

Association between Thrombophilia and Repeated Assisted Reproductive Technology Failures

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ABSTRACT

Purpose: This study was performed to investigate the incidence of thrombophilic gene mutations in repeated assisted reproductive technology (ART) failures. **Methods:** The prevalence of mutated genes in the patients with a history of three or more previous ART failures was compared with the patients with a history of successful pregnancy following ARTs. The study group included 70 patients, 34 with three or more previously failed ARTs (A) and control group consisted of 36 patients with successful pregnancy following ARTs (B). All patients were tested for the presence of mutated thrombophilic genes including factor V Leiden (FVL), Methylenetetrahydrofolate reductase (MTHFR) and Prothrombin (G20210A) using real-time polymerase chain reaction (RT-PCR). **Results:** Mutation of FVL gene was detected in 5.9% women of group A (2 of 34) compared with 2.8% women (1 of 36) of control group ($P = 0.6$). Mutation of MTHFR gene was found in 35.3% (12 cases) as compared with 50% (18 cases) of control (35.3% versus 50%; $P = 0.23$). Regarding Prothrombin, only control group had 5.6% mutation ($P = 0.49$). No significant differences were detected in the incidences of FVL, Prothrombin and MTHFR in the study group A compared with the control group B. **Conclusion:** The obtained results suggest that thrombophilia does not have a significant effect in ART failures.

Introduction

Infertility affects couples ranged from 3.5% to 16.7% in developed countries and from 6.9% to 9.3% in developing nations.¹ There are many known causes such as tubal factor, unovulation, uterine abnormality, male factors as well as unexplained reasons which make infertile couples are not able to conceive naturally. Assisted reproductive technologies (ARTs) provide hopes for these couples; however the average pregnancy rate following ARTs despite transfers of good-quality embryos is 30-40%^{2,3} and the main cause might be an implantation failure.^{4,5}

Association between inherited thrombophilia and unexplained infertility reported before.⁶⁻⁹ Both acquired and inherited thrombophilic gene mutations can be accepted as risk factors for recurrent pregnancy loss.¹⁰⁻¹⁵ However the effect of thrombophilia on implantation failure is a controversial issue and there are conflicting results about this observation.^{12,16-21} Findings regarding the positive effects of heparin therapy in women with recurrent IVF-embryo transfer (ET) failure and caring thrombophilic defect²²⁻²⁴ make association between

thrombophilia and reproductive failure to be investigated further.

The present study was carried out to compare the prevalence of three thrombophilic gene mutations in patients with a history of three or more implantation failures with patients with successful pregnancy following ARTs (IVF/intracytoplasmic sperm injection (ICSI) – ET, IVF/ICSI-ET) cycles at a Fertility Centre of AL-Zahra Hospital of Tabriz University, Tabriz, Iran.

Materials and Methods

This study was carried out in the AL-Zahra Hospital, Fertility Centre of Tabriz University, Tabriz and Iran. Patients underwent IVF/ICSI-ET were contacted by phone and invited voluntarily without any cost to take part in this study and no intervention has been made to select patients. The participants completed the consent form according to the Ethical Committee of Tabriz University of Medical Sciences. A total of 70 infertile women, aged 20-35, underwent ART cycles, based on

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inclusion criteria were divided into two groups: thirty four patients with a history of 3 or more unsuccessful ARTs (group A) and thirty six patients with a history of successful ARTs (group B) verified by positive β HCG as well as clinical pregnancy proven using transvaginal ultrasonography. Inclusion criteria included male factor, unexplained infertility, infertile females with tubular factors, lack of ovulation, ovaries reserve test result of FSH and estradiol in the normal range and lack of uterine cavity abnormality, endometriosis and hydrosalpinges as well as embryo grades of A or B for transfer.

All infertile participants had undergone ARTs according to clinical and laboratory protocols. Embryos had been scored as grade 1-5. Transferred embryos in all cases were grades A and B. The serum β HCG level had been measured two weeks after embryo transfer.

In addition, we collected the following information from women participating in this study: age, type of infertility treatment protocol, a history of previous successful pregnancies, a history of systemic diseases, education and body mass index (BMI).

To show association between thrombophilia and repeated IVF/ICSI-ET failure cycles, mutations of factor V gene (V Leiden), methylenetetrahydrofolate reductase C677T enzyme gene (MTHFR) and Prothrombin or coagulation factor II (G20210A) gene were investigated in the patients of group A and B. For this aim, three millilitres of peripheral venous blood from each patient were collected in a tube including EDTA as anticoagulant under sterile conditions. EDTA-anticoagulant blood samples were centrifuged by 2500 rpm and transferred to 1.5 ml sterile tubes using RNase and DNase free tips. Genomic DNA was extracted from buffy coat section of blood using the QIAamp DNA blood Mini kit (50) from QIAGEN (Cat no. 51304) according to the manufacturer's instructions. The quantity of genomic DNA was measured by Nano-drop (Thermo Scientific). Extracted genomic DNA was stored in -80 for analysing. Factor V Leiden, prothrombin G20210A and MTHFR C677T polymorphisms were tested with real-time PCR using a Rotor-Gene 6000 (Corbett Life Science, Mortlake, Australia) and analysed using Rotor-Gene 6000 Series Software version 1.7.61 (Corbett Life Science, Australia).

