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Chapter 4

The Means of Progress in Improving the Results of *in vitro* Fertilization Based on the Identification and Correction of the Pathology of Hemostasis

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Additional information is available at the end of the chapter

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1. Introduction

In most developed countries the problem of infertility has acquired not only medical and sociodemographic, but also economic significance. One of the key and vigorously developing trends in modern obstetrics is aimed at overcoming this problem and requires a multidisciplinary approach. The 2010 Nobel Prize was awarded to Robert Edwards, 85-year-old British researcher and recognized the methods of assisted reproductive technologies (ARTs) which introduced a new era in human demography. ARTs are used to solve the problem of infertility; they use all treatment methods and procedures which support in vitro processing of human oocytes, sperm or embryos to become pregnant. These technologies involve in vitro fertilization (IVF) and transcervical embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, embryo intrafallopian transfer, gamete and embryo cryopreservation, oocyte and embryo donation, and surrogacy (Current Practices and Controversies in Assisted Reproduction. Report of.., 2001).

25 July 1978 was marked by a significant event in the history of ARTs. On that day, the first "test tube baby" was born in an obstetrics and gynecology clinic located in Oldham, North West England. This was also the birth date of modern assisted reproduction. In Russia, the first baby born by this method was delivered in 1986 in the Research Center for Obstetrics, Gynecology and Perinatology, the division of the Russian Academy of Medical Sciences. Other leading centers in Russia are “Fertimed “Center for Reproduction and Genetics (Moscow), “ART-ECO” Clinic for Reproductive Health (Moscow), “ECO” Center for the

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Treatment of Infertility, LLC (Moscow), International Center for Reproductive Medicine (Saint-Petersburg), the Baltic Institute of Human Reproductology (Saint-Petersburg), etc.

2. In vitro fertilization and known reasons that reduce its efficacy

In vitro fertilization of preovulatory oocytes and transfer of cleaving embryos into the patient's uterine cavity has become the prevalent method to overcome the problem of infertility (Pioneers in in vivo Fertilisation .., 1995). The IVF method was initially devised for those women whose Fallopian tubes were removed for one reason or another. However, at present, IVF is the most effective treatment for virtually all types of infertility, including endometriosis, polycystic ovary syndrome, oocyte donation (in infertile patients with oogenesis depletion), fertilization of an egg with a single sperm in cases of virtually absolute forms of male infertility (intracytoplasmic sperm injection - ICSI), carriage of an embryo by a voluntary egg recipient when a woman cannot carry the pregnancy due to some somatic or other diseases (Maheshwari et al., 2008). Despite all achievements, the IVF pregnancy rate is comparatively low. It ranges from 25% to 30% and has not changed considerably in recent years (Nyboe Andersen et al., 2009). This rate relates to a number of diverse factors that affect the reproductive process. Implantation failure following embryo transfer is the major problem in IVF (Bischof et al., 2006; Christiansen et al., 2006). IVF failures may be caused by a variety of factors: diminished ovarian reserve, maternal and paternal age, excessive body weight, endocrine disorders in the hypothalamus-pituitary-ovary system, as well as in the suprarenal and thyroid systems, diminished endometrial receptivity, quantity and quality of transferred embryos, number of transfers, and thrombophilic disorders.

Ovarian reserve. Ovarian reserve is one of the factors that determine the efficacy of IVF (Gregory, 1998; Navot et al., 1987; Scheffer et al., 2003). Assessing the ovarian reserve, specialists draw conclusions based on the prospects of ovarian stimulation in a particular patient. Conclusions may be used to define a specific procedure and further treatment prospects, as well as to make the right choice of the ovarian stimulation scheme and the quantity of drugs of human menopausal gonadotropin or follicle-stimulating hormone (FSH), which are necessary for an adequate response. The routine method for assessing the ovarian reserve measures basal FSH level on the 3rd or 4th day of the menstrual cycle and estimates the quantity of antral follicles with ultrasonography. However, at present, the prognostic significance of ultrasonography is considered less informative, even though it reflects the quantity and quality of oocytes (Damti et al., 2008). The role of new factors capable of reflecting the functional status of the ovary in a more precise manner is under discussion. (Gregory, 1998).

Maternal and paternal age. Lintsen et al. (2007) concluded that the most important prognostic indicator to define the probability of pregnancy after IVF and ICSI is maternal age (more frequently observed positive results -in 30-year-old women, less frequently - in women under 35, and least frequently - in women over 35). Physiological process of the gradual decline of ovarian function is one of the key obstacles for the efficacy of IVF, which depends on maternal age, current condition of the ovarian reserve and to a lesser extent on chosen

schemes of ovulation induction. The cases of women under 41 are treated as relatively promising, to reason the use of donated oocytes in older women (Maheshwari et al., 2008). Paternal age also affects the conception rate: it shrinks with men after 35 due to the quality of sperm to have been deteriorated by this age (Saleh et al., 2002).

Excessive body weight. Menstrual dysfunction, polycystic ovary syndrome, hyperplastic processes in endometrium, infertility, miscarriage, gestoses, fetal hypotrophies, high rate of operative deliveries make up an incomplete list of reproductive disorders typical of obese women. 40% of women seeking for treatment of infertility in medical centers have excessive body weight; over 15% of such women are obese. The IVF program is preferable to start after the patient's body weight has become normalized; therefore, patients often fail to meet the required standards (Ku et al., 2006; Lintsen et al., 2005; Mc Clamrock, 2008; Megan et al., 2008). Status of the hypothalamus-pituitary-ovary system, suprarenal and thyroid systems. Interaction of the two key pituitary hormones - FSH and luteinizing hormone (LH) - is essential for the adequate growth of follicles, as well as for the formation of viable oocytes. Studies of ovulation induction in hypogonadotropic patients showed that exogenous FSH stimulates the growth of follicles up to the preovulatory stage and its synthesis primarily depends on LH, i.e. adequate maturation of follicles takes place due to this gonadotropin. Insufficient concentration of LH disturbs paracrine mechanisms regulating granulosa cells, as well as endometrial proliferation, and results in inadequate luteal phase (Alviggi et al., 2009; Balasch et al., 1995; Hull et al., 1994). Excessive concentration of LH also negatively affects the growth of follicles to be the result of suppressed aromatase activity, accompanied by fertilization disorders, decreased pregnancy rate decrease and increased miscarriage rate (Hillier, 1994). Thus, the threshold concentration (1-10 IU/l) is optimal for adequate folliculogenesis (Howles et al., 2006). It has been noted that low estradiol level in the blood serum (<200 pmole/l) on the 3rd day of the patient's menstrual cycle is a positive prognostic indicator of successful implantation in the IVF cycle. At the same time, some reports state that basal estradiol level was not a significant indicator of ovarian response to stimulation and did not correlate with the IVF result (Friedler et al., 2005). In recent years, researchers and clinicists have given a lot of consideration to the problem of thyroid gland dysfunction in infertile women (Bellver et al., 2008). Female reproductive system consists of interrelated structural elements: hypothalamus, pituitary gland, ovaries, other endocrine glands and target organs facilitating reproductive function. Thyroid gland is a chief part of the neuroendocrinal system; it significantly affects reproductive function. The hypothalamus-pituitary-gonadal and hypothalamus-pituitary-thyroid systems are closely related due to the presence of common central regulating mechanisms. For example, the spread of thyroid gland dysfunction diagnosed at the examination in women, who seek clinical diagnosis and treatment of infertility, ranges from 2.5 to 38.3% (Lazarus & Premawardhana, 2005). In addition to gonadotropic hormones, ovarian function is determined by adrenal hormones produced under impact of ACTH. When a patient has developed any genetic defects in the enzyme systems, cortisol synthesis in adrenal glands decreases with the increase of level of ACTH followed by the increased production of androgens under normal synthesis. This condition may be typical of congenital adrenal hyperplasia. As a result of adrenal

hyperandrogenism, the suppression of ovarian function takes place, which leads to the development of a number of disorders in the menstrual cycle accompanied by anovulation.

Diminished endometrial receptivity. After high-quality embryos have been transferred into the uterine cavity and all evident causes for the failure of the IVF program have been eliminated, the unsuccessful IVF cycle is regarded as a result of disorders that occurred during the embryo implantation stage. A few years ago a new term - "repeated implantation failure" - was introduced (Margalioth et al., 2006; Tan et al., 2005). Recent years have shown that, despite the selection of obviously normal embryos for the transfer, only 20% of human embryos transferred in IVF cycles have been implanted in the uterus (International Committee for Monitoring Assisted Reproductive Technology (ICMART), 2002; Nyboe Andersen et al., 2009). This condition is considered to be based on the endometrial dysfunction occurring on the mollecular-cellular level. Lately, as a result of the tendency to transfer one or two embryos inside of an uterus, the method to determine repeated implantation failure has been modified. Margalioth et al. (2006) concluded that detailed examination should be done after 3 unsuccessful IVF cycles. Thus, the main causes are the factors which diminish endometrial receptivity: anatomical defects in the uterus, chronic endometritis, non-correspondence between the endometrial thickness and the day of embryo transfer, combined gynecologic pathology (adenomyosis, uterine fibroid), somatic diseases (including autoimmune diseases), and thrombophilias (Margalioth et al., 2006; Tan et al., 2005).

Quantity of transferred embryos. Due to the absence of conventional clinical guidelines for the treatment of infertility with the IVF method, there are on-going discussions regarding the elective transfer of one embryo to patients under 40. In 2009, a mathematical model was drawn to prove that the transfer of only one embryo shall decrease the pregnancy rate by 20% (Gelbaya et al., 2009).

Embryo transfer on the stages of cleavage or blastocyst. The data obtained during the systematic review and meta-analysis (Papanikolaou et al., 2008) of 1654 patients (blastocyst transferred to 815 patients, cleaving embryo transferred to 839 patients) showed that live birth rate was higher with embryos transferred on the blastocyst stage as compared to the rate at the cleavage stage. Multiple gestation rates were the same for both study groups.

3. Data on the IVF results as affected by pathologies of hemostasis

As we have already noted, unsuccessful IVF cycles are caused by many factors, including thrombogenicity of the medical technology itself due to the high estrogen-gestagen rate and frequent presence of thrombogenic risk factors and predisposition to intravascular coagulation (thrombophilia) in women that need IVF. According to up-to-date conceptions, the term "thrombophilia" means predisposition to arterial or venous thrombosis as a result of several hereditary or acquired disorders in the systems of blood coagulation, anticoagulation, or fibrinolysis (Bates et al., 2008; Heit, 2007). We use this term in a different sense, which makes most of the described thrombophilias no more than thrombogenic risk factors that may or may not become evident during the lifespan of a human being.

According to our conceptions, thrombophilia should be detected in case of everpresent thrombosis risk factors (thromboses) or miscarriage syndrome in the individual medical history. In order to prove it, we note the fact that, according to the guidelines of the International Society on Thrombosis and Haemostasis (ISTH), diagnosis of antiphospholipid syndrome (APS) shall be considered invalid unless at least one or more clinical implications of this pathology match the results of special laboratory assays (lupus anticoagulant effects, antiphospholipid antibodies in the diagnostic titer) (Harris & Pierangeli, 2008).

Some publications indicate data on typical changes in the system of hemostasis that occurs during the IVF cycle. In particular, demonstration has shown that hormonal stimulation of the ovaries is accompanied by the increased von Willebrand factor, factors V and VIII, fibrinogen, enhanced APC resistance, and the decreased activity of principal physiological anticoagulants - antithrombin, proteins C and S (Andersson, 1997; Biron et al., 1997; Chan & Dixon, 2008; Curvers et al., 2001b; Nelson, 2009). Relationship between the predisposition to intravascular coagulation (thrombophilia) and unsuccessful ART results is actively discussed in current publications; however, mechanisms to produce the impact that increases thrombotic readiness on IVF are not absolutely clear. It is reported that women with thrombophilia may have increased risks for spontaneous abortion, preclinical pregnancy loss and recurrent implantation failure (Christiansen et al., 2006; Coulam et al., 2006b; Curnow et al., 2006; Many et al., 2001; Seghatchian et al., 1996; Stern & Chamley, 2006; Urman et al., 2005; Wichers et al., 2009; Younis et al., 2000). Presently, most studied and prevalent thrombophilias include APS and such risk factors as hereditary antithrombin III deficiency, factor V Leiden mutation, prothrombin mutation, polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, platelet glycoproteins ITGA2, ITGB3, and some others. Beer and Kwak (2000) treated unsuccessful IVF programs as the evident indication for assays capable of detecting hereditary and acquired thrombophilias. In 2004, Azem et al. (2004) demonstrated higher occurrence of hereditary thrombophilia in women with multiple IVF failures as compared to the group of fertile women who became pregnant after the first IVF cycle. In the research conducted by Qublan et al. (2006), 69% of women with recurrent IVF failures had at least one hereditary or acquired thrombogenic risk factor as compared to 25% of women in the group where this reproductive technology was successful. In the publication presented by Grandone et al. (2001), factor V Leiden mutation prevailed (14.4%) in women with recurrent IVF failures as compared to the controls (1%). Recently, Coulam and Jeyendran (2009b) have shown that frequency of genetic polymorphisms has been 1.6 times higher in infertile women with IVF failures as compared to the fertile group; thus, polymorphism of the MTHFR gene has prevailed. 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 (Coulam et al., 2006b).

Mechanisms of hemostasis and implantation pathologies typical of some thrombophilias (carriage of thrombogenic risk factors):

- factor V Leiden mutation (1691G>A). As long as activated protein C (APC) blocks activated factor V in regular conditions, resistance of the latter to APC can cause the increase of thrombinemia (Bertina et al., 1994; Dahlbäck et al., 1993). It is considered that patients who experience this mutation have higher risks for thrombosis and subsequent pregnancy loss. (Ridker et al., 1998; Urman et al., 2005; Younis et al., 2000);
- prothrombin mutation (20210G>A). This mutation is accompanied by the increased synthesis of prothrombin in the liver and increased risk for venous thrombosis (Girolami & Vianello, 2000; Poort et al., 1996);
- MTHFR gene polymorphism (C677>T) potentially causes increase in the level of homocysteine in blood (> 15 μmole/l), which affects human reproductive function (Nelen et al., 1998). In their publication Berker et al. (2009), interconnection between high level of homocysteine in the follicular fluid and decreased quality of oocytes and embryos in IVF programs has been discovered. High level of homocysteine in blood plasma relates to the decreased diameter of blood vessels located in the chorionic villi and miscarriage (Jerzak et al., 2003; Nelenet al., 2000). Thrombogenic action of homocysteine is based on the damage of endothelial cells, inhibition of prostacyclin synthesis, and increased platelet aggregation;
- PAI-1 gene polymorphism (5G>4G; 4G/4G) is related to the increased expression of PAI-1 in the blood plasma and endometrium, which disturbs the reaction of plasminogen activation into plasmin (Anteby et al., 2004; Buchholz et al., 2003; Ebish IM, et al., 2008; Kim et al., 1997);
- antiphospholipid syndrome. Damaging effect of antiphospholipid antibodies (APLAs) on oocytes and embryos on the early development stages has been indicated (Matsubayashi et al., 2006). Women with APS demonstrate high level of NK cells in the peripheral blood and endometrium. It is possible that disturbed synthesis of mediator molecules which participate in embryo adhesion and invasion by NK cells is one of the causes of disturbed signaling interaction between the blastocyst and endometrium on the earliest implantation stages (Quenby et al., 2009; Sher et al., 2000). APLAs stimulate PAI-1 synthesis that may cause disturbed degradation of intercellular matrix and decreased depth of blastocyst invasion.

