Original Article

Combination of Thrombophilic Gene Polymorphisms as a Cause of Increased the Risk of Recurrent Pregnancy Loss

Raheleh Torabi [1], Saeed Zarei [2], Hojjat Zeraati [3], Amir Hassan Zarnani [4, 5], Mohammad Mehdi Akhondi [6], Reza Hadavi [2], Elham Savadi Shiraz [6], Mahmood Jeddi-Tehrani [2*]

1- Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
2- Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3- Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4- Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
5- Immunology Research Center, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
6- Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Abstract

Background: Recurrent pregnancy loss is (RPL) a heterogeneous condition. While the role of acquired thrombophilia has been accepted as an etiology for RPL, the contribution of specific inherited thrombophilic gene polymorphisms to the disorder has been remained controversial.

Methods: One hundred women with a history of two or more consecutive abortions and 100 women with at least two live births and no miscarriages were included in the study and evaluated for the presence of 11 thrombophilic gene polymorphisms (Factor V LEIDEN, Factor V 4070 A/G, Factor V 5279 A/G, Factor XIII 103 G/T, Factor XIII 614 A/T, Factor XIII 1694 C/T, PAI-1 -675 4G/5G, ITGB3 1565 T/C, β-Fibrinogen -455G/A, MTHFR 677 C/T, MTHFR 1298 A/C) using PCR-RFLP technique. The data were statistically analyzed using Mann-Whitney test and logistic regression model.

Results: There was no relation between factor XIII 103G/T gene polymorphism with increased risk of RPL. However, the other 10 gene polymorphisms were found to be associated with increased/decreased risk of RPL. Multiple logistic regression model for analyzing the simultaneous effects of these polymorphisms on the risk of RPL showed that six of these 11 polymorphisms (Factor V 1691G/A, Factor V 5279A/G, Factor XIII 614A/T, β-Fibrinogen -455G/A, ITGB3 1565T/C, and MTHFR 1298A/C) were associated with RPL.

Conclusion: It is possible to calculate the risk of abortion in a patient with RPL by determining only six of the 10 polymorphisms that are individually associated with RPL.

** Corresponding Author:*
Mahmood Jeddi-Tehrani, Department of Immunochemistry, Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, P.O. Box: 19835-1177, Daneshjo Blvd., Velenjak, Tehran, Iran
E-mail:
mahjed@yahoo.com

Received: Dec. 13, 2011
Accepted: Feb. 18, 2012

Keywords: PCR-RFLP, Recurrent pregnancy loss, Thrombophilic gene.
To cite this article: Torabi R, Zarei S, Zeraati H, Zarnani AH, Akhondi MA, Hadavi R, et al. Combination of Thrombophilic Gene Polymorphisms as a Cause of Increased the Risk of Recurrent Pregnancy Loss. J Reprod Infertil. 2012;13(2):89-94.

Introduction

Inherited thrombophilia is a risk factor for pregnancies leading to reproductive disorders and recurrent pregnancy loss (RPL). A number of thrombophilic gene polymorphisms that are suspected to associate with RPL, have been reported (1).

The most common polymorphism studied for association with RPL is Factor V (1q23) (FV) Leiden (2). FV Leiden may cause thrombosis by decreasing the sensitivity of FV to inactivation by activated protein C, leading to increased generation of thrombin. Activated protein C resistance (APCR) is the inability of protein C to cleave Factor Va, which allows for longer duration of

thrombin degeneration and may lead to a hypercoagulable state (3). There are many studies that have confirmed the association of this polymorphism with RPL, although there are other reports that challenge the idea (2).

In addition to FV Leiden, there are two other FV polymorphisms that are candidates for increasing the risk of RPL: FV 4070A/G, FV 5279A/G. These two polymorphisms are associated with lowered FV activity and concentration (4) and may associate with APCR (5). In addition there are some reports that correlate these polymorphisms with FV Leiden (6, 7).

Factor XIII (A subunit (6p25-p24), and B subunit (1q31-q32.1)) is also an important coagulation factor (6). FXIII deficiency results in serious bleeding complications, inefficient wound healing and a high risk of miscarriage in deficient women (8).

The most common polymorphism in FXIII gene is FXIII 103G/T variant that may modify FXIII activity. This polymorphism may interfere with fibrin cross-linking and regulation of fibrinolysis and may, therefore, contribute to early pregnancy loss (9).

