Mattila M, Vagnini LD, et al. Fresh embryos versus freeze-all embryos - transfer strategies: Nuances of a meta-analysis. JBRA Assist Reprod. 21 (2017) 260-272.
14. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 4 (2006) 295–306.
15. Gopel W, Ludwig M, Junge AK, Kohlmann T, Diedrich K, Moller J. Selection pressure for the factor-V-Leiden mutation and embryo implantation. Lancet. 358 (2001) 1238–1239.
16. Rudick B, Su HI, Sammel MD, Kovalevsky G, Shaunik A, Barnhart K. Is factor V Leiden mutation a cause of in vitro fertilization failure? Fertil Steril. 92 (2009) 1256–1259.
17. van Mens TE, Joensen UN, Bochdanovits Z, Takizawa A, Peter J, Jørgensen N, et al. Factor V Leiden is associated with increased sperm count. Hum Reprod. 32 (2017) 2332-2339.
18. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet. 361 (2003) 901-908.
19. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med. 340 (1999) 9-13.
20. Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. Arch Intern Med. 164 (2004) 558-563.

21. Howley HE, Walker M, Rodger MA. A systematic review of the association between factor V Leiden or prothrombin gene variant and intrauterine growth restriction. Am J Obstet Gynecol. 192 (2005) 694–708.

22. Rodger MA, Betancourt MT, Clark P, Lindqvist PG, Dizon-Townson D, Said J, et al. The association of Factor V Leiden and prothrombin gene tuition and placenta-mediated pregnancy complications: A systematic review and meta-analysis of prospective cohort studies. PloS Medicine. 7 (2010) e1000292.

23. Rodger MA, Walker MC, Smith GN, Wells PS, Ramsay T, Langlois NJ, et al. Is thrombophilia associated with placenta-mediated pregnancy complications? A prospective cohort study. J Thromb Haemost. 12 (2014) 469–478.

24. Hansen AT, Kesmodel US, Juul S, Hvas AM. No evidence that assisted reproduction increases the risk of thrombosis: a Danish National cohort study. Human Reproduction. 27 (2012) 1499–1503.

25. Henriksson P, Westerlund E, Wallén H, Brandt L, Hovatta O, Ekbom A. Incidence of pulmonary and venous thromboembolism in pregnancies after in vitro fertilisation: cross sectional study. BMJ. 346 (2013) e8632. doi: 10.1136/bmj.e8632.

26. Sennström M, Rova K, Hellgren M, Hjertberg R, Nord E, Thurn L, et al. Thromboembolism and in vitro fertilization - a systematic review. Acta Obstet Gynecol Scand. 96 (2017) 1045-1052.

27. Chan W-S, Ginsberg JS. A review of upper extremity deep vein thrombosis in pregnancy: unmasking the 'ART' behind the clot. J Thromb Haemost. 4 (2006) 1673–1677.

28. Rao AK, Chitkara U, Milki AA. Subclavian vein thrombosis following IVF and ovarian hyperstimulation: a case report. Hum Reprod. 20 (2005) 3307–3312.

29. Dentali F, Grandone E, Rezoagli E, Ageno W. Efficacy of low molecular weight heparin in patients undergoing in vitro fertilization or intracytoplasmic sperm injection. J Thromb Haemost. 9 (2011) 2503–2506.

30. Dentali F, Ageno W, Rezoagli E, Rancan E, Squizzato A, Middeldorp S, et al. Low-dose aspirin for in vitro fertilization or intracytoplasmic sperm injection: a systematic review and a meta-analysis of the literature. J Thromb Haemost. 10 (2012) 2075–2085.

