British Journal of Medicine & Medical Research
5(5): 626-632, 2015, Article no.BJMMR.2015.067
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

Thrombophilic Mutations and Folate Gene Polymorphisms and Plasminogen Activator Inhibitor-1 in Russian Women with Unexplained Recurrent Early Spontaneous Abortion

Tatiana Ye. Belokrinitskaya[1*], Nataly I. Frolova[1], Nataliya N. Strambovskaya[2] and Anton A. Petrov[3]

[1]Department of Obstetrics and Gynaecology, Chita State Medical Academy, Chita, Russia.
[2]Molecular-Genetic Laboratory, Chita State Medical Academy, Chita, Russia.
[3]Department of Normal Physiology, Chita State Medical Academy, Chita, Russia.

Authors' contributions

This work was carried out in collaboration between all authors. Author TYB designed the study, performed statistical analyses and wrote the first draft of the manuscript. Author NIF maintained the study database and performed statistical analyses. Authors NNS and AAP performed genetic examinations. Author NNS made critical revisions to the manuscript. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/BJMMR/2015/12980
Editor(s):
(1) Jimmy T. Efird, Department of Public Health, Epidemiology and Outcomes Research East Carolina Heart Institute, Brody School of Medicine, Greenville, North Carolina, USA.
Reviewers:
(1) Viviane Alessandra Capelluppi Tófano, Clinical Dept., Marília University and Marília Medicine Faculty (FAMEMA), Brazil.
(2) Mariano Martin-Loeches de la Lastra, Human Reproduction, Hospital Universitario San Carlos, Denia (Alicante), Spain.
(3) Anonymous, Rinehart Center for Reproductive Medicine, Evanston, IL, USA.
(4) Anonymous, Sanjay Gandhi Post Graduate Institute, India.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=672&id=12&aid=6277

Original Research Article

Received 26th July 2014
Accepted 11th September 2014
Published 30th September 2014

ABSTRACT

Aims: To assess the association between FVL G1691A, FII G20210A, MTHFR A1298C, C677T and PAI-1 5G/675/4G gene polymorphisms among women with unexplained recurrent early spontaneous abortion (URESA)
Materials and Methods: This study included two groups of Russian women: 50 currently non-pregnant women with a history of 2-5 unexplained recurrent early spontaneous abortion and unknown causes of miscarriages (URESA group), and 50 currently non-pregnant women with a

**Corresponding author: Email: tanbell24@mail.ru;*

history of having given birth to at least one live baby and without a history of spontaneous abortion, prematurity, stillbirth, eclampsia and other pregnancy complications (control group). Gene polymorphisms were detected by the technique of polymerase chain reaction-real time (PCR-RT). We have analyzed the frequencies, $\chi 2$ test, odds ratio (OR) and its 95% confidence interval (CI), Hardy-Weinberg equilibrium.

Results: Significant association between heterozygotes genotype FVL 1691G/A and URESA was found (OR=3.1). Heterozygous genotype FII20210G/A was associated mainly with recurrent spontaneous abortions (4% vs 0%). The PAI-1 5G/4G genotype was significantly associated with URESA (OR=2.3). Heterozygotes with MTHFR 677C/T genotype had high risk of early recurrent pregnancy loss (OR=4.6). Heterozygotes with MTHFR 1298A/C genotype showed low association with pregnancy loss (OR=1.2). We did not observe increased risk of early pregnancy loss in mutant homozygotes with MTHFR 677C/C and 1298C/C genotypes (OR=1.0). The presence of the PAI-1 gene 5G/4G genotype together with the MTHFR 677C/T or MTHFR 1298A/C or FVL 1691G/A genotypes was found to be a risk factor for URESA (OR=4.5; OR=2.3, respectively). Combined PAI-1 5G/4G// FVL 1691G/A genotypes was detected only in patients (2% vs 0%). Women carrying combined PAI-1 5G/4G//MTHFR 677C/T//MTHFR 1298A/C genotypes had an increased frequency of recurrent early spontaneous abortion (OR=1.4).