Other polymorphic sites related to FXIII activity are FXIII 614A/T and FXIII 1694C/T polymorphisms which have been associated with decreased plasma factor XIII concentrations and lower activity rates, making these FXIII variants candidates for the pathogenesis of thrombotic disorders (8, 10–12). Some investigators have found an association between the FXIII 614A/T polymorphism with RPL (8), but others have not (10). However, the role of FXIII 1694C/T variant in RPL has previously been assessed in only a few articles that could not strongly confirm this association (10).

Two common polymorphisms of methylenetetrahydrofolate reductase (1p36.3) (MTHFR), the MTHFR 677C/T and MTHFR 1298A/C, result in the most common forms of hyperhomocystinemia and increased thrombotic tendency. In the presence of these two polymorphisms the activity of the encoded enzyme is reduced, although the decrease in the enzyme activity in the case of MTHFR 1298A/C allele is less than that of the other polymorphism (13). Although most of studies show the association of these two polymorphisms with RPL (14–18), their compound heterozygosity in women with RPL has not been investigated thoroughly (19).

Deficiencies in fibrin stabilization and fibrinolysis are other risk factors for thrombosis associated with RPL. One of the most important polymorphisms related to these deficiencies is a single nucleotide deletion/insertion in the 675 nucleotide upstream of plasminogen activator inhibitor 1 (7q21.3-q22) (PAI-1) promoter which produces two alleles containing either four (4G) or five (5G) consecutive guanosine nucleotides (PAI-1 -675 4G/5G), resulting in unbalanced fibrin deposition. In successful implantation, PAI-1 controls maternal tissue during trophoblast invasion and its polymorphism leads to insufficient trophoblast invasion (20).

Beta-fibrinogen (Ensembl: 4q31.3) (BF)−455 G/A promoter region polymorphism is associated with 7–10% higher plasma fibrinogen levels and it could enhance concentration driven enzyme-substrate interactions between thrombin, fibrinogen, and platelets, and thus result in increased intravascular fibrin deposition followed by promotion of placental thrombosis that may lead to increased risk of RPL (21).

A 1565T/C transition in exon 2 of the integrin beta 3 (Ensembl: 17q21.32) (ITGB3) gene which is associated with conformational changes, also increases platelet aggregability resulting in intervillous or spiral artery thrombosis and inadequate placental perfusion (22–24).

In the present study, we compared the frequencies of FV Leiden, FV 4070A/G, FV 5279A/G, FXIII 103G/T, FXIII 614A/T ,FXIII 1694C/T, BF -455G/A, PAI-1-4G/5G, ITGB3 1565T/C, MTHFR 677C/T, and MTHFR 1298A/C polymorphisms and their combinations in patients with RPL and the control group.

Methods

Participants: One hundred women with at least two recurrent pregnancy losses, as the case group and 100 healthy women with at least two successful deliveries and no miscarriages, as the control group were included in the study. For women with RPL, anatomic, hormonal and chromosomal abnormalities, as well as antiphospholipid antibodies, were ruled out. The pregnancy losses had happened before the 20th week of gestation based on the last menstrual period. The study was approved by the Ethics Committee for Medical Research at Avicenna Research Institute and written consent was obtained from all participant.

Blood Sample Collection and Isolation of Genomic

DNA: Blood samples (5 *ml*) were obtained from the participants in tubes containing EDTA and genomic DNA was extracted using salting out method.

Genotype Analysis: Polymerase chain reaction (PCR) amplification of DNA samples was performed using FV Leiden, FV 4070A/G, FV 5279 A/G, FXIII 103G/T, FXIII 614A/T , FXIII 1694C/T, BF -455G/A, PAI-1 -4G/5G, ITGB3 1565T/C, MTHFR 677C/T, and MTHFR 1298A/C polymorphism specific primers (7, 25, 26). PCR products were then confirmed by electrophoresis on 1.5% agarose gel. PCR product variants were then digested by specific restriction enzymes, and analyzed on 1.5% agarose- or Poly Acrylamide-Gel Electrophoresis (PAGE) (7, 25, 26).