31. Sher G, Feinman M, Zouves C, Kuttner G, Maassarani G, Salem R, et al. High fecundity rates following in-vitro fertilization and embryo transfer in antiphospholipid antibody seropositive women treated with heparin and aspirin. Hum Reprod. 9 (1994) 2278-2283.
32. Sher G, Matzner W, Feinman M, Maassarani G, Zouves C, Chong P, et al. The selective use of heparin/aspirin therapy, alone or in combination with intravenous immunoglobulin G, in the management of antiphospholipid antibody positive women undergoing in vitro fertilization. Am J Reprod Immunol. 40 (1998) 74-82.
33. Qublan H, Amarin Z, Dabbas M, Farraj AE, Beni-Merei Z, Al-Akash H, et al. Low-molecular-weight heparin in the treatment of recurrent IVF-ET failure and thrombophilia: a prospective randomized placebo-controlled trial. Hum Fertil. 11 (2008) 246-253.
34. Urman B, Ata B, Yakin K, Alatas C, Aksoy S, Mercan R, et al. Luteal phase empirical low molecular weight heparin administration in patients with failed ICSI embryo transfer cycles: a randomized open-labeled pilot trial. Hum Reprod. 24 (2009) 1640-1647.



**Table 1. Baseline characteristics of study population**

| Characteristic | N = 687 |
|---|---|
| Age, years, mean (SD) | 34.6 (3.2) |

| | |
|---|---|
| Body mass index, kg/m$^2$, mean (SD) | 22.9 (4.1) |
| Smoking | |
| Current | 99 (14.4) |
| Previous | 11 (1.6) |
| Previous venous thromboembolism | 0 |
| Family history positive for venous thromboembolism | 4 (0.6) |
| Aspirin | 6 (0.9) |
| Indication for IVF | |
| Ovulatory | 59 (8.6) |
| Tubaric | 153 (22.3) |
| Endometriosis | 55 (8.0) |
| Male | 197 (28.7) |
| Unexplained | 145 (21.1) |
| Uterus | 12 (1.7) |
| Recurrent abortion | 5 (0.7) |
| Multiple | 61 (8.9) |
| Previous IVF cycles | |
| 0 | 417 (60.9) |
| 1 | 153 (22.3) |
| ≥2 | 115 (16.8) |
| Previous pregnancies | |
| Spontaneous | 61 (8.9) |
| Following ICSI | 60 (8.7) |
| Previous ectopic pregnancy | 35 (5.1) |
| Previous abortion | |
| Before week 12 | 31 (4.5) |
| After week 12 | 6 (0.9) |
| Polycistic ovarian syndrome | 16 (2.3) |
| Number of transferred grade A embryos | |
| 0 | 36 (5.2) |
| 1 | 95 (13.8) |
| ≥2 | 556 (80.9) |
| Number of transferred grade B embryos | |
| 0 | 510 (74.2) |
| 1 | 128 (18.6) |
| 2 or 3 | 49 (7.2) |
| Number of transferred grade C embryos | |
| 0 | 671 (97.7) |
| 1 | 12 (1.7) |
| 2 or 3 | 2 (0.2) |

Data are reported as number of patients (%) or mean (± standard deviations).

ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization

**Table 2. Inherited and acquired thrombophilia in study population**

| Type of thrombophilia | Patients | | |
|---|---|---|---|
| | Positive | Tested | % |
| *Inherited thrombophilia* | | | |
| Protein S deficiency | 15 | 629 | 2.4 |
| Protein C deficiency | 8 | 624 | 1.3 |
| Antithrombin deficiency | 4 | 502 | 0.8 |
| Hyperhomocysteneimia | 32 | 626 | 5.1 |
| Factor II mutation (G20210A) | 21 | 626 | 3.4 |
| Heterozygous | 21 | | |
| Homozygous | 0 | | |
| Factor V mutation | 34 | 624 | 5.5 |
| Heterozygous | 33 | | 5.3 |
| Homozygous | 1 | | 0.2 |
| *Acquired thrombophilia* | | | |
| Lupus anticoagulant | 20 | 597 | 3.4 |
| Anti-cardiolipin antibodies | 31 | 622 | 5.0 |
| IgM | 15 | | 2.4 |
| IgG | 12 | | 1.9 |
| IgM and IgG | 4 | | 0.7 |
| Sapporo criteria | | | |
| IgM | 2 | | 0.3 |
| IgG | 0 | | 0 |
| Anti-beta2 glycoprotein antibodies | 12 | 467 | 2.6 |
| IgM | 4 | | 0.8 |
| IgG | 4 | | 0.8 |
| IgM and IgG | 4 | | 0.8 |
| Sapporo criteria | | | |
| IgM | 3 | | 0.6 |
| IgG | 0 | | 0 |
| Lupus anticoagulant, anti-cardiolipin or anti-beta2 glycoprotein antibodies | 57 | 655 | 8.7 |
| Single positive | 51 | | 7.8 |
| Double positive | 6 | | 0.9 |
| Triple positive | 0 | | 0 |