Conclusion: The genetic polymorphisms of FVL 1691G/A, FII 20210G/A, MTHFR 677C/T, MTHFR 1298A/C, and PAI-1 4G/4G, and the PAI-1 5G/4G genotypes are associated with URESA. The patients carrying combined heterozygous genotypes PAI-1 5G/4G//MTHFR 677C/T or PAI-1 5G/4G//MTHFR 1298A/C or PAI-1 5G/4G//FVL 1691G/A or PAI-1 5G/4G//MTHFR 677C/T//MTHFR 1298A/C have higher risks of URESA. These results might indicate a genetic influence on pathogenesis of URESA.

Keywords: Thrombophilic mutations; folate gene polymorphisms; plasminogen activator inhibitor-1; recurrent early spontaneous abortion.

1. INTRODUCTION

Factor V Leiden (FVL G1691A), prothrombin (FII G20210A) and methylenetetrahydrofolate reductase (MTHFR C677T) gene mutations are commonly inherited thrombophilias. The presence of mutations that promote thrombophilia in the genes responsible for folate metabolism and for plasma coagulation is often associated with pregnancy failure and may be the underlying cause in some cases. Incidentally, the FVL G1691A and FII G20210A gene mutations are associated mainly with recurrent spontaneous abortions [1-3].

Folic acid is essential for normal embryogenesis and the physiological course of pregnancy. The MTHFR gene C677T polymorphism and the MTRR gene A66G polymorphism are associated with early and late miscarriages [1-3].

Plasminogen activator inhibitor-1 (PAI-1) is important for maintaining pregnancy. Aberrantly increased PAI-1 levels may contribute to thrombosis and inflammation, leading to pregnancy loss [4]. The association between PAI-1 5G/675/4G gene polymorphism and early pregnancy loss has been reported but with controversial results [4-6].

Objective of this study was to assess the association between FVL G1691A, FII G20210A, MTHFR A1298C, C677T and PAI-1 5G/675/4G gene polymorphisms among women with unexplained recurrent early spontaneous abortion (URESA).

2. MATERIALS AND METHODS

The present study was approved by the Ethical Committee of the Chita State Medical Academy (Chita, Russia, Protocol No 44 of 19.11.2012). All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

This small pilot study included two groups of Russian women: 50 currently non-pregnant women with a history of 2-5 unexplained recurrent early spontaneous abortion (URESA group), and 50 currently non-pregnant women with a history of having given birth to at least one live baby and without a history of spontaneous miscarriages early spontaneous abortions, prematurity, stillbirth, eclampsia and other pregnancy complications (control group). The patients with the antiphospholipid syndrome,

genital infections, genital inflammatory diseases, infective agents and TORCH-infections, uterine abnormalities, endocrine disorders and other verified cause factors of recurrent miscarriages were excluded from this study [7]. Median age of URESA group women was 31.0±3.3 years (range 21-35 years), and 31.3±2.9 years (range 21-35 years) in healthy controls (p> 0,05).

2.1 Genetic Analysis

Peripheral venous blood from patients and controls was collected in standard collection tubes containing Ethylene-Diamine-Tetra-Acetic acid (EDTA) as the anticoagulant. Genomic DNA was isolated from peripheral blood leukocyte with a standard express-extraction method using the PREP-RAPID-GENETICS DNA Extraction Kit ("DNA-Technology", Moscow, Russia). The extracted DNA was stored at 4ºC until analysis. The Taq-AT-polymerase was stored at -20ºC during the storage period. Gene polymorphisms were detected by the technique of Real-time polymerase chain reaction followed by melting curve analysis (DTlite-96 Real-Time PCR System, "DNA-Technology", Moscow, Russia). Genomic DNA was obtained using a PREP-RAPID-GENETICS kits (Thrombophilic Risk Real-Time SNP genotyping Kit, Folate Metabolism REAL-TIME PCR genotyping Kit, "DNA-Technology", Moscow, Russia), according to the manufacturer's instructions [8].

2.2 Statistical Analysis

Differences between the clinical and control groups were assessed by the $\chi 2$ test and the Fisher's exact test. Genotypes for the tested groups and the controls were assessed for departures from the Hardy-Weinberg equilibrium ($G^2+2GA+A^2=1$). For various genotypes and haplotypes, an odds ratio (OR) and its 95% confidence interval (95% CI) were calculated.