The prevalence of thrombophilic mutations was assessed in the infertile women undergoing ARTs in both groups, A and B. The embryological data of the patients undergoing ARTs were analysed per patient basis.

Statistical analysis

Statistical analysis was performed using by SPSS™ 15 software. Data was expressed as mean \pm SD and also frequency and percentage and analyzed using Samples T-test and the Chi-Square statistics or Fisher's exact test, as appropriate. In all investigated cases, the results were statistically significant in case of $P \leq 0.05$.

Results

Seventy infertile couples, divided in two groups of group A (with unsuccessful pregnancy) and group B, which their pregnancy verified by positive β HCG as well as clinical pregnancy proven using transvaginal ultrasonography, did not have statically significant differences regarding, age, a history of previous successful pregnancy, type of infertility treatment protocol, education; however BMI was significantly lower in group (B) with successful pregnancy ($p=0.02$) than that of the other group (A). The clinical characteristics of both groups are summarized in Table 1.

In both groups, mutations in three inherited thrombophilia genes: FVL, MTHFR and Prothrombin factor were investigated and the results of two groups were compared. Heterozygous Factor V Leiden mutation was found in two patients (5.9%) of group A and one patient (2.8%) of group B. There were no statically significant difference between these two groups ($p = 0.6$). Regarding MTHFR gene mutation, there were 12 cases (35.3%) in group A, two cases with homozygous genotypes and ten patients with heterozygous mutation and in group B, there were 18 cases (50%): 17 patients with heterozygous genotypes and 1 with homozygous mutation. There were no significant differences between these two groups ($p = 0.23$). Regarding Prothrombin gene mutation, there was not any case in group A, but in group B there were 2 cases (5.6%) with heterozygous genotype. There were no statically significant differences between the two groups ($p = 0.49$), as well (Figure 1).

Discussion

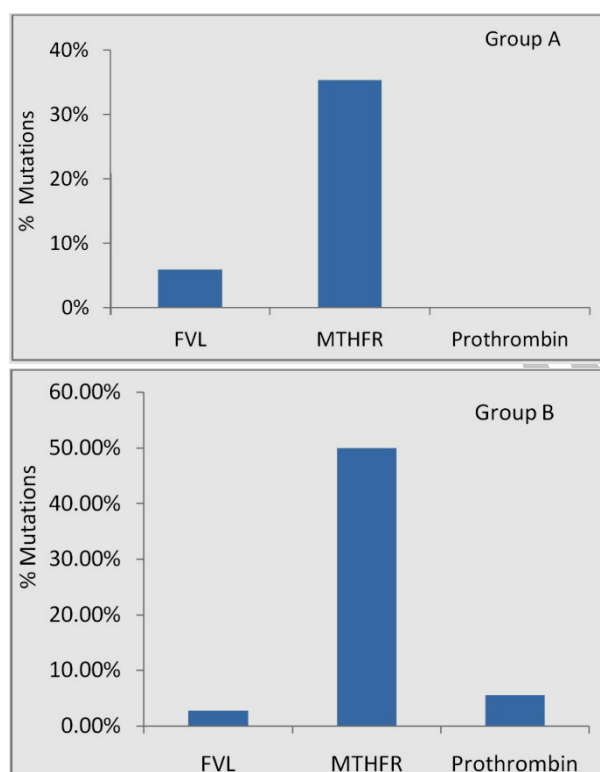
This paper describes the incidence of thrombophilic gene mutations of FVL, MTHFR and Prothrombin in patients with 3 or more IVF/ICSI-ET failure cycles compared with patients with successful IVF/ICSI-ET. The results revealed that there are no statically significant differences among women of two groups regarding these three mutated genes. In addition, in our study, the patient who had a combination of heterozygous MTHFR and FVL mutations and the other who had a combination of heterozygous MTHFR and Prothrombin mutations resulted in successful implantation. Moreover, two patients with homozygous MTHFR mutations had successful pregnancy.

These findings support the study carried out by Simur and coworkers²⁵ and Martinelli and coworkers²⁰ which reported no association between inherited thrombophilia and embryo implantation failures and Casadei and coworkers⁷ which mentioned it might be thrombophilia is not a risk factor for infertility; however several studies have shown an association between inherited thrombophilia and repeated IVF failures.^{12,19,21,26} The reason might be that there are other factors such as sample size and a familial history of prior thrombosis affecting implantation.

