DETERMINING THE RISK OF MISCARRIAGE IN GENETIC FORMS OF THROMBOPHILIA

Tetiana Loskutova, Albina Petulko

The aim: *to study the distribution and influence of coagulation factor gene polymorphisms and endothelial dysfunction on the development of recurrent pregnancy loss.*

Materials and methods: *a prospective case-control study included 109 pregnant women in the 1st trimester with habitual miscarriage and 34 conditionally healthy pregnant women with an uncomplicated obstetrical anamnesis without risk factors for miscarriage. Genetic polymorphisms of coagulation and fibrinolysis factors (1691 G→A FVL, 20210 G→A prothrombin, 675 5G/4G PAI-1, 455 G→A fibrinogen β), as well as endothelial dysfunctions (192 Q→R PON-1, 677 C → T MTHFR) were investigated using allele-specific polymerase chain reaction.*

Results: *Pathological polymorphisms of the genes of the hemostasis system and endothelial dysfunction play a significant role in the development of miscarriage, namely such pathological genotypes as 1691 GA of factor V Leiden – increases the risk by 5.3 times (95 % CI 1.5–18.5), 20210 GA of prothrombin – by 26.47 times (1.6–445.7), 675 4G/4G PAI-1 – by 7, 5 times (1.7–33.79), -455AA fibrinogen β – 9.7 times (1.3–74.16), 677 CT MTHFR – 2.6 times (1.0–6.2), 677 TT MTHFR – 21.7 times (1.3–368.6). Multigenic forms of thrombophilia predominate in the majority of patients with miscarriage and account for 76.1 % (p<0.001, OR=12.31, 95 % CI 4.8–31.55). It was determined that the simultaneous presence of two pathological polymorphisms increases the risk of miscarriage by 3.88 times (OR 3.38; 95 % CI 1.26–9.97), and three ones – more than 2.5 times (OR 2.66; 95 % CI 1.02–7.19).*

Conclusions: *the course of pregnancy against the background of pathological polymorphisms of the genes of the hemostasis system and endothelial dysfunction significantly increases the risk of habitual miscarriage, which should be considered when planning a pregnancy in women with habitual miscarriage*

Keywords: *habitual miscarriage, genetic thrombophilia, pregnancy complications, prognosis, genes polymorphisms*

1. Introduction

Unfavourable dynamic changes in demographic indicators make preserving the population's reproductive health one of the most important and priority areas of modern medicine [1]. About 15 % of pregnancies are terminated spontaneously [2], and repeated miscarriages significantly deepen the stress experienced by the family [3]. The risk of miscarriage increases each time, reaching 40 % after the third miscarriage [4]. At the same time, reducing the number of spontaneous miscarriages in the early stages of gestation should be considered one of the reserves for preventing perinatal losses and increasing the birth rate in our country.

It is known that habitual miscarriage or recurrent pregnancy loss (RPL) has a multifactorial genesis that includes genetic, immune, infectious, anatomical, endocrine, and thrombophilic components [3, 5]. None of the factors can fully explain the occurrence of reproductive losses, and in 40 % of cases, the PL remains without an established cause after all possible factors have been excluded [6].

In turn, thrombophilia is attributed to the etiological factors of habitual miscarriage [2], as well as obstetric complications such as preeclampsia, fetal growth retardation, and placental abruption. Non-thrombogenic mechanisms of mutations and polymorphisms of thrombophilia genes disrupt normal implantation processes, which creates conditions for the development of obstetric complications. At the same time, the prevalence of this pathology (the type of pathological polymorphisms and their combinations) in women with repeated miscarriages has not been finally determined until today. It should also be borne in mind that the development, course, and complications of thrombophilia may depend on defects in various components of the hemostatic system and external factors, vary in degree of manifestation and depends on the interaction and specifics of the combination of these disorders. According to Musters, A. (2013) [7],

33

"couples suffering from recurrent pregnancy loss require individualised management that includes appropriate support, and in this context, testing for relevant factors can help reduce anxiety and manage expectations." All of the above determined the choice of the topic and purpose of the study.

The aim – to study the distribution and influence of coagulation factor gene polymorphisms and endothelial dysfunction on the development of recurrent pregnancy loss.