3. RESULTS AND DISCUSSION

3.1 Results

The distribution of FVL G1691A, FII G20210A, PAI-1 5G/4G, MTHFR C677T and MTHFR A1298C genotypes in comparison groups are shown in Table 1.

FVL G1691A and FII G20210A SNP-mutations were not found either in patients or in controls. The frequency of normal homozygotes genotype FVL1691G/G was significantly higher in both groups than heterozygotes genotype FVL1691G/A (98% and 94%, 2% and 6%, respectively, p >0.05).

All of controls had normal homozygotes genotype FII20210G/G. Of the patients, 96% were GG homozygous (p>0.05) and 4% were GA heterozygous (p>0.05) carriers of FII G20210A genotype.

Table 1. The distribution of FVL G1691A, FII G20210A, PAI-1 5G/4G, MTHFR C677T and A1298C genotypes in comparison groups

Genotype	URESA-group[a] (n=50)	Controls[a] (n=50)	χ2	*p* Value[b]
FVL G1691A				
GG	47 (94%)	49 (98%)	0.26	0.6098
GA	3 (6%)	1 (2%)	0.26	0.6098
AA	0 (0%)	0 (0%)		
G	97(0,97)	98(0,98)		
A	3 (0,03)	2 (0,02)	0.00	1.000
FII G20210A				
GG	48 (96%)	50 (100%)	0.51	0.4751
GA	2 (4%)	0 (0%)	0.51	0.4751
AA	0 (0%)	0 (0%)		
G	98(0,98)	100 (1,00)		
A	2(0,02)	0,00	0.51	0.4773
PAI-1 5G/4G				
5G/5G	1 (2%)	11 (22%)	7.67	0.0056
5G/4G	36 (72%)	16 (32%)	14.46	0.0001
4G/4G	13 (26%)	23 (46%)	3.52	0.0608
5G	38 (0,38)	38 (0,38)		
4G	62 (0,62)	62 (0,62)	0.00	1.000
MTHFR C677T				
CC	17 (34%)	22 (44%)	0.67	0.4122
CT	31 (62%)	23 (46%)	1.97	0.1602
TT	2 (4%)	5 (10%)	0.61	0.4331
C	65 (0,65)	67 (0,67)		
T	35 (0,35)	33 (0,33)	0.02	0.8813
MTHFR C1298A				
AA	23 (46%)	26 (52%)	0.16	0.6891
AC	23 (46%)	20 (40%)	0.16	0.6862
CC	4 (8%)	4 (8%)	0.00	1.000
A	69 (0,69)	62 (0,62)		
C	31 (0,31)	28 (0,28)	0.02	0.8883

a Hardy-Weinberg equilibrium, b Fisher's exact T-test

The frequency of heterozygous PAI-1 5G/4G genotype was significantly higher in URESA group than in normal controls (72% and 32%; $\chi 2=14.46$; p=0.0001).

The frequencies of normal MTHFR 677C/C genotype for the patients and controls are 34% and 44%, respectively (p>0.05).There were no significant differences in genotype frequencies for heterozygous MTHFR 677C/T genotype (62% vs 46%, p>0.05) and mutant homozygous

MTHFR 677T/T genotype (4% vs 10%, p>0.05) in both groups.

The overall frequencies of normal MTHFR 1298A/A genotype for the patients and controls are 46% and 52%, respectively (p>0.05). The allele and genotype frequencies of the A1298C mutation did not differ significantly between comparison group. Heterozygous MTHFR 1298 A/C genotype was determined in 46% and 40%, respectively (p>0.05). The mutant alleles with the MTHFR 1298C/C genotype were found significantly less frequently when compared with the MTHFR 1298A/A and the MTHFR 1298A/C genotypes – in 8% of the cases (p>0.05).