Thus, making a brief summary, different forms of thrombophilia refer to different pathogenesis of implantation failures. Fixed and common conception for all thrombophilias is that pathology mechanisms are revealed in the earliest stages of pregnancy and are caused due to microcirculation and hemostasis disorders, as well as vessel wall pathology. Some researchers deny the impact of mutations of certain genes within the hemostasis system or isolated asymptomatic increase in the level of phospholipid antibodies on implantation. However, combination of several factors is believed to considerably increase individual risk of possible implantation failures and miscarriage. While estimating the occurrence of APS and mutations of genes participating in the hemostasis system in infertile women and women with IVF failures, specialists actively investigate qualitative changes on the level of endometrial structures and vascular endothelium accompanied by thrompophilia (Anteby et al., 2004; Coulam & Roussev, 2009a). Local mechanisms in the basis for implantation failures which occur along with thrombophilias are under

investigated. Excessive activation of coagulation, imbalance in the coagulation system, endotheliopathy, local haemorrhages and microthrombi in the area of blastocyst invasion are common elements in the mechanism of implantation failures caused by thrombophilias. Thus, different forms of thrombophilia result in disorders on different stages of the coagulation cascade and fibrinolysis.

Analysis of publications demonstrates diverse expert opinions on the role of some mutations, polymorphisms of genes which are part of the hemostasis system and APS role in the development of infertility and ART failures. The American Society of Reproductive Medcine does not find it necessary to examine women who participate in IVF programs in order to detect risk factors for thrombophilia (American Society of Reproductive Medcine .., 2008). On the contrary, approach of the American Society for Reproductive Immunology to treat the same problem is completely opposite (American Society for Reproductive Immunology Antiphospholipid Antibody Committee .., 2000; Gleicher et al., 2002). Inconsistency of the opinions mentioned above may be explained by the absence of vast multicentral studies, use of diverse methodological approaches to diagnosing hemostasis pathologies and interpretation of examination results.

We believe that the most perspective approach in this area may be found in simultaneous consideration of thrombogenic risk factors and monitoring of the results collected with "global" methods which are capable of detecting disorders in the natural balance of pro- and anticoadulants in blood plasma under controlled ovarian hyperstimulation. This approach offers specialists the potential for the thrombin generation assay (TGA). This assay is known to define the dynamics and intensity of thrombin development; thrombin is the key hemostatic enzyme and relates to the group of integral indexes of the coagulation system (Hemker et al., 2000; Hemker et al., 2003; Hemker et al., 2006; Regnault et al., 2003; Wielders et al., 1997). This methodological approach has been successfully tested as a part of the complex estimation of the hemostasis system during pregnancy (Dargaud et al., 2010), preeclampsia (Macey et al., 2010) and oral contraceptive intake (Tchaikovski et al., 2007). Recent publication presented interesting data on the specifics of thrombin generation in blood plasma within IVF cycle (Westerlund et al., 2012). Shifts detected in 31 women were interpreted as the result of estrogen load and ovarian hyperstimulation syndrome and estimated to be vital for thrombosis prediction and IVF monitoring.

Besides, a number of researchers raise a great interest in the decrease of fibrinolytic blood activity, which is often detected in recurrent miscarriages, APS, deep vein thrombosis of lower extremities, oral contraceptive intake, myocardial infarction, and malignant neoplasms (Bertina, 1997; Birkenfeld et al., 1994; Curnow et al., 2006; Dmowski et al., 1995; Egbase et al., 1999; Lisman et al., 2005; Meltzer et al., 2009; Meltzer et al., 2010; Triplett, 1989; Wichers et al., 2009). The system of fibrinolysis, as well as the system of coagulation, is a complex system which gives characteristics to fibrinolytic responses and its central element that plays role in the activation of plasminogen into plasmin. Lately, its pathologies have been treated as new approach that explains the mechanisms of thrombosis pathogenesis (Zorio et al., 2008). The analysis of the fibrinolysis system during the IVF procedure shows the decrease in fibrinolytic responses due to several reasons (Andersson et

al., 1997; Aune et al., 1991; Kim et al., 1981; Many et al., 2001; Martinez-Zamora et al., 2011; Meltzer et al., 2010; Nelson, 2009; Rice et al., 1993; Sarto et al., 2000). One of them is a decreased activity of tissue plasminogen activator (t-PA), increased level of its inhibitor - plasminogen activator inhibitor-1 (PAI-1), and increased level of thrombin-activatable fibrinolysis inhibitor (TAFI) dependent on the response of vascular endothelium (Bouma & Meijers, 2003; Martınez-Zamora et al., 2010; Martinez-Zamora et al., 2011; Meltzer et al., 2010).

4. Potential methods for the correction of hemostasis and fibrinolysis pathologies within the IVF program

Analysis of publications shows that correction of imbalanced homeostatic and fibrinolytic responses may be used in case of hormonal load within the IVF program accompanied by thrombophiia or present thrombogenic risk factors in a patient (Martinez-Zamora et al., 2011; Nelson & Greer, 2008; Rova et al., 2012; Urman et al., 2005). This is reasonable to determine thrombotic readiness, which becomes evident through the increase of general coagulation activity and thrombinemia and/or fibrinolysis suppression identified, for example, with the help of the thrombin generation assay upon detecting markers of thrombinemia and estimating fibrin clot lysis time for the fibrin obtained from euglobulins (Lisman et al., 2005; Wichers et al., 2009).

The use of heparins may become one of the methodologies aimed at the decrease of thrombogenicity and increase of IVF efficacy (Nelson & Greer, 2008; Urman et al., 2009). Still, there are no clear indications for the selection of women that need heparin prophylaxis within the IVF cycle.

Correction of hypofibrinolysis within IVF cycle also offers some difficulties for there are no published evidence of any successful drug therapy. Moreover, the hypothetic possibility to use pharmaceutical drugs - fibrinolysis activators (streptokinase, urokinase, and tissue plasminogen activator) - cannot be considered due to the absence of acute thrombosis. In the study conducted by Bjornsson et al. (1989), regular intake of aspirin in high doses (650 mg every 12 hours) caused the acceleration of fibrinolysis. But the mechanism of this effect is not absolutely clear, whereas the use of acetylsalicylic acid in high doses is unsafe due to the potential ulcerogenic effect. Nevertheless, it has been known for about 50 years that some stimuli (venous occlusion, physical load, desmopressin) lead to the acceleration of fibrinolytic responses facilitated by the fast increase of t-PA in blood due to its enhanced secretion by vascular endothelium. The effects of intermittent pneumatic compression (IPC) used to decrease the occurrence of postoperative venous thrombosis became our interests(Browse et al., 1977; Jacobs et al., 1996; Januszko et al., 1967; Holemans, 1963; Keber et al., 1979; Tarnay et al., 1980; Turpie et al., 1977; Weitz et al., 1986). Macdonald et al. (2003) published the results of their randomized pilot study demonstrating the efficacy of heparin prophylaxis combined with IPC in the course of neurosurgical invasions. The study conducted by Tarney et al. (1980) showed that intermittent compression of the calf, along with the increase in linear blood velocity and the decrease in venous stasis, increases local and systemic fibrinolytic potential (according to the shortened fibrin clot lysis time) in

patients with acute myocardial infarction and prolonged movement disorders. Thus, the larger the volume of the compressed tissue gets, the more apparent became the response. The increase in blood and t-PA fibrinolytic activity after mechanical exposure on blood vessels is supported by the results presented by many authors (Bjornsson et al., 1989; Christen et al., 1997; Jacobs et al., 1996; Pandolfi et al.,1968; Salzman et al., 1987; Tarnay et al., 1980). However, we were not able to find any published data on the dynamics of PAI-1 activity. In the meantime, the correlation of the activities presented by these participants of the fibrinolysis system determines its overall efficacy. Some publications relate the mechanism of the IPC antithrombotic effect to the inhibition of coagulation cascade due to the expression of the tissue factor pathway inhibitor (TFPI) into blood flow and the decrease in the level of factor VIIa (Chouhan et al., 1999; Christen et al., 1997). Currently IPC is used worldwide for thromboprophylaxis in patients with strokes, after arthroplasty and a number of other operative invasions, in medical emergency,and applicable, firstly, in cases when administration of anticoagulants is dangerous due to the development of haemorrhage (Geerts & Selby, 2003; Gordon et al., 2012). As a rule, IPC is performed on the lower extremities, though some publications present positive results for the upper extremities compression (Knight & Dawson, 1976). Despite the fact that legs weigh more than arms, it was proved that forearm veins have considerably more t-PA than leg veins (Pandolfi et al., 1968). In our study this form of IPC was used to activate fibrinolytic responses within the IVF program and in the presence of relevant indications.

5. Original researches

This publication is based on the clinical study carried out to define the role of pathologies in the coagulation and fibrinolysis systems facilitating IVF failures, as well as to estimate the results of their correction.

In the framework of prospective analysis we collected data on 327 women who have been visiting the Center for Saving and Recovering the Reproductive Function, a subdivision of the Clinical Regional Hospital (Barnaul), from 2010 to 2012, to participate in the IVF program due to infertility. This study was approved by the Regional Ethics Committee of the Altai Medical University, and all the participants under study expressed their informed consent.

At the first visit women were interviewed about their obstetric, gynecological, and thrombotic history, possible diabetes, pathologies in the thyroid gland, heart and blood vessels. We have conducted ultrasonography of the genitals in order to detect organic pathologies of the pelvic organs and estimate the ovarian reserve (according to the quantity of antral follicles), aspiration biopsy and histologic examination of the endometrium, as well as to detect infections, including sexually transmitted diseases. We have also conducted general and special laboratory assays, including hormone panel assessment, blood chemistry panel, thrombogenic mutation and polymorphism carriage, coagulation profiles, and homocysteine presence. Then, based on the obtained results, women received professional consultation by obstetrician-gynecologists, physicians, and hematologists.

The study was chronologically conducted in two stages. At the first (observational) stage, we examined a random sampling of 163 women in their IVF cycle. At the second stage, we examined a random sampling of 164 women, 98 of which underwent the correction of the hemostasis and fibrinolysis systems in the presence of relevant indications - increased thrombin generation and/or decreased fibrinolytic activity of blood plasma (controlled group or group with the therapeutic effect on hemostasis and fibrinolysis) (Fig. 1).

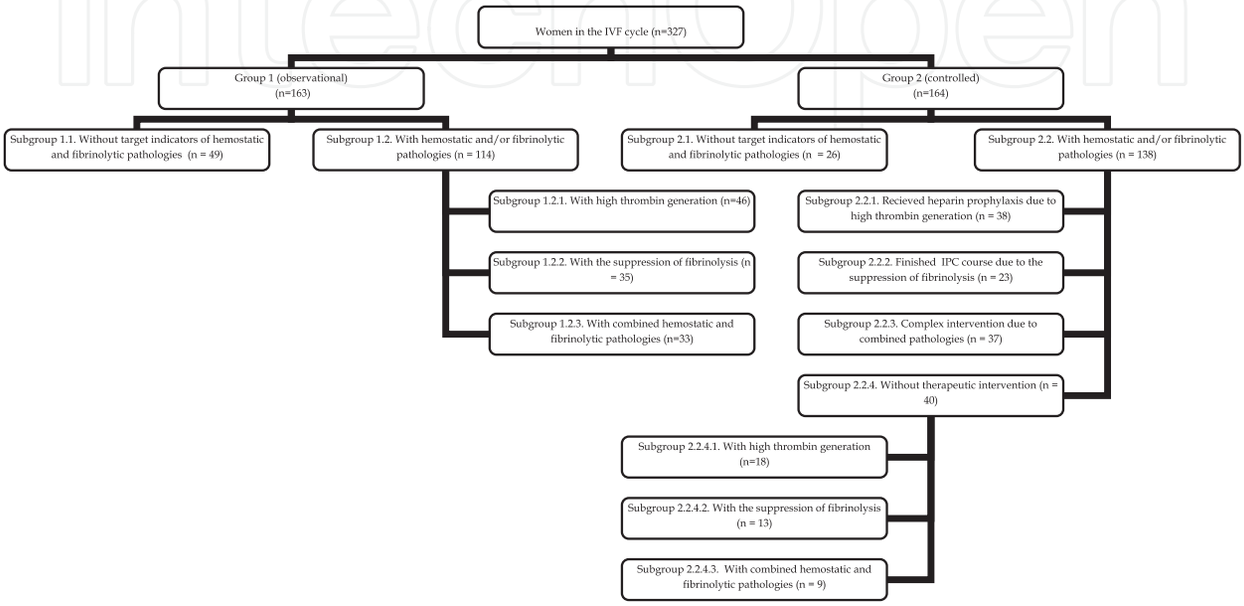


Figure 1. Division of women participating in the study into groups and subgroups

The selection criterion for the patients was any form of infertility non-responsive to traditional treatment. The exclusion criteria were somatic diseases serving as contraindications for carrying a pregnancy and delivery, congenital malformations or acquired deformations of the uterine cavity that make embryo implantation or carrying of a pregnancy impossible, ovarian tumors, benign uterine tumors that require operative invasion, malignant neoplasms.

All patients are representatives of the Caucasian race, their age ranged from 21 to 42 years (Table 1)

There was a difference in clinical profiles of women representing the two groups. The second group of patients suffers from chronic endometritis and has lower ovarian reserve. Besides, male infertility factor was more frequent in this group.

