Genetic and hemostasiological predictors of IVF pregnancy

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ABSTRACT
The performed clinical laboratory and instrumental research involved 93 pregnant women after IVF: 36 with progressive pregnancy and 57 women with missed abortion at term 6–8 weeks. The following parameters were evaluated: family history of thrombosis, menstrual function, gynecological and other diseases, duration of infertility treatment, pelvic organs sonography, hemostasis evaluation and thrombophilia genes investigation. The association of polymorphic allele 455 A of gene FGB with the risk of nondeveloping pregnancy after IVF was revealed. The genotype presence of allele 455 A of gene FGB increases the risk of miscarriage after IVF. The research has also revealed the association of polymorphic allele 4 G of gene PAI-1 in polymorphic locus 675:4 G/5 G with the risk of miscarriage. The received data on hemostatic system at an early term of pregnancy after IVF demonstrate enough stability and consistent thrombocyctic and coagulative hemostasis of all examined women. Patients with progressive pregnancy have a considerably lower level of platelets than the patients with miscarriage. The average content of fibrinogen in women with progressive pregnancy is considerably higher than that in the patients in other groups. APTT is positively higher in patients of group A. The average content of SFMC in patients of group A was positively higher. The patients with non-developing pregnancy after IVF showed a considerably lower SFMC content.

Introduction
The data on associations between genetic markers of thrombophilia, IVF and ET failures and reproductive losses, determined by defective ovum implantation and miscarriage [1,2], are quite contradictory [3,4]. On the one hand, there is enough data on considerable decrease in effectiveness of IVF in thrombophilia prone women, while on the other, H. Qublan [5] claims that LMH prescription in IVF protocols for women with at least one thrombophilia will definitely increase the frequency of pregnancy and child birth and decrease the possibility of miscarriage. Russian and foreign scientists [3–5] recommend to include LMH in preconception period for patients with inborn thrombophilia and failed IVF in anamnesis. However, there is neither standard preconception examination to identify genetic thrombophilia nor effective monitoring of hemostatic system during state funded IVF [6–10].

Research objective
Improving the algorithm of examination, preparation and selection of patients for IVF, developed on the basis of studying the role of gene polymorphism predisposing for thrombophilia.

Methods
In accordance with the objectives, the researchers conducted clinical laboratory and instrumental examination that involved 93 women divided into two study groups. All the patients were examined in the diagnostic department and treated in the gynecological department of FSBI ‘Federal State Budgetary Institution Urals Scientific Research Institute for Maternal and Child Care’ of Ministry of Healthcare of the Russian Federation from 2015 to 2016. The research was carried out with consent of the patients. The criteria for selection were the following: the IVF/IVF + ICSI programs, the standard protocol with antagonist of gonadotropin releasing hormone was followed. The second phase of the patients’ menstrual cycle enacted the prescription of vaginally injected micronized progesterone in a daily dose of 600 mg. The patients of all groups did not receive medications of estrogen group (estradiol valerate). All the patients were prescribed to take folic acid in a dose of 400 mg/day and potassium iodide in a dose of 200 mg/day until pregnancy was confirmed. Each patient was prescribed to take a...
pregnancy test 14 days after the IVF/IVF + ICSI procedure. When pregnancy was confirmed, the groups for prospective research were formed.

The main group A included 36 patients with progressive pregnancy after ART the average age of the patients being 33.18 ± 0.49 years.

The second group B included 57 patients with nondeveloping pregnancy after IVF at term of 6–8 weeks, the average age being 33.93 ± 0.57. The research did not include the patients with cardiac defects, second-degree obesity and more (BMI ≥35), blood disorders, polycyesis pregnancy, ovary hyperstimulation syndrome and after cryo protocols.

The research included the family thrombotic analysis, the rhythm, the character and the duration of menstruation, its intensiveness and timeline, gynecological diseases, length of infertility treatment and somatic pathology. The ultrasound of pelvic organs was conducted using Hawk 2102 EXL Ultrasound Scanner (Denmark) with the multi-frequency abdominal transducer of 3.5–5 Hz.