As shown in Table 2, significant association between heterozygotes genotype FVL 1691G/A and URESA was found (OR=3.1). Heterozygous genotype FII 20210G/A was associated mainly with recurrent spontaneous abortions (4% vs 0%). The PAI-1 5G/4G genotype was associated with URESA (OR=2.3). We did not observe increased risk of early pregnancy loss in mutant homozygotes with MTHFR 677C/C and MTHFR 1298C/C genotypes (OR=1.0). Heterozygotes with MTHFR 677C/T genotype had high risk of early recurrent pregnancy loss (OR=4.6). Heterozygotes with MTHFR 1298A/C genotype showed low association with pregnancy loss (OR=1.2).

In addition, the presence of the PAI-1 gene 5G/4G genotype together with the MTHFR 677C/T or MTHFR 1298A/C or FVL 1691G/A genotypes was found to be a risk factor for URESA (OR=4.5; OR=2.3, respectively). Combined PAI-1 5G/4G// FVL 1691G/A genotypes was detected only in patients (2% vs 0%). Women carrying combined PAI-1 5G/4G//MTHFR 677C/T//MTHFR 1298A/C genotypes had an increased frequency of recurrent early spontaneous abortion (OR=1.4).

3.2 Discussion

The presence of the FVL G1691A and FII G20210A gene mutations are associated mainly with recurrent spontaneous miscarriages [9-11]. In our study, FVL G1691A and FII G20210A SNP-mutations were not found either in patients or in controls. Probably, these gene mutations are rare in Russian women, and have no significance in URESA. Our data has indicated statistically significant associations between heterozygotes genotypes FVL 1691G/A and FII 20210G/A and URESA. Souza SS et al. in 1999 raised the possibility that heterozygous with FVL 1691G/A and FII 20210G/A genotypes had increased risk of recurrent miscarriage [12].

We also found a significant association between the PAI-1 5G/4G genotype and URESA. These observations are in contrast with a previous report on the literature. Jeddi-Tehrani M et al. concluded, that homozygosity but not heterozygosity for PAI-1 4G/5G polymorphism was significantly higher in patients with recurrent pregnancy loss [13]. Guan LX et al. also had shown, that high risk of recurrent early spontaneous abortion was associated with the PAI-1 4G/4G genotype (OR = 4.8) [5]. According to the results of present study and findings of Parveen F et al. [6], PAI-1 mutations showed no significance on URESA. This difference may be explained by differences in the populations or by using low numbers of samples.

Table 2. Comparison of haplotype frequencies and combined genotypes of the FVL G1691A, FII G20210A, PAI-1 5G/675/4G, MTHFR C677T and A1298C polymorphisms between the UERSA-group and controls

Gene polymorphisms	Patients (n=50)	Controls (n=50)	Odds Ratio (95% CI)	χ^2	p Value a
FVL 1691G/A	3 (6%)	1 (2%)	3.1(1.1-9.1)	0.26	0.6098
FII 20210G/A	2 (4%)	0		0.51	0.4751
PAI-1 5G/4G	36 (72%)	16 (32%)	2.3 (1.0-3.4)	14.46	0.0001
MTHFR 677C/T	23 (46%)	5 (10%)	4.6 (1.8-6.6)	14.34	0.0002
MTHFR1298A/C	23 (46%)	20 (40%)	1.2 (0,85-1,6)	0.16	0.6862
MTHFR 1298C/C	4 (8%)	4 (8%)	1.0	0.000	1.000
PAI-1 5G/4G// MTHFR 677C/T	9 (18%)	2 (4%)	4.5 (0,1-8,1)	3.68	0.0552
PAI-1 5G/4G// MTHFR 1298A/C	7 (14%)	3 (6%)	2.3 (1.2-5.8)	1.00	0.3173
PAI-1 5G/4G// FVL 1691G/A	1 (2%)	0		0.00	1.00
PAI-1 5G/4G// MTHFR 677C/T //MTHFR 1298A/C	7 (14%)	5 (10%)	1.4 (2.0-3.9)	0.09	0.7583

a - p value for Fisher's exact T-test

Many studies in the literature have discussed the subject of the role of the MTHFR gene mutations as a risk factor for recurrent spontaneous early abortions. Some of these studies have demonstrated a relationship between MTHFR C677T and A1289C mutations and URESA [14-16], whereas others have been unable to confirm these results [17-19]. Cao Y et al. [20], had demonstrated a significant association between MTHFR C677T mutation and recurrent spontaneous early abortions in the East Asian subgroup and mixed subgroup, but no significance in MTHFR A1298C mutation.