We used standard protocols to induce superovulation. In 72.4% (237 patients) of the whole population we used the "prolonged" protocol with diphereline (Ipsen) 0.1 mg or decapeptyl (Ferring) 0.1 mg and gonadotropic preparations - puregon (MSD) 150-250 IU, menopur (Ferring) 225 IU, or gonal (Merck Serono) 225 IU. In 27.6% (90 patients) of the whole population we stimulated superovulation using the same gonadotropic preparations and an antagonist - cetrotide (Merck Serono) 0.25 mg. Transvaginal follicle puncture was conducted

with ultrosonic guidance using Medison Sonoace X8 machine. After we counted obtained oocytes and estimated their quality with the conventional scale (normal oocytes of good quality - 4-5 points, modified oocytes - less than 4 points), oocytes and embryos were cultivated in 6-well plates in the IVF medium (Vitrolife, Sweden), at 37 degrees Centigrade, in humid atmosphere containing 5% of CO2. Sperm processing was conducted with the Sil-Select Plus medium (FertiPro, Belgium). The ICSI procedure consisted of fertilization with the injection of a single sperm into the oocyte (177 married couples) in case of decreased sperm mobility or irregular sperm morphology. Embryo transfer was conducted with ultrasonic guidance on the 3rd day of cultivation. Embryos with the highest quality rating (A, AB) were selected for the transfer. Pregnancy was diagnosed two weeks after the embryo transfer by means of detecting b-human chorionic gonadotropin (b-hCG). After three weeks we defined quantity and location of the implanted embryos with ultrasonography (in 124 patients).

Indication	Total (n=327)	Group 1 (n=163)	Group 2 (n=164)	P-value*
Age, years (mean ± SD)	33.7 ± 4.1	33.2 ±3.6	34.4 ± 3.9	> 0.5
BMI > 25 kg/m^2, n (%)	61 (18.7)	34 (20.9)	27 (16.5)	0.323
Genital pathology:				
- Chronic endometritis, n (%)	**108 (33.0)**	**42 (25.8)**	**66 (40.2)**	**0.006**
- Endometriosis, n (%)	55 (16.8)	28 (17.2)	27 (16.5)	0.883
- Myoma, n (%)	45 (13.8)	25 (15.3)	20 (12.2)	0.426
IVF failure registered in the history, n (%)	36 (11.0)	19 (11.7)	17 (10.4)	0.727
Low ovarian reserve, n (%)	**55 (16.8)**	**19 (11.7)**	**36 (21.9)**	**0.017**
Extragenital pathology:				
- Hypothyroidism, n (%)	49 (15.0)	21 (12.9)	28 (17.1)	0.352
- Arterial hypertension, n (%)	6 (1.8)	2 (1.2)	4 (2.4)	0.684
Infertility causes:				
- Tubal factor, n (%)	119 (36.4)	61 (37.4)	58 (35.4)	0.730
- Male factor, n (%)	**138 (42.2)**	**59 (36.2)**	**79 (48.2)**	**0.033**
- Endocrinal factor, n (%)	29 (8.9)	14 (8.6)	16 (9.8)	0.848
Combination of female and male factors, n (%)	41 (12.5)	23 (14.1)	17 (10.7)	0.316

P-value* for within-group comparison (Fisher,s exact test); SD, standart deviation

Table 1. Clinical characteristic of the patients examined in the IVF cycle.

Technique of laboratory assays for hemostatic and fibrinolytic profiles.

Examination was conducted three times: 1-2 days before the start of controlled ovarian hyperstimulation and an IVF program (1st observation point), 2-3 days before the puncture of ovarian follicles (2nd observation point), and on the 12th-14th day after the embryo transfer (3rd observation point), when the outcome in terms of pregnancy was defined by estimating the level of b-hCG.

Sampling of venous blood was in the cubital vein in VACUETTE test tubes with the buffer solution of sodium citrate with the proportion of 9:1 (9NC Coagulation sodium citrate 3.2%). Blood was centrifuged at 1400 g and room temperature for 15 minutes. Plasma samples were generally studied within the two hours after being obtained. Prior to conducting immune-enzyme assays and estimating the endogenous thrombin potential, plasma was stored at -40 degrees Centigrade for the period of time ranging from 24 hours to 1 month.

Measuring of the endogenous thrombin potential was made as a part of the thrombin generation assay (TGA). We believe that this method may be regarded as a historical modification of the two-stage self-coagulogram suggested by Berkarda et al. (1965) and developed to pursue similar goals. It was demonstrated that TGA allows experts to measure the dynamics of thrombin generation and inactivation with high precision (Hemker et al., 2003; Hemker et al., 2006). To perform calibrated automated thrombography, the Fluoroskan Ascent microplate fluorometer was applied (Thermo Fisher Scientific, Finland) equiped with a dispenser with Thrombinoscope 3.0.0.26 software. Coagulation of plasma under study was conducted in the presence of tissue factor (5 μM) and phospholipids (4 μM); thrombin generation was continually registered by measuring the signal of fluorogenic substrate (Z-Gly-Gly-Arg-AMC). The following parameters were considered: endogenous thrombin potential (ETP, nM×min), calculating the area under the thrombin generation curve and taking into account specifics of the enzyme inactivation, and peak thrombin concentration - Peak thrombin (nM/l), maximal thrombin concentration per time unit.

Activity of t-PA and PAI-1 was defined by means of the immune-enzyme analysis with sets of reagents t-PA Combi Actibind ELISA Kit and Actibind PAI-1 ELISA (Technoclone, Austria), while the collected data was estimated by mutual comparison. Due to the fact that these important participants of fibrinolytic responses are antagonistic to each other and have a common origin (vascular endothelium), we calculated the index of endothelial ability to activate fibrinolysis (EAAF index). To do that we applied the following formula:

$$\text{EAAF index, \%} = \frac{\text{Activity of t} - \text{PA, un / ml}}{\text{Activity of PAI} - 1\text{, un / ml}} \times 100\%.$$

Defining clot lysis time (CLT). Many authors refer this method to the group of global assays for fibrinolysis assessment. It can be conducted in a variety of ways (Lisman et al., 2005; Martinez-Zamora et al., 2011; Wichers et al., 2009).

Method to define spontaneous lysis time of a fibrin clot obtained from plasma euglobulins was described by Kowarzyk and Buluk (1954). It is based on the precipitation of euglobulin fraction stabilized with sodium citrate in the acid medium with the simultaneous removal of fibrinolysis inhibitors. Then, following this methodology, specialists promote clot formation by recalcifying the reconstituted euglobulin solution and register period for its complete dissolution at fixed temperature (+37 degrees Centigrade). Martinez-Zamora et al. (2011) have recently published original CLT results obtained in women participating in IVF cycles using another method described by Lisman et al. (2002). In particular, this method implies the study of CLT for clots obtained from blood plasma by means of activitaing fibrinolysis with

exogenous t-PA. For our study we use a modified method suggested by Kowarzyk and Buluk (1954) and used kaolin that activates the contact phase of coagulation and starts activation cascade. Factor XIIa → kallikrein → plasmin. Description of this method was given earlier by Barkagan and Momot [Barkagan Z.S., Momot A.P. Diagnosis and controlled therapy of hemostasis pathologies. / M., Publisher: Nyudiamed-AO, 2001. - 296 P.]. Range of normal CLT variations in this modification is 8-12 minutes.

Definition of the D-dimer concentration in blood plasma was conducted with the help of reagent set D-dimer Red-700 (Helena Bioscience, UK) and blood coagulation analyzer Sysmex CA-1500 (Sysmex, Japan). This parameter was considered in accordance with conventional conceptions as a global marker for the completion of fibrin generation and fibrinolysis of stabilized fibrin.

Definition of gene mutations and polymorphisms, predisposing to thrombosis, was conducted with the method of polymerase chain reaction. Thus, we detected the carriers of factor V Leiden (1691G>A) and factor II (20210G>A) mutations, MTHFR (C677>T) and PAI-1 gene polymorphisms related to potential activation of coagulation or decreased plasmin generation, as well as to IVF failures and miscarriages (Coulam et al., 2006a).

5.1. Statistical analysis

Statistical processing of the obtained data was made with the following software: Microsoft Office Excel 2003, Statistica 6.1, and Medcalc 12.2.1. Validity of the differences in mean values was defined with the Student's t-test (t). Group distribution normalcy was estimated with the Shapiro-Wilk test. In cases when the distribution deviated from the norm, we used the non-parametric Mann-Whitney U test for two independent groups and the Spearman's rank correlation coefficient (R). For experimental data presented in percentages or rates the Fisher's exact test was used. Odds ratio (OR) and log-linear rate analysis were calculated as a measure of predictor impact. To estimate the accuracy of obtained values, we defined a 95% confidence interval. Differences $P<0.05$ were considered statistically significant. We assessed the efficacy of the chosen treatment methods with conventional criteria applied in evidence-based medicine, including Absolute Risk Reduction (ARR), Relative Risk (RR), Relative Risk Reduction (RRR), Number Needed to Treat (NNT), and Confidence Interval (CI).

5.2. Study results

5.2.1. Estimation of hemostatic and fibrinolytic indications in the IVF cycle and definition of threshold values occurring in the assay results for the selection of women who need therapeutic intervention during the controlled ovarian hyperstimulation

At the beginning of the study, it was important to investigate shifts in the hemostatic and fibrinolytic systems that may affect IVF outcomes (in terms of the pregnancy rate) and to define their quantitative level that may help in selecting those women who need the correction of disturbed hemostatic and fibrinolytic responses. As a result, we defined that

thrombin generation in the observational group had considerably increased after the start of controlled ovarian hyperstimulation in response to a sharp increase in the level of estrogens in blood, which is consistent with recently published data (Westerlund et al., 2012). We have also found that the degree of the increase in thrombin generation is different at successful and unsuccessful IVF outcomes (Table 2). In particular, the values of such thrombin generation factors as ETP and peak thrombin concentration at the 2nd observation point were higher in case of IVF failures (P<0.001) as compared with the similar data in patients with pregnancies.

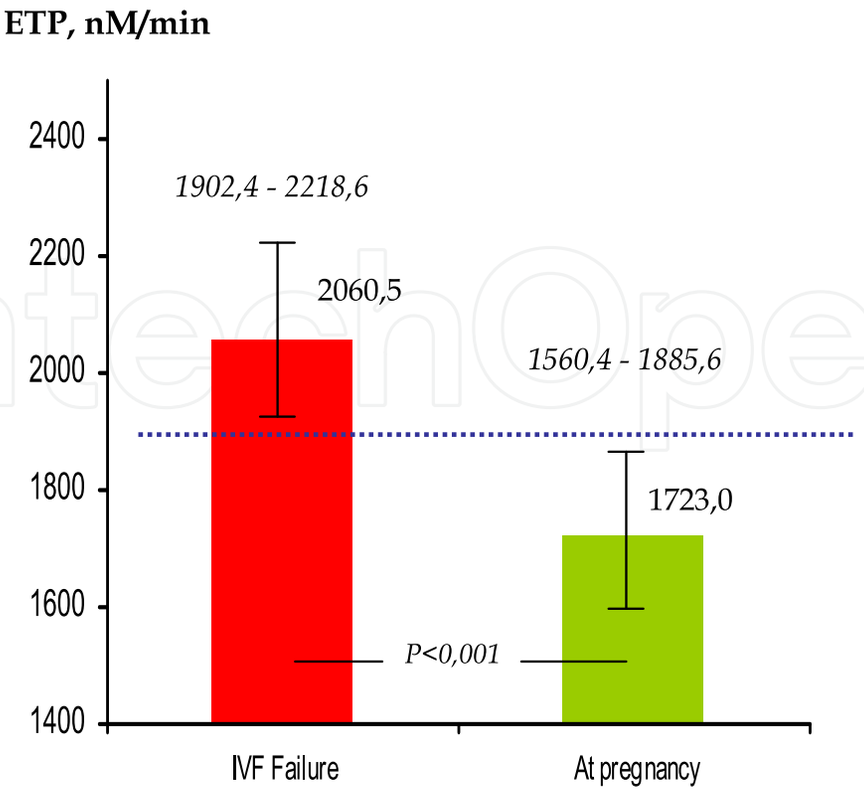
Indication	At IVF failure (n=107)			At pregnancy (n=56)		
	1st observation point	2nd observation point	3rd observation point	1st observation point	2nd observation point	3rd observation point
ETP, nM/min	1655±39.2**	2060.5±52.7**	1853.9±55.6**	1574.8±36.9	1723±54.2	1632.7±56.4
Peak thrombin concentration, nM/l	328.6±24.8**	394.1±25.6**	378.4±25.5**	313.2±24.6	338.3±25.5	310.1±26.0
t-PA, activity, un/ml	0.36±0.16	0.37±0.18	0.38±0.18	0.37±0.14	0.36±0.16	0.35±0.17
PAI-1, activity, un/ml	4.22±2.98	4.08±2.76	4.10±2.16	3.67±2.68	3.41±2.87	3.25±2.31
EAAF index, %	8.5±3.4	9.1±4.1	9.3±4.2	10.0±4.1	10.5±4.9	10.7±4.6
Clot lysis time, min	13.5±3.7*	14.3±3.9*	14.9±3.8*	11.2±3.5	11.9±2.9	12.5±3.3
D-dimers, ng/ml	223.7±31.7**	266.4±27.8*	321.0±31.4**	198.5±25.4	251.6±26.1	298.4±28.4

t – test; * - in this table, as well as in tables 5, 8-11, the validity of differences P<0.05 between the groups characterized by different outcomes occurring within the same observation periods of the IVF cycle; ** - the same, P<0.01.

Table 2. Quantitative level and change dynamics of hemostatic and fibrinolytic indications (mean ± SD) in women of the 1st (observational) group (n=163)

The most significant adverse indication for this reproductive technology was ETP value, which range limits at different IVF outcomes in the middle of the cycle did not intersect even with the M ± 3SD limit (Fig. 2). Peack thrombin concentration also changed, but its values in the compared groups did not intersect within the M ± SD limit.

With regard to the conducted studies, the threshold value for the positive decision on administering heparin prophylaxis was set according to the following criteria: ETP exceeds 1900 nM/min and/or increased Peack thrombin concentration of over 360 nM/l.