The research included complex evaluation of hemostatic system in pregnant women: the number of platelets in blood was calculated with the help of automatic analyzer ABX Micros 60 by ‘Horiba’ (France). To evaluate plasmatic element of hemostatic system, screening coagulative (clotting) tests were carried out using hemostatic analyzer Helena AC-4 with certified reagents and consumables (HELENA BioSciences Europe, UK, certificate of validation FS №2006/1412; №2006/1411).

The following indicators were found: photofluorography – fibrinogen level (g/l); APTT – activated partial thromboplastin time (sec); TT – thrombin time (sec); PT – Quick’s prothrombin time (%); thrombocytopenia markers – D-dimers (mg/ml) n SFMC – soluble fibrin monomer complexes (mg%) n antithrombin III – main blood anticoagulant.

Genetic thrombophilia proclivity was estimated by determining polymorphic gene variants which code the components of hemostatic system: FGB -455 G > A, F2 20210 T > A, F5 1691 G > A, F7 10976 G > A, F13 163 (100) G > T (Val34Leu), ITG2A 807 C > T, ITGB3 1565 T > C, PAI-1 -675 G > A. To make genotyping DNA samples were retrieved from the cells of buccal epithelium after which the real-time polymerase chain reaction was conducted using reagents and protocols of R and D company ‘DNK-Tekhnologiya’ (‘DNA-Technology’) (Russia). The DT-96 device software produced by the same company realized the detection and interpretation of the results.

The credibility of differences between the study groups in genotype and allele frequency was confirmed with the help of $\chi^2$ criterion according to the standard formula with Yates correction for paired comparison. The strength of association was estimated with odd ratio (OR). The OR and 95% confidence intervals (95% CI) were calculated using the program of ‘Calculator for confidence intervals or odds ratio’. The differences at $p < .05$ were considered statistically significant.

The test of genotype distribution compliance with Hardy–Weinberg’s law in both samplings was conducted using $\chi^2$ criterion ($p > .05$).

General statistical analysis was made using a set of applied programs Statistica 10 (Microsoft World, Palo Alto, CA). The results were processed using the methods of variation statistics and presented as M±m. The differences of averages and relative ratios were assessed with the help of t-test (Student’s t-test). The level of significance in the research is fixed at $p < .05$.

**Results**

The first group A included 36 patients with progressive pregnancy after ART, the average age of the patients being 33.18 ± 0.49 years. The second group B included 57 patients with nondeveloping pregnancy after ART at term of 6–8 weeks, the average age was 33.93 ± 0.57. Statistically no significant differences were found between the groups neither in this regard ($p = .1617$) nor regarding the body mass index with 23.1 ± 0.29 kg/mö in group A and 22.8 ± 0.34 kg/mö ($p = .54$) in group B. The analysis of menarche age of women under study revealed the following data: 13.32 ± 0.12 years for the first group and 13.11 ± 0.15 years ($p = .09$) for the second one. There were no statistically significant differences between the groups: average infertility period of the first group comprised 4.9 ± 0.32 years, while the second was 4.5 ± 0.32 years at the duration of infertility.

Allele and genotype frequency distribution according to polymorphic gene variants in women of all studied groups showed no deviation from the Hardy–Weinberg’s law. The group of women with nondeveloping pregnancy after IVF (Group B) had more carriers of variant allele A of polymorphism 455 G > A of gene FGB in genotype (OR = 2.20, CI95% 0.49–5.16, $p < .05$) than the women with progressive pregnancy after IVF (Table 2).

The analysis of the dominant model showed that the genotype -5G5G of gene FGB has a protective effect against the risk of miscarriage (OR = 0.30, 95%CI 0.93–0.1; $p < .03$). The presence of at least one variant allele -455 A of gene FGB in the genotype increases the chances of miscarriage (OR = 3.37; 95%CI 1.08–10.51; $p < .03$) (Table 2).

The similar tendency was revealed during the analysis of 4G/5G polymorphism occurrence in gene PAI-1: the frequency of variant allele 6754 G in the group of women with nondeveloping pregnancy after IVF was considerably higher than among women with pregnancy prolongation after IVF (OR = 2.90; 95%CI: 1.62–5.19; $p < .01$) (Table 1). The dominant model analysis showed that the genotype 5G5G of gene PAI-1 has an evident protective effect in pregnancy prolongation (OR = 3.55; 95%CI: 1.50–8.39; $p < .04$), while at least one alternative allele 6754 G of gene PAI-1 in the genotype decreases the chance of full-term pregnancy (OR = 0.28; 95%CI: 0.67–0.12; $p < .04$), which allows for the conclusion about the association of allele --6754 G of gene PAI-1 with the risk of miscarriage (Table 2).