2. Materials and methods

The study was conducted at Dnipro State Medical University, Dnipro, Ukraine, in 2018–2020. A prospective cohort study covered 143 women in the first half of pregnancy. The diagnosis of habitual miscarriage was based on Order No. 624 of the Ministry of Health of Ukraine and the ESHRE, 2017 guidelines "Recurrent Pregnancy Loss" and determined that habitual miscarriage is the result of two or more consecutive pregnancies that ended in miscarriage. The exclusion criteria for the study were the presence of Anti-Phospholipid Syndrome (APS), istmic-cervical insufficiency, anatomical malformations, and submucosal leiomyoma of the uterine body (FIGO type 0-II).

Examination of patients was performed if parents provided written informed consent. The management of the study was conducted in full compliance with the ethical principles contained in the "Human Rights Declaration" adopted in Helsinki, which follows the Good Practice Rules in the Clinical Study and Legal Regulations and with the approval of the Ethics Committee of the Dnipro State Medical University (protocol No. 5 dated September 13, 2018).

The main cohort (M) consisted of 109 women with recurrent pregnancy loss. The control group (C) was formed by 34 conditionally healthy pregnant women with a non-complicated obstetrical anamnesis and without risk factors for miscarriage. All women underwent a clinical and laboratory examination, analysis of complaints, study of obstetric, gynaecological, somatic, hereditary anamnesis, and instrumental examination (ultrasound).

Genetic polymorphisms of coagulation factors and fibrinolysis (1691 G→A FVL, 20210 G→A prothrombin, 675 5G/4G PAI-1, 455 G→A fibrinogen β), endothelial dysfunction (192 Q→R PON-1, 677 C→T MTHFR) were studied with the help of allele-specific polymerase chain reaction, followed by detection by electrophoresis in 3 % agarose gel. A set of reagents "SNP-Express" (Litech SPF) was used. DNA from leukocytes of blood, which was isolated using the reagent "DNA-express blood" (Litech SPF,) was used for analysis.

The concentration of homocysteine in blood plasma was determined by enzyme-linked immunosorbent assay using Axis® reagents "Axis-Shield AS" (Norway) on a Stat-Fax device (United States of America).

To exclude APS, the acquired forms of thrombophilia were studied; for this purpose, total antibodies to cardiolipin were determined using an enzyme-linked immunosorbent assay system manufactured by Granum (Ukraine). Determination of Ig M and Ig G to β2 GP1, prothrombin and annexin V was performed by indirect solid-phase ELISA (ELISA analyser "Stat-Fax", USA) in blood serum using reagents manufactured by "Orgentec

Diagnostika GmbH" (Germany). An elevated level of antibodies to cardiolipin was considered if the response index was greater than 2. The number of Ig M and Ig G antibodies to annexin V, antibodies of Ig M, Ig G, and Ig A classes to β2 GP1 is increased if it exceeds 8 U/ml, and the level of antibodies of Ig M, Ig G, Ig A classes to prothrombin is more than 20 U/ml.

Statistical processing of the study results was performed using licensed computer programs Microsoft Excel 2010 and Graph Pad Prism 5 using methods of parametric and nonparametric statistics. The normality of the distribution of quantitative traits was assessed using Shapiro-Wilk, and Kolmogorov-Smirnov criteria, analysis of variance, odd t-test, Mann-Whitney test, χ^2 test with conjugation of conjugation tables and Yates correction, Fisher's exact test was used. Spearman and Pearson correlation coefficients (r) were used to assess the relationship between the indicators. To assess the relationship between impact and outcome, relative risk (RR) and odds ratio (OR) assessments were performed at 95 % confidence interval (CI). The difference between the values was considered significant by $p < 0.05$.

3. Research results

It was found that the average age of pregnant women in the main group (M) exceeded that of the control group and was 30.7±0.52 years (95 % CI: 29.7–31.7) versus 25.8±0.85 (95 % CI: 24.1–27.5) in the control group (p=0.001). This is due to the fact that this pregnancy occurred after several unsuccessful pregnancies and/or infertility treatment.