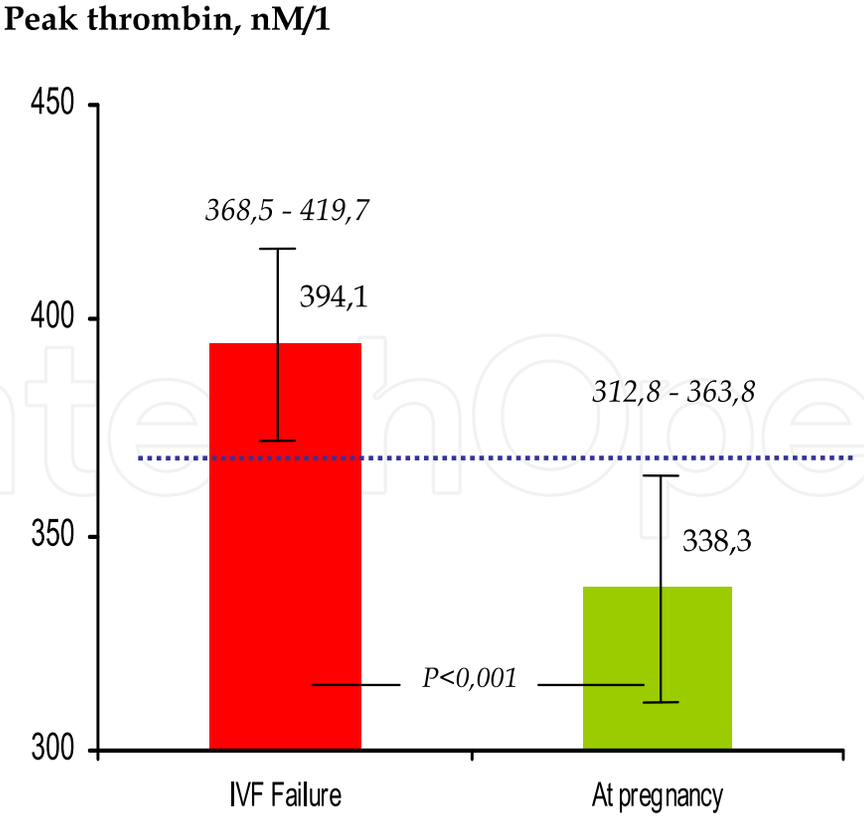


Figure 2. Definition of the threshold values for TGA indications (ETP and peak thrombin concentration) at different IVF outcomes in the 1st (observational) group (at the 2nd observation point).

The level of D-dimers, a known marker for fibrin generation and fibrinolysis, showed less distinctive differences in subgroups 1.1 and 1.2, even though it was slightly decreased, according to the mean data, in case of IVF failures (Table 2). Mean values of this indication in virtually healthy women of fertile age registered in our Center were equal to 205.3 ng/ml, with M ± 2SD 148.5 - 262.1 ng/ml. Respectively, registered results of the D-dimer level at the 2nd observation point were within the allowed value limits or slightly exceeded them.

It was more difficult to define the threshold values that reflect the decrease in the activity of fibrinolytic responses and allow specialists to select patients in need of hypofibrinolysis correction. Recent important publications devoted to this field demonstrate that hypofibrinolysis may be typical of some women who participate in the IVF procedure, but this pathology is original and not triggered in the course of controlled hormonal stimulation as a part of the IVF program (Martinez-Zamora et al., 2011; Westerlund et al. 2012). We received similar data proving that the suppression of fibrinolytic responses was actually typical of a number of women before the beginning of the IVF cycle. Suppression was steady throughout the cycle. Dynamics analysis of the changes in t-PA and PAI-1 activities, their EAAF index, and clot lysis time revealed more dramatic shifts at IVF failures, though the difference between the mean values of the parameters under study turned out to be invalid ($P<0.05$) (Table 2). Nevertheless, we have recorded two facts. First, mean values of the EAAF index defined in the group of 10 virtually healthy female volunteers (20-23 years) were equal (M±SD) to 11.0±3.3%. Second, in case of IVF failures we registered decreased EAAF indexes (less than 11%) in 93.5% (43 out of 46) of women in the 1st (observational) group before the start of the IVF program (1st observational point) as compared to 4,1% (2 out of 49) of women after successful impregnation ($P<0.000001$) (Table 3).

We used the values of the applied CLT assay to be the method of general fibrinolysis monitoring and refused to consider it as a potential criterion for the selection of women in need of therapeutic invasion in relation to its laboratory standardization. Thus, to select females to undergo IPC procedure we chose EAAF index calculation rate, which records the correlation between t-PA and PAI-1 activities, with the value of less than 11%.

Back to the data presented above and obtained during the study of the hemostasis and fibrinolysis systems in the patients of the 1st or observational group (n=163), one can see significant correlation between the detected pathologies and certain IVF outcomes (Table 3). Adverse shifts in these systems had records in 114 out of 163 women (70%) in the 1st group. In general, the IVF cycle efficacy at this stage of the study was equal to 34.4%, however, certain hemostatic and fibrinolytic pathologies facilitated the decrease in the number of successful impregnations from 95.9% (47 out of 49 women in subgroup 1.1) to 7.9% (9 out of 114 women in subgroup 1.2), i.e. in 12.1 times ($P<0,000001$).

5.2.2. Results of the therapeutic invasion in the hemostatic and fibrinolytic systems administered for the increase in the number of successful IVF outcomes

Correction of the hemostatic system at the excessive thrombin generation initially arranged the administration of heparin prophylaxis by means of subcutaneous introduction of

nadroparin calcium (Sanofi-Aventis): 0.3 ml twice a day for 12-14 days. Decision to begin the therapy was based on marking a suprathreshold increase in the major indications of thrombin generation - ETP (over 1900 nM/min) and/or Peack thrombin concentration (over 360 nM/l) at the 2nd observation point.

Patient subgroups	Abs. (n=163)	% of the whole study population	Impregnation	
			Abs. (n=56)	% of pregnant women
1.1. Without target indicators of hemostatic and fibrinolytic pathologies	49	30.1	47	83.9
1.2. With hemostatic and fibrinolytic pathologies	114	69.9	9	16.1
1.2.1. With ETP exceeding 1900 nM/min and/or increased Peack thrombin concentration of over 360 nM/l (at the 2nd observational point)	46	28.2	3	5.4
1.2.2. With decreased EAAF index of less than 11% and prolonged clot lysis time of over 12 min. (at the 1st observational point)	35	21.5	4	7.1
1.2.3. With ETP exceeding 1900 nM/min and/or increased Peack thrombin concentration of over 360 nM/l (at the 2nd observational point) and decreased EAAF index of less than 11% (at the 1st observational point)	33	20.2	2	3.6

Table 3. IVF results in women of the 1st (observational) group depending on the presence or absence of hemostatic and fibrinolytic pathologies

Impact on the vessel wall to increase fibrinolytic activity was made by means of IPC. In the publication byKakkos et al. (2005) a comparative description of the two widely used compression machines - SCD Express™ Compression System (Tyco Healthcare Group LP, Mansfield, MA, USA) was introduced with a rapid inflation device that delivers uniform compression and VenaFlow® (Aircast Inc, Summit, NJ, USA). However neither of them is normally equipped with proper braces to provide mechanical invasion for arm vessels. Still, we found it important to compress this vessel area to exclude even the hypothetic possibilty

Subgroups examined	Abs. (n=164)	% of total number of women	Impregnation	
			Abs. (n=68)	% of number of pregnant
2.1. Without the required signs of pathology of hemostasis and fibrinolysis	26	15,8	21	30,9
2.2. Without the required signs of pathology of hemostasis and fibrinolysis	138	84,2	47	69,1
- including an increase in ETP over 1900 nM/min and/or with Peack thrombin more than 360 nM/l (2nd point of observation)	50	30,5	19	27,9
- including those with reduced EAAF index less than 11% (on the 1st point of observation)	40	24,4	14	20,6
- including an increase in ETP over 1900 nM/min and/or increased Peack thrombin more than 360 nM/l (2nd point of observation) and a decrease of the index EAAF less than 11% (on the 1st point of observation)	48	29,3	14	20,6
Received treatment against diseases of hemostasis, including:	98	59,8	42	61,8
2.2.1. After heparin prophylaxis	38	23,2	15	22,1
2.2.2. After IPC course	23	14,0	10	14,7
2.2.3. When combined IPC course with heparin prophylaxis	37	22,6	17	25
2.2.4. Those in need of treatment, but did not receive it	40	24,4	5	7,3

Table 4. Results of IVF in women of the 2nd (controlled) group in relation to the presence or absence of disorders in hemostasis and fibrinolysis

of the pulmonary artery thromboembolia (e.g. in the presence of clinically non-evident iliofemoral thrombosis) and due to the obtained data on the increased content of t-Pa in endotelial vessels of the upper extremities (Pandolfi et al., 1968). That is why we chose pneumatic massaging device PM-01 (Russia) to apply a 7-chamber compression brace in the upper arm area using the mode of wave compression with the following parameters:

chambers from 30 to 150 mm. Hg. Art., 45 cycles of compression wave with memory for 30 minutes to maintain the pressure in the cuff chambers from 5 to 90., the pressure of compressed air supplied to the compression performed in a course of 8 sessions (twice a week) with 30-minute cuff device to the left or right hand. The starting point of this therapy was to reduce EAAF index below 11% measured in a number of patients just prior to IVF program (1st point of observation).

Studies have found that the total number of favorable outcomes of IVF in the 2nd (controlled) group was 41.5%, an increase of only 7.1%, compared with those in group 1 (34.4%; P <0,21) (Table 3 and Table 4).

However, please note that 40 patients (subgroup 2.2.4) failed our proposed therapy, although prescribed by the above criteria. If to sum up the outcome of IVF in all samples in the study of women who had indications for treatment but did not receive it for a number of reasons (sub 1.2 and 2.2.4; 154 cases) we indicate positive results in 9.1%, whereas in the treated patients (subgroup 2.2.1, 2.2.2 and 2.2.3, 98 cases) - in 42.9%, or 4.71 times more likely (P<0,000001).

Effect of treatment produced on the dynamics of hemostasis and fibrinolysis during IVF is shown in Table 5. You can see the previous trends in Table 2, but the women in group 2, compared to the 1 st group demonstrated lower intensity of thrombin generation and improvement of fibrinolysis regardless of the outcome of IVF.

D-dimer levels in blood plasma has indicated growing trend - the concentration of this indicator has been consistently higher regardless of the period of the survey in patients with unsuccessful IVF outcome (Tables 2 and 5). However, the value of D-dimer levels was within the range of normal variation or slightly exceeded them, hardly matching the identified changes parameters under study (Table 6).

In this regard, we have not put the emphasis on the results of this test upon the following analysis.

Other risk factors of unsuccessful IVF which did not depend on the characteristics of hemostatic and fibrinolytic had its effect on IVF success (see Section 2, Table 13, 14), as well as the fact of receiving or not receiving therapeutic intervention aimed at reducing the thrombogenic potential and increasing fibrinolytic activity of blood. Calculations and observations in tables 7-12 demonstrate a better pattern in this regard. In particular, it appeared that an approved prescription of low-molecular heparin at high thrombin generation at the 2nd stage of the study contributed to the increase in the incidence of pregnancy in 6.4 times (P<0,0001), isolated IPC course application -in 3.0 times (P<0,007), and the combination of IPC course with heparin prophylaxis - in 6.5-times (P<0,0001) (Table 7).

Indication	At failure in IVF cycle (n=96)			At pregnancy (n=68)		
	First point of observation	Second point of observation	Third point of observation	First point of observation	Second point of observation	Third point of observation
ETP, nM/min	1825,3±96,8*	1922,1±64,7*	1785,4±±89,5	1645,6±54,9	1762,1±79,8	1711,2±84,3
Peack thrombin, nM/l	342,1±26,3**	381,5±25,7**	364,4±24,3**	310,7±27,4	329,1±29,3	326,4±25,7
t-PA, activity un/ml	0,35±0,14	0,51±0,15	0,48±0,13	0,35±0,15	0,47±0,16	0,44±0,14
PAI-1, activity un/ml	3,55±1,75	2,64±1,91	2,32±1,58	3,48±2,11	1,95±0,75	1,86±0,57
EAAF index, %	9,9±4,28	19,3±3,88*	20,6±4,53*	10,0±3,97	24,1±4,39	23,6±4,77
Clot lysis time, min	15,5±3,65	12,3±3,86	11,4±4,04	13,4±3,85	11,8±3,85	10,1±3,11
D-dimers, ng/ml	236,4±26,2*	258,6±25,1*	305,6±28,5*	194,7±32,4	233,4±24,5	211,7±28,6

Table 5. The dynamics of hemostasis and fibrinolysis (M ± SD) in women of second (controlled) group (n = 164), t - test

Indication	Rank correlation	P value
ETP, nM/min	0,09	0,017
Peack thrombin, nM/l	0,125	0,002
t-PA, activity, un/ml	0,118	0,0003
PAI-1, activity, un/ml	0,124	0,0004
EAAF index, %	0,107	0,0006
Clot lysis time, min	0,111	0,0005

Table 6. Pair correlation between the level of D-dimers (by Spearman) and the study of hemostasis and fibrinolysis in different periods of the IVF cycle, regardless of its outcome

Modality	In need of treatment, but did not receive it (sub-1.2 and 2.2.4; n = 154)			In need of treatment, and treated (sub 2.2.1, 2.2.2 and 2.2.3; n = 98)		
	Abs.	Became pregnant	%	Abs.	Became pregnant	%
1. Heparin Prophylaxis	64	4	6,2	38	15	39,5
2. IPC course	48	7	14,6	23	10	43,5
3. Combined effect	42	3	7,1	37	17	45,9

Table 7. The influence of the methods of correction of hemostatic and fibrinolytic responses to the effectiveness of IVF (with data 1 and 2 stage of the research)

Criteria such as Absolute Risk Reduction (ARR), Relative Risk (RR), Relative Risk Reduction (RRR), Number Needed to Treat (NNT), Confidence Interval (CI) were determined for further evaluation of the effectiveness of the treatment (Table 8).