We did not observe increased risk of early pregnancy loss in mutant homozygotes with MTHFR 677C/C and MTHFR 1298C/C genotypes. However, heterozygotes with MTHFR 677C/T genotype had high risk of early recurrent pregnancy loss. On the other hand, heterozygotes with MTHFR 1298A/C genotype showed a low association with first-trimester pregnancy loss.

The findings of the earlier studies had shown that the combined presence of the MTHFR 677T/T and 1298C/C mutant genotypes highly increased the risk of pregnancy loss [21]. It has been also noted that the connection of thrombogenic risk factors with recurrent miscarriages and repeated implantation failures after IVF is mainly evident in the simultaneous carriage of several thrombogenic mutations and polymorphisms [22]. We found that women carrying combined PAI-1 5G/4G// MTHFR 677C/T or PAI-1 5G/4G// MTHFR 1298A/C or PAI-1 5G/4G// FVL 1691G/A or PAI-1 5G/4G// MTHFR 677C/T //MTHFR 1298A/C heterozygous genotypes had an increased frequency of recurrent early spontaneous abortion.

4. CONCLUSION

The genetic polymorphisms of FVL 1691G/A, FII 20210G/A, MTHFR 677C/T, MTHFR 1298A/C, and PAI-1 4G/4G, and the PAI-1 5G/4G genotypes may be associated with URESA. The patients carrying combined heterozygous genotypes PAI-1 5G/4G//MTHFR 677C/T or PAI-1 5G/4G// MTHFR 1298A/C or PAI-1 5G/4G//FVL 1691G/A or PAI-1 5G/4G// MTHFR 677C/T //MTHFR 1298A/C have higher risks of URESA. These results might indicate a genetic influence on pathogenesis of URESA.

CONSENT

Not applicable.

ETHICAL APPROVAL

This study was approved by the Ethical Committee of the Chita State Medical Academy (Chita, Russia). All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration ofHelsinki.

ACKNOWLEDGMENTS

We acknowledge Glotova E. Yu. (Chief of Polyclinic Department of Transbaikal Regional Perinatal Center, Russia), Maltceva T.V. (doctor of Medical Center "Health Academy", Russia), Zolotaryova A.A. (doctor of Transbaikal Regional Medical Diagnostic Center, Russia) for the organizing and partial support of clinical examinations of patients that were included in our study.

We thank Moamar Al-Jefout, MD, Ph.D. (Assistant Professor in Reproductive Medicine, Obstetrics & Gynaecology Department at Mutah Medical Faculty, Mutah University, Jordan) for his assistance in editing the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Govindaiah V, Naushad SM, Prabhakara K, Krishna PC, Radha Rama Devi A. Association of parental hyperhomocysteinemia and C677T Methylene tetrahydrofolater eductase (MTHFR) polymorphism with recurrent pregnancy loss. Clin Biochem. 2009;42(4-5):380-6.
2. Nadir Y, Hoffman R, Brenner B. Association of homocysteine, vitamin B12, folic acid, and MTHFR C677T in patients with a thrombotic event or recurrent fetal loss. Ann Hematol. 2007;86(1):35-40.
3. Parén L, Palmqvist L, Barkhordar GS, Hellgren M, Zetterberg H. Pregnancy and a rare MTHFR haplotype. Acta Obstet Gynecol Scand. 2012;91(5):635-6.
4. Jeon YJ, Kim YR, Lee BE, Choi YS, Kim JH, Shin JE, et al. Genetic association of five plasminogen activator inhibitor-1 (PAI-

1) polymorphisms and idiopathic recurrent pregnancy loss in Korean women. Thromb Haemost. 2013;110(4):742-50.