Thus, the given research reveals the association of the polymorphic allele 455 A of gene FGB with the risk of nondeveloping pregnancy after IVF. The presence of allele 455 A of gene FGB in the genotype increases the risk of miscarriage after IVF (OR = 3.37; 95%CI 1.08–10.51; $p < .03$). The literature data prove that A-allele in polymorphic locus 455 G > A of gene FGB is associated with the increased level of fibrinogen in blood and the increased risk of thrombosis; however, the present research shows that the level of fibrinogen in women with nondeveloping pregnancy after IVF was considerably lower than in the experimental group.

The research proved the association of polymorphic allele 4 G of gene PAI-1 in polymorphic locus 6754 G/5 G with the risk of nondeveloping pregnancy (OR = 2.90; 95%CI: 1.62–5.19; $p < .01$). The presence of at least one alternative allele 6754 G of gene PAI-1 in the genotype decreases the chances of pregnancy prolongation after ART (OR = 0.28; 95%CI: 0.67–0.12; $p < .04$). Some authors believe that 4 G-allele is connected with the increased inhibitor of plasminogen activator in blood and consequently it increases the risk of thrombophilia 1.7-fold because of arterial and venous thromboses. Geterozygous carrierhip 5 G/4 G
promotes the risk of complications during pregnancy as this polymorphism is inherited according to autosomal dominant type [2,9].

The received data on hemostatic system at an early term of pregnancy after IVF (represented in Table 2) demonstrate enough stability and consistent thrombocytic and coagulative hemostasis of all examined women. The number of platelets was within normal physiological limits: 213.25 ± 7.01 thous/mkl and 244.20 ± 7.34 thous/mkl in groups A and B, respectively. However, the patients with progressive pregnancy had a considerably lower level of platelets than the patients with non-developing pregnancy (p < .05).

The study of plasmic element of clotting revealed different indexes in the groups. For example, the average content of fibrinogen in women from group A amounted to 4.85 ± 0.16 g/l, while in group B, it was considerably lower – 3.12 ± 0.11 g/l (p < .001).

The same differences were revealed after APTT-test (activated partial thromboplastin time), which is sensitive to coagulative disorders in intrinsic coagulation pathway: 37.76 ± 1.34 s – in group A, 31.01 ± 0.93 s (p < .001) – in group B.

The indicators of thrombin time, characterizing the third stage of clotting, is the speed with which fibrinogen turns into fibrin, and Quíck’s prothrombin which was revealed during the screening test of extrinsic blood coagulation, were within normal limits and considerably the same in both groups.

Significant differences were observed in the content of soluble fibrin monomer complexes (SFMC) in blood plasma. The system of fibrinolysis is known to be activated in reaction to the intensified of intravascular blood coagulation, so the increased SFMC is a marker of thrombinemia of one of the main symptoms of disseminated intravascular blood coagulation (DIC syndrome). Pregnant women have a higher concentration of SFMC in comparison with nonpregnant starting with the 1st trimester. The average content of SFMC in patients of group A was positively higher and comprised 15.22 ± 0.18 mg/ml during pregnancy after IVF revealed the symptoms of acti-

Table 1. Allele and genotype frequency distribution according to polymorphic gene variants in women after ART with nonde-

