Prothrombotic states in women with infertility and psychosomatic disorders

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Introduction

In the second decade of the new century, infertility remains a widespread common global condition. According to WHO, infertility is inability of a woman of reproductive age to become pregnant over a year of regular sexual life without contraception. Infertility is a complex medical-social problem of a global level, the extent of which affects the demographic parameters and has no tendency towards decrease. According to different assessments, infertility affects 8 to 12% of reproductive couples around the world, at the same time the indicators of infertility are much higher (up to 30%) in some regions of the world, including Central and Eastern Europe (Mascarenhas et al., 2012).

There are various reasons for infertility, but most often, a complex impact of 2–5 factors is observed. There is rising interest in the psychological factors behind infertility, which many researchers consider a psychosomatic disease (Boivin & Gameiro, 2015; Lampe & Schüßler, 2015). Infertility has deep social, economic, psychological and physical consequences, especially for women, significantly decreasing the quality of life. Experiencing infertility significantly worsens the psychological life of a person, which manifests in emotional reactions, psychological stress, sense of loss of control, impact on self-esteem, identity and social relations. Depressive symptoms occur in women with infertility twice as often as women with children. The impact of infertility is related to the factors such as depression, somatization and anxiety.

Assisted reproductive technologies (ART) are being actively developed, and are gradually become a routine procedure for solving the problem of producing children in case of infertility in marriage. Around 5 million children have been born thanks to ART, which equals 1% to 4% of all children born in the world (Ishihara et al., 2015). The main method of ART is extracorporeal fertilization (ECF). Most often, ECF is used in cases of obstruction or absence of uterine tubes; in cases of unclear reasons of infertility, decrease in total number of spermatozoids, disorders of their motility in men. However, despite initial successes, the efficiency of programmes of extracorporeal fertilization (ECF) has stagnated at the level of 20–35%, that is most attempts remain unsuccessful, and these failures are repeated among some patients (Simon & Laufer, 2012), which motivates further search for reasons for failure and the possibility of increasing the efficiency of ECF (Messerlian & Gaskins, 2017). Some researchers have identified a relationship between recurrent failures of ECF and psychological factors (Coughlan et al., 2014). A number of authors consider that failures of ECF can be related to increased ability of blood coagulation, prothrombotic conditions and antiphospholipid antibodies circulation (APA) (Mashkova, 2015; Stuleva et al., 2015). The presence of one or more types of APA causes a three-fold increase in the risk of failures of ECF (Sauer et al., 2010), though some authors deny such a relationship (Di Nisio et al., 2011).

Hypercoagulation syndrome is a relevant multi-disciplinary problem of the last decade, which is considered to be an inherited (primary) or obtained (secondary) condition of increased thrombogenesis in the arte-
ries and/or veins. Disorder of hemostasis, which causes hypercoagula-
tion syndrome, is manifested in different diseases in neurology, midwi-
fery, rheumatology, surgery. Antiphospholipid syndrome (APLS) is the
commonest form of hypercoagulation syndrome, which usually develops
at a young age, among children and even newborns, 5 times more often
in females than males. APLS is a symptom complex characterized by
venous or arterial thrombosis or thrombocytopenia, which develop fol-
lowing the synthesis of antibodies to phospholipids.

The presence of APLS can be accompanied by different clinical
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The diagnosis of determined antiphospholipid syndrome is made in
the presence of one of the clinical criteria and one of the laboratory
criteria in cases when the first laboratory examination was performed
over 12 weeks since the clinical manifestations.

Clinical criteria.
1. Vascular thrombosis. One (or more) clinical episode of arterial,
venous thrombosis or thrombosis of small vessels providing blood to
any organ or tissue. Presence of thrombi must be proved by methods of
visualization or Doppler test or proved pathohistologically, except for
thrombosis of the superficial veins. During proving of the diagnosis using
pathohistological investigation, thrombosis should be present without
significant inflammatory processes in the vessel wall.
2. Pathology of pregnancy:
(a) one or more cases of undetermined death of a morphologically
normal fetus in the 10th or later week of pregnancy, during presence of
normal morphology of the fetus, recorded by ultrasound study or direct
examination, or
(b) one or more early deliveries of a morphologically normal new-
born before the 34th week of pregnancy as a result of (a) eclampsia or
severe preeclampsia, determined according to the standard criteria, or
(c) clear signs of fetoplacental insufficiency, or
(e) three or more unexplained successive spontaneous abortions before
10th week of pregnancy, at excluding anatomical or hormone disorders
in the mother and chromosome anomalies in father and mother.

Patients who have one type of pregnancy pathology are strongly
recommended to be separated into group a, b or c.

Laboratory criteria.
1. Anticardiolipid antibodies of IgG or IgM isotypes in the serum or
blood plasma, present on average or high titer, found on 2 or more
cisions during 12 weeks using the standardized method of immune-

2. Lupus anticoagulant in blood plasma, found on 2 or occasions
during 12 weeks using the method which corresponds to the regulations
of the International Society of Thrombosis and Hemostasis (Scientific
Subcommission of Antikoagulant of Lupus erythematosus / phospholipid-
dependent Antibodies).
3. Antibodies to β2-glycoprotein I IgG and (or) IgM isotypes, found
in blood serum or plasma on 2 or more occasions during 12 weeks
using the standardized method of immune-enzymatic analysis in

New models of quantitative assessment of risk of thromboses and
midwifery processes in patients with APLS are in the process of deve-
lopment. Their main goal is detecting patients with higher possibility of
development of new thromboses with a view to their timely preven-
tion. Apart from laboratory indicators, the global anti-phospholipid synd-
rome score (GAPPS) includes other variables such as cardio-vascular
risk factors (Sciascia et al., 2013). It is expected that GAPPS will make
it possible to provide quantitative assessment of risk of thrombosis and
midwifery pathology in patients with APLS.

Disorders in the system of hemostasis can not only be one of the
reasons for infertility, but also condition the successlessness of ART,
hormostasis system, and is accompanied by increased thrombotic risk. Within two weeks, the estradiol concentration increases by over 100 times, and its level correlates with the level of fibrinogen, D-dimer and activated protein C. Stimulation of ovulation is accompanied by increases in some circulating coagulation factors: factor V, fibrinogen, Von Willebrand factor, thrombophilia markers, disorder in the endogenous anticoagulants – antithrombin III and protein S. Factually, in parallel, the process of chronic intravascular blood coagulation is activated. Women with excessive reactions to exogenous gonadotropins can develop ovarian hyperstimulation syndrome (OHSS) which is associated with deeper changes in the system of hemostasis and clear hypercoagulation (Chan, 2009; Kasurn et al., 2014).

Presence of undiagnosed processes of activation of blood coagulation before ECF programmes are conducted is a risk factor of ovarian hyperstimulation syndrome and thromboses (Levy & Lucidi, 2011; Ro-vaet et al., 2012). Risk of venous thromboembolism after ECF increases by 20–30 times compared to the general population (Rao et al., 2005), which indicates the necessity of assessing such risk during preparation for ART and taking necessary preventive measures (Nelson, 2013; Sennström, 2017).

Presence of psychosomatic disorders in a woman with infertility can also be related to APLS, for currently antiphospholipid syndrome (APLS) is understood as a symptom complex which combines clinical signs and laboratory data: circulation of APA in combination with arterial and venous thromboses, syndrome of fetal loss, immune thrombocytopenia or neurological disorders (Negrini, 2017). Neurological disorders are among the most common manifestations of APLS. The first description of the disease, made by G. R. Hughes, included pathology of the nervous system. The possibility is foreseen that APA can directly connect with nervous tissues, thus causing disorders in their function. Although in the recent updated criteria of APLS classification (2006), the neurological manifestations cover only transient ischemic attacks and strokes, there are known various "non-critical" neurologic disorders or manifestations: headache, migraine, bipolar disorder, myelopathy, dementia, chorea, epileptic seizures, multiple sclerosis, psychosis, cognitive disorders, Tourette syndrome, Parkinsonism, dystonia, transient global amnesia, disorders of eyegaze etc. (Islam et al., 2016; Yelnik et al., 2016).

Therefore, the objective of our study was determining the role of antiphospholipid antibodies in the effectiveness of treating infertility using the methods of reproductive technologies on women with psychosomatic disorders.

Materials and methods

In the clinic of reproductive technologies of the Ukrainian National Institute of Reproductology of Shupyk National Academy of Postgraduate Education, 93 women with tubular-peritoneal factor of infertility were examined, who were prescribed the treatment of infertility using the methods of ART (main group). The control group comprised 30 fertile women with newborn children, and who had contacted the women consultation service of the Kyiv Center of Reproductology and Perinatology for preconception preparation before planned parenthood.

All women were examined by therapist, psycho-neurologist. For the assessment of psychological and social status, we used a specially developed questionnaire, where the women answered the questions about the family condition, occupation, reproductive anamnesis, presence of bad habits.

The syndrome of vegetative dysfunction (SVD) was diagnosed using the A. M. Vein questionnaire (1998) with assessment of the vegetative symptoms expressed in points (11 parameters). For the quantitative assessment of SVD signs, expert assessment was performed by assigning a corresponding point (1 to 10) in relation to the specific weight in the total of the SVD symptoms. If the total amount of the points reaches 15 and higher, the SVD is diagnosed.

Assessment of sleep disorders was made in accordance to the A. M. Vein Table which includes 6 questions, in accordance to which the respondent assesses the time of falling asleep, duration of sleep, night awakenings, dreams, quality of sleep, quality of awakening, by choo-
using endonuclease of HindIII restriction. The condition of amplified and restricted fragments was analyzed using the horizontal electrophoresis in 2% agarose gel (160 V for 40 minutes). The obtained results were visualized using a transilluminator and the patient’s genotype was determined in relation to the molecular weight of the DNA fragments of the examined genes.

The results were statistically analyzed using Statistica 6.0 program packs (StatSoft Inc., USA). The statistical reliability of the difference between the selections and qualitative parameters which had normal distribution was assessed according to Student’s t-test, for the parameters expressed in the shares, we used the criterion of Fisher’s angle transformation, the differences were considered significant at P < 0.05. At the normal distribution law, the parameters are presented as mean arithmetic value, mean standard error (x ± SE).

Results

Increased level of APA (over 10 GPL-U-ml for IgG and over 10 MPL-U-ml for IgM) was found only in 2 (6.7%) women of the control group and in one third of the women with infertility (34 patients, 36.6%, P < 0.05), i.e. over five time increase of this parameter was determined, which indicates the possibility of autoimmune and thrombo-vascular disorders which condition the risk of ART inefficiency and premature birth (Fig. 1). At the same time, in the range of the determined APA, the dominant part was anticardiolipid antibodies (76.5% against 35.8% and 27.2% APA to PE and PS respectively).

The frequency of significant titers of APA in the 1st group equaled 18.9%, while in the control group it was 9.6% against 24.5% in the women in the second group. The mutant variant of polymorphism related to C148T gene of β-2-GPI were found in 18 (19.4%) patients and one (3.3%) woman in the control group (P < 0.05).

Analysis of determined APA depending on the presence and extent of manifestation of psychosomatic disorders indicated (Fig. 1) that the frequency of significant titers of APA in the 1st group equaled 18.9%, and in women with psychosomatic disorders, it was more than twice as high, equalling 44.3% (P < 0.05). The frequency of finding β-2-GPI in the first group was 9.6% against 24.5% in the women in the second group. An integrated analysis of anamnestic data indicated that only 7 (11.5%) women of the second group had an increased level of APA combined with a significant level of β-2-GPI and/or one or several clinical criteria of APA (cases of instrumentally or morphologically proven vascular thrombosis, loss of morphologically normal fetus after 10 weeks of pregnancy, premature birth before the 34th week of pregnancy as a result of preeclampsia or clear placental insufficiency, three or more subsequent cases of spontaneous abortions before the 10th week of pregnancy without anatomic anomalies of the womb or chromosomal disorders). In these cases, APLS diagnosis was considered proven, for the other 23 (37.7%) of women of the second group and all 7 (18.9%) of the women of the first group, a clinically unproven increased APA level was determined as "doubtful APLS".

The study of the hemostasis system (Table 1) of the patients with infertility and psychosomatic disorders revealed an increase in the aggregation of thrombocytes following relative thrombocytopenia (number of thrombocytes 231.4 ± 15.5 against 278.2 ± 17.4 and 341.3 ± 18.4 10⁷/l in women of the first and control group, P < 0.05), and also, compared to the first group and in the control, significantly higher level of fibrinogen, soluble fibrin-monomer complexes (8.4 ± 1.2 against 4.8 ± 0.76 and 3.7 ± 0.32 mg/ml respectively, P < 0.05), significantly extended APTT (to 34.9 ± 1.2 ± ± 1.0 and 26.3 ± 1.2 s in the women of the 1st and the control groups, P < 0.05) at significantly high level of D-dimer (328.1 ± 37.6 in relation to 186.2 ± 15.4 and 158.3 ± 18.4 ng/ml, P < 0.05). Such changes indicate certain vulnerability to hypercoagulation and thrombus formation and often are often associated with APLS. At the same time, there was recorded hyperhomocysteinemia among some of the patients from this group: mean value of concentration of the parameter equaled 10.5 ± 0.36 µmol/l against 5.7 ± 0.32 and 5.0 ± 0.24 µmol/l in the first and the control groups (P < 0.05), which indicates the possibility of damaging endothelium of the vessel and is associated with APLS.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group of women</th>
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<tr>
<td></td>
<td>first group (women with infertility without psychosomatic disorders, n = 32)</td>
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<tr>
<td>Number of thrombocytes, 10⁷/l</td>
<td>(278.2 ± 17.4)* (231.4 ± 15.5)% # 341.3 ± 18.4</td>
</tr>
<tr>
<td>Index of ATP-induced aggregation, %</td>
<td>48.3 ± 6.2 (58.8 ± 4.6)*</td>
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<tr>
<td>Fibrinogen, g/d</td>
<td>3.0 ± 0.21 (4.2 ± 0.26)*</td>
</tr>
<tr>
<td>Prothrombin index, %</td>
<td>92.2 ± 8.5 (107.4 ± 13.6)</td>
</tr>
<tr>
<td>APTT, s</td>
<td>29.2 ± 1.0 (34.9 ± 1.2)*</td>
</tr>
<tr>
<td>SFMC-test, mg/ml</td>
<td>4.8 ± 0.76 (8.4 ± 1.2)*</td>
</tr>
<tr>
<td>D-dimer, ng/ml</td>
<td>186.2 ± 15.4 (329.1 ± 37.6)*</td>
</tr>
<tr>
<td>Homocysteine, µmol/l</td>
<td>5.7 ± 0.32 (10.5 ± 0.36)*</td>
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Notes: * – difference is significant in relation to the parameter of women of the control group (P < 0.05); # – difference is significant in relation to the parameter of women of the first group (P < 0.05).

According to the obtained data on the women with infertility and psychosomatic disorders, we determined higher frequency of pathologial polymorphisms of C148T gene of β-fibrinogen (Fig. 2).

### Notes

**Fig. 1.** Frequency of APA occurrence among the women with infertility depending on the presence and extent of psychosomatic disorders.  
- difference is statistically significant in relation to the parameter of women in the control group (P < 0.05); # – difference is statistically significant in relation to the parameter of women in the first group (P < 0.05).

**Fig. 2.** Frequency of genotypes of polymorphism of C148T of β-fibrinogen gene. * – difference is significant in relation to the parameter of the women from the first group (P < 0.05)

The normal variant of polymorphism in homozygous form CC occurred in the half (54.1%) of the examined patients against 75.0% of the women with infertility without psychosomatic signs (P < 0.05). The heterozygous form of polymorphism of CT was found in 21.9% of the women from the first group and one third (29.5%) of the women from the second group. The mutant variant of polymorphism related to
increase of the risk of thromboses and other pathologic conditions was found in homozygous form only in 1 (3.7%) woman of the first group, and in 10 women in the second group, which equaled 16.4% (P < 0.05).

After conducting a study on the distribution of frequencies of the variants of genotypes in relation to 455GA polymorphism of β-fibrinogen gene among the women with infertility and liver disorders, we determined a certain decrease in the frequency of the protective allele G and increase in the frequency of allele A which is responsible for the risk of development of thromboses and midwifery complications (Fig. 3) mostly due to the development of the heterozygous form (GA), the percentage of the carriers of which in the first group equaled 31.3%. The homozygous variant AA, quite rare in the general population, was observed in 9.8% women of the second group.

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### Table 2

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<tbody>
<tr>
<td></td>
<td>the first group (women with infertility without psychosomatic disorders, n = 32)</td>
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<tr>
<td>Minor allele T of polymorphic locus C148T</td>
<td>number of patients</td>
</tr>
<tr>
<td>Minor allele A of polymorphic locus 455GA</td>
<td>8</td>
</tr>
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<td></td>
<td>10</td>
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Note: * = difference is significant in relation to the women of first group (P < 0.05).

Analysis of the effectiveness of using ART on the patients of the examined groups revealed the following: pregnancy occurred almost in half of the women of the first group (46.8%) and only in 14 (23.0%) patients with psychosomatic disorders (P < 0.05), which can be conditioned by the high frequency of their determined prothrombotic parameters associated with APLS and which can be used as predicting risk factors of ART inefficiency (Fig. 4):

- increase in the level of APA (odds ratio OR = 2.4; confidence interval CI 1.0–7.7, P < 0.05);
- increase in the frequency of allele T of polymorphic locus C148T of β-fibrinogen (OR = 4.2; CI 1.0–16.9, P < 0.05).

As we see from the data of risk and calculation of the odds ratio, among the analyzed factors, the most significant effect on the ART efficiency among the women with infertility and psychosomatic disorders belongs to the genetic conditionality – presence of minor allele T of polymorphic locus C148T of β-fibrinogen gene (OR = 4.2; CI 1.0–16.9, P < 0.05).

### Discussion

We determined a more than five times increase in the frequency of finding significant titers of APA among the women with infertility, which indicates the possibility of autoimmune and thrombovascular disorders, which condition the risk of ART inefficiency and premature birth, which corresponds to the results of our studies (Sauer et al., 2010; Mashkova, 2015; Stuleva et al., 2015; Saccone et al., 2017; Song et al., 2017).

Analysis of manifestations of APA depending on the presence and manifestation of psychosomatic disorders indicated that the frequency of significant APA titers in patients with infertility and psychosomatic disorders was two times higher and equaled 44.3% against 18.9% (P < 0.05). We found no similar data in the available literature, but such result can be explained by close relationship with various somatic pathology, particularly endocrine and cardiovascular pathology (Meledi et al., 2011; da Silva et al., 2014).

An integrated analysis of anamnesis data showed that only 11.5% of those women had the APA level combined with one or several clinical APLS criteria and/or high level of antibodies to cofactor APA β-GP1. In such cases, the APLS diagnosis is considered proven (Chigizola et al., 2015). In 18.9% of the women, a clinically unproven level of APA is defined by the term "doubtful APLS", but the authors have demonstrated (Chernyshev et al., 2011) that such cases concern "cofactor antiphospholipid antibodies" associated with heightened risk of typical reproductive and midwifery complications. Such changes indicate certain immune-regulatory disorders and risk of autoimmune changes in future.

The study of the system of hemostasis revealed that the patients with infertility and psychosomatic disorders have a certain predisposition to hypercoagulation and thrombus formation, which is associated with APLS (Masliash-Planchon & Darnige, 2012; Bao et al., 2017; Fabris et al., 2017). At the same time, some of the patients of this group were observed to have hyperhomocysteinemia which indicates possible damage to the endothelium of the vessels (Lai & Kan, 2015) and is also associated with APLS (de Souza, 2007).

In the course of the study of the polymorphic loci C148T and 455GA of β-fibrinogen gene, which according a number of researchers are related
to APLS and reproductive disorders of women (Alessio et al., 2008; Jeddi- Tehrani et al., 2011; Tataruchak et al., 2015), we determined that the group of women with infertility without psychosomatic disorders had a frequency of minor allele of these loci within the values reported in the literature sources (16–29%) (Rupert et al., 1999; Madjunkova et al., 2012; Osrajca et al., 2012), whereas in the patients with psychosomatic disorders it exceeded 40%.

After the analysis of odds ratio of the prothrombotic parameters we studied, we demonstrated their impact on the effectiveness of ART among women with infertility and psychosomatic disorders, which coincides with the data of other researchers, which, however, did not consider the presence of psychosomatic disorders (Sauer et al., 2010; Mashkova, 2015; Stuleva et al., 2015). The most significant among the analyzed parameters, according to our data, is genetic conditionality – presence of minor allele T and polymorph locus C148T of β-fibrinogen gene (OR = 4.2; CI 1.0–16.9, P < 0.05).

Conclusions

The women with infertility and psychosomatic disorders were characterized by a high frequency of increase in the level of antiphospholipid antibodies, antibodies to β2-glycoprotein and other prothrombotic conditions (changes in the system of hemostasis towards thrombus formation and increase in coagulation potential, hyperhomocysteinemia, carrying mutant alleles of polymorphic loci of β-fibrinogen gene) associated with antiphospholipid syndrome. There the factors were distinguished which negatively affect the effectiveness of the use of assistive reproductive technologies on the patients with psychosomatic disorders: increase in APA level (OR = 2.4); decrease in the number of erythrocytes (OR = 3.1); increase in the index of ATP-induced aggregation (OR = 2.8); increase in the level of fibrinogen (OR = 3.4), prolongation of APTT (OR = 3.1); increase in the level of D-dimer (OR = 1.3); increase in the level of homocysteine (OR = 2.8); presence of minor allele T of polymorph locus C148T of β-fibrinogen gene (OR = 4.2). The most significant among the analyzed parameters, according to our data, is the genetic conditionality – presence of minor allele T of polymorph locus C148T of β-fibrinogen gene. The abovementioned parameters may be predicting markers of ineffectiveness of assistive reproductive technologies among patients with psychosomatic disorders. Presence of prothrombotic conditions associated with APLS should be taken into account during the preparation for assistive reproductive technologies and correction must be made for them.

References