**Table 3. Association between live birth and multiple or individual thrombophilia**

| Thrombophilia | Live birth | | OR (95% CI) |
|---|---|---|---|
| | No | Yes | |
| Multiple thrombophilia (≥2) | 19/546 | 3/138 | 0.62 (0.18 to 2.11) |
| Any thrombophilia (≥1) | 126/546 | 23/138 | 0.67 (0.41 to 1.09) |
| Protein S deficiency | 11/506 | 4/121 | 1.54 (0.48 to 4.92) |
| Protein C deficiency | 7/499 | 1/121 | 0.59 (0.07 to 4.81) |
| Hyperhomocysteneimia | 28/498 | 4/126 | 0.55 (0.19 to 1.60) |
| Prothrombin mutation | 19/502 | 2/122 | 0.42 (0.10 to 1.84) |
| Factor V Leiden | 27/504 | 6/118 | 0.92 (0.38 to 2.21) |
| Antithrombin deficiency | 4/394 | 0/105 | NA |
| Positivity to Lupus anticoagulant | 16/477 | 4/118 | 1.01 (0.33 to 3.08) |
| Positivity to Anti-cardiolipin antibodies | 28/499 | 3/120 | 0.43 (0.13 to 1.44) |
| Positivity to Anti-beta2 glycoprotein antibodies | 12/362 | 0/104 | NA |
| Positivity to lupus anticoagulant, Anti-cardiolipin antibodies or beta2 glycoprotein antibodies | 50/524 | 7/128 | 0.54 (0.25 to 1.15) |

CI = confidence intervals; ; OR = odds ratio

**Highlights**

- The presence of two or more thrombophilic defects was infrequent in women undergoing in-vitro fertilization
- The effects of multiple thrombophilic defects on outcomes of in-vitro fertilization remain unclear.
- Live birth was lower with one or more thrombophilia, albeit differences were not significant.

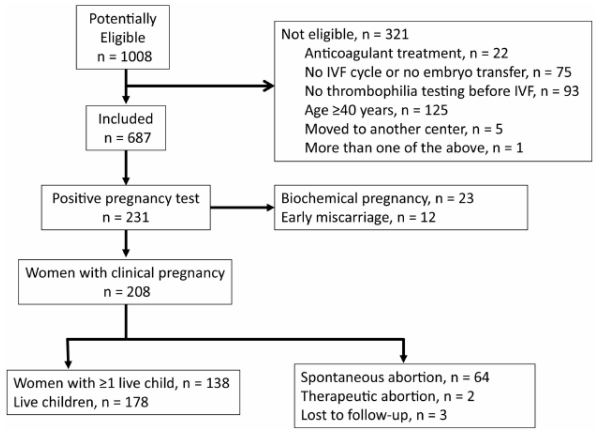
Potentially Eligible n = 1008
Not eligible, n = 321
Anticoagulant treatment, n = 22
No IVF cycle or no embryo transfer, n = 75
No thrombophilia testing before IVF, n = 93
Age ≥40 years, n = 125
Moved to another center, n = 5
More than one of the above, n = 1
Included n = 687
Positive pregnancy test n = 231
Biochemical pregnancy, n = 23
Early miscarriage, n = 12
Women with clinical pregnancy n = 208
Women with ≥1 live child, n = 138
Live children, n = 178
Spontaneous abortion, n = 64
Therapeutic abortion, n = 2
Lost to follow-up, n = 3

Figure 1