| Allele | Group A | Group B | Chi-square | p | OR | 95% CI |
|--------|---------|---------|------------|---|----|--------|
| FGB -455 G | 0.59 | 0.67 | 3.34 | .05 | 0.45 | 1.07 (0.19 |
| FGB -455 A | 0.41 | 0.33 | 2.20 | 0.49 | 5.16 |
| FGB -455 GG | 0.39 | 0.50 | 4.56 | .03 | 0.30 | 0.93 (0.10 |
| FGB -455 GA + AA | 0.61 | 0.50 | 3.37 | 1.08 | 10.51 |
| PAI-1 -675 GG | 0.53 | 0.28 | 8.51 | .04 | 0.28 | 0.67 | 0.12 |
| PAI-1 -675 SGG | 0.22 | 0.50 | 6.25 | .01 | 0.60 | 0.90 (0.41 |
| PAI-1 -675 SG4G + 4G4G | 0.78 | 0.50 | 3.55 | 1.50 | 8.39 |
| ITGA2 807C | 0.35 | 0.58 | 7.42 | .01 | 0.41 | 0.78 (0.21 |
| ITGA2 807T | 0.65 | 0.43 | 2.90 | 1.62 | 5.19 |
| ITGB3 1565T | 0.18 | 0.35 | 2.45 | 0.25 | 1.28 | 4.70 |
| ITGB3 1565G | 0.82 | 0.65 | 3.35 | .07 | 0.39 | 1.08 (0.14 |
| F13 C | 0.14 | 0.06 | 2.54 | 0.93 | 6.94 |
| F7: 10976 G | 0.64 | 0.56 | 2.35 | .13 | 1.37 | 0.92 | 2.05 |
| F7: 10976 A | 0.36 | 0.44 | 0.73 | 1.09 | 0.49 |
| F13 G | 0.72 | 0.74 | 0.73 | 1.09 | 0.49 |
| F13 T | 0.28 | 0.26 | 1.08 | 0.69 | 1.68 |

Table 2. The state of hemostatic system in patients after ART.

| Parameter | Group A (IVF, preg) n = 36 | Group B (IVF, regress) n = 57 | T-test |
|-----------|----------------------------|----------------------------|--------|
| Age       | 33.18 ± 0.49               | 33.93 ± 0.57               | 0.1617 |
| Platelets, ×10/l | 213.25 ± 7.01   | 244.20 ± 7.34               | 0.0025 |
| Fibrinogen, g/l | 4.85 ± 0.16            | 3.12 ± 0.11                | 0.0000 |
| SFMC, mg/dl | 15.22 ± 1.18             | 4.72 ± 0.29               | 0.0000 |
| TT, s      | 15.04 ± 0.26              | 15.19 ± 0.39               | 0.3927 |
| APTT, s    | 37.76 ± 1.34              | 31.01 ± 0.93               | 0.0000 |
| Quick’s PT, % | 101.3 ± 2.3           | 95.9 ± 2.9                | 0.0726 |
| INR (International normalized ratio) | 0.94 ± 0.01 | 0.94 ± 0.01 | 0.2273 |
| AT III, %  | 100.56 ± 3.95            | 96.42 ± 5.29              | 0.2785 |

Discussion

Thus, the conducted comparative study of hemocoagulation system during pregnancy after IVF revealed the symptoms of activated intravascular blood coagulation, most evident in patients with progressive pregnancy (most considerable decrease in platelets, rise of fibrinogen and SFMC). The patients with regressive pregnancy showed hemostatic indexes corresponding to those of nonpregnant (in fibrinogen, SFMC).

The conducted research demonstrates no direct connection of alleles carried in the genotype with the risk of thrombogenic complications and hemostatic symptoms, which may be explained by a poor effect of certain single nucleotide polymorphisms (SNPs) on the development of the disease without their connection with the rest of genome and regardless of environmental impact. The lack of evident associated trait of procoagulant genotypes may also be explained by early miscarriage.

A wider genetic association with developing pathologies may require the consideration of total number of genes involved in
pathogenesis as well as the environmental factors with the help of bioinformatic method of Multifactor Dimensionality Reduction (MDR) to analyze intergenic interactions.

It has been found that the IVF program involves pathological activation of hemostatic system which affects the process of implanting, it may disturb the development of fetoplacental complex from the moment of impregnation and lead to complicated induced pregnancy. Genetically determined changes in the activity of hemostatic system may present additional ethiopathogenetic factors of the faulty ovum implantation into the endometrium that results in the IVF failure. The introduction of thrombophilia associated genetic polymorphisms into the routine examination of patients before IVF appears rational and effective especially in CMI programs (Complete Medical Insurance) with thorough hemostatic control at all the stages of both IVF program and pregnancy.

Disclosure statement
No potential conflict of interest was reported by the authors.

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