Statistical Analysis: Genotype distributions of each polymorphism among different groups were compared by Mann–Whitney test. The homozygote and heterozygote groups were unified and a new group, namely, composed of those with the polymorphism was created. Multiple logistic regression was used for calculating the odds ratios (ORs) as a measure of risk for RPL in association with the presence of each of the polymorphisms. Moreover, multiple logistic regression model was used to analyze the simultaneous effects of the polymorphisms on the risk for RPL. For controlling interactions, we computed measure of agreement (Kappa), and used McNemar test. We considered probable collinearity in regression models, and the p <0.05 was considered significant.

Results

Factor V polymorphisms: The frequencies of FV Leiden polymorphism in the case and control groups were 13% and 4%, respectively (Table1). The frequencies of FV 4070A/G and FV 5279A/G were 14% and 37% in the case and 4% and 7% in the control groups, respectively. Statistical analysis showed significant difference between the case and control groups in these polymorphisms (p <0.05), (Table 1).

Factor XIII polymorphisms: The frequency of FXIII 103G/T polymorphism was 29% in the case group and 17% in the control group, (p >0.05), (Table 1). On the other hand, the frequencies of FXIII 614A/T and FXIII 1694C/T were 84% and 68% in the case and 46% and 31% in the control groups, respectively (p <0.001), (Table 1). The latter two polymorphisms were also found to be associated with an increased risk for RPL.

MTHFR polymorphisms: The proportions for homozygous and heterozygous polymorphisms of MTHFR 677C/T were 15% and 42% in the case group and 9% and 25% in the control group, respectively (Table 1). The frequencies of heterozygous and homozygous polymorphisms of MTHFR 1298A/C in the case group were 27% and 4%, and in the control group 6% heterozygosity and no homozygosity were seen. There were also significant (p <0.001) differences between the case and the control groups for MTHFR 677C/T (p <0.01) and MTHFR 1298A/C polymorphisms (Table 1).

PAI-1-675 4G/5G: Thirty-one patients (31%) with heterozygous polymorphism of PAI-1 -675 4G/5G gene and 9 patients (9%) with its homozygous polymorphism were seen in the case group while the frequencies in the control group consistent of 27 women (27%) with heterozygous and one (1%) with homozygous polymorphisms of the gene (Table 1); the difference between the groups was

Table 1. Prevalence of the 11 thrombophilic polymorphisms in the case and control groups

Polymorphism	Case			Control			P-value *
	Homozygote %	Heterozygote %	Normal %	Homozygote %	Heterozygote %	Normal %	
FV LEIDEN	1	12	87	0	4	96	<0.05
FV 4070 A/G	2	12	86	0	4	96	<0.05
FV 5279 A/G	1	36	63	0	7	93	<0.001
FXIII 103 G/T	4	25	71	2	15	83	>0.05
FXIII 614 A/T	66	18	16	30	16	54	<0.001
FXIII 1694 C/T	12	56	24	10	21	69	<0.001
PAI-1 -675 4G/5G	9	31	60	1	27	72	<0.05
ITGB3 1565 T/C	17	1	82	15	27	58	<0.05
BF -455 G/A	3	33	64	1	11	88	<0.001
MTHFR 677 C/T	15	42	43	9	25	66	<0.01
MTHFR 1298 A/C	4	27	69	0	6	94	<0.001

* The p-value was derived from Mann-Whitney test for homozygote, heterozygote and normal genotypes

Table 2. Multiple logistic regression models used for analyzing the simultaneous effect of polymorphisms on the risk of RPL

Polymorphism	P-value	OR	95.0% C.I. for OR	
			Lower	Upper
FV LEIDEN	0.016	6.920	1.443	33.177
FV 5279 A/G	<0.001	6.503	2.323	18.206
FXIII 614 A/T	<0.001	6.267	2.756	14.249
ITGB3 1565 T/C	<0.001	0.127	0.051	0.319
BF -455 G/A	0.001	5.213	1.970	13.798
MTHFR 1298 A/C	0.001	7.147	2.313	22.084

statistically significant ($p < 0.05$).

BF -455G/A: In the case group, the frequencies for the heterozygous and homozygous polymorphisms of BF -455 G/A were 33% and 3%, respectively, while in the control group, the frequencies for the heterozygous and homozygous polymorphisms were 11% and 1%, respectively (Table 1). The two groups were also significantly different in this regard ($p < 0.001$).