Table 1. Clinical characteristics of the patients in each group.

		Group A (34)	Group B (36)	P value
Age	20 - 25 years	3 cases (8.8%)	4 cases (11.1%)	0.16
	25 – 30 years	19 cases (55.9%)	12 cases (32.3%)	
	30 – 35 years	12 cases (35.3%)	20 cases (55.6%)	
Mean BMI (kg/m ²)		26.16 ± 3.10	24.44 ± 3.15	0.02
A history of systemic diseases		2 cases (6%)	2 cases (5.5%)	
A history of previous successful pregnancy		1 case (3%)	2 cases (5.5%)	0.55
Type of infertility treatment protocol	Agonist protocol (Long protocol)	32 cases (94%)	35 cases (97%)	0.55
	Antagonist and short protocol	2 cases (6%)	1 case (3%)	
Education	Under diploma	24 cases (70.6%)	18 cases (50%)	0.15
	High education	10 cases (29.4%)	18 cases (50%)	

BMI: Body Mass Index

**Figure 1.** The percentage of mutations of thrombophilic genes: FVL, MTHFR, and Prothrombin in Group A (unsuccessful pregnancy) and Group B (successful pregnancy).

FVL mutation is the most common thrombophilia²⁷ and the rate of preeclampsia syndrome is high in women with FVL mutation.²⁸ Di Nisio and coworkers⁴ reported association between FVL mutation and repeated ART failures. The results of our study showed that the prevalence of FVL mutation was higher in patients with the repeated IVF/ICSI failures (5.9%) than that of the group B (2.8%); however this difference was not statically significant.

There are factors such as age, personal and/or family history of systemic diseases, number of antral follicles before stimulation, basal hormonal levels, previous pregnancy, endometrial thickness, embryo grading, ET technique and length and position of the uterus²⁹⁻³⁴ which influence success or failure rate of IVF/ICSI-ET, perhaps affect the patients with thrombophilia, thus thrombophilia might not be a solely reason for the repeated ART failures.

Thrombophilia has been associated with poor obstetrical outcomes, local micro- thrombosis of maternal vessels at implantation site may affect syncytiotrophoblast invasion and might be resulted in implantation failure; however due to the development of intervillous space at the end of first trimester of gestation, it is possible that thrombophilia has less effects on implantation failure than that of other risk factors.

In summary, our findings suggest that there is not a relationship between solely thrombophilia and repeated implantation failures. However, implantation is a complex process and further research in large samples of infertile women might be needed.

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Conflict of interest

The authors report no conflicts of interest.