The analysis of the premorbid background, obstetric and gynaecological and somatic anamnesis data revealed that the risk factors for miscarriage include age over 35 years (OR=5.43, 95 % CI 1.02–60.9), history of preterm birth (5.22, 1.66–41.6), dysmenorrhea (18.39; 2.42–139.66), background cervical disease (11.33; 3.27–39.27), overweight (7.88; 1.02–60.9), hypertensive disorders (8.74; 1.13–67.36), varicose veins of the lower extremities (9.74; 1.27–74.8). In addition, the data of hereditary history, namely hypertension in parents (OR=7.17, 95 % CI 3.09–16.73), lipid metabolism disorders (32.4; 4.28–245.4), carbohydrate metabolism disorders (9.09; 2.62–31.5), cardiovascular incidents (heart attacks, strokes under the age of 50) in first-line relatives (21.5; 2.83–163.08), thyroid disease (16.27; 2.17–123.8), and miscarriage (3.81; 1.46–9.94). Patients with miscarriage more often (p<0.05) had the following gestational complications fetal growth retardation 26 (29.2 %) (14.19, 1.85–109.08), oligohydramnios 22 (20.2 %) (5.75, 1.05–31.44), preeclampsia 26 (23.9 %) (21.9, 1.3–369.5), threatened miscarriage (230.6, 48.9–1086.11), surgical delivery (3.75, 1.29–10.89).

The average weight of newborns in group M ((2744.0±83.0) g) was 1.27 times less than in group K ((3485.6±79.5) g, p<0.05). The height of newborns in the M group ((48.0±0.62) cm) is 1.09 times less compared to the K group ((52.1±0.39) cm, p<0.05). The Apgar score in the M group was significantly lower compared to the C group (p<0.05): at the 1st minute in the O group, 42.2 % had a score of ≥7 points (C=85.3 %, OR=7.32; 95 % CI 2.73–19.63), and at the 5th minute in the O group – 71.6 % (C=100 %, OR 27.69, 95 % CI 1.65–

465.5). Miscarriage in past medical history has a significant effect on the weight and height of the newborn (r_{Sp}=0.680, r_{Sp}=0.636, respectively, p<0.001) and on the Apgar score (at the 1st minute r_{Sp}=0.470, at the 5th minute r_{Sp}=0.480, p<0.001), so the condition of children of mothers with miscarriage deserves attention both during intrauterine growth and after birth. Analysis of the results of tests of genes that regulate the hemostasis system, "endothelial system" revealed a high frequency of pathological polymorphisms in patients with RPL (Table 1).

The results of testing for the presence of polymorphisms in the gene PON-1 192 Q→R did not reveal significant changes between the studied groups (Table 1).

Table 1

Frequency of genotypes and alleles of thrombophilia and endothelial dysfunction genes in pregnant women from study groups, n (%)

Study group	Genotype			Alleles	
Prothrombin 20210 G→A					
	GG	GA	AA	20210G	20210A
M (n=109)	77 (70.6)*	30 (27.5)*	2 (1.8)	184 (84.8)*	34 (15.6)*
C (n=34)	34 (100.0)	0 (0.0)	0 (0.0)	68 (100.0)	0 (0.0)
Factor V Leiden 1691 G→A					
	GG	GA	AA	1691G	1691A
M (n=109)	71(65.1)*	37 (33.9)*	1 (0.9)	179 (82.1)*	39 (17.9)*
C (n=34)	31 (91.2)	3 (8.8)	0 (0.0)	65 (95.6)	3 (4.4)
PAI-1 5G/4G					
	5G/5G	5G/4G	4G/4G	5G	4G
M (n=109)	18 (16.5)*	56 (51.4)	35 (32.1)*	92 (42.2)*	126 (57.8)*
C (n=34)	19 (55.9)	13 (38.2)	2 (5.9)	5 (75.0)	17 (25.0)
Fibrinogen β -455 G→A					
	GG	GA	AA	-455G	-455A
M (n=109)	38 (34.9)*	44 (40.4)	27 (24.8)*	120 (55.0)*	98 (45.0)*
C (n=34)	25 (73.5)	8 (23.5)	1 (2.9)	58 (85.3)	10 (14.7)
MTHFR 677C→T					
	CC	CT	TT	677C	677T
M (n=109)	40 (36.7)*	48 (44.0)*	21 (19.3)*	128 (58.7)*	90 (41.3)*
C (n=34)	26 (76.5)	8 (23.5)	0 (0.0)	60 (88.2)	8 (11.8)
PON 1 192 Q→R					
	QQ	QR	RR	192Q	192Q
M (n=109)	71 (65.1)	30 (27.6)	8 (7.3)	172 (78.9)	46 (21.1)
C (n=34)	25 (73.5)	7 (20.6)	2 (5.9)	57 (83.8)	11 (16.2)