ITGB3 1565T/C: The ITGB3 1565T/C genotype distribution significantly differed between the case and control groups, However, the variant genotypes were more frequent in the control group ($p < 0.001$), (Table 1). Considering the more frequent variant genotypes of ITGB3 1565T/C in the control group, it seems to be unrelated to an increased risk for RPL or it may have a protective effect against.

Multiple logistic regression: Using multiple logistic regression model for analyzing the simultaneous effect of the polymorphisms on the risk for RPL, it was shown that among the eleven polymorphisms, only six including FV Leiden (OR: 6.920, 95% CI for OR: 1.443–33.177), FV 5279 A/G (OR: 6.503, 95% CI for OR: 2.323–18.206), FXIII 614 A/T (OR: 6.267, 95% CI for OR: 2.756–14.249), BF -455 G/A (OR: 5.213, 95% CI for OR: 1.970–13.798), ITGB3 1565 T/C (OR: 0.127, 95% CI for OR: 0.051–0.319) and MTHFR 1298 A/C (OR: 7.147, 95% CI for OR: 2.313–22.084), remained in the model (Table 2). Considering the aforementioned odds ratios, five of these polymorphisms tended to increase the risk of RPL considerably, while ITGB3 1565 T/C polymorphism seemed to have a protective role against RPL (OR: 0.127).

Discussion

RPL is a multifactorial syndrome which seems to increase in frequency by thrombophilic gene polymorphisms (2). We carried out multiple analyses to find combinations of polymorphisms that could lead to an increased risk for RPL.

Hohlagschwandtner et al. were the first to simultaneously investigate on the frequency and the interrelations of the MTHFR 677 C/T, MTHFR 1298A/C, FV Leiden, factor II prothrombin 20210 G/A, ITGB3 1565T/C and APO B R3500Q polymorphisms in a Caucasian population with a history of RPL. They fell short of showing any association between either the examined polymorphisms and RPL or a significant impact of the interrelations of these polymorphisms on RPL (27).

Sotiriadis et al. compared the prevalence of five common thrombophilic polymorphisms (FV Leiden, FV 4070 A/G, Factor II prothrombin 20210 G/A, MTHFR 677C/T and MTHFR 1298 A/C) and their combinations in women with recurrent miscarriage and a control group. They reported that combinations of all or some of the five thrombophilic polymorphisms were uncommon events with heterogeneous patterns without significantly increasing the risk for miscarriage (28).

By prospective evaluation of the prevalence of three common thrombophilic polymorphisms; FV Leiden, MTHFR 677C/T and factor II prothrombin 20210 G/A in 76 women with apparently unexplained fetal loss and 106 healthy controls, Brenner et al. showed that combinations of these polymorphisms increased the risk of fetal loss. They also detected combined thrombophilic polymorphisms in 6 (8%) of the 76 patients compared to 1 (1%) in 106 controls (OR=9.0, 95% CI: 1.1–76, p=0.02) (29).

Sarig et al. also found thrombophilia in the majority (66%) of women with idiopathic pregnancy loss. They also reported that combined thrombophilia was a frequent finding in women with pregnancy loss and concluded that thrombophilia was associated with increased frequency of late pregnancy wastage (30).

Coulam et al. compared the prevalence of 10 thrombophilic gene polymorphisms (FV Leiden, FV 4070A/G, FV 5279A/G, Factor II prothrombin 20210 G/A, FXIII 103G/T, BF-453G/A, PAI-1 4G/5G, ITGB3 1565T/C, MTHFR 677C/T, MTHFR 1298A/C) in 75 patients with a history of recurrent miscarriage with 14 fertile control women and found no differences between the two groups. However, the prevalence of total gene

polymorphisms among patients with recurrent miscarriage was significantly higher than that of the controls. They also reported that more than three gene polymorphisms, among the 10 studied genes, existed in 68% of women with recurrent miscarriage and in 21% of the controls (Relative risk: 0.4073, 95% CI 0.2653–0.6253, p <0.001) (31).

In addition, Goodman et al. reported that women with a history of RPL demonstrated significantly higher numbers of polymorphism than the controls (p <0. 001) (32).