References

- Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for

- infertility medical care. *Hum Reprod* 2007;22(6):1506-12.
2. Kuperman A, Micco PD, Brenner B. Fertility, infertility and thrombophilia. *Women Health* 2011;7(5):545-53.
 3. Margalioth EJ, Ben-Chetrit A, Gal M, Eldar-Geva T. Investigation and treatment of repeated implantation failure following IVF-ET. *Hum Reprod* 2006;21(12):3036-43.
 4. Di Nisio M, Rutjes AWS, Ferrante N, Tiboni GM, Cuccurullo F, Porreca E. Thrombophilia and outcomes of assisted reproduction technologies: a systematic review and meta-analysis. *Blood* 2011;118(10):2670-8.
 5. Sharif KW, Ghunaim S. Management of 273 cases of recurrent implantation failure: results of a combined evidence-based protocol. *Reprod Biomed Online* 2010;21(3):373-80.
 6. Fatini C, Conti L, Turillazzi V, et al. Unexplained infertility: Association with inherited thrombophilia. *Thromb Res* 2012;129(5):185-8.
 7. Casadei L, Puca F, Privitera L, Zamaro V, Emidi E. Inherited thrombophilia in infertile women: implication in unexplained infertility. *Fertil Steril* 2010;94(2):755-7.
 8. Bianca S, Barrano B, Cutuli N, et al. Unexplained infertility and inherited thrombophilia. *Fertil Steril* 2009;92(1):4.
 9. Coulam CB, Jeyendran RS. Thrombophilic gene polymorphisms are risk factors for unexplained infertility. *Fertil Steril* 2009;91(4):1516-7.
 10. Torabi R, Zarei S, Zeraati H, et al. Combination of Thrombophilic Gene Polymorphisms as a Cause of Increased the Risk of Recurrent Pregnancy Loss. *J Reprod Infert* 2012;13(2):89-94.
 11. Yokus O, Balçık OS, Albayrak M, et al. Thrombophilic risk factors in women with recurrent abortion. *J Clin Exp Invest* 2010;1(3):168-72.
 12. Coulam BC, Jeyendran R, Fishel LA, Roussev R. Multiple thrombophilic gene mutations are risk factors for implantation failure. *Reprod Biomed Online* 2006;12(3):322-327.
 13. Kovalevsky GG. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: A meta-analysis. *Arch Intern Med* 2004;164(5):558-63.
 14. Finan RR, Tamim H, Ameen G, Sharida HE, Rashid M, Almawi WY. Prevalence of factor V G1691A (factor V-Leiden) and prothrombin G20210A gene mutations in a recurrent miscarriage population. *Am J Hematol* 2002;71(4):300-5.
 15. Foka ZJ, Lambropoulos AF, Saravelos H, et al. Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. *Hum Reprod* 2000;15(2):458-62.
 16. Bellver J, Soares SR, Alvarez C, et al. The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. *Hum Reprod* 2008;23(2):278-84.
 17. Ricci G, Bogatti P, Fischer-Tamaro L, et al. Factor V Leiden and prothrombin gene G20210A mutation and in vitro fertilization: prospective cohort study. *Hum Reprod* 2011;26(11):3068-77.
 18. Qublan HS, Eid SS, Ababneh HA, et al. Acquired and inherited thrombophilia: implication in recurrent IVF and embryo transfer failure. *Hum Reprod* 2006;21(10):2694-8.
 19. Azem F, Many A, Yovel I, Amit A, Lessing JB, Kupfermanc MJ. Increased rates of thrombophilia in women with repeated IVF failures. *Hum Reprod* 2004;19(2):368-70.
 20. Martinelli I, Taioli E, Ragni G, et al. Embryo implantation after assisted reproductive procedures and maternal thrombophilia. *Haematologica* 2003;88(7):789-93.
 21. Grandone E, Colaizzo D, Lo Bue A, Checchia MG, Cittadini E, Margaglione M. Inherited thrombophilia and in vitro fertilization implantation failure. *Fertil Steril* 2001;76(1):201-2.
 22. Lodigiani C, Di Micco P, Ferrazzi P, et al. Low-molecular-weight heparin in women with repeated implantation failure. *Womens Health (Lond Engl)* 2011;7(4):425-31.
 23. Qublan H, Amarin Z, Dabbas M, et al. Low-molecular-weight heparin in the treatment of recurrent IVF-ET failure and thrombophilia: A prospective randomized placebo-controlled trial. *Hum Fertil (Camb)* 2008;11(4):246-53.
 24. Nelson SM, Greer IA. The potential role of heparin in assisted conception. *Hum Reprod Update* 2008;14(6):623-45.
 25. Simur A, Ozdemir S, Acar H, et al. Repeated in vitro fertilization failure and its relation with thrombophilia. *Gynecol Obstet Invest* 2009;67(2):109-12.
 26. Bare SN, Poka R, Balogh I, Ajzner E. Factor V Leiden as a risk factor for miscarriage and reduced fertility. *Aust N Z J Obstet Gynaecol* 2000;40(2):186-90.
 27. Ridker PM, Miletich JP, Buring JE, et al. Factor V Leiden Mutation as a Risk Factor for Recurrent Pregnancy Loss. *Ann Intern Med* 1998;128:1000-3.
 28. Karimi S, Yavarian M, Azinfar A, Rajaei M, Kootenaee MA. Evaluation the frequency of factor V Leiden mutation in pregnant women with preeclampsia syndrome in an Iranian population. *Iran J Reprod Med* 2012;10(1):59-66.
 29. Qublan HS, Malkawi HY, Tahat YA, et al. In-vitro fertilisation treatment: Factors affecting its results and outcome. *J Obstet Gynaecol* 2005;25(7):689-93.
 30. Kovacs P, Matyas S, Boda K, Kaali SG. The effect of endometrial thickness on IVF/ICSI outcome. *Hum Reprod* 2003;18(11):2337-41.
 31. Akande VA, Fleming CF, Hunt LP, Keay SD, Jenkins JM. Biological versus chronological ageing of oocytes, distinguishable by raised FSH levels in

- relation to the success of IVF treatment. *Hum Reprod* 2002;17(8):2003-8.
32. Schoolcraft WB, Surrey ES, Gardner DK. Embryo transfer: techniques and variables affecting success. *Fertil Steril* 2001;76(5):863-70.
33. Egbase PE, Al-Sharhan M, Grudzinskas JG. Influence of position and length of uterus on implantation and clinical pregnancy rates in IVF and embryo transfer treatment cycles. *Hum Reprod* 2000;15(9):1943-6.
34. Templeton A, Morris JK, Parslow W. Factors that affect outcome of in-vitro fertilisation treatment. *Lancet* 1996;348(9039):1402-6.

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