Note: * – the statistical significance of differences of the indicator relative to the C group (p<0.05), the χ^2 test and Fisher's exact test are used

4. Discussion

The frequency and structure of genetic polymorphisms and mutations in genes that regulate the hemostasis system and endothelial dysfunction in pregnant women with pregnancy loss have been studied. Analysing the frequency of prothrombin gene genotypes (20210 G→A), it has been found that the heterozygous variant 20210 GA is unique to the group with RPL (p<0.001, OR=26.47; 95 % CI 1.6–445.7), and the homozygous variant 20210 GG has projective properties (p<0.001, OR=0.03; 95 % CI 0.002–0.58). Similar changes apply to the polymorphism of factor V Leiden. Carriers of the heterozygous variant 1691 GA factor V Leiden were 3.8 times more likely to be observed in group M (p<0.05, OR=5.3; 95 % CI 1.5–18.5), and genotype 1691 GG in 1, 4 times more often registered in group K (p <0.05, OR=0.18; 95 % CI 0.05–0.63). The correlation between RPL and mutation of the prothrombin gene was r=0.361, with the mutation of factor V Leiden – r=0.287 (p<0.05). The consequence of the FV Leiden mutation is the impaired functioning of protein C as the most important representative of the natural anticoagulant mechanism. The presence of this mutation increases the risk of developing a number of pregnancy complications: miscarriage (the risk increases 3 times), fetal growth retardation, gestosis, and fetoplacental insufficiency [8, 9]. According to the literature, heterozygous carriage of the prothrombin 20210G→A mutation is associated with placental abnormalities and an increased risk of miscarriage up to 12 times and is a risk factor for miscarriage in early pregnancy [8]. In the case of gene polymorphism, the level of prothrombin in the blood plasma can be increased by 30 % due to more stable mutant matrix RNA, which provokes the appearance of an excessive amount of fibrin clots and increases the risk of venous thrombosis and leads to fetal loss, mainly in the first trimester [10]. The study indicates that the relative risk of MC increases to 3.2-3.3 in carriers of FV and prothrombin mutations [11, 12].

Analysis of the frequencies of MTHFR 677 C→T genotypes revealed a decrease in the frequency of the normal CC genotype in the M group. Its frequency is reduced by 2.1 times compared with the K group (p<0.001, OR=0.18, 95 % CI 0.07–0.43). The number of heterozygotes 677 CT MTHFR in the M group exceeded the value of the K group 1.9 times (p<0.05, OR=2.6; 95 % CI 1.0–6.2). Carriers of pathological homozygote 677 TT were registered only in group M (p<0.05, OR=21.7; 95 % CI 1.3–368.6). The correlation between the polymorphisms of the fibrinogen gene β -455 G→A, MTHFR 677 C→T and RPL were r=0.399 and r=0.409, respectively (p<0.05).

Comparing the frequencies of PAI-1 5G / 4G genotypes, it has been determined that the 5G / 5G genotype has protective properties against the development of RPL, and is 3.4 times more common in pregnant women of group K (p<0.001, OR=0.16, 95 % CI 0,07–0.36) than in the M group. Carriers of the pathological homozygote of the PAI-1 4G / 4G gene have been registered 5.4 times more often in the M group (p<0.05, OR=7.5; 95 % CI 1.7–33.39). The correlation between PAI-1 5G / 4G and RPL was r=0.438 (p<0.05). The data we obtained partially agreed with a study [11] in which it was deter-

mined that both mutations in factor V Leiden and MTHFR C677T polymorphisms were significantly associated with recurrent pregnancy loss (RPL) in Bosnian women while prothrombin G20210A and PAI-1 4G/5G polymorphisms did not show strongly significant association.