Modality	Indication					
	ARR	NNT	OR	CI 95% (RRR)	CI 95% (OR)	RRR%
Heparin Prophylaxis	0,27	3,7	0,22	0,52-0,92	0,07-0,69	31
IPC Course	0,31	3,2	0,19	0,44-0,94	0,05-0,65	35
Heparin prophylaxis combined with IPC course	0,55	1,8	0,24	0,23-0,60	0,02-0,22	63

Table 8. The effectiveness of different methods of therapy in women in a cycle of IVF

Indication	In need of treatment, but did not receive it (subgroup 1.2 и 2.2.4; n=154)			In need of treatment, and treated (subgroup 2.2.1, 2.2.2 и 2.2.3; n=98)		
	First point of observation	Second point of observation	Third point of observation	First point of observation	Second point of observation	Third point of observation
ETP, nM/min	1459,4±85,1	1912,5±108,6**	1872,8±85,1**	1490,6±84,1	1729,8±89,5	1566,4±72,7
Peack thrombin, nM/l	307,1±31,8	391,2±25,3**	385,4±22,5**	313,1±19,7	362,8±23,1	341,3±25,6
t-PA, activity, un/ml	0,32±0,15	0,34±0,16	0,33±0,15	0,34±0,12	0,48±0,15	0,49±0,14
PAI-1, activity, un/ml	3,18±2,32	3,22±1,66	3,26±1,75	3,22±1,71	2,76±1,51	2,68±1,67
EAAF index, %	10,0±3,2	10,5±4,3**	10,1±4,1**	10,5±3,5	17,3±4,4	18,2±4,2
Clot lysis time, min	13,1±3,6	14,2±3,9	15,4±3,8**	12,2±4,4	10,1±4,5	9,2±3,4

Table 9. The dynamics of hemostasis and fibrinolysis (M ± SD) in women in a cycle of IVF when indicated for the correction of hemostasis (n = 138), t - test

The choice was made towards absence of pregnancy after conducted treatment as the negative outcome of IVF. The control group included 154 women who had revealed violations of blood coagulation and fibrinolysis and did not receive treatment (subgroups 1.2 and 2.2.4). The intervention group had patients who received one of three therapies

aimed at correcting identified violations in the hemostatic system and hypofibrinolysis (subgroup 2.2.1, 2.2.2, 2.2.3). It was discovered that all of the treatment reduced the risk of a negative outcome of IVF. In particular, in order to prevent one adverse outcome, you need to treat 2 women by the combined treatment option (1.8), using the IPC - 3 women (3.2), and heparin prophylaxis - 4 women (3.7). The relative risk reduction (RRR) in all cases was greater than 25%, which corresponded to clinical effect, and upon combined RRR therapy more than 50%, indicated a pronounced clinical effect.

Evolution of indicators reflecting defects of hemostatic and fibrinolytic reactions in patients in need of therapeutic intervention are shown in Table 9. Obviously, undertaken treatment has a beneficial effect on the rate of thrombin generation and fibrinolysis, and the index EAAF which reflects fibrinolysis-activation ability of the vascular wall indicated the increase in 2 times.

We also made separate calculations of laboratory parameters in women with high thrombin generation in need of heparin prophylaxis (Table 10). As a result, it was found that low molecular weight heparin with a mid-cycle IVF significantly reduces the generation of thrombin. In particular, the background rate nadroparin ETP between the 2nd and 3rd observation points decreased by 18.1%, compared to 3.6% in women who did not receive anticoagulant. The similar dynamics had Peack thrombin, which decreased, respectively, by 13.2% and 1.8%.

Indication	In need of treatment, but did not receive it (subgroup 1.2.1. и 2.2.4.1; n=64)			At heparin prophylaxis (subgroup 2.2.1; n=38)		
	First point of observation	Second point of observation	Third point of observation	First point of observation	Second point of observation	Third point of observation
ETP, nM/min	1461,2±81,6	1849,3±89,2	1782,4±93,5**	1489,5±85,5	1861,2±94,4	1524,6±88,9
Peack thrombin, nM/l	310,1±23,1	382,4±19,5	375,6±25,2**	321,2±22,3	386,3±23,5	335,2±20,2
t-PA, activity, un/ml	0,30±0,16	0,33±0,15	0,31±0,15	0,33±0,12	0,41±0,16	0,42±0,17
PAI-1, activity, un/ml	2,40±1,13	2,50±1,22	2,44±1,98	2,84±1,45	3,61±1,75	3,50±1,71
EAAF index, %	12,5±3,1	13,2±4,2	12,7±3,6	11,62±3,2	11,35±4,2	12,0±3,9
Clot lysis time, min	9,4±3,0	8,7±3,3	10,1±3,5	8,4±3,7	8,7±2,8	9,7±3,2

Table 10. The dynamics of hemostasis and fibrinolysis (M ± SD) in the second group of women in the presence of indications for heparin prophylaxis (n = 102), t – test

It should be noted that the indicators of fibrinolytic activity has not changed and remained stable without regard to heparin and duration of the study.

Indication	In need of treatment, but did not receive it (subgroup 1.2.2. и 2.2.4.2; n=48)			IPC Course recipients (subgroup 2.2.2.2; n=23)		
	First point of observation	Second point of observation	Third point of observation	First point of observation	Second point of observation	Third point of observation
ETP, nM/min	1534,4±87,2**	1575,7±85,6	1589,6±96,4	1415,1±87,7	1562,3±86,1	1632,3±93,8
Peack thrombin, nM/l	314,2±30,3*	322,4±28,2	327,7±25,7**	294,5±24,4	322,1±31,1	353,3±25,9
t-PA, activity, un/ml	0,36±0,14	0,39±0,15	0,37±0,14*	0,35±0,16	0,51±0,15	0,48±0,15
PAI-1, activity, un/ml	3,98±1,67	4,11±2,32	4,05±2,54*	3,55±1,88	2,64±1,06	2,32±1,32
EAAF index, %	9,0±3,2	9,5±3,6**	9,1±4,0**	9,9±3,2	19,3±3,4	20,6±4,5
Clot lysis time, min	13,8±4,3	14,4±4,6*	14,8±3,2**	14,3±4,1	11,4±3,7	9,0±3,3

Table 11. The dynamics of hemostasis and fibrinolysis (M ± SD) in the second group of women in the event of Table readings for IPC course (n = 71), t – test

Indication	In need of treatment, but did not receive it (subgroup 1.2.3 и 2.2.4.3; n=42)			At heparin prophylaxis and IPC course recipients (subgroup 2.2.3; n=37)		
	First point of observation	Second point of observation	Third point of observation	First point of observation	Second point of observation	Third point of observation
ETP, nM/min	1538,6±78,3	1770,4±85,6	1756,6±95,7**	1566,7±86,3	1764,4±95,7	1542,1±81,3
Peack thrombin, nM/l	322,3±32,4	379,3±29,1	374,5±31,6**	324,7±27,6	371,2±33,8	335,6±30,7
t-PA, activity, un/ml	0,37±0,17	0,39±0,15	0,36±0,16	0,31±0,16	0,49±0,15	0,46±0,14
PAI-1, activity, un/ml	3,64±2,05	4,12±1,77	3,45±1,98	3,02±1,69	2,29±1,14	2,09±0,91
EAAF index, %	10,2±2,9	9,5±3,1**	10,4±3,3**	10,2±2,6	21,4±4,5	22,0±4,3
Clot lysis time, min	15,4±3,9	13,2±4,2**	15,7±3,4**	13,8±2,3	10,0±3,8	9,1±3,9

Table 12. The dynamics of hemostasis and fibrinolysis (M ± SD) in the second group of women when indicated for combination therapy (n = 79), t – test

Similar calculations were performed in women with hypofibrinolysis as well as with the combination of low fibrinolytic activity with excessive generation of thrombin (Tables 11 and 12). It was found that the isolated effects of IPC in women with original, prior to the IVF

cycle hypofibrinolysis indicated a sharp increase in EAAF index combined with the decrease in CLT. Interestingly, vases compression led to the significant increase of t-PA activity as well as to the reduction of PAI-1activity at the end of the IVF cycle. It was also discovered that the application of IPC led to increased thrombin generation which Peack thrombin factor clearly demonstrated.

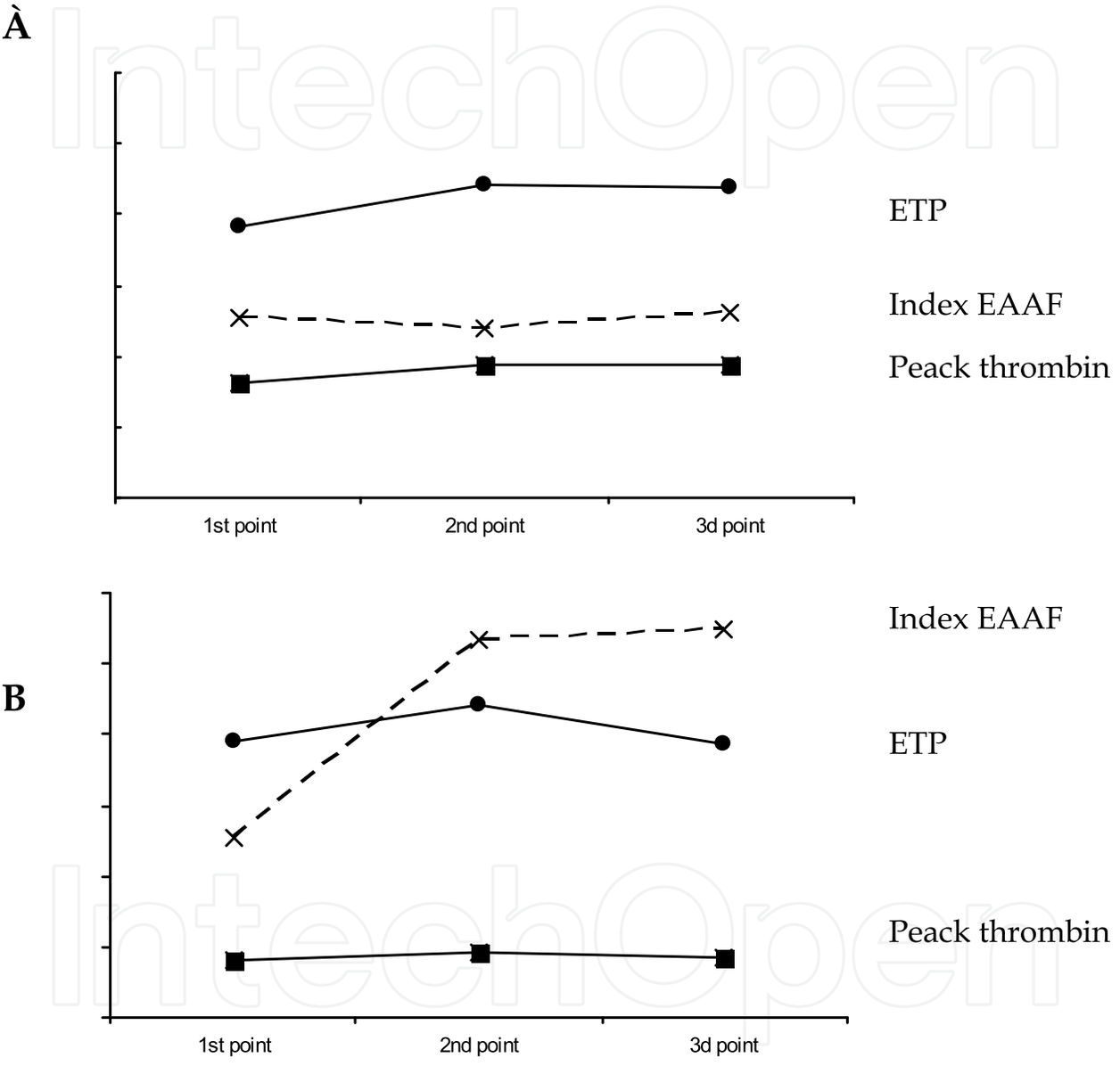


Figure 3. The evolution of laboratory parameters of hemostasis and fibrinolysis in women who require concomitant therapy, but do not receive it (A) during such therapy (B)

Combined application of IPC and low molecular weight heparin produced complex beneficial effect on hemostasis and fibrinolysis to be in correspondence with the maximum increase in the number of IVF successful outcomes (Tables 4, 7, 8 and 12).

In Fig. 3 the dynamics of the main parameters studied in the course of the treatment for the correction of hemostasis and fibrinolysis is highlighted.

5.2.3. Comparative analysis of the prognostic value of a number of factors contributing to the failure of IVF

This section has recorded and compared the effect of risk factors in the failure of IVF in 1st (observation) group and women of (2nd) group. We studied a wide range of adverse prognostic factors which are well-known in Reproduction and discussed in this publication: markers of thrombogenic risk and blood fibrinolytic activity reduction as well as hyperhomocysteinemia, thrombogenic mutations and polymorphisms carriers, presence of an inflammatory response (Bates et al., 2008; Coulam & Jeyendran, 2009b; Heit, 2007; Qublan & Eid, 2006), "0" blood type negative factor [Canonico et al., 2008; Ohira et al., 2007.)

Assessing the reasons for the failure of pregnancy in IVF cycles in 163 patients of the 1st (observation) group based on the analysis of the odds ratio (OR), 9 out of 27 (33.3%) factors became the most important adverse factors or symptoms to be rated as fairly significant (Table 13). Importantly, indicators reflecting increased thrombin generation and inhibition of fibrinolytic reactions, respectively, 2nd and 3d entered the adverse factors as well. Hypo fibrinolysis factors with high reliability proved to be at the 5th and 6th adverse factors ranking. Consequently, in addition to well-known factors listed in Section 2, increased ability to thrombosis, in response to the stress estrogen and inhibition of fibrinolytic reactions are among the leading causes of failure of IVF. It is interesting to note that such a well-known and widely used in clinical practice marker of thrombinemia as high D-dimer plasma levels did not vary in frequency of occurrence in impregnate women at all stages of observation (OR 0,99; 0,95% CI 0,42-2,31 – 21 rank).

Carriage of thrombogenic mutations and polymorphisms were identified in the majority of our patients (71.2%). By the rare mutations - Factor V Leiden and prothrombin in only 4 cases (2.4%), we cannot judge the significance of their influence. However, the combination of polymorphisms MTGFR and PAI-1 was found in a slightly larger percentage of cases with unsuccessful IVF (24.3% vs. 16.0%) to be, however, insignificant. Interestingly, blood type “not 0”did not prove to be protrombogen/unfavorable by nature in our observation as well as a number of variants of virus infection carriers and the manifestations of inflammation (fibrinosis, leukocytosis).

The number of factors contributing to the failure of pregnancy in IVF changed dramatically after exposure to therapeutic correction of hemostasis and fibrinolysis, used in the present publication. In accordance with the data in Table 14, traditional reasons for Reproduction are among the leaders: early hyperstimulation, male factor, and others. Hyperhomocysteinemia (OR 3,45; 0,95% CI 1,16-10,2) became one of the hemostatic reasons at the 4th rank to be the result of obvious lack of attention to the problem of metabolic methionine in preparation for IVF protocol. The significance level of manifestation of high thrombin generation shifted from the 2nd and 3rd rank to 8th and 9th rank whereas EAAF index - from 6th to 15th rank to be the further proof of the effectiveness of our methods of applied therapeutic intervention. Please, note that the calculations in this table exclude 40 patients with disorders of hemostasis and fibrinolysis who did not receive treatment for a number of reasons (subgroup 2.2.4).