Our work is unique in that we applied a multiple logistic regression model for analyzing the simultaneous effects of 11 polymorphisms on the risk for RPL. This kind of analysis facilitates the evaluation of the risk for RPL due to thrombophilic gene polymorphisms by genotyping the six genes reported in this study. For instance, if any of two polymorphisms, homozygote or heterozygote, is detected in a woman, the OR for the increased risk of RPL will be calculated by multiplying the OR of the individual genes. For example, a woman with an OR of 44.79 for a polymorphism in MTHFR 1298A/C and FXIII 614 A/T will have a 44.79-fold increase in the risk for RPL (Table 2).

Conclusion

In conclusion, the data presented in this study suggests the importance of determining six thrombophilic gene polymorphisms that are most effective in increasing the risk of RPL in women experiencing recurrent abortion losses.

Acknowledgement

The authors are grateful to women who accepted to enter this study. We are indebted to the personnel of Avicenna Infertility Clinic for their assistance in the sample collection.

This work was supported by a grant (86–47) from Avicenna Research Institut, ACECR.

References

1. Coulam CB, Jeyendran RS, Fishel LA, Roussev R. Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. Am J Reprod Immunol. 2006;55(5): 360-8.
2. Robertson L, Wu O, Langhorne P, Twaddle S, Clark P, Lowe GD, et al. Thrombophilia in pregnancy: a systematic review. Br J Haematol. 2006;132(2):171-96.
3. Seligsohn U, Lubetsky A. Genetic susceptibility to venous thrombosis. N Engl J Med. 2001;344(16): 1222-31.
4. Lunghi B, Iacoviello L, Gemmati D, Dilasio MG, Castoldi E, Pinotti M, et al. Detection of new polymorphic markers in the factor V gene: association with factor V levels in plasma. Thromb Haemost. 1996;75(1):45-8.
5. Bernardi F, Faioni EM, Castoldi E, Lunghi B, Castaman G, Sacchi E, et al. A factor V genetic component differing from factor V R506Q contributes to the activated protein C resistance phenotype. Blood. 1997;90(4):1552-7.
6. Folsom AR, Cushman M, Tsai MY, Aleksic N, Heckbert SR, Boland LL, et al. A prospective study of venous thromboembolism in relation to factor V Leiden and related factors. Blood. 2002;99(8):2720-5.
7. Jeddi-Tehrani M, Torabi R, Zarnani AH, Mohammadzadeh A, Arefi S, Zeraati H, et al. Analysis of plasminogen activator inhibitor-1, integrin beta3, Beta fibrinogen, and methylenetetrahydrofolate reductase polymorphisms in Iranian women with recurrent pregnancy loss. Am J Reprod Immunol. 2011;66(2):149-56.
8. Anwar R, Gallivan L, Edmonds SD, Markham AF. Genotype/phenotype correlations for coagulation factor XIII: specific normal polymorphisms are associated with high or low factor XIII specific activity. Blood. 1999;93(3):897-905.
9. Dossenbach-Glaninger A, van Trotsenburg M, Dossenbach M, Oberkanins C, Moritz A, Krugluger W, et al. Plasminogen activator inhibitor 1 4G/5G polymorphism and coagulation factor XIII Val34Leu polymorphism: impaired fibrinolysis and early pregnancy loss. Clin Chem. 2003;49(7):1081-6.
10. Barbosa HC, Carvalho EC, Barini R, Siqueira LH, Costa DS, Annichino-Bizzacchi JM. Tyr204Phe and Val34Leu polymorphisms in two Brazilian ethnic groups and in patients with recurrent miscarriages. Fertil Steril. 2004;82(5):1455-7.
11. Gallivan L, Markham AF, Anwar R. The Leu564 factor XIIIA variant results in significantly lower plasma factor XIII levels than the Pro564 variant. Thromb Haemost. 1999;82(4):1368-70.
12. Kohler HP, Futers TS, Grant PJ. Prevalence of three common polymorphisms in the A-subunit gene of factor XIII in patients with coronary artery disease. Thromb Haemost. 1999;81(4):511-5.
13. van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-

tube defects? Am J Hum Genet. 1998;62(5):1044-51.