Analysing the relationship between pathological gene polymorphisms and the coagulation link of hemostasis, it was found that the correlation between mutations in the PAI-1 5G/4G gene and INR, aPTT level of D-dimer was weak and amounted to –0.177, –0.259 and 0.252, respectively (p<0.05). The correlation between the fibrinogen β-455 G→A gene polymorphism and INR, aPTT and D-dimer was 0.209, 0.286, and 0.183 (p<0.001), respectively. Regarding the mutation in the prothrombin gene 20210 G→A, the r=-0.234 (p<0.05) with aPTT and r=0.279 (p<0.05) with activated recalcification time. Mutations in the MTHFR and FVL genes were associated only with D-dimer levels r=0.293 and r=0.174 (p<0.05), respectively.

Analysis of homocysteine levels revealed significant differences between the control (7.11±0.56, n=15) and the main groups (11.75±0.54, n=109) (p<0.05). In addition, in group M in a significantly larger number of women, the level of homocysteine exceeded 15 μmol 35 (53.3 %) compared with control 0 (0 %, p<0.001, OR 32.88, 95 % CI: 1.96–551.77). The cause of hyperhomocysteinemia is a mutation in MTHFR 677 C→T, which is confirmed by the correlation (r=0.267, p=0.042) between the level of homocysteine and the polymorphic variant of the gene.

RPL is a multifactorial disease and not only pathological polymorphisms of individual genes but also their combined effect, which reveals the potentiation of their action play a role in its occurrence. As combinations of unfavourable genotypes have been considered: homo- and heterozygous mutations of the prothrombin gene 20210 GA, AA, gene FV Leiden 1691 GA, AA, homo and heterozygous polymorphisms PAI-1 genes 5 G / 4G, 4G / 4G, FGB -455 GA, AA, monozygous PON – 1 192RR and MTHFR 677 TT. Analysing the distribution of combinations of pathological variants of genes, we have found that they were more common in pregnant women with RPL. The simultaneous existence of two or more pathological polymorphisms has been determined in 83 (76.1 %) women of group M against 7 (20.5 %) of group K (p<0.001, OR=12.31, 95 % CI 4.8–31.55). The presence of two pathological polymorphisms (33 % vs. 14.7 %) increases the chances of pregnancy loss by 2.66 times (95 % CI 1.02–7.19), and the presence of three pathological polymorphisms (28.4 % vs. 5.9 %) increases the chances of developing RPL by 4.99 times (p<0.05, 95 % CI 1.29–19.29). There was a significant difference in the combinations of allelic variants of the genes PAI-1 5G / 4G, 4G / 4G and FGB – 455 GA, –455 AA between women with RPL and the control group, which separately (25.7 % vs. 14.7 %) or in combination with other pathological polymorphisms in women of group M 58 (53.2 %) were probably more common than in group K (7 (20.5 %), p <0.05, OR=4.17, 95 % CI 1.71–10.14). The combination of PAI-1 5G / 4G or 4G / 4G with MTHFR 677 TT and other polymorphisms was more likely to occur more frequently in the

main group 16 (14.7 % vs. 0 % in K, p=0.039), which increases the chances of pregnancy loss in 12,18 times (95 % CI 7.1–208.5).

Thus, taking into account the current data and the results of many studies, it should be understood that although thrombophilia is not the main cause of pregnancy complications, it still contributes to the risks of pregnancy loss and habitual miscarriage, as well as worsening the possible effects of other concomitant pathology during pregnancy, and therefore should be considered in the context of examinations of such patients.

According to the latest guidelines on RPL [1, 2, 13], routine screening for genetic thrombophilia is not performed except for women with miscarriage and thrombotic risks and for scientific purposes. This approach is explained by the fact that there is currently no method of medical treatment for RPL and genetic forms of thrombophilia with proven effectiveness. Heparin and aspirin are not routinely indicated but used only in the context of thromboprophylaxis in women at risk for thromboembolic complications.