Criterion	Failure of IVF (n=107)		Success of IVF (n=56)		Odds ratio (0,95% CI)	P-value
	Abs.	%	Abs.	%		
1. Hyperstimulation (early stage)	22	20,5	0	0	29,7 (1,76-500)	< 0,00001
2. ETP more than 1900 nM/min (2nd point of observation)	73	68,2	4	7,1	27,9 (9,33-83,4)	< 0,00001
3. Peack thrombin more than 360 nM/L (2nd point of observation)	74	69,1	5	8,9	22,8 (8,36-62,5)	< 0,00001
4. Oligozoospermia (moderate and severe)	45	42,0	2	3,5	19,5 (4,53-84,6)	< 0,00001
5. Clot lysis time of over 12 minutes (1st point of observation)	69	64,5	5	8,9	18,5 (6,81-50,3)	< 0,000001
6. EAAF index less than 11% (1st point of observation)	62	57,9	6	10,7	11,5 (4,53-29,1)	0,00005
7. Defective embryo	23	21,4	3	5,3	4,83 (1,38-16,9)	0,011
8. Insufficient number of embryos transferred in IVF cycles (1-2)	38	35,5	6	10,7	4,58 (1,80-11,6)	0,0007
9. Mutation FV (G/A, A/A)	3	2,8	0	0	3,78 (0,19-74,5)	0,319
10. Difficult embryo transfer	32	29,9	6	10,7	3,55 (1,38-9,12)	0,006
11. Hypoplasia of the endometrium	11	10,3	3	5,3	2,02 (0,54-7,57)	0,383
12. Unsuccessful IVF attempt in history	20	18,7	6	10,7	1,91 (0,72-5,08)	0,260
13. Homocysteine in blood of more than 15 mM/l (1st point of observation)	22	20,5	7	12,5	1,81 (0,72-4,54)	0,280
14. Age (36-40 years)	19	17,7	6	10,7	1,79 (0,67-4,80)	0,262

Criterion	Failure of IVF (n=107)		Success of IVF (n=56)		Odds ratio (0,95% CI)	P-value
	Abs.	%	Abs.	%		
15. High dose-protocol	35	32,7	12	21,4	1,78 (0,83-3,79)	0,148
16. The combination of polymorphisms MTHFR (C/T, T/T) and PAI-I (5 G/4 G, 4 G/4 G)	26	24,3	9	16,0	1,67 (0,72-3,87)	0,315
17. Low ovarian reserve	19	17,7	7	12,5	1,51 (0,59-3,84)	0,500
18. Polymorphism of PAI-I (5 G/4 G, 4 G/4 G)	42	39,2	20	35,7	1,16 (0,59-2,27)	0,735
19. Cytomegalovirus infection	11	10,3	5	8,9	1,16 (0,38-3,54)	> 1,00
20. Fibrinosis greater than 5.0 g/l (for 2nd point of observation)	14	13,0	7	12,5	1,05 (0,39-2,78)	> 1,00
21. D-dimer levels over 500 ng/mL (2nd point of observation)	19	17,7	10	17,8	0,99 (0,42-2,31)	> 1,00
22. Herpes type 1 and 2	35	32,7	21	37,5	0,81 (0,41-1,59)	> 1,00
23. Hypothyroidism	6	5,6	4	7,1	0,77 (0,20-2,85)	0,737
24. Leukocytosis over $11,0\times10^9$/l (1st point of observation)	23	21,5	15	26,8	0,74 (0,35-1,58)	> 1,00
25. Blood group - is not "0"	85	79,4	47	83,9	0,74 (0,31-1,73)	0,535
26. Polymorphism of MTHFR (C/T, T/T)	30	28,0	23	41,0	0,55 (0,28-1,10)	0,113
27. Mutation of FV Leiden (G/A)	1	0,9	1	1,7	0,51 (0,03-8,45)	> 1,00

Table 13. Factors contributing to the failure of pregnancy in IVF cycles in the 1 st. observation group (n = 163)

Criterion	Failure of IVF (n=61)		Success of IVF (n=63)		Odds ratio (0,95% CI)	P-value
	Abs	%	Abs	%		
1. Hyperstimulation (early stage)	12	19,6	0	0	32,0 (1,85-555)	< 0,0001
2. Oligozoospermia (moderate and severe)	26	42,6	4	6,3	10,9 (3,53-34,0)	0,0002
3. Insufficient number of embryos transferred in IVF cycles (1-2)	23	37,7	8	12,7	4,16 (1,68-10,28)	0,003
4. Homocysteine in blood of more than 15 nM/l (on the 1st point of observation)	14	22,9	5	7,9	3,45 (1,16-10,2)	0,043
5. Difficult embryo transfer	18	29,5	7	11,1	3,34 (1,28-8,74)	0,013
6. Mutation FII (G/A, A/A)	1	1,6	0	0	3,14 (0,12-78,8)	0,491
7. Defective embryo	17	27,8	8	12,7	2,65 (1,04-6,72)	0,044
8. Peak thrombin more than 360 mM/l (for 2nd point of observation)	43	70,4	32	50,8	2,31 (1,10-4,84)	0,028
9. ETP more than 1900 nM/min (2nd point of observation)	41	67,2	31	49,2	2,11 (1,02-4,38)	0,047
10. Unsuccessful IVF attempt in anamnesis	11	18,0	6	9,5	2,09 (0,72-6,06)	0,198
11. D-dimer levels over 500 ng/mL (2-nd point of observation)	13	21,3	8	12,7	1,86 (0,71-4,87)	0,476
12. Age (36-40 years)	8	13,1	5	7,9	1,75 (0,53-5,68)	0,392
13. Polymorphism of PAI-I (5 G/4 G, 4 G/4 G)	26	42,6	20	31,7	1,59 (0,76-3,32)	0,265
14. Cytomegalovirus infection	3	4,9	2	3,1	1,57 (0,25-9,78)	1,677
15. EAAF index less than 11% (1st point of observation)	33	54,0	27	42,8	1,57 (0,77-3,19)	0,280
16. The combination of polymorphisms MTHFR (C/T, T/T) and PAI-I (5 G/4 G, 4 G/4 G)	17	27,8	13	20,6	1,48 (0,64-3,40)	0,706
17. Fibrinosis greater than 5.0 g/l (for the 2nd point of observation)	8	13,1	6	9,8	1,43 (0,46-4,40)	0,580

Criterion	Failure of IVF (n=61)		Success of IVF (n=63)		Odds ratio (0,95% CI)	P-value
	Abs	%	Abs	%		
18. High-dose protocol	6	9,8	5	7,9	1,26 (0,36-4,38)	0,760
19. Blood group - is not "0"	48	78,6	47	74,6	1,24 (0,54-2,89)	0,673
20. Low ovarian reserve	11	18,0	10	15,8	1,16 (0,45-2,98)	0,813
21. Clot lysis time of over 12 minutes (1st point of observation)	35	57,4	34	53,9	1,14 (0,56-2,33)	0,720
22. Leukocytosis over $11,0\times10^9$/l (1st point of observation)	13	21,3	12	19,0	1,15 (0,47-2,77)	0,824
23. Hypoplasia of the endometrium	4	6,5	4	6,3	1,03 (0,24-4,33)	> 1,00
24. Hypothyroidism	4	6,5	4	6,3	1,03 (0,24-4,33)	> 1,00
25. Herpes type 1 and 2	7	11,4	9	14,3	0,77 (0,27-2,23)	0,790
26. Polymorphism of MTHFR (C/T, T/T)	12	19,6	25	39,6	0,37 (0,16-0,83)	0,018

Table 14. Factors contributing to the failure of pregnancy in IVF cycles in the 2nd group (n = 124)

In our publication we studied a number of women with the known risk factors of IVF failures to be less significant regardless of the ongoing correction of hemostasis and hypo fibrinolysis. We also record such risk factors as manifestations of inflammatory reaction (leukocytosis, fibrinosis) of virus (herpes, cytomegalovirus), the pathology of the thyroid gland and a number of others.

We are particularly interested in the reasons for the failure of IVF which remained relevant after therapeutic correction aimed at hemostasis and fibrinolysis. The list is given in Table. 15.

As noted earlier (Table 14), in addition to gynecological risk factors, hyperhomocysteinemia, and manifestations of excessive thrombin generation became the causes of the failure of IVF. Consequently, despite treatment some female patients maintained the trend to intravascular coagulation. Earlier, Table 11 demonstrated that isolated course of IPC to treat hypo fibrinolysis and other effects led to increased thrombin generation whereas the combination of IPC with dalteparin did not lead to such a shift (Table 12). Considering that we used three variants of therapeutic intervention our attempt was to determine the frequency of each of them under different outcomes of IVF in women with a high ETP in thrombin generation test.

Criterion	Failure of IVF (n=56)		Success of IVF (n=42)		P-value
	Abs.	%	Abs.	%	
1. Hyperstimulation (early stage)	10	17,8	0	0	0,004
2. Oligozoospermia (moderate and severe)	22	39,3	4	9,5	< 0,001
3. Insufficient number of embryos transferred in IVF cycles (1-2)	18	32,1	5	11,1	0,029
4. Homocysteine in blood of more than 15 nM/l (on the 1st point of observation)	17	30,3	4	9,5	0,039
5. Difficult embryo transfer	19	33,9	6	14,2	0,035
6. Defective embryo	18	32,1	5	11,1	0,029
7. ETP more than 1900 nM/min (2nd point of observation)	39	69,6	17	40,5	0,006
8. Peak thrombin more than 360 mM/l (for 2nd point of observation)	42	75,0	19	45,2	0,003

Table 15. Causes for the failure of IVF under conducted therapeutic correction of hemostasis and fibrinolysis (n = 98)

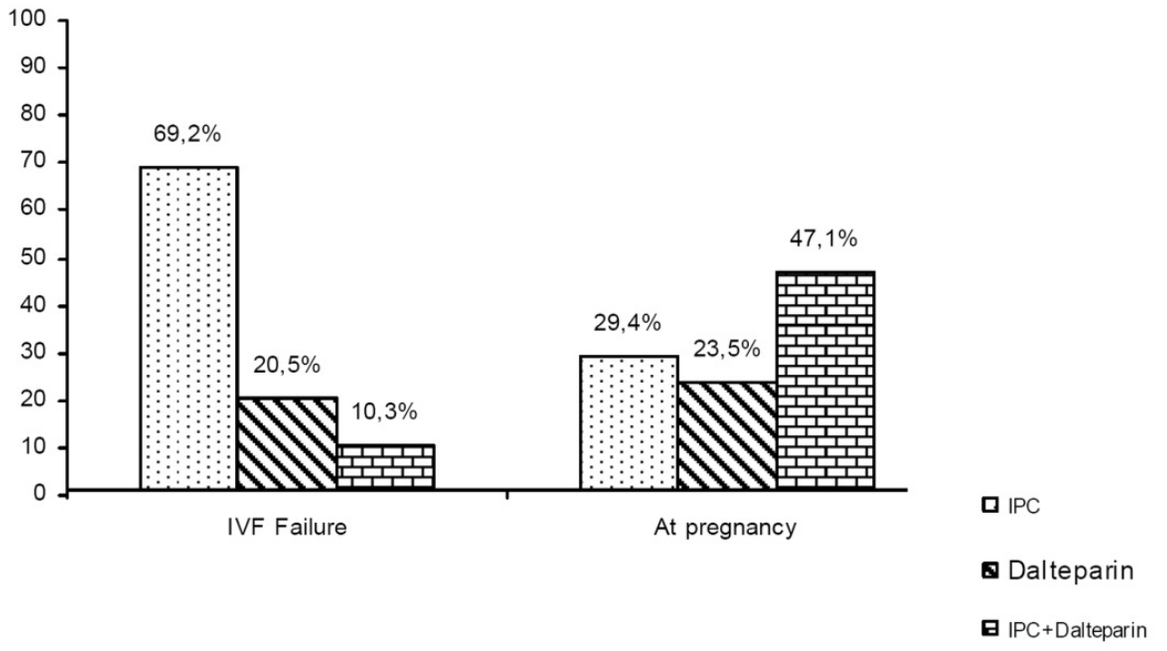


Figure 4. Analysis of the effectiveness of different therapies with excessive thrombin generation (ETP to 1900 nM/min in the 2nd point of observation)

The data in Figure 4 show that the high generation of thrombin and IVF failure is more common in the course of isolating IPC and less - in combination therapy.

6. Discussion

To sum up the results we note that excessive thrombin generation and a fibrinolytic inhibition fatal reaction (without correction) reduces the effectiveness of IVF and has a comparable value compared to the traditional risk factors of reproduction. Thus, our study proves a number of recent statements devoted to this problem (Martinez-Zamora et al., 2011; Meltzer et al., 2010; Nelson & Greer, 2008; Rova et al., 2012; Westerlund et al., 2012). However, we have made further progress due to suprathreshold values of laboratory parameters which allow to monitor the increased propensity to blood clotting and / or hypo fibrinolysis and therefore to identify patients with high risk of IVF failure in order to conduct therapeutic correction of disorders . In particular, by the calibrated test of thrombography administration of dalteparin was authorized by its indicators (ETP, Peack thrombin) to lead to the effective reduction of thrombin generation as well as the significant increase in positive outcomes of IVF (6.4 times).

We first proposed and tested method and mode of correction hypo fibrinolysis IPC for women in a cycle of IVF. It was shown that vases compression in these cases leads to the increased activity of t-PA and reduced PAI-1 activity, which is clearly manifested by the sharp increase in the calculated EAAF index , accelerated clotlysis time and the increase in the number of positive outcomes for assisted reproductive technology in 3 times. However, there were no obvious reasons to reduce the dynamic activity of PAI-1 during the course of IPC, even though it appeared to be a favorable result of the non-drug therapy. A negative consequence of the IPC was the phenomenon increasing the generation of thrombin which did not have the prior record. The calculations showed that the IPC in all cases should be combined with heparin prophylaxis to obtain the best clinical results. In this publication, combining vases compression with prophylactic doses of LMWH (low molecular weight heparin) really helped to increase the number of pregnancies in 6.5 times. In our opinion, this non-pharmacological approach to correcting hypo fibrinolysis demonstrates great potential for use in a number of clinical situations, including pregnancy period. In this chapter, we do not include the results of vases compression in women with low fibrinolytic activity after IVF in the first 12 weeks of pregnancy. However, the results are encouraging and will be published later.

The leading role of increased thrombin generation and hypo fibrinolysis in negative consequences of this reproductive technology has been proved in comparative evaluation of the significance of the risk factors of IVF failure. Its value appeared to be comparable with such risk factors as ovarian hyperstimulation syndrome, male factor, poor embryo or small quantities, or hard to bear embryos. In the meantime, the range of risk factors and their significance has changed dramatically after the treatment and correction of disorders of hemostasis and fibrinolysis. In particular, the list of relevant factors was reduced significantly to hypo fibrinolysis (rated by EAAF index) whereas indicators of excessive thrombin generation remained, though in less prominent positions. In addition, risk factors such as male factor, insufficient and difficult embryo transfer, as well as their low quality became more significant. The publication indicates the important role of hyperhomocysteinemia in the failure of IVF. As you know, it refers to the controllable risk

factors which can and must be eliminated by recognized medical methods (by taking vitamins B6, B12, folic acid) at pre-gravid preparation.

In our research, we tested only 7 women with rare mutations F V Leiden (1691 G>A) and FII (20210 G>A), associated with thrombosis, pregnancy failure and reproductive technologies. Therefore, we were unable to prove their relevance to IVF outcomes. A common gene polymorphisms MTHFR (C 677> T) and gene PAI 1 (5G>4G) compared with the results of Coulam and Jeyendran (2009b) did not prove the significance.

7. Conclusion

The research marks the opportunities to progress and improve outcomes of IVF based on the identification and correction of the pathology of hemostasis and fibrinolysis. The research data may serve as the basis for the development of guidelines and standards which allow improving the efficiency of modern reproductive technologies. The marked problem requires interdisciplinary approach, joint efforts by obstetricians and hematologists and reward by better efficiency of IVF despite the increased cost of diagnosis and treatment of disorders of hemostasis and fibrinolysis.

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8. References

Alviggi C, Humaidan P, Howles CM, Tredway D. & Hillier S.G. (2009) Biological versus chronological ovarian age: implications for assisted reproductive technology. Reprod Biol Endocrinol. 2009;22:101-13.

American Society for Reproductive Immunology Antiphospholipid Antibody Committee: A rational basis for antiphospholipid antibody testing and selective immunotherapy in assisted reproduction: a rebuttal to the American Society for Reproductive Medicine Practice Committee Opinion. Fertil Steril. 2000;74:631-4.

American Society of Reproductive Medcine. Birmingham. Alabama. Antiphospholipid Antibodies Do Not Affect IVF Success. Fertil Steril. 2008;90:S172-3.

Andersson O, Blombäck M, Bremme K. & Wramsby H. (1997) Prediction of changes in levels of haemostatic variables during natural menstrual cycle and ovarian hyperstimulation. Thromb Haemost 1997;77:901-4.

Anteby Y, Greenfield C. & Natanson-Yaron S. (2004) Vascular endothelial growth factor, epidermal growth factor and fibroblast growth factor-4 and -10 stimulate trophoblast plasminogen activator system and metalloproteinase-9. Mol. Hum Reprod. 2004;10:229-35.

Aune B, Hoie KE, Oian P, Holst N. & Osterud B. (1991) Does ovarian stimulation for in-vitro fertilization induce a hypercoagulable state? Hum Reprod 1991;6:925-7.

Azem F, Many A, Yovel I, Amit A, Lessing JB. & Kupfermic MJ. (2004) Increased rates of thrombophilia in women with repeated IVF failure. Hum Reprod. 2004;19:368- 70.

Balasch J, Miró F, Burzaco I, Casamitjana R, Civico S, Ballescá JL, Puerto B. & Vanrell JA. (1995) The role of luteinizing hormone in human follicle development and oocyte fertility: evidence from in vitro fertilization in a woman withlong-standing hypogonadotrophic hypogonadism and using recombinant human follicle stimulating hormone. Hum Reprod. 1995;10:1678-83.

Баркаган З.С., Момот А.П. *Диагностика и контролируемая терапия нарушений гемостаза.* / М., *Изд-во*: "Ньюдиамед-АО", 2001. - 296 С.

Bates SM, Greer IA, Pabinger I, Sofaer S. & Hirsh J. (2008) Venous Thromboembolism, Thrombophilia, Antithrombotic Therapy, and Pregnancy American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008;133:844-86.

Beer AE, Kwak J. (2000) Reproductive medicine program Finch University of I lealth Science. Chicago Med School. 2000;96.

Bellver J, Soares SR, Alvarez C, Muñoz E, Ramirez A, Rubio C, Remohi J. & Pellicer A. (2008) The role of trombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. Hum Reprod. 2008;23(2):278-84.

Berkarda B, Akokan G. & Derman U. (1965) Self-coagulogram. Thromb Diath Haemorrh. Mar. 1965; 15(13):297-303.

Berker B, Kaya C, Aytac R. & Satiroglu H. (2009) Homocystein concentrations in follicular fluid are associated with poor oocyte and embryo qualities in polycystic ovary syndrome patients undergoing assisted reproduction. Hum Reprod. 2009;24(9):2293-302.

Bertina R.M. (1997) Factor V Leiden and other coagulation factor mutations affecting thrombotic risk (1997) Clin Chem. 1997;43(9):1678-83.

Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van Velden PA. & Reitsma PH. (1994) Mitation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369(6475):64-7.

Birkenfeld A, Mukaida T, Minichiello L, Jackson M, Kase NG. & Yemini M. (1994) Incidence of autoimmune antibodies in failed embryo transfer cycles. Am J Repro Immunol. 1994;31(2-3):65-8.

Biron C, Galtier-Dereure F, Rabesandratana H, Bernard I, Aguilar-Martinez P, Schved JF. & Hedon B. (1997) Hemostasis parameters during ovarian stimulation for in vitro fertilization: results of a prospective study. Fertil Steril. 1997;67(1):104-9.

Bischof P, Aplin JD, Bentin-Ley U, Brannstrom M, Casslen B, Castrillo JL, Classen-Linke I, Critchley HO, Devoto L, D'Hooghe T, Horcajadas JA, Groothuis P, Ivell R, Pongrantz I, Macklon NS, Sharkey A, Vicovac L, White JO, Winterhager E, von Wolff M, Simon C. & Stavreus-Evers A. (2006) Implantation of the human embryo: Research lines and models. From the implantation Research Network 'Fruitful'. Gynecol Obstet Invest. Jun. 2006;62(4):206-16.

Bjornsson TD, Schneider DE. & Berger H. (1989) Aspirin acetylates fibrinogen and enhances fibrinolysis. Fibrinolytic effect is independent of changes in plasminogen activator levels. J Pharmacol Exp Ther. 1989;250:154-61.

Bouma BN, Meijers JC. (2003) Thombin-activatable fibrinolysis inhibitor (TAFI, plasma procarboxypeptidase B, procarboxypeptidase R, procarboxypectidase U). Thromb Haemost. 2003;1: 1566-74.

Browse NL, Gary L, Jarrett PEM. & Morland M. (1977) Blood and vein-wall fibrinolytic activity in health and vascular disease. Br Med J. 1977;1:478-81.

Buchholz T, Lohse P, Rogenhofer N, Kosian E, Pihusch R. & Thaler CJ. (2003) Polymorphisms in the ACE and PAI-1 genes are associated with recurrent spontaneous miscarriages. Hum Reprod. 2003;18(11):2473-7.

Canonico M, Olie V, Carcailon L, Tubert-Bitter P. & Scarabin P-Y. on behalf of the Estrogen and Thromboembolism Risk (ESTHER) Study Group (2008) Synergism between non-O blood group and oral estrogen in the risk of venous thromboembolism among postmenopausal women: The ESTHER study. Thromb Haemost. 2008; 99(1):246-8.

Chan WS, Dixon ME. (2008) The "ART' of thromboembolism: A review of assisted reproductive technology and thromboembolic complications. Thromb Res. 2008:121:713-26.

Chouhan V, Comerota AJ, Sun I, Harada R, Gaughan JP. & Rao AK. (1999) Inhibition of tissue factor pathway inhibitor during intermittent pneumatic compression: a possible mechanism for antithrombotic effect. Arterioscler Thromb Vasc Biol. 1999; 19:2812-7.

Christen Y, Wutschert R, Weimer D, de Moerloose P, Kruithof EK. & Bounameaux H. (1997) Effects of intermittent pneumatic compression on venous haemodynamics and fibrinolytie activity. Blood Coagul Fibrinolysis. 1997;8:185-90.

Christiansen OB, Nielsen HS. & Kolte AM. (2006) Future directions of failed implantation and recurrent miscarriage research. Reprod Biomed Online. 2006;13:71-83.

Coulam C, Roussev R. (2009a) Antiphospholipid antibodies inhibit angiogenesis. Am J Reprod Immunol. 2009;81(2):149.

Coulam CB, Jeyendran D. (2009b) Trombophilic gene polymorphisms are risk factors for unexplained infertility. Fertil Steril 2009b;91(4):1516-7.

Coulam CB, Jeyendran RS, Fishel LA. & Roussev R. (2006a) Multiple thrombophilic gene mutations are risk factors for implantation failure. Reprod BioMed Online. 2006a;12:322-7.

Coulam CB, Jeyendran RS, Fishel LA. & Roussev RG. (2006b) Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. Am J Reprod Immunol. 2006b; 55:360-8.

Curnow JL, Morel-Kopp M-C, Roddie C, Aboud M. & Ward CM. (2006) Reduced fibrinolysis and increased fibrin generation can be detected in hypercoagulable patients using the overall hemostatic potential assay. Thromb Haemost. 2006;5:528-34.

Current Practices and Controversies in Assisted Reproduction. Report of a meeting on «Medical, Ethical and Social Aspects of Assisted Reproduction» held at WHO Headquarters in Geneva, Switzerland, 17-21 Sept 2001. WHO, Geneva, 2002:396 P.

Curvers J, Nap AW, Thomassen MC, Nienhuis SJ, Hamulyak K, Evers JL, Tans G. & Rosing J. (2001) Effect of in vitro fertilization treatment and subsequent pregnancy on the protein C pathway. Br J Haematol. 2001;a115:400-7.

Dahlbäck B, Carlsson M. & Svensson PJ. (1993) Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of cofactor to protein C. Proc Natl Acad Sci U.S.A. 1993;90(3):1004-8.

Damti OB, Sarid O, Sheiner E, Zilberstein. & Cwikel J. (2008) Stress and distress in infertility among women. Harefuah. 2008;147(3):256-60.

Dargaud Y, Hierso S, Rugeri L, Battie C, Gaucherand P, Négrier C. & Trzeciak MC. (2010) Endogenous thrombin potential, prothrombin fragment 1 + 2 and D-dimers during pregnancy. Thromb Haemost. 2010; 103(2):469 -71.

Dmowski WP, Rana N, Michalowska J, Friberg J, Papesirniak C. & El-Roeiy A. (1995) The effect of endometriosis, its stage and activity and of autoantibodies on in vitro fertilization/ Embryo Transfer Success Rates. Fertil Steril. 1995;63:555-62.

Ebish IM, Thomas CM, Wetzels AM, Willemsen WN, Sweep FC. & Steegers-Theunissen RP. (2008) Review of the role of the plasminogen activator system and vascular endothelial growth factor in subfertility. Fertil Steril. Dec. 2008;90(6):2340-50.

Egbase PE, Al Sharhan M, Diejomaoh M. & Grudzinskas JG. (1999) Antiphospholipid antibodies in infertile couples with two consecutive miscarriages after in-vitro fertilization and embryo transfer. Hum Reprod. 1999;14(6):1483-6.

Friedler S, Zimerman A, Schachter M, Raziel A, Strassburger D. & Ron El R. (2005) The midluteal decline in serum estradiol levels is drastic but not deleterious for implantation after in vitro fertilization and embryo transfer in patients with normal or high responses. Fertil Steril. 2005;83:54- 60.

Geerts W, Selby R. (2003) Prevention of venous thromboembolism in the ICU. Chest. 2003;124:357S-63S.

Gelbaya TA, Tsoumpou I, Nardo LG (2009). "The likelihood of live birth and multiple birth after single versus double embryo transfer at the cleavage stage: a systematic review and meta-analysis". Fertil. Steril. 2009;94(3): 936-45.

Girolami A, Vianello F. (2000) The significance or nonsignificance of the G to A 2010 prothrombin polymorphism. Clin Appl Thromb Hemost. 2000;6:239-40.

Gleicher N, Vidali A. & Karande V. (2002) The immunological «Wars of the Roses»: disagreemants amongst reproductive immunologists. Hum Reprod. 2002;17:539-42.

Gordon H, Elie A, David D. & Holger J. (2012) Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012; 141(2Suppl):7S-47S.

Grandone E, Colaizzo D, Lo Bue A, Checola MG, Cittadini E. & Margaglione M. (2001) Inherited thrombophilia and in-vitro fertilization implantation failure. Fertil Steril. 2001;176:201-2.

Gregory L. (1998) Ovarian markers of implantation potential in assisted reproduction. Hum Reprod.1998;13:117-32.

Harris E, Pierangeli S. (2008) Primary, secondary and catastrophic antiphospholipid syndrome: what's in a name. Semin Thromb Hemost. 2008;34:219-26.

Heit J.A. (2007) Thrombophilia: clinical and laboratory assessment and management. Consultative Hemostasis and Thrombosis. - 2nd ed. Philadelphia, PA: Saunders Elsevier, 2007;213-44.

Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedr E, Wagenvoord R, Lecompte T. & Béguin S. (2003) Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiol Haemost Thromb. 2003;33:4-15.

Hemker HC, Giesen PLA, Ramjee M, Wagenvoord R. & Béguin. (2000) The thrombogramm: Monitoring thrombin generation in platelet-rich plasma. Thromb Haemost. 2000;83(4):589-91.

Hemker HC Al Dieri R, De Smalt F. & Beguin S. (2006) Thrombin generation, a function test of the haemostatic-thrombotic system. Thromb Haemost 2006;96(5):553-61;

Hillier S.G. (1994) Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. Hum Reprod. 1994;9:188-91.

Holemans R. (1963) Increase in fibrinoiytic activity by venous occlusion. J Appl Physiol. Nov. 1963;18:1123-9.

Howles CM, Kim CH. & Elder K. (2006) Treatment strategies in assisted reproduction for women of advanced maternal age. Int Surg. 2006;91:S37-S54.

Hull M, Corrigan E, Piazzi A. & Loumaye E. (1994) Recombinant human luteinizing hormone: an effective new gonadotropin preparation. Lancet. 1994;344:334-5.

International Committee for Monitoring Assisted Reproductive Technology (ICMART), de Mouzon J, Lancaster P, Nygren KG, Sullivan E, Zegers-Hochschild F, Mansour R, Ishihara O. & Adamson D. (2009) World collaborative report on assisted reproductive technology, 2002. Hum Reprod. Sep;2009;24(9):2310-20.

Jacobs DG, Piotrowski JJ, Hoppensteadt DA, Salvator AE. & Fareed J. (1996) Hemodynamie and fibrinolytie consequences of intermittent pneumatic compression: preliminary results. J Trauma. 1996; 40:710-6.