14. Altomare I, Adler A, Aledort LM. The 5, 10 methylenetetrahydrofolate reductase C677T mutation and risk of fetal loss: a case series and review of the literature. Thromb J. 2007;5:17.
15. Ciacci C, Tortora R, Scudiero O, Di Fiore R, Salvatore F, Castaldo G. Early pregnancy loss in celiac women: The role of genetic markers of thrombophilia. Dig Liver Dis. 2009;41(10):717-20.
16. Govindaiah V, Naushad SM, Prabhakara K, Krishna PC, Radha Rama Devi A. Association of parental hyperhomocysteinemia and C677T Methylene tetrahydrofolate reductase (MTHFR) polymorphism with recurrent pregnancy loss. Clin Biochem. 2009;42(4-5):380-6.
17. Ivanov P, Kovacheva K, Komsa-Penkova R, Konova E, Simeonova M, Popov I, et al. [Genetic variant C677T in the MTHFR in women with recurrent early fetal loss]. Akush Ginekol (Sofiia). 2007;46 (4):19-22. Bulgarian.
18. Altmäe S, Stavreus-Evers A, Ruiz JR, Laanpere M, Syvänen T, Yngve A, et al. Variations in folate pathway genes are associated with unexplained female infertility. Fertil Steril. 2010;94(1):130-7.
19. Gebhardt GS, Scholtz CL, Hillermann R, Odendaal HJ. Combined heterozygosity for methylenetetrahydrofolate reductase (MTHFR) mutations C677T and A1298C is associated with abruptio placentae but not with intrauterine growth restriction. Eur J Obstet Gynecol Reprod Biol. 2001;97(2):174-7.
20. Dossenbach-Glaninger A, van Trotsenburg M, Dossenbach M, Oberkanins C, Moritz A, Krugluger W, et al. Plasminogen activator inhibitor 1 4G/5G polymorphism and coagulation factor XIII Val34Leu polymorphism: impaired fibrinolysis and early pregnancy loss. Clin Chem. 2003;49(7):1081-6.
21. Kerlin B, Cooley BC, Isermann BH, Hernandez I, Sood R, Zogg M, et al. Cause-effect relation between hyperfibrinogenemia and vascular disease. Blood. 2004;103(5):1728-34.
22. Newman PJ, Derbes RS, Aster RH. The human platelet alloantigens, PlA1 and PlA2, are associated with a leucine33/proline33 amino acid polymorphism in membrane glycoprotein IIIa, and are distinguishable by DNA typing. J Clin Invest. 1989; 83(5):1778-81.
23. Ruzzi L, Ciarafoni I, Silvestri L, Semeraro ML, Abeni D. Association of PLA2 polymorphism of the ITGB3 gene with early fetal loss. Fertil Steril. 2005;83(2):511-2.
24. Yilmaz S, Bayan K, Tüzün Y, Batun S, Altintaş A. A comprehensive analysis of 12 thrombophilic mutations and related parameters in patients with inflammatory bowel disease: data from Turkey. J Thromb Thrombolysis. 2006;22(3):205-12.
25. Jeddi-Tehrani M, Torabi R, Mohammadzadeh A, Arefi S, Keramatipour M, Zeraati H, et al. Investigating association of three polymorphisms of coagulation factor XIII and recurrent pregnancy loss. Am J Reprod Immunol. 2010;64(3):212-7.
26. Torabi R, Ostad Karampour M, Mohammadzadeh A, Arefi S, Keramatipour M, Zarei S, et al. The Relationship between Polymorphisms of Blood Coagulation Factor V Gene and Recurrent Pregnancy Losses. J Reprod Infertil. 2009;9(4):305-16.
27. Hohlagschwandtner M, Unfried G, Heinze G, Huber JC, Nagele F, Tempfer C. Combined thrombophilic polymorphisms in women with idiopathic recurrent miscarriage. Fertil Steril. 2003;79(5):1141-8.
28. Sotiriadis A, Vartholomatos G, Pavlou M, Kolaitis N, Dova L, Stefos T, et al. Combined thrombophilic mutations in women with unexplained recurrent miscarriage. Am J Reprod Immunol. 2007;57 (2):133-41.
29. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N. Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Haemost. 1999;82(1):6-9.
30. Sarig G, Younis JS, Hoffman R, Lanir N, Blumenfeld Z, Brenner B. Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. Fertil Steril. 2002;77(2):342-7.
31. Coulam CB, Jeyendran RS, Fishel LA, Roussev R. Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. Am J Reprod Immunol. 2006;55 (5):360-8.
32. Goodman CS, Coulam CB, Jeyendran RS, Acosta VA, Roussev R. Which thrombophilic gene mutations are risk factors for recurrent pregnancy loss? Am J Reprod Immunol. 2006;56(4):230-6.