Although there is no reasonable treatment for RPL, couples evaluate opportunities for subsequent pregnancy. Before becoming pregnant, couples and clinicians try to find an explanation for pregnancy loss and choose appropriate treatment tactics to prevent a recurrence, especially in cases with modifiable risk factors such as thyroid disorders and APS. That is why most recommendations advise investigating the causes of miscarriage. However, there is no consensus on when to investigate risk factors in spouses with RPL.

According to Musters A. (2013) [4], couples suffering from RPL need individualised management that includes appropriate support, and, in this context, testing for relevant factors can help reduce anxiety and manage expectations. Therefore, at this stage of scientific development, screening for polymorphisms in the genes for thrombophilia and endothelial dysfunction is a matter of personalised medicine.

Study limitations:

1. Small sample size

2. Different distribution of genetic forms of thrombophilia depending on the region of residence

Prospects for further research. The results of the study can be used to develop a model for predicting miscarriage and creation a personalised management algorithm to prevent pregnancy complications.

5. Conclusions

Thus, molecular diagnostics is recognised as the most promising in the modern world, as it can provide clear and specific information about the possible development of a disease, taking into account the individual structure of the human genome and the peculiarities of the metabolic processes of the body. In the case of a certain disease (in our case, RPL), several genes have different degrees of involvement in its development, but by combining their roles, it is possible to predict the possibility of this pathology in a particular patient, i.e. to predict the development of miscarriage and create a personalised management algorithm to prevent pregnancy complications.

1. The main risk factors for miscarriage are the age of the pregnant woman over 35 years (OR 5.43; 95 % CI 1.02–60.9), history of preterm birth (5.22; 1.66–41.10), overweight (7.88; 1.02–60.91), hypertensive disorders (8.74; 1.13–67.36), and varicose veins of the lower extremities (9.74; 1.27–74.83). It was found that the hereditary factors of miscarriage are hypertension of the proband mother (5.81; 2.15–12.52), the father (23.2; 3.06–175.9), lipid metabolism disorders (6.32; 2.61–15.34), carbohydrate metabolism disorders (9.09; 2.62–31.51), cardiovascular incidents (heart attacks, strokes) under the age of 50 (21.5; 2.83–103.08).

2. Women with a history of miscarriage are at risk for obstetric complications, including preeclampsia (OR=21.9; 95 % CI 1.3–369.5), fetal growth retardation (14.19; 1.85–109.08), and hydramnion (5.75; 1.05–31.44).

3. Pathological polymorphisms of the genes of the hemostasis system and endothelial dysfunction play a significant role in the development of miscarriage, namely such pathological genotypes as 1691 GA of factor V Leiden – increases the risk by 5.3 times (95 % CI 1.5–18.5), 20210 GA of prothrombin – by 26.47 times (1.6–445.7), 675 4G/4G PAI–1 – by 7, 5 times (1.7–33.79), – 455AA fibrinogen β – 9.7 times (1.3–74.16), 677 CT MTHFR – 2.6 times (1.0–6.2), 677 TT MTHFR – 21.7 times (1.3–368.6).

4. Multigenic forms of thrombophilia predominate in most patients with miscarriage and account for 76.1 % (p<0.001, OR=12.31, 95 % CI 4.8–31.55). It was determined that the simultaneous presence of two pathological polymorphisms increases the risk of miscarriage by 3.88 times (OR 3.38; 95 % CI 1.26–9.97), and three ones – more than 2.5 times (OR 2.66; 95 % CI 1.02–7.19).

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

Data availability

Data will be made available on reasonable request.

References

1. Toth, B., Würfel, W., Bohlmann, M., Zschocke, J., Rudnik-Schöneborn, S., Nawroth, F. et al. (2018). Recurrent Miscarriage: Diagnostic and Therapeutic Procedures. Guideline of the DGGG, OEGGG and SGGG (S2k-Level, AWMF Registry Number 015/050). Geburtshilfe Und Frauenheilkunde, 78 (4), 364–381. doi: https://doi.org/10.1055/a-0586-4568

2. Bender Atik, R., Christiansen, O. B., Elson, J., Kolte, A. M., Lewis, S., Middeldorp, S. et al. (2018). ESHRE guideline: recurrent pregnancy loss. Human Reproduction Open, 2018 (2). doi: https://doi.org/10.1093/hropen/hoy004

3. Homer, H. A. (2018). Modern management of recurrent miscarriage. Australian and New Zealand Journal of Obstetrics and Gynaecology, 59 (1), 36–44. doi: https://doi.org/10.1111/ajo.12920

4. Magnus, M. C., Wilcox, A. J., Morken, N.-H., Weinberg, C. R., Håberg, S. E. (2019). Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. BMJ, 364, l869. doi: https://doi.org/10.1136/bmj.l869

5. Li, Y. H., Marren, A. (2018). Recurrent pregnancy loss: A summary of international evidence-based guidelines and practice. Australian Journal of General Practice, 47 (7), 432–436. doi: https://doi.org/10.31128/ajgp-01-18-4459

6. van Dijk, M. M., Kolte, A. M., Limpens, J., Kirk, E., Quenby, S., van Wely, M., Goddijn, M. (2020). Recurrent pregnancy loss: diagnostic workup after two or three pregnancy losses? A systematic review of the literature and meta-analysis. Human Reproduction Update, 26 (3), 356–367. doi: https://doi.org/10.1093/humupd/dmz048

7. Musters, A. M., Koot, Y. E., van den Boogaard, N. M., Kaaijk, E., Macklon, N. S., van der Veen, F. et al. (2013). Supportive care for women with recurrent miscarriage: a survey to quantify women's preferences. Human reproduction, 28 (2), 398–405. doi: https://doi.org/10.1093/humrep/des374

8. Sergi, C., Al Jishi, T., Walker, M. (2014). Factor V Leiden mutation in women with early recurrent pregnancy loss: a meta-analysis and systematic review of the causal association. Archives of Gynecology and Obstetrics, 291 (3), 671–679. doi: https://doi.org/10.1007/s00404-014-3443-x

9. Liu, X., Chen, Y., Ye, C., Xing, D., Wu, R., Li, F., Chen, L., Wang, T. (2021). Hereditary thrombophilia and recurrent pregnancy loss: a systematic review and meta-analysis. Human Reproduction, 36 (5), 1213–1229. doi: https://doi.org/10.1093/humrep/deab010

10. Croles, F. N., Nasserinejad, K., Duvekot, J. J., Kruip, M. J., Meijer, K., Leebeek, F. W. (2017). Pregnancy, thrombophilia, and the risk of a first venous thrombosis: systematic review and bayesian meta-analysis. BMJ, 359, j4452. doi: https://doi.org/10.1136/bmj.j4452

11. Jusic, A., Balic, D., Avdic, A., Podanin, M., Balic, A. (2018). The association of factor V G1961A (factor V Leiden), prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G polymorphisms with recurrent pregnancy loss in Bosnian women. Medicinski glasnik, 15 (2), 158–163. doi: https://doi.org/10.17392/948-18

12. Favaloro, E. J. (2019). Genetic Testing for Thrombophilia-Related Genes: Observations of Testing Patterns for Factor V Leiden (G1691A) and Prothrombin Gene "Mutation" (G20210A). Seminars in Thrombosis and Hemostasis, 45 (7), 730–742. doi: https://doi.org/10.1055/s-0039-1694772

13. Arachchillage, D. J., Mackillop, L., Chandratheva, A., Motawani, J., MacCallum, P., Laffan, M. (2022). Thrombophilia testing: A British Society for Haematology guideline. British Journal of Haematology, 198 (3), 443–458. doi: https://doi.org/10.1111/bjh.18239

Tetiana Loskutova*, Doctor of Medical Science, Professor, Department Obstetrics and Gynecology, Dnipro State Medical University, Volodymyra Vernadskoho str., 9 Dnipro, Ukraine, 49044

Albina Petulko, PhD, Assistant, Department Obstetrics and Gynecology, Dnipro State Medical University, Volodymyra Vernadskoho str., 9 Dnipro, Ukraine, 49044

*Corresponding author: Tetiana Loskutova, e-mail: loskutovata@gmail.com