5. Guan LX, Du XY, Wang JX, Gao L, Wang RL, Li HB, et al. Association of genetic polymorphisms in plasminogen activator inhibitor-1 gene and 5,10-methylenetetrahydrofolate reductase gene with recurrent early spontaneous abortion. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2005;22(3):330-3.
6. Parveen F, Tuteja M, Agrawal S. Polymorphisms in MTHFR, MTHFD, and PAI-1 and recurrent miscarriage among North Indian women. Arch Gynecol Obstet. 2013;288(5):1171-7.
7. The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage. Green-top Guideline No. 17. Royal College of Obstetricians and Gynaecologists (RCOG) (April 2011). Retrieved 2 July 2013.
8. Available:http://www.dna-technology.ru/eng/
9. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: A meta-analysis. Lancet. 2003;361:901–8.
10. Pasińska M, Soszyńska K, Runge A, Dabrowska A, Juraszek A, Janiszewska T, et al. Molecular diagnostic tests for thrombophilia in patients referred to genetic counseling clinic because due to recurrent pregnancy failure. One center's experience. Ginekol Pol. 2012;83(3):178-82. [Article in Polish].
11. Rodger MA, Betancourt MT, Clark P, Lindqvist PG, Dizon-Townson D, Said J, et al. The association of factor V Leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: A systematic review and meta-analysis of prospective cohort studies. PLoS Med. 2010;7(6):e1000292.
12. Souza SS, Ferriani RA, Pontes AG, Zago MA, Franco RF. Factor V Leiden and factor II G20210A mutations in patients with recurrent abortion. HumReprod. 1999;14:2448-50.
13. 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 ReprodImmunol. 2011;66(2):149-56.
14. Mtiraoui N, Zammiti W, Ghazouani L, Braham NJ, Saidi S, Finan RR, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphism and changes in homocysteine concentrations in women with idiopathic recurrent pregnancy losses. Reproduction. 2006;131(2):395-401.
15. Goodman CS, Coulam CB, Jeyendran RS, Acosta VA, Roussev R. Which thrombophilic gene mutations are risk factors for recurrent pregnancy loss? American Journal of Reproductive Immunology. 2006; 56(4):230–236.
16. 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].
17. Carp H, Salomon O, Seidman D, Dardik R, Rosenberg N, Inbal A. Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. Human Reproduction. 2002;17(6):1633–1637.
18. Wiwanitkit V. Roles of methylenetetrahydrofolate reductase C677T polymorphism in repeated pregnancy loss. Clin Appl Thromb Hemost. 2005;11(3):343-5.
19. Poursadegh Zonouzi A,Chaparzadeh N, Asghari Estiar M, Mehrzad Sadaghiani M, Farzadi L, Ghasemzadeh A, et al. Methylenetetrahydrofolate Reductase C677T and A1298C mutations in women with recurrent spontaneous abortions in the Northwest of Iran. ISRN Obstet Gynecol. 2012;2012:945486. doi: 10.5402/2012/945486. Epub 2012 Nov 14.
20. Cao Y, Xu J, Zhang Z, Huang X, Zhang A, Wang J, et al. Association study between methylenetetrahydrofolate reductase polymorphisms and unexplained recurrent pregnancy loss: a meta-analysis. Gene. 2013;514(2):105-11. doi: 10.1016/j.gene.2012.10.091. Epub 2012 Nov 29.
21. Zetterberg H, Regland B, Palmér M, Ricksten A, Palmqvist L, Rymo L, et al. Increased frequency of combined methylenetetrahydrofolate reductase C677T and A1298C mutated alleles in

spontaneously aborted embryos. Eur J Hum Genet. 2002;10(2):113-8.

22. Momot A, Lydina I, Tsyvkina L, Borisova O, Serdyuk G. The means of progress in improving the results of *in vitro* fertilization based on the identification and correction of the pathology of hemostasis. In: "Enhancing Success of Assisted Reproduction", Ed. by Atef M.M. Darwish, ISBN 978-953-51-0869-6: In Tech, 2012;77-116. Available from: http://www.intechopen.com/books/enhancing-success-of-assisted-reproduction/the-means-of-progress-in-improving-the-results-of-in-vitro-fertilization-based-on-the-identification

© 2015 Belokrinitskaya et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=672&id=12&aid=6277