Januszko T, Reutt H, Malofiejew M. & Buluk K. (1967) Plasminogen activator in the course and after physical exercise. Acta Physiol Pol. 1967;18:272-9.

Jerzak M, Putowski L. & Baranowski W. (2003) Homocystein level in ovarian follicular fluid or serum as predictor of successful fertilization. Ginekol Pol. 2003;74(9):949-52.

Kakkos SK., Nicolaides AN, Griffin M. & Geroulakos G. (2005) Comparison of two intermittent pneumatic compression systems. A hemodynamic study. International Angiology. 2005;24(4):330-5.

Keber D, Stegnar M, Keber I. & Acceito B. (1979) Influence of moderate and strenuous daily physical activity on fibrinolytic activity of blood: possibility of piasminogen activator stores depletion. Thromb Haemost. 1979;41:745-55.

Kim DK, Kim JW, Kim S, Gwon HC, Ryu JC, Huh JE, Choo JA, Choi Y, Rhee CH. & Lee WR. (1997) Polymorphism of angiotensin converting enzyme gene is associated with circulating levels of plasminogen activator inhibitor-1. Arterioscler Thromb Vasc Biol. 1997;17(11):3242-7.

Kim HC, Kemmann E, Shelden RM. & Saidi P. (1981) Response of blood coagulation parameters to elevated endogenous 17 beta-estradiol levels induced by human menopausal gonadotropins. Am J Obstet Gynecol. 1981;140:807-10.

Knight MTN, Dawson R. (1976) Effect of intermittent compression of the arms on deep venous thrombosis in the legs. Lancet. 1976; 2:1265-7.

Kowarzyk H, Buluk K. (1954) Trombina, proteasa trombinowa i plazmina. Acta physiol Pol. 1954;5(1):35-56.

Ku SY, Kim SD, Jee BC, Suh CS, Choi YM, Kim JG, Moon SY. & Kim SH. (2006) Clinical efficacy of body mass index as predictor of in vitro fertilization and embryo transfer outcomes. J Korean Med Sci. 2006;21:300-3.

Lazarus JH, Premawardhana LD. (2005) Screening for thyroid disease in pregnancy. J Clin Pathol. 2005;58:449-52.

Lintsen AME, Eijkemans MJC, Hunault CC, Bouwmans CAM, Hakkaart L, Habbema JDF. & Braat DDM Predicting ongoing pregnancy chances after IVF and ICSI: a national prospective study. Hum Reprod. 2007;22(9):2455-62.

Lintsen AM, Pasker-de Jong PC, de Boer EJ, Burger CW, Jansen CA, Braat DD. & van Leeuwen FE. (2005) Effects of subfertility cause, smoking and body weight on the success rate of IVF. Hum Reprod. 2005;20(7):1867-75.

Lisman T, Leebek FWG, Meijer K, Van Der Meer J, Nieuwenhuis K. & De Groot P. (2002) Recombinant factor VIIa improves clot formation but not fibrinolytic potential in patients with cirrhosis and during liver transplantation. Hepatology. 2002;35:616-21.

Lisman T, deGroot PG, Meijers JCM. & Rosendaal FR. (2005) Reduced plasma fibrinolytic potential is a risk for venous thrombosis. Blood. 2005;105:1102-5.

Macdonald RL, Amidei C, Baron J, Weir B, Brown F, Erickson RK, Hekmatpanah J. & Frim D. (2003) Randomized, pilot study of intermittent pneumatic compression devices plus dalteparin versus intermittent pneumatic compression devices plus heparin for prevention of venous thromboembolism in patients undergoing craniotomy. Surg Neurol. 2003;59(5):363-72.

Macey MG, Bevan S, Alam S, Verghese L, Agrawal S, Beski S, Thuraisingham R. & MacCallum PK. (2010) Platelet activation and endogenous thrombin potential in preeclampsia. Thromb Res. 2010;125(3):e76-e81.

Maheshwari A, Hamilton M. & Bhattchary S. (2008) Effect of female age on the diagnostic categories of infertility. Hum Reprod. 2008;23(3):538-42.

Many A, Schrieber L, Rosner S, Lessing JB, Eldor A. & Kupferminc MJ. (2001) Pathologic features of the placenta in women with severe pregnancy complications and thrombophilia. Obstet Gynecol. 2001;98(6):1041-4.

Margalioth E, Ben-Chetrit A, Gal M. & Eldar-Geva T. (2006) Investigation and treatment of repeated implantation failure following IVF-ET. Hum Reprod. 2006;21(12):3036-43.

Martinez-Zamora MA, Creus M, Tassies D, Reverter JC, Civico S, Carmona F. & Balasch J. (2011) Reduced plasma fibrinolytic potential in patients with recurrent implantation failure after IVF and embryo transfer. Hum Reprod. 2011;26 (3):510-6.

Martınez-Zamora MA, Creus M, Tassies D, Bové A, Reverter JC, Carmona F. & Balasch J. (2010) Thrombin activatable fibrinolysis inhibitor and clot lysis time in women with recurrent miscarriage associated with the antiphospholipid syndrome. Fertil Steril. 2010;94:2437-40.

Matsubayashi H, Sugi, T, Arai T, Shida M, Kondo A, Suzuki T, Izumi, S. & McIntyre JA. (2006) IgG-antiphospholipid antibodies in follicular fluid of IVF-ET patients are related to low fertilization rate of their oocytes. Am J Reprod Immunol May 2006;55(5):341-8.

Mc Clamrock H.D. (2008) The great weight debate: do elevations in body mass index (BMI) exert a negative extraovarian effect on in vitro fertilization outcome? Fertil Steril. 2008;89:1609-10.

Megan L, Meike L, Uhter H, Edward G, John J, Kevin J. & Angeline N. (2008) Body Mass Index: Impact on IVF Success Appears. Hum Reprod 2008;23:1835-9.

Meltzer ME, Doggen CJ, de Groot PG, Rosendaal FR. & Lisman T. (2009) Reduced plasma fibrinolytic capacity as a potential risk factor for a first myocardial infarction in young men. Br J Haematol. 2009;145:121-7.

Meltzer ME, Lisman T, De Groot PG, Meijers JCM, Le Cessie S, Doggen CJM. & Rosendaal FR. (2010) Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. Blood. 2010;116:113-21.

Navot D, Rosenwaks L. & Margalioth E. (1987) Prognostik assessment of female fecundity. Lancet. 1987;2:645-7.

Nelen W, Bulten J. & Steegers E. (2000) Maternal homocysteine and chorionic vascularization in recurrent early pregnancy loss. Hum Reprod. 2000;15:954-60.

Nelen WL, Blom HJ, Thomas CM, Steegers EA, Boers GH. & Eskes TK. (1998) Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. J Nutrition. 1998;128:1336-41.

Nelson SM. (2009) Prophylaxis of VTE in women - during assisted reproductive techniques. Thromb Res. 2009;123:8-15.

Nelson SM, Greer IA (2008) The potential role of heparin in assisted conception. Hum Reprod. Update. Nov.-Dec. 2008;14:623-45.

Nyboe Andersen A, Goossens V, Bhattacharya S, Ferraretti AP, Kupka MS, de Mouzon J, Nygren KG. & The European IVF-monitoring (EIM) Consortium, for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology and intrauterine inseminations in Europe, 2005: results generated from European registers by ESHRE: ESHRE. The European IVF Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). Hum Reprod 2009;24(6):1267-87.

Ohira T, Cushman M, Tsai MY, Zhang Y, Heckbert SR, Zakai NA, Rosamond WD. & Folsom AR. (2007) ABO blood group, other risk factors and incidence of venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE). Thromb Haemost. 2007;5:1455-61.

Pandolfi M, Robertson B, Isacson S. & Niisson IM. (1968) Fibrinolytic activity of human veins in arms and legs. Thromb Diath Haemorrh. 1968;20:247-55.

Papanikolaou G, Evangelos G. 1, Kolibianakis E, Efstratios M, Tournaye H, Venetis CA, Fatemi H, Tarlatzis B. & Devroey P. (2008) Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. Hum Reprod. 2008;23(1):91-9.

Pioneers in in vivo Fertilisation. Ed. A.Th. Alberda, R.A. Gan, H.M. (1995) Vemer. London-New York:The Parthenon Publishing Group 1995;114.

Poort SR, Rosendaal FR, Reitsma PH. & Bertina RM. (1996) A common genetic variation in the 3- prime-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996;88:3698-703.

Qublan H, Eid S. (2006) Acquired and inherited thrombophilia: implication in recurrent IVF and embryo transfer failure. Hum Reprod. 2006;21(10):2694-8.

Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J. & Bulmer J. (2009) Uterine natural killer cells and angiogenesis in recurrent reproductive failure. Hum Reprod. 2009;24:45-54.

Regnault V, Beguin S. & Lecompte T. (2003) Calibrated automated thrombin generation in frozen-thawed platelrich plasma to detect hypercoagulability. Pathophysiol Haemost Thromb. 2003;33:23-9.

Rice VC, Richard-Davis G, Saleh AA, Ginsburg KA, Mammen EF, Moghissi K. & Leach R. (1993) Fibrinolytic parameters in women undergoing ovulation induction. Am J Obstet Gynecol. 1993; 169 (6):1549-53.

Ridker PM, Miletich JP, Buring JE, Ariyo AA, Price DT, Manson JE. & Hill JA. (1998) Factor V Leiden mutation as a risk factor for recurrent pregnancy loss. Annals of Internal Medicine. 1998;128:1000-3.

Rova K., Passmark H. & Lindqvist P.G. (2012) Venous thromboembolism in relation to in vitro fertilization: an approach to determining the incidence and increase in risk in successful cycles. Fertil Steril. (2012);97(1):95-100.

Saleh RA, Agarwal A, Sharma RK, Nelson DR. & Thomas Jr AJ. (2002) Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. Fertil Steril. 2002; 78:491-9.

Salzman EW, McManama GP, Shapiro AH, Robertson LK, Donovan AS, Blume HW, Sweeney J, Kamm RD, Johnson MC & Black PM. (1987) Effect of optimization of hemodynamics on fibrinolytie activity and antithrombotic efficacy of external pneumatic calf compression. Ann Surg. 1987;206:636-41.

Sarto A, Rocha M, Martinez M. & Pasqualini R. (2000) Hypofibrinolysis and other hemostatic defects in women with antecedents of early reproductive failure. Medicina. 2000;60:441-7.

Scheffer GJ, Broekmans FJ, Looman CW, Blankenstein M, Fauser BC, teJong FH. & teVelde ER. (2003) The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. Hum Reprod. 2003;18:700-6.

Seghatchian MJ, Samama MM. & Hecker SP. (1996) Hypercoagulable states. Fundamental aspects, acquired disorders, and congenital thrombophilia. C.R.C. Press. Boca Raton e.a. 1996;462 P.

Sher G, Fisch JD, Maassarani G, Matzner W, Ching W. & Chong P. (2000) Antibodies to phosphatidylethanolamine and phosphatidylserine are associated with increased natural killer cell activity in non-male factor infertility patients. Hum Reprod. 2000;15(9):1932-6.

Stern C, Chamley L. (2006) Antiphospholipid antibodies and coagulation defects in women with implantation failure after IVF and miscarriage. Reprod Biomed Online. 2006;13:29-37.

Tan BK, Vandekerckhove P. & Kennedy R. (2005) Investigation and current management of recurrent IVF treatment failure in the UK. BJOG. 2005;112:773.

Tarnay TJ, Rohr PR, Davidson AG, Stevenson MM, Byars EF. & Hopkins GR. (1980) Pneumatic calf compression, fibrinolysis, and the prevention of deep venous thrombosis. Surgery. 1980;88:489-96.

Tchaikovski SN, van Vliet HA, Thomassen MC, Bertina RM, Rosendaal FR. Sandset PM, Frans M Helmerhorst FM, Tans G. & Rosing J. (2007) Effect of oral contraceptives on thrombin generation measured via calibrated automated thrombography. Thromb Haemost. 2007;98(6):1350-6.

Triplett D.A. (1989) Antiphospholipid antibodies and recurrent pregnancy loss. Am Reprod Immun. 1989;20:52-7.

Turpie AG, Gallus A, Beattie WS. & Hirsh J. (1977) Prevention of venous thrombosis in patients with intracranial disease by intermittent pneumatic ompression of the calf. Neurology. 1977;27:435-8.

Urman B, Ata B, Yakin K, Alatas C, Aksoy S, Mercan R,. & Balaban B. (2009) Luteal phase empirical low molecular weight heparin administration in patients with failed ICSI embryo transfer cycles: a randomized open-labeled pilot trial. Hum Reprod. Jul 2009;24(7):1640-7.

Urman B, Yakin K. & Balaban B. (2005) Recurrent implantation failure in assisted reproduction: how to counsel manage. A. General considerations and treatment options that may benefit the couple. Reproductive BioMedicine Online. 2005;11:382-91.

Weitz J, Michelsen J, Gold K, Owen J. & Carpenter D. (1986) Effects of intermittent pneumatic calf compression on postoperative thrombin and plasmin activity. Thromb Haemost. 1986;56:198-201.

Westerlund E, Henriksson P, Wallén H, Hovatta O, Wallberg KR. & Antovic http://www.sciencedirect.com/science/article/pii/S0049384811006189 - af0005 A. (2012) Detection of a procoagulable state during controlled ovarian hyperstimulation for in vitro fertilization with global assays of haemostasis. Thromb Res. Apr. 2012;46(4):417-25.

Wichers IM, Tanck MW, Meijers JC, Lisman T, Reitsma PH, Rosendaal FR, Büller HR. & Middeldorp S. (2009) Assessment of coagulation and fibrinolysis in families with unexplained thrombophilia. Thromb Haemost. 2009;101:465-70.

Wielders S, Mukherjee M, Michiels J, Rijkers DT, Cambus JP, Knebel RW, Kakkar V, Hemker HC. & Béguin S. (1997) The routine determination of the endogenous thrombin potential, first results in different forms of hyper- and hypocoagulabillity. Thromb Haemost. 1997;77(4):629-36.

Younis JS, Brenner B, Ohel G, Tal J, Lanir N. & Ben-Ami M. (2000) Activated protein C resistance and factor V Leiden mutation can be associated with first-as well as second-trimester recurrent pregnancy loss. Am J Reprod Immunol. 2000;43(1):31-5.

Zorio E, Gilabert-Estellés J, España F, Ramón LA, Cosin R. & Estellés A. (2008) Fibrinolysis: the key to new pathogenetic mechanisms. Curr Med Chem. 2008;15:923-9.