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Departament of Midwifery Care

Dr. Zlatko Kirovakov

Prevention of reproductive failures and postnatal complications in women with genetically determinet Thrombophilia

ABSTRACT

of a dissertation

to acquire an educational and scientific degree **"Doctor"**

Doctoral programe "Obsterics and Gynaecology"

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The dissertation consists of 181 standard typed pages and is illustrated with 22 figures, 52 tables, and one appendix. The literature sources used include 377 titles, 11 of which are in Cyrillic and 366 in Latin script. The figure and table numbers in the abstract do not correspond to the numbers in the dissertation.

The author is a doctoral student in the independent study form at the Department of "Health Care," Faculty of "Health Care," Medical University - Pleven, and was enrolled by order No. 789/09.03.2023. He works as an obstetrician-gynecologist in the Maternity Ward of "UMHAT - Burgas" and at the "Prime Clinic" Medical Center - Burgas.

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ABBREVIATIONS USED

ACE	Angiotensin-converting enzyme
APC	Activated protein C
APS	Antiphospholipid syndrome
ART	Assisted reproductive technology
AT III	Antithrombin III
BMI	Body mass index
CβS	Cystathionine beta synthase
FVL	Factor V Laiden
IR	Insulin resistance
IUGR	Intrauterine growth restriction
MTHFR	Methylenetetrahydrofolate reductase
PE/E	Preeclampsia/Eclampsia
PTE	Pulmonary thromboembolism
PTG	Prothrombin Gene
RP	Reproductive problems
RPL	Recurrent pregnancy loss
SA	Spontaneous abortion
t-PA	Tissue plasminogen activator
UA-PI	Uterine artery – Pulsatility Index
VTE	Venous thromboembolism

TABLE OF CONTENTS

I. INTRODUCTION

The problem of reproductive failures continues to be one of the most pressing issues in modern obstetrics. The discovery in the 1960s of new genetic forms of thrombophilia—such as Factor V Leiden, G20210A mutation in the Prothrombin gene, the C677T genetic variant in the Methylenetetrahydrofolate Reductase (MTHFR) gene, the genetic variant in the Plasminogen Activator Inhibitor 1 (PAI-1) gene (carriage of the 4G/4G genotype), Angiotensin-Converting Enzyme (ACE) D/D genotype, and others—and the progress in understanding the molecular mechanisms of thrombophilia have contributed to clarifying the causes of development and the emergence of new perspectives on the pathogenesis of reproductive losses.

Reproductive failures (infertility, recurrent pregnancy loss, pregnancy complications, and unsuccessful in vitro fertilizations) are a serious medical, demographic, and emotional problem affecting an increasing number of couples in developed countries.

It is believed that infertility (the inability to conceive after one year of regular attempts for women under 35 years old and after 6 months for women over 35 years old, according to WHO) affects approximately 1 in 6 couples, with this frequency increasing with age. Despite the continuous improvement of medical technologies and the enhancement of the quality of obstetric and gynecological care, the frequency of spontaneous pregnancy losses is not inclined to decrease and accounts for 10 to 20% of all clinically diagnosed pregnancies. This pathology continues to be the main cause of reproductive losses, making it one of the most urgent problems in modern medicine, not only in our country but also worldwide. Clinical losses vary depending on the gestational age and the age of the woman and can be categorized as:

- Biochemical pregnancy: 8 to 33% in the general population
- Early fetal losses (up to 12 weeks of gestation): on average 15%, varying with age from 10% (ages 20-24) to 51% (ages 40-44)
- Late losses (12-20 weeks of gestation): approximately 4%.

Some couples experience recurrent pregnancy losses, which, according to the American Society for Reproductive Medicine (ASRM, 2012), include the loss of two or more consecutive clinical pregnancies up to 20 weeks of gestation. According to the European Society of Human Reproduction and Embryology (ESHRE, 2017), recurrent losses are defined as two or more losses up to 24 weeks of gestation, including biochemical pregnancies. The frequency of clinical losses alone is 0.8% to 1.4%, and when biochemical pregnancies are included, it rises to 2% to 3%.

II. OBJECTIVE AND TASKS

Objective:
The aim of this dissertation is to develop a therapeutic and preventive algorithm of management to prevent recurrent pregnancy losses, postnatal, and thromboembolic complications associated with the carriage of inherited genetic factors for thrombophilia in women with a history of reproductive failures.

Tasks:
In relation to the aim of the dissertation, the following main tasks were set:

2.1 To determine the demographic characteristics, anamnesis data, and exclusion factors to characterize the clinical group of women studied with reproductive failures who carry one or more of the following five genetic factors associated with thrombophilia: Factor V Leiden; G20210A mutation in the Prothrombin gene; C677T genetic variant in the Methylenetetrahydrofolate Reductase (MTHFR) gene; Genetic variant in the Plasminogen Activator Inhibitor 1 (PAI-1) gene (carrying the 4G/4G genotype); and Angiotensin-Converting Enzyme (ACE D/D, I/D) genotype.

2.2. To analyze the frequency of the studied gene mutations in the clinical group of women with reproductive failures.

2.3. To determine the accompanying pathology and family history in the clinical and control groups of women studied.

2.4. To assess the dynamics in the Doppler velocimetry of the uterine arteries, with the mean PI from the right and left uterine arteries of pregnant women (at 6-7 weeks, $11\text{-}13^{+6}$, 20-22 weeks, and 35-36 weeks) in the clinical and control groups.

2.5. To monitor the dynamics of deviations in complete blood count (CBC) and hemostasis during pregnancy—at 4-5 week

intervals after 12 weeks of gestation—in the clinical and control groups.

2.6. To track the outcomes of pregnancy, childbirth, and the puerperal period, as well as the condition of the fetus in the early neonatal period, in the groups of women studied.

2.7. To develop a therapeutic and preventive management algorithm according to the specificity and severity of genetic mutations, with the aim of preventing frequent recurrent pregnancy losses, thromboembolic, and postnatal complications.

III. CLINICAL MATERIAL, METHODS OF RESEARCH. METHODOLOGY AND STATISTICAL ANALYSIS OF THE RESULTS

III.1 CLINICAL MATERIAL

The study is both prospective and retrospective. It was conducted at "Prime Clinic - Dr. Kirovakovi" Medical Center in Burgas, "St. Elisaveta" KIRM Medical Center in Pleven, and the Maternity Ward of UMBAL Burgas. The study covers the period from January 2021 to December 2023. A total of 459 pregnant women were included in the study.

Clinical Characteristics of the Studied Patient Groups
III.1.1. Clinical Group

Patients with confirmed inherited thrombophilia — (n=309) pregnant women registered at the prenatal care clinic who are carriers of one or more genetic thrombophilic factors and have a history of reproductive failures. The following analyses were performed for these patients: the genetic spectrum of thrombophilic mutations; anamnesis data on personal thrombotic incidents; family history; accompanying pathology; obstetric history; course of past pregnancies; course of the current pregnancy; childbirth and the puerperal period.

To analyze the course of pregnancy, childbirth, and the postpartum period, the clinical group was divided into two main subgroups depending on the carriage of PAI-1 and ACE:

- **Group 1** - Women with a mutation in the 4G/5G PAI-1 gene (heterozygous or homozygous carriage) and a normal genotype (I/I) of ACE (n=225).
- **Group 2** - Women with a mutation in the PAI-1 gene (heterozygous or homozygous carriage) + homo or

heterozygous mutation (D/D, I/D) in the ACE gene (n=84).

Exclusion Criteria for the Clinical Group:

Age > 40 years
Patients with acquired thrombophilia (antiphospholipid syndrome)
Confirmed chromosomal or other genetic anomaly
Infections during pregnancy
Anatomical abnormalities
Multiple pregnancy

III.1.2. Control Group

Pregnant women without a history of thrombophilia, reproductive failures, or thrombotic incidents, registered at the prenatal care clinic, with normal pregnancies and deliveries in the past (n=150).

II.2. RESEARCH METHODS

To determine the frequency of thrombophilic mutations, identify pregnancy complications, establish the prognostic value, Pulsatility Index of the Uterine Arteries, and coagulation status, analyze the course of pregnancy, gestational age, and mode of delivery, as well as assess the condition of the fetus after birth and monitor the puerperal period, four groups of methods were used:

1. Clinical methods
2. Instrumental methods
3. Paraclinical methods
4. Epidemiological methods

III.2.1. Clinical Methods

III.2.1.1. General status, obstetric status, and obstetric examination upon the registration of patients in prenatal care (PNC).

Upon registering each pregnant woman in prenatal care, an obstetric examination was performed. The Body Mass Index (BMI) was determined at the beginning and end of the pregnancy.
BMI = Body weight (kg) / Height squared (in meters). Obesity is defined when the Body Mass Index is greater than 30 kg/m². Increased body weight is considered if the BMI is between 25 and 30 kg/m².

Blood pressure was recorded monthly using validated automatic blood pressure monitors on both arms after a 5-minute rest, with the patient in a seated position.

Upon admission of each pregnant woman to the hospital, an obstetric examination was performed. The obstetric status was determined by assessing the position of the cervix, its consistency, the dilation of the cervical canal, the shortening of the cervix, the exact position of the presenting part of the fetus relative to the linea interspinalis, and the condition of the amniotic sac.

III.2.1.2. Determining the Apgar Score of the Newborn

The assessment of the newborn's condition immediately after birth is performed by determining the Apgar score by the attending neonatologist. This is a quick assessment method that provides information about the newborn's condition and the need for resuscitation. Five criteria are evaluated: breathing, heart rate, skin color, muscle tone, and reflex response. Each criterion is scored with 0, 1, or 2 points, which are then summed to form

the Apgar score. The Apgar score at 1 minute indicates the need for initial resuscitation, while the Apgar score at 5 minutes can be used for a longer-term assessment of the newborn's condition.

III.2.2. Instrumental Methods

III.2.2.1. Imaging Diagnostics - Ultrasound Method

Ultrasound examinations were performed on all women during pregnancy to assess the condition of the fetus, placenta, and amniotic fluid monthly up to 30 weeks of gestation, and thereafter every two weeks.

This method provided information on the gestational age of the fetus, allowed identification of fetuses with hypotrophy (we considered the fetus hypotrophic if its weight was below the 10th percentile), and measurements of BPD, HC, AC, FL were taken to calculate the average expected fetal weight. The position of the fetus in the uterus, the maturity and position of the placenta, and the amount of amniotic fluid were determined. Ultrasound was used to diagnose specific complications such as missed abortion, blighted ovum, placental insufficiency, intrauterine growth retardation (IUGR), fetal death, oligohydramnios, polyhydramnios, and increased resistance of uterine vessels according to the nomogram for the respective gestational age.

The average Pulsatility Index of the uterine arteries was measured using Doppler velocimetry at 6-7 weeks, 11-13^{+6} weeks, 20-22 weeks, and 30-32 weeks. High-quality ultrasound machines with high resolution from GE and Mindray were used for examining blood flow in the uterine arteries. Pulsed and color mapping were employed. Flow velocity waveforms were recorded with 3.5 MHz and 5 MHz using a transabdominal convex transducer with a 3.2 MHz Doppler frequency.

The examination is performed transabdominally with the ultrasound probe placed longitudinally in the suprapubic area, with the patient lying on her back. The transducer is positioned parallel to the anterior uterine wall in the isthmic portion. The common iliac artery is traced to its bifurcation, and then the transducer is directed medially in the same plane to visualize the uterine artery. The insonation window is positioned approximately 1 cm medially from where the uterine artery crosses the external iliac artery. The insonation angle should be less than 30 degrees, with an acoustic window of 2 mm width to cover the entire vessel. The maximum systolic velocity in the vessel should be no less than 60 cm/s to accurately assess the correct vessel rather than its branch. At this point, the recording of the waveforms begins.

III.2.2.2. Fetal Anthropometry

Anthropometric methods were used for all newborns immediately after birth. The weight was measured with an electronic scale in grams, and the height was measured in centimeters for all newborns.

III.2.3. Paraclinical Methods

III.2.3.1. Laboratory Tests

For each woman, a complete blood count, coagulation status, vitamin D levels, TSH, FT4, anti-TPO (Microsomal antibodies), and anti-Tg were tested during preconception preparation, as well as genetic testing for mutant genes.

The following parameters were evaluated: hemoglobin, hematocrit, erythrocytes, leukocytes, platelets, and hemostatic indicators: fibrinogen, D-dimer, APTT, bleeding time, and coagulation time, every 3-4 weeks. Using the values of hemoglobin, hematocrit, and platelets, we identified cases of

anemia and thrombocytopenia during pregnancy, as well as increased and decreased blood coagulability due to hemoconcentration and hemodilution.

To assess the vaginal and cervical microbiome, microbiological examination of the vaginal discharge and PCR-DNA testing from cervical secretions for Ureaplasma, Mycoplasma, and Chlamydia were performed in the first and third trimesters of pregnancy.

III.2.4. Epidemiological Methods

III.2.4.1. Informational Method

In cases where personal communication with the patient was not possible, data were obtained from documentation such as the "Pregnancy Exchange Card," "Medical History," and "Discharge Summary."

III.2.4.2. Survey Method

This method was used with all pregnant women. The questions concerned the course and outcome of previous pregnancies that ended in early or late fetal loss, early preeclampsia, intrauterine growth restriction, as well as pre- and postpartum complications such as varicose veins of the lower limbs, superficial and deep thrombophlebitis, as well as the course of the current pregnancy, initiation of anticoagulant and antiplatelet therapy, bleeding during the current pregnancy, and the presence of subjective complaints.

III.3. STATISTICAL ANALYSIS

Statistical Methods

The data were collected and recorded in Microsoft Excel and analyzed using R Studio 4.2.2. The libraries stats, tidyverse, ggplot, and rstatix were used to perform descriptive statistics, analysis of variance, and post-hoc analyses.

Descriptive Statistics: Key parameters of the variables of interest in the different groups of examined women were presented, including mean, median, standard deviation, and variance, along with their frequency distribution.

Non-parametric Hypothesis Testing Methods:

- χ^2 **Analysis**: Used to determine the presence of dependency between categorical variables based on their frequency distribution.
- **Mann-Whitney Test**: Used to identify statistically significant differences between two groups for a dependent variable with non-normal distribution.

Analysis of Variance:

- **One-way ANOVA**: Employed for different measures relative to the groups of examined women.
- **Repeated Measures ANOVA**: Used to test for statistically significant differences across different periods (gestational weeks) for a specific quantitative parameter.

Post-hoc Tukey Test: Conducted to test the statistical significance of individual comparisons between pairs of groups/periods to track differences between specific pairs.

Post-hoc Bonferroni Test: Conducted to test the statistical significance of individual comparisons between pairs of groups/periods to track differences between specific pairs.

IV. RESULTS AND DISCUSSION

1.1 Demographic Characteristics and Medical History

To characterize the clinical group of women with reproductive failures and carriers of one or more of the following five genetic factors associated with thrombophilia—Factor V Leiden; G20210A mutation in the Prothrombin gene; C677T genetic variant in the Methylenetetrahydrofolate reductase gene; genetic variant in the Plasminogen Activator Inhibitor 1 (PAI-1) gene (carrying genotype 4G/4G); Angiotensin-Converting Enzyme (ACE) D/D genotype—the following aspects were assessed:

Table 1. Demographic Profile of the Study Women

	Clinical group A (n=309)				Control group B (n=150)				Mann-Whitney 'U' test	
	Shapiro-wilk		M	S.D.	Shapiro-Wilk		M	S.D.	Statistic	P-value
	Statistic	P-value			Statistic	P-value				
Age	0.95	0.001*	31.75	5.89	0.95	0.001*	30.75	5.53	1.64	0.101
BMI	0.97	0.006*	25.19	3.38	0.98	0.027*	24.99	2.49	0.03	0.977

The results from Table 1 show the descriptive statistics for women in Group A (clinical group, n=309) and Group B (control group, n=150) with respect to age and BMI, as well as the Mann-Whitney nonparametric test for differences between the two groups regarding these two demographic characteristics. In both groups, the pregnant women are approximately 31 years old with a standard deviation of ±5.5-6 years. The average BMI is around 25 for both groups, but there is a larger standard deviation in Group A, indicating that the values in Group A vary slightly more than those in Group B. Both variables in the two groups do not follow a normal distribution, which is why the test for differences in means is the nonparametric Mann-Whitney test. The p-value for the test for both variables is greater than the significance level of p=0.05, indicating no significant difference in means between the two groups for both variables. Therefore, the women in Groups A and B have similar average ages and BMI.

Table 2 provides descriptive statistics for sociodemographic factors—smoking, marital status, number of pregnancies, number of births, and clinical conditions—such as varicose veins, anemia, pregnancy complications, family history of thrombosis, and family history of diabetes.

Table 2. Sociodemographic factors, descriptive statistics and chi – square analysis

	Clinical group A n=309	**%**	**Control group B n=150**	**%**	**χ^2 Statistics**	**P-value**
Smocers						
Yes	122	39.5	36	24	10.87	0.001*
No	187	60.5	114	76		
Material status						
Married	168	54.4	64	42.7	5.53	0.019*
Non-married	141	45.6	86	57.3		
Gravidity						
	n	%	n	%	120.06	0.001*
Primigravida	88	28.50	68	52.00		
Secundagravida	116	35.70	47	24.70		
Multigravida	105	34.00	35	23.30		

Parity						
Did not give birth	247	79.90	81	54.00	34.48	3.26E-08*
One	58	18.80	61	40.70		
Two or more	4	1.30	8	5.30		
Miscarriages in the past						
Early pregnancy loss <10g.w.						
One loss	62	20.065	0	0		
More than two	234	75.728	0	0		
Late pregnancy loss 12-24g.w.	13	4.207	0	0		

Smoking Status: In Group A, 39.5% of women are smokers compared to 60.5% who are non-smokers, whereas in Group B, 76% are non-smokers and 24% are smokers. The χ^2 test for independence between the two categorical variables shows that there is a difference in the expected values of smokers in Group A versus Group B (p-value < 0.05). This indicates that women in Group B are more likely to be non-smokers compared to those in Group A. Among married women, Group A has a higher percentage (55.4%) compared to Group B (42.7%). The χ^2 test indicates that the mean frequencies in the two groups are different, showing a statistically significant difference, which means the expected values for married versus unmarried women differ between the groups. The expected number of pregnancies differs statistically between the two groups according to the χ^2 analysis, which is also supported by the observed frequency differences. Pregnant women in Group A show a higher frequency of pregnancies, with 34% and 32% having more than 3 and more than 2 pregnancies, respectively. Cumulatively, 65% of women in Group A have had more than one pregnancy, 28.5% have had only one pregnancy, and 5.5% have never been pregnant. In Group B, the distribution is different: 52% of women have had only one pregnancy, and the remaining 48% are divided as follows: 24.7% with two pregnancies, 23.3% with three or more pregnancies. There are no women in Group B who have never been pregnant.

Looking at the number of births after the 6th month, 79.9% of women in Group A have not had any live births, 18.8% have one child, and only 1.8% have more than two children. This leads to the conclusion that 93.11% of women in this group who did not have a live birth have experienced pregnancies and miscarriages. In Group B, 54% of women have no living children, meaning 54% of pregnant women have not had a live birth, which is a significantly smaller percentage compared to Group A (93.3%). Additionally, in Group B, the highest percentage of women have had only one pregnancy, with 40.7% having one child and 5.5% having two or more. The χ^2 test confirms statistically significant

differences between the frequencies of the number of births in the two groups. Finally, Table 2 examines past spontaneous abortions. There are no women with a history of spontaneous abortion in Group B, whereas in Group A, women are categorized as having one loss (20.065%) or more than two losses (75.728%), with a few cases of late losses up to 24 weeks gestation (only 4.207%). Early losses and more than two losses predominate in Group A.

Table. 3 Structure of the accompanying pathology in the clinical (A) and control group (B)

Structure of the accompanying pathology in the clinical (A) and control group (B)						
	Group A		**Group B**			
	N=309	**%**	**N=150**	**%**	**χ^2**	**P-value**
					Statistics	
Varicose vein store						
No	162	52.43%	134	89.33%	60.99	5.711E-14*
Varicose veins (lower limbs)	102	33.01%	14	9.33%		
Varicose veins (vulva and vagina)	45	14.56%	2	1.33%		
Hypertensive disease						
Yes	54	17.48%	13	8.67%	6.286	0.012*
No	255	82.52%	137	91.33%		

Insulin resistence and Diabetes						
No	78	25.24%	150	100%	225.75	1.087E-47*
Insulin resistence	135	43.69%	0	0%		
Diabetes I type	4	1.29%	0	0%		
Diabetes II type	16	5.18%	0	0%		
PCOS	76	24.60%	0	0%		
Diseases of the Thyroid gland						
No	239	73.3%	150	100%	77.28	1.97E-09*
Subclinical hypothyroidism	67	21.68%	0	0%		
Morbus Basedow	3	0.97%	0	0%		
Anemia						
No	257	83.17%	134	89.3%	3.05	2.18E-01*
Iron deficiency anemia	45	14.56%	14	9.3%		
Thalassemia	7	2.27%	2	1.4%		

The condition of varicose veins is categorized as follows: absence, presence on the lower extremities, and presence on the vulva and vagina. The results in Table 3 show that 33.01% of women in Group A have varicose veins on the lower extremities, 14.56% have them on the vulva and vagina, and 52.43% do not have this pathology. Group B shows a markedly different trend—89.33% of women do not have varicose veins, 9.33% have them on the lower extremities, and 1.3% have them on the vulva and vagina. As expected, the chi-square test indicates a statistically significant difference in the presence of varicose veins between the two groups of women (p-value = 5.711E-14).

In Group A, 17.48% of women have hypertensive disease and 82.52% do not. In contrast, in Group B, only 8.67% have hypertensive disease and 91.33% do not. The chi-square test confirms a statistically significant difference in the presence of hypertensive disease between the experimental and control groups (p-value = 0.012).

In Group A, 25.24% of women do not exhibit insulin resistance, 43.69% have insulin resistance, 1.29% have Type I diabetes, 5.18% have Type II diabetes, and 24.60% have PCOS. In Group B, no women exhibit insulin resistance. The chi-square test confirms a significant difference in the presence of this pathology between the two groups of women (p-value = 1.087E-47). Considering thyroid diseases, it is found that 73.3% of women in Group A do not have any thyroid disease, 21.68% have subclinical Hashimoto's thyroiditis, and only 0.97% have Graves' disease. In Group B, there are no women with developed thyroid disease. The chi-square test confirms a statistically significant difference between the control and experimental groups regarding thyroid disease (p-value = 1.97E-09). The results for the presence of anemia show more similar frequencies between the two groups across different categories, but the chi-square test still reveals statistically significant differences between the two groups. The percentages of women without any form of anemia are 83.17% in Group A and 89.3% in Group B.

The respective percentages of women with iron-deficiency anemia are 14.56% in Group A and 9.3% in Group B. The percentages of women with Thalassemia are 2.27% in Group A and 1.4% in Group B.

Table 4. Family history for hereditary

Family history for hereditary						
	Group A		**Group B**		**χ^2**	**P-value**
	N	**%**	**N**	**%**	**Statistic**	
Family history for thrombosis, myocardial infarction, or Stroke						
Yes	43	13.9%	10	6.67%	5.1956	0.023*
No	266	86.1%	140	93.33%		
Family history for hypertension						
Yes	62	20.06%	17	11.3%	5.4	0.02*
No	247	79.94%	133	88.7%		
Family history for diabetes						
Yes	58	18.77%	16	10.67%	4.9	0.027*
No	251	81.23%	134	89.33%		

The family history for thrombosis, myocardial infarction, and/or stroke shows that 13.9% of women in Group A have such a family history, while 86.1% do not. In contrast, only 6.67% of women in Group B have a family history of thrombosis, myocardial infarction, and/or stroke, and 93.33% do not. Again, the chi-square test confirms significant differences between the distributions of women in the two groups regarding family history of thrombosis (p-value = 0.023). The second type of family history of burden presented in Table 4 is family history of hypertension, where 20.06% of women in Group A have this history, and 79.94% do not. In Group B, 11.3% have such a family burden, and 88.7% do not. The chi-square test for independence between the two groups shows a statistically significant difference (p-value = 0.02). Regarding family history of diabetes, 18.77% of women in Group A have such a history, while only 10.67% of women in Group B do. The chi-square test confirms differences in the expected frequencies of family history of diabetes between the two groups (p-value = 0.027).

Table 5. Method of conception during the current pregnancy

Method of conception during the current pregnancy						
	Group A N	**%**	**Group B N**	**%**	**χ^2 Statistic**	**P-value**
Spontaneus	211	68.28%	138	92	32.107	1.0669E-07*
After ART	83	26.86%	12	8		
After insemination	15	4.854%	0	0		

The data for the method of conception during the current pregnancy in the two studied groups show that 68.28% of the women in Group A conceived spontaneously, 26.86% through ART (Assisted Reproductive Technology), and 4.85% through insemination. In Group B, 92% of the women conceived spontaneously, and only 8% conceived through ART, with no pregnancies resulting from insemination. The chi-square test confirms the presence of statistically significant differences between Groups A and B regarding conception methods with a 95% confidence level (p-value = 1.067E-07).

Table 6 and Fig.2 presents the results of the cytogenetic examination (chromosomal analysis, karyotyping) of both partners in women with more than two pregnancy losses. All examined couples had normal female and male karyotypes.

Table 6. Karyotype test of the both partners trom the experimental group with one or more miscarriages

Karyotype test of the partners trom the experimental group with one or more miscarriages		
Karyotype	**N=234**	**%**
Conducted	194	82.91%
Not conducted	40	17.09%

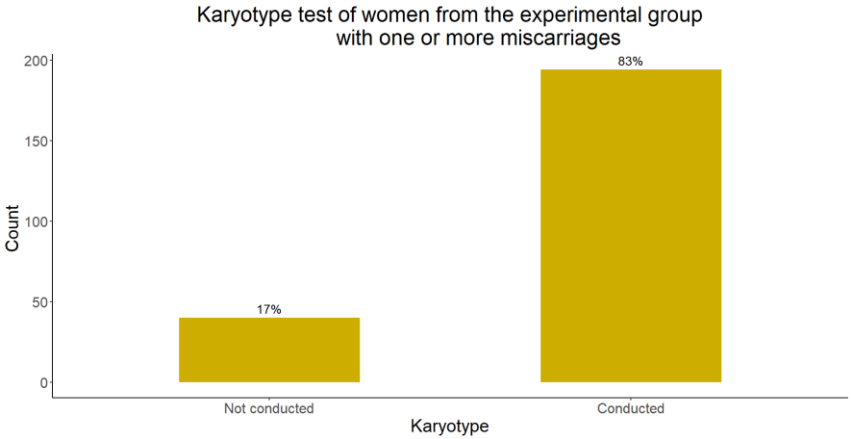

Figure 1. Karyotype test of the partners trom the experimental group with one or more miscarriages

The results show that among women in Group A with more than two pregnancy losses (234 women), 82.91% underwent karyotyping, while 17.09% did not.

Table 7. Results from Genetic Mutation Testing Associated with Thrombophilia in the clinical group

Thrombophilic Spectrum	**N = 309**	**%**
Factor V Leiden Mutation		
Homozygous	**7**	**2.27%**
Heterozygous	**31**	**10.03%**
Mutation in the Prothrombin gene G20210A		
Homozygous	**12**	**3.88%**
Heterozygous	**27**	**8.74%**
Mutation in MTHFR C677T		
Homozygous	**92**	**29.77%**
Heterozygous	**172**	**55.66%**
Mutation in Plasminogen Activator Inhibitor 1 (PAI-1)		
Homozygous (4G/4G)	**74**	**23.95%**
Heterozygous (5G/4G)	**182**	**58.89%**
Normal allel (5G/5G)	**45**	**14.56%**
Mutation in Angeotensin – Converting Enzyme		
Homozygous (ACE D/D)	**38**	**12.29%**
Heterozygous (ACE I/D)	**46**	**14.89%**
Normal allel (ACE I/I)	**225**	**72.82%**

The mutation in Factor V Leiden is present in 12.30% of the women in Group A, with 2.27% being homozygous and 10.03% heterozygous. The mutation in the prothrombin gene G20210A is found in 12.62% of the women, divided into 3.88% homozygous and 8.74% heterozygous. The MTHFR C677T mutation is present in 85.43% of the women in Group A, with 29.77% being homozygous and 55.66% heterozygous. The Plasminogen Activator Inhibitor 1 (PAI-1) 4G/4G homozygous is observed in 97.40% of the women, with 23.95% being homozygous, 58.89% heterozygous, and 14.56% having normal alleles. The Angiotensin-Converting Enzyme (ACE) mutation is found in 27.18% of the women, with 12.29% having the ACE D/D genotype, 14.89% having the ACE I/D genotype, and 39.81% having the ACE I/I genotype (normal genotype).

The following graph illustrates the results from Table 7, showing the percentage distribution of women in Group A.

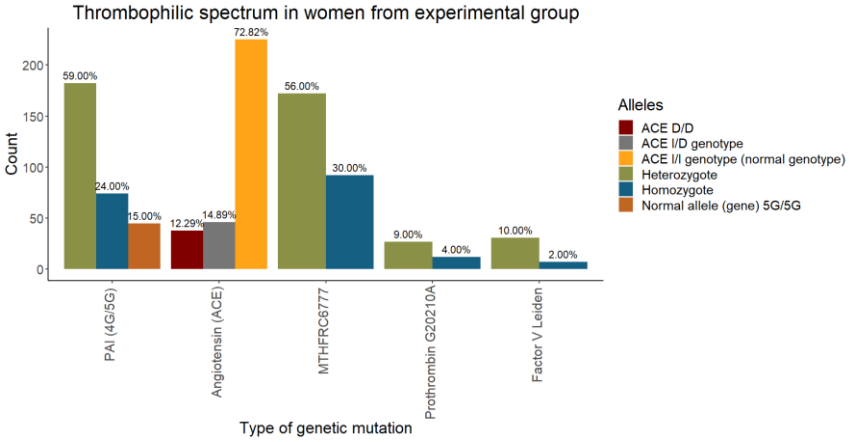

Figure 2. Thrombophilic Spectrum in women from the Studied Group

Evaluation of the dynamics in Doppler velocimetry of the uterine arteries, averaged PI from the right and left uterine arteries of pregnant women (measured three times – at 11-13+6 weeks of gestation, 20-22 weeks of gestation, and 35-36 weeks of gestation) in both the clinical and control groups.

Table 8 (a). Uterine artery Pulsatility index within group comparison

G.W.	A		B	
	M	**S.D.**	**M**	**S.D.**
6-7 g.w.	2.90	0.52	2.18	0.49
11-13 g.w.	2.70	0.42	2.01	0.48
20-22 g.w.	2.22	0.58	1.52	0.38
30-33 g.w.	1.88	0.74	1.42	0.40

Table 8(b). Uterine artery pulsatility index ANOVA within group comparison by repeated measures of analysis of variance

	df	**F**	**P-value**
A	3,1232	413.38	0.001*
B	3,596	104.58	0.001*

Table 8(c). Uterine artery pulsatility Index post-Hoc Analysis

G.W.	Group A		Group B	
	M.D.	P	M.D.	P
6-7 g.w.				
11-13 g.w.	0.2	0.42	0.17	0.48
20-22 g.w.	0.68	0.58	0.66	0.38
30-33 g.w.	1.02	0.74	0.76	0.4
11-13 g.w.				
20-22 g.w.	0.48	0.001*	0.49	0.001*
30-33 g.w.	0.82	0.001*	0.59	0.001*
20-22 g.w.				
30-33 g.w.	0.34	0.001*	0.1	0.054

In Table 8(a), the average arithmetic mean of UtA-PI (uterine artery pulsatility index) for women in the clinical group (Group A) is 2.90 ± 0.52 at 6-7 weeks of gestation, whereas in the control group (Group B), the mean is 2.18 ± 0.49. Between 11-13 weeks of gestation, UtA-PI shows a decrease in values for Group A, m = 2.70 ± 0.42, and a decreasing trend is also observed in Group B, 2.01 ± 0.48. The arithmetic mean of UtA-PI is 2.22 ± 0.58 in the clinical group during 20-22 weeks of gestation, while in the control group, it is reduced to 1.52 ± 0.38 during the same gestational weeks. At 30-33 weeks of gestation, the mean UtA-PI is 1.88 ± 0.74 in the experimental group and

1.42 ± 0.40 in the control group. In conclusion, we can state that women in the clinical group show higher average values of UtA-PI throughout all gestational weeks of measurement compared to women in the control group.

Table 8(b) presents the results of the repeated measures ANOVA of UtA-PI for the control and clinical groups across different measurement periods as shown in Table 5(a). The model tests the within-group temporal differences in UtA-PI for the two studied groups concerning different gestational weeks of index measurement. According to the results in the table, there is a statistically significant difference at 95% confidence level between the means in both groups across different measurement periods, $F(3,1232) = 413.38$, $p = 0.001 < 0.05$ for Group A and $F(3,596) = 104.58$, $p = 0.001 < 0.05$ for Group B. We can assert that in 95% of cases, UtA-PI changes in both groups of women across different gestational weeks of pregnancy.

Table 8(c) contains the results from the post-hoc Tukey test, which evaluates the statistical significance of differences in the means between various pairs of gestational periods for both studied groups of women. According to the results, there is a statistically significant difference in UtA-PI between the gestational weeks 11-13 and 20-22, as well as between 11-13 and 30-33, with $p < 0.05$ in the experimental group. Additionally, there is a statistically significant difference in the arithmetic means of UtA-PI between the gestational weeks 20-22 and 30-33 in the clinical group.

In the control group, statistically significant differences are observed between the arithmetic means of UtA-PI between the gestational weeks 11-13 and 20-22, as well as between 11-13 and 30-33 weeks.

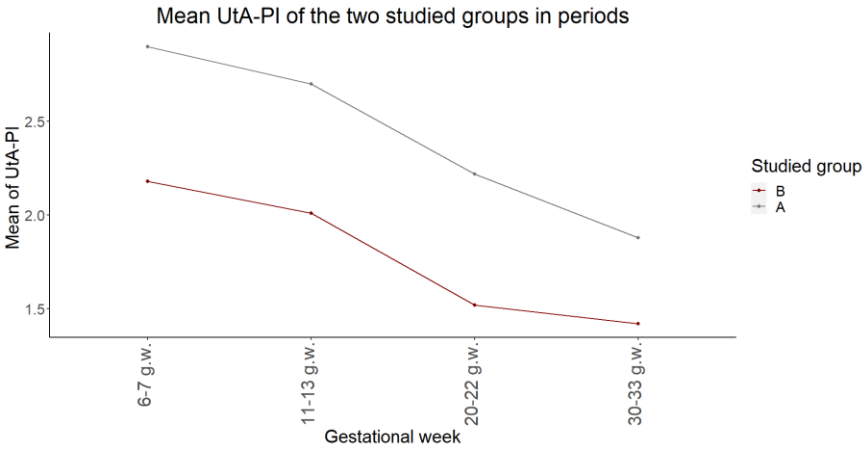

Figure 3. Mean UtA-PI of the studied groups in periods

Figure above shows a significant decrease in the average UtA-PI in both groups of women between the periods of 6-7 weeks and 30-33 weeks of gestation, with a more pronounced drop observed between the periods of 11-13 weeks and 20-22 weeks. This finding supports the results of the ANOVA analysis. It is also evident that in group A (the clinical group), the UtA-PI values are higher at each period compared to the control group, group B.

Dynamics of Hematological Parameters and Hemostasis during Pregnancy and the Postpartum Period – a 4-5 week interval after 12 weeks of gestation in both clinical and control groups

Table 9 presents the data on hemoglobin levels in both groups of women, along with the results of the ANOVA for differences between the testing periods given at different gestational weeks and the levels of hemoglobin in the two studied groups.

Table 9(a) contains the arithmetic means and standard deviations of hemoglobin levels at various gestational weeks for the experimental and control groups.

Table 9(b) presents the results from the repeated measures ANOVA on hemoglobin levels in the two studied groups of women relative to the different measurement periods. The results are supported by the post-hoc Tukey test for pairwise comparisons between different periods in the two groups, as presented in Table 9(c).

Table 9(a). Hemoglobin Comparison at different study times in pregnancy within group analysis

Hb g/l	**Group A**		**Group B**	
	M	**S.D.**	**M**	**S.D.**
10-12 g.w.	135.48	6.46	131.69	4.83
16-19 g.w.	110.35	1.87	113.43	3.48
20-22 g.w.	100.31	2.23	100.49	2.37
24-26 g.w.	103.20	2.77	103.65	2.92
30-32 g.w.	104.18	4.89	104.43	4.86
34-36 g.w.	105.76	3.40	106.05	3.42

Table 9(b). Hemoglobin within group analysis – repeated measures of ANOVA

	df	**F**	**P**
A	5,1842	3362.74	0.001*
B	5,894	1395.69	0.001*

Table 9(c). Hemoglobin comparison post-Hoc Analysis

	A		B	
	M.D.	**P**	**M.D.**	**P**
10-12 weeks				
16-19 weeks	25.13	0.001*	18.12	0.001*
20-22 weeks	35.17	0.001*	31.12	0.002*
24-26 weeks	32.28	0.001*	28.02	0.001*
30-32 weeks	31.30	0.001*	27.26	0.001*
34-36 weeks	29.71	0.001*	25.64	0.001*
16-19 weeks				
20-22 weeks	10.04	0.001*	13.00	0.001*
24-26 weeks	7.15	0.001*	9.90	0.001*
30-32 weeks	6.17	0.001*	9.14	0.001*
34-36 weeks	4.58	0.001*	7.52	0.001*
20-22 weeks				
24-26 weeks	2.89	0.001*	3.10	0.001*
30-32 weeks	3.87	0.001*	3.87	0.001*
34-36 weeks	5.45	0.001*	5.48	0.001*
24-26 weeks				
30-32 weeks	0.98	0.004*	0.76	0.051
34-36 weeks	2.56	0.001*	2.38	0.001*
30-32 weeks				
34-36 weeks	1.58	0.001*	1.62	0.001*

According to Table 9(a), the highest hemoglobin levels, accompanied by the greatest variation, are observed at 10-12 weeks of gestation in both groups, with Group A showing m=135.48±6.46m and Group B showing m=131.69±4.83m.

The average hemoglobin is the lowest in both groups at 20-22 weeks: Group A m=100.31±2.23 and Group B m=100.49±2.37. At 34-36 weeks of gestation, the average hemoglobin is 105.76±3.40 for Group A and 106.05±3.42 for Group B.

Therefore, it should be noted that there is a decrease in hemoglobin levels from 10-12 to 20-22 weeks of gestation in both groups, followed by an increase in weeks 24-36. Both groups of women show similar hemoglobin levels and similar standard deviations, with an average standard deviation of 3.6 for all periods.

Table 9(b) shows that there is a statistically significant difference between the various periods for hemoglobin levels in both studied groups: Group A - $F = (5, 1842) = 3362.74$, $p = 0.001 < 0.05$**;** Group B - $F= (5,894) = 1395.69$, $p = 0.001 < 0.05$**..**

According to the post-hoc Tukey test presented in Table 9(c), there are statistically significant differences in hemoglobin levels between all pairs of periods in both groups (p-value < 0.05). However, in the control group, there is no statistically significant difference with 95% confidence between hemoglobin levels in gestational weeks 24-26 and 30-32.

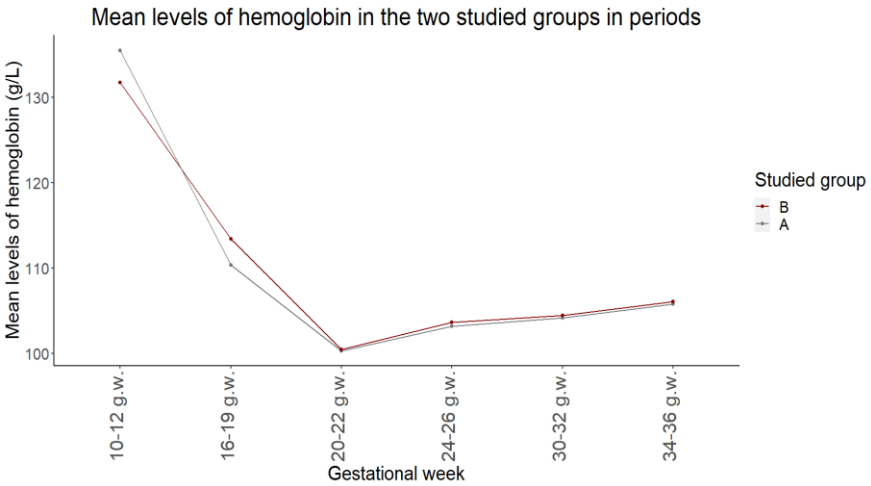

Figure. 4 Mean levels of hemoglobin in two studied groups in paeriod

Table 10 shows, in a similar manner, the descriptive statistics for red blood cells across different gestational weeks for pregnant women in both the clinical and control groups, as well as the conducted analysis of variance and post-hoc Tukey test for pairwise comparison across periods.

Table 10(a). Hemoglobin Comparison at different study times in pregnancy within group analysis

RBC (mCL)	Group A		Group B	
	M	S.D.	M	S.D.
10-12 g.w.	3.79	0.29	4.06	0.35
16-19 g.w.	3.29	0.17	3.40	0.15
20-22 g.w.	3.14	0.17	3.27	0.14
24-26 g.w.	3.25	0.15	3.04	0.13
30-32 g.w.	3.48	0.24	3.44	0.13
34-36 g.w.	3.41	0.12	3.36	0.24

Table 10(b). Hemoglobin within group analysis – repeated measures of ANOVA

	df	**F**	**P**
A	5,1842	400.45	0.001*
B	5,894	412.86	0.001*

Table 10(c). Hemoglobin comparison post-Hoc Analysis

G.W.	**A**		**B**	
	M.D.	**P**	**M.D.**	**P**
10-12 g.w.				
16-19 g.w.	0.50	0.001*	0.66	0.001*
20-22 g.w.	0.64	0.001*	0.77	0.001*
24-26 g.w.	0.54	0.001*	1.02	0.001*
30-32 g.w.	0.31	0.001*	0.62	0.001*
34-36 g.w.	0.37	0.001*	0.70	0.001*
16-19 g.w.				
20-22 g.w.	0.14	0.001*	0.13	0.001*
24-26 g.w.	0.04	0.001*	0.36	0.001*
30-32 g.w.	0.19	0.001*	0.04	0.069*
34-36 g.w.	0.13	0.001*	0.03	0.138*
20-22 g.w.				
24-26 g.w.	0.10	0.001*	0.23	0.001*
30-32 g.w.	0.33	0.001*	0.17	0.001*
34-36 g.w.	0.27	0.001*	0.09	0.001*

24-26 g.w.				
30-32 g.w.	0.23	0.001*	0.40	0.021*
34-36 g.w.	0.16	0.001*	0.32	0.001*
30-32 g.w.				
34-36 g.w.	0.06	0.009*	0.32	0.001*

The results from Table 10(a) show that the mean red blood cell (RBC) count is highest during the 10-12 weeks of gestation for both groups: Group A has M = 3.79 ± 0.29 mCL, and Group B has M = 4.06 ± 0.35 mCL. Additionally, the RBC level is lowest during the 20-22 weeks of gestation for Group A, with M = 3.14 ± 0.17 mCL, and during the 24-26 weeks for Group B, with M = 3.04 ± 0.13 mCL. During the 34-36 weeks of gestation, the mean RBC level is 3.41 ± 0.12 mCL for Group A and 3.36 ± 0.24 mCL for Group B.

There is a statistically significant difference in RBC counts across different measurement periods for pregnant women in both groups: Group A has $F(5,1842) = 400.45$, $p = 0.001 < 0.05$, and Group B has $F(5,894) = 412.86$, $p = 0.001 < 0.05$. The post-hoc Tukey analysis also shows that there are statistically significant differences between all period pairs, observed in both groups of women studied.

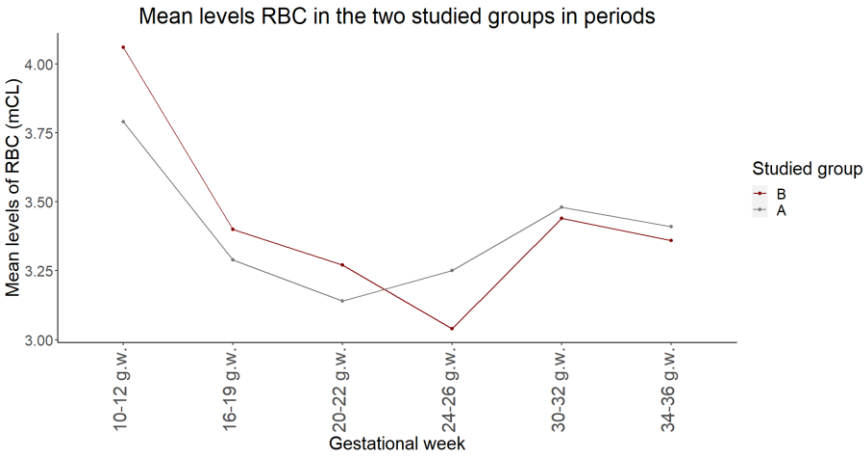

Figure 5. Mean levels RBC in the two studied groups in periods

The figure above illustrates the results from Table 10(a), showing that during the 10-12 weeks of gestation, the mean red blood cell (RBC) count is highest in both groups, with slightly higher values in Group B. A trend of slightly higher RBC levels in Group B is observed up to the 20-22 weeks of gestation, after which the trend reverses from the 24th week onwards.

Table 11(a). Hemotocrit comparison at different study times in pregnancy with group analysis

HCT L/L	A		B	
	M	S.D.	M	S.D.
10-12 g.w.	0.40	0.03	0.39	0.04
16-19 g.w.	0.35	0.02	0.34	0.02
20-22 g.w.	0.29	0.01	0.30	0.01
24-26 g.w.	0.31	0.01	0.32	0.01
30-32 g.w.	0.28	0.01	0.29	0.06
34-36 g.w.	0.31	0.01	0.32	0.02

Table 11(b). Hemotocrit within group analysis – repeated measures ANOVA

	df	**F**	**P**
A	5,1842	1388.99	0.001*
B	5,894	419.30	0.001*

Table 11(c). Hemotocrit comparison Post-Hoc analysis

G.W.	**A**		**B**	
	M.D.	**P**	**M.D.**	**P**
10-12 g.w.				
16-19 g.w.	0.05	0.001*	0.05	0.001*
20-22 g.w.	0.11	0.001*	0.09	0.001*
24-26 g.w.	0.09	0.001*	0.07	0.001*
30-32 g.w.	0.12	0.001*	0.09	0.001*
34-36 g.w.	0.09	0.001*	0.07	0.001*
16-19 g.w.				
20-22 g.w.	0.06	0.001*	0.04	0.001*
24-26 g.w.	0.04	0.001*	0.02	0.001*
30-32 g.w.	0.07	0.001*	0.05	0.001*
34-36 g.w.	0.04	0.001*	0.02	0.001*
20-22 g.w.				
24-26 g.w.	-0.02	0.001*	0.02	0.001*
30-32 g.w.	0.01	0.001*	0	0.242

34-36 g.w.	-0.02	0.001*	0.02	0.001*
24-26 g.w.				
30-32 g.w.	0.03	0.001*	0.03	0.021*
34-36 g.w.	0	0.154	0	0.824
30-32 g.w.				
34-36	-0.03	0.001*	0.03	0.001*

Table 11(a) shows that the average hematocrit levels are highest during the 10-12 weeks of gestation for both groups, with M=0.40±0.03 for Group A and M=0.39±0.04 for Group B. The lowest average hematocrit levels are observed at 30-32 weeks of gestation, with M=0.28±0.01 for Group A and M=0.29±0.001 for Group B. Hematocrit levels rise again at 34-36 weeks of gestation, with averages of 0.31±0.01 for Group A and 0.32±0.02 for Group B.

The repeated dispersion analysis presented in Table 11(b) indicates a statistically significant difference in average hematocrit levels between different periods for both groups, with $F(5,1842) = 1388.99$, $p = 0.001 < 0.05$ for Group A and $F(5,894) = 419.30$, $p = 0.01 < 0.05$ for Group B. The post-hoc Tukey test reveals statistically significant differences between all pairs of periods except between 24-26 weeks and 34-36 weeks, where the average difference between the two groups is zero, indicating no difference in hematocrit levels between these two periods for both groups.

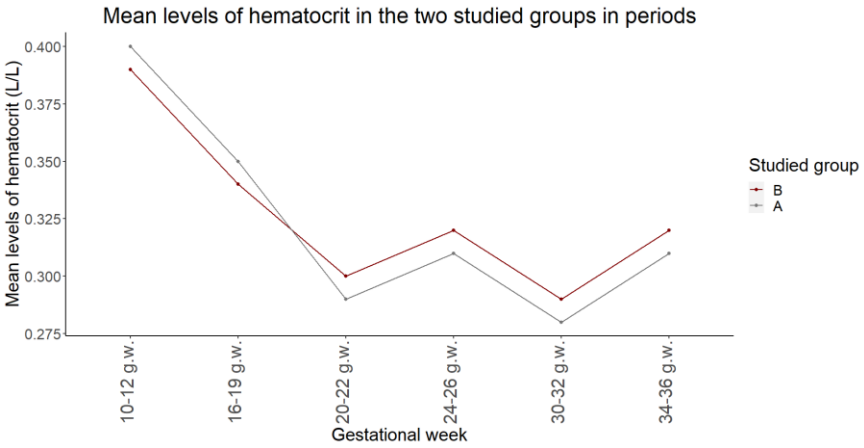

Figure 6. Mean levels of hematocrit in two studied groups in period

Tables 12 present the mean values and their deviations of leukocyte levels by periods for the two studied groups—12(a), the analysis of variance with repeated measurements of leukocyte levels in different periods for the two groups—12(b), and the post-hoc Tukey test for pairwise comparisons of leukocyte levels between different periods—12(c).

Table 12(a). Leukocyte comparison at different study times in pregnancy with group analysis

Leukocyte (μ/L)	A		B	
	M	S.D.	M	S.D.
10-12 weeks	9.02	1.29	7.80	1.48
16-19 weeks	110.70	1.21	7.30	0.86
20-22 weeks	11.62	1.24	8.47	0.54
24-26 weeks	12.26	0.91	10.52	9.06
30-32 weeks	11.17	1.71	9.51	1.96
34-36 weeks	11.96	1.70	11.88	1.82

Table 12(b). Leukocyte within group analysis – repeated measures ANOVA

	df	F	P
A	5,1842	212.62	0.001*
B	5,894	253.62	0.001*

Table 12(c). Leukocyte comparison Post-Hoc analysis

Leukocyte	A		B	
	M.D.	P	M.D.	P
10-12 weeks				
16-19 weeks	1.67	0.001*	0.51	0.001*
20-22 weeks	2.60	0.001*	0.66	0.001*
24-26 weeks	3.24	0.001*	2.71	0.001*
30-32 weeks	2.14	0.001*	1.81	0.001*
34-36 weeks	2.86	0.001*	4.16	0.001*
16-19 weeks				
20-22 weeks	0.93	0.001*	1.17	0.001*
24-26 weeks	1.57	0.001*	3.22	0.001*
30-32 weeks	0.48	0.001*	2.32	0.001*
34-36 weeks	0.19	0.001*	4.67	0.001*
20-22 weeks				
24-26 weeks	0.64	0.001*	2.05	0.001*
30-32 weeks	0.46	0.001*	1.15	0.001*

34-36 weeks	0.25	0.023*	3.50	0.001*
24-26 weeks				
30-32 weeks	1.09	0.001*	0.90	0.002*
34-36 weeks	0.38	0.002*	1.45	0.001*
30-32 weeks				
34-36 weeks	0.71	0.001*	2.35	0.001*

According to the results from Table 12(a)**,** the average leukocyte level is highest at 24-26 weeks of gestation in women from Group A, with M=12.26±0.91 and highest at 34-36 weeks in women from Group B, with M=11.88±1.82. The average leukocyte level is lowest at 10-12 weeks in the clinical group (Group A) with M=9.02±1.29, and at 16-19 weeks in the control group (Group B) with M=7.30±0.86. At 34-36 weeks, the average leukocyte level is 11.96±1.70 in Group A and peaks, as previously mentioned, in women from Group B (M=11.88±1.82). Leukocyte levels are notably higher in Group A compared to Group B across all periods, with convergence from 24-26 weeks onwards due to a spike in leukocytes in Group B.

The descriptive analysis conducted between groups and periods for different leukocyte levels in the blood of the studied women confirms a statistically significant difference between different periods in leukocyte levels for both groups ($F(5, 1842) = 212.62$, $p=0.001<0.05p$ for Group A and $F(5, 894) = 253.62$, $p=0.001<0.05p$ for Group B). The post-hoc Tukey analysis confirms the results of the ANOVA, showing that there are statistically significant differences in leukocyte levels between all pairs of periods in both groups of studied women. The greatest difference in average leukocyte levels is observed between 10-12 weeks and 24-26 weeks in Group A, and between 16-19 weeks and 30-36 weeks in Group B.

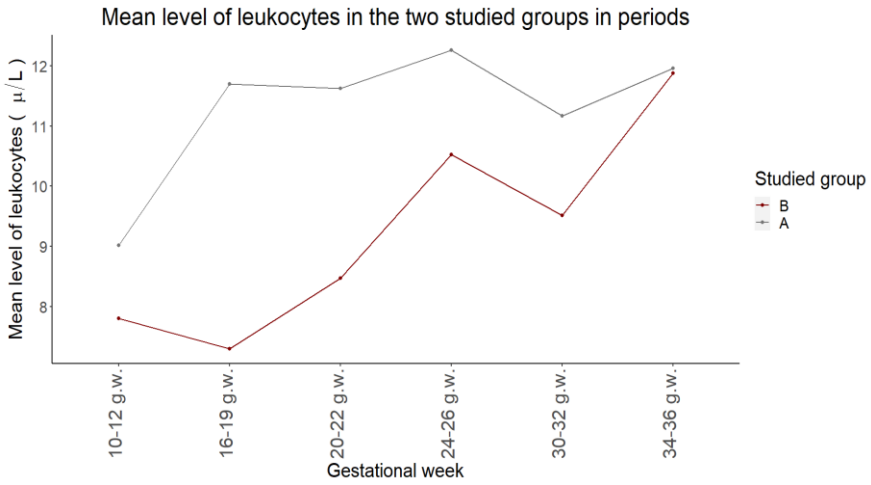

Figure 7. Mean level of leukocytes in two studied groups in period

The results from Table 13(a) show that the highest level of platelets is observed during 10-12 weeks of gestation in both groups: M = 444.52 ± 22.94 mcL in Group A and M = 407.68 ± 33.92 mcL in Group B. The observed average platelet levels are lowest during 34-36 weeks of gestation in Group A (M = 155.65 ± 15.74 mcL) and during 24-26 weeks of gestation in Group B (M = 228.20 ± 41.35 mcL). In Group A, there is a decreasing trend in platelet levels as pregnancy progresses. A similar trend is observed in Group B, but there is a slight increase in platelet levels during 30-32 weeks, followed by a decline in the later gestational weeks.

The results from Table 13(b) indicate that the repeated measures analysis of variance shows a statistically significant difference in the average platelet levels across different measurement periods in both groups (Group A: $F(5,1842) = 8678.33$, $p = 0.001 < 0.05$; Group B: $F(5,894) = 265.18$, $p = 0.001 < 0.05$).

Table 13(c) presents the Tukey post-hoc test, which confirms the statistically significant differences in platelet levels across the different periods for both studied groups. The greatest difference in mean platelet values in Group A is observed between 10-12 weeks and 34-36 weeks of gestation, and in Group B between 10-12 weeks and 24-26 weeks of gestation.

Table 13(a). Platelet count comparison at different study times in pregnancy with group analysis

Platelet count	**A**		**B**	
	M	**S.D.**	**M**	**S.D.**
10-12 weeks	444.52	22.94	407.68	33.92
16-19 weeks	297.25	12.02	328.78	39.49
20-22 weeks	281.26	44.42	280.58	48.55
24-26 weeks	155.65	15.74	228.20	41.35
30-32 weeks	128.07	18.08	263.74	43.32
34-36 weeks	118.05	15.92	240.44	81.01

Table 13(b). Platelet count within group analysis – repeated measures ANOVA

	df	**F**	**P**
A	5,1842	8678.33	0.001*
B	5,894	265.18	0.001*

Table 13(c). Platelet count comparison Post-Hoc analysis

Platelet count (mCL)	A		B	
	M.D.	P	M.D.	P
10-12 weeks				
16-19 weeks	147.27	0.001*	78.90	0.001*
20-22 weeks	163.25	0.001*	127.10	0.001*
24-26 weeks	288.86	0.001*	179.48	0.001*
30-32 weeks	316.45	0.001*	143.94	0.001*
34-36 weeks	326.46	0.001*	167.24	0.001*
16-19 weeks				
20-22 weeks	15.98	0.001*	48.20	0.001*
24-26 weeks	141.59	0.001*	100.58	0.001*
30-32 weeks	169.17	0.001*	65.04	0.001*
34-36 weeks	179.19	0.001*	88.34	0.001*
20-22 weeks				
24-26 weeks	125.60	0.001*	52.38	0.001*
30-32 weeks	153.19	0.001*	16.84	0.004*
34-36 weeks	163.20	0.001*	40.14	0.001*
24-26 weeks				

30-32 weeks	27.58	0.001*	35.54	0.007*
34-36 weeks	37.59	0.001*	12.24	0.035*
30-32 weeks				
34-36 weeks	10.62	0.001*	23.30	0.001*

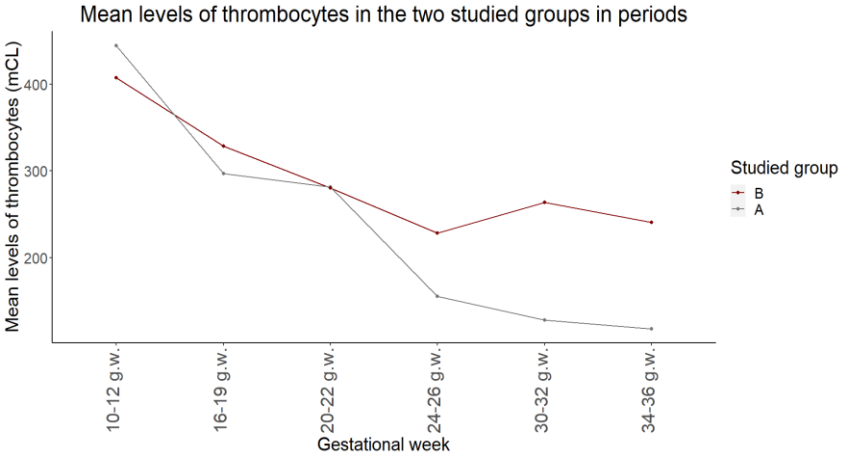

Figure 8. Mean level of Thrombocytes (Platelets) in two studied groups in periods

From the figure above, it is clearly observed that the average platelet levels in the clinical Group A during the initial measurement period (10-12 weeks of gestation) are higher than those in the control Group B. After this point, the average platelet levels in the experimental group drop below those of the control group and remain lower throughout the rest of the studied periods.

Tables 14 present the statistical analysis conducted on the fibrinogen levels in the two groups of women studied.

Table 14(a). Fibrinogen comparison at different study times in pregnancy with group analysis

Fibrinogen (mg/dL)	**A**		**B**	
	M	**S.D.**	**M**	**S.D.**
10-12 weeks	3.71	0.29	3.67	0.29
16-19 weeks	4.18	0.42	4.07	0.42
20-22 weeks	4.78	0.52	4.72	0.53
24-26 weeks	4.95	0.48	4.88	0.49
30-32 weeks	5.51	0.38	5.41	0.29
34-36 weeks	5.99	0.44	5.71	0.33

Table 14(b). Fibrinogen within group analysis – repeated measures ANOVA

	df	**F**	**P**
A	5,1842	1167.68	0.001*
B	5,894	556.49	0.001*

Table 14(c). Fibrinogen comparison Post-Hoc analysis

Fibrinogen	A		B	
	M.D.	P	M.D.	P
10-12 weeks				
16-19 weeks	0.47	0.001*	0.41	0.001*
20-22 weeks	1.07	0.001*	1.05	0.001*
24-26 weeks	1.24	0.001*	1.21	0.001*
30-32 weeks	1.80	0.001*	1.74	0.001*
34-36 weeks	2.28	0.001*	2.07	0.001*
16-19 weeks				
20-22 weeks	0.59	0.001*	0.64	0.002*
24-26 weeks	0.76	0.001*	0.80	0.001*
30-32 weeks	1.33	0.001*	1.33	0.001*
34-36 weeks	1.81	0.001*	1.66	0.001*
20-22 weeks				
24-26 weeks	0.17	0.001*	0.16	0.001*
30-32 weeks	0.73	0.001*	0.69	0.001*
34-36 weeks	1.21	0.001*	1.02	0.001*
24-26 weeks				
30-32 weeks	0.56	0.001*	0.53	0.001*
34-36 weeks	1.04	0.001*	0.86	0.001*
30-32 weeks				
34-36 weeks	0.48	0.001*	0.33	0.001*

According to the results from Table 14(a), the average fibrinogen levels are lowest during the 10-12 weeks of gestation in both groups—M = 3.71 ± 0.29 mg/dL for Group A and M = 3.67 ± 0.29 mg/dL for Group B. The observed trend is an increase in fibrinogen levels as the gestational week's progress in both groups, with a peak measured during the 34-36 weeks of gestation—M = 5.99 ± 0.44 mg/dL in Group A and M = 5.71 ± 0.33 mg/dL in Group B. In all measurements, women in Group A show higher average fibrinogen levels compared to those in Group B, with the difference being most significant during the final measurement period (34-36 weeks) and almost negligible during the 10-12 weeks.

The repeated measures analysis of variance, presented in Table 14(b), reveals a statistically significant difference in fibrinogen levels across the different measurement periods for both groups ($F(5,1842) = 1167.68$, $p = 0.001 < 0.05$ for Group A and $F(5,894) = 556.49$, $p = 0.001 < 0.05$ for Group B). The subsequent Tukey post-hoc test shown in Table 14(c) confirms statistically significant differences between all pairs of studied periods in fibrinogen levels, with the greatest absolute difference in mean fibrinogen levels observed between 10-12 weeks and 34-36 weeks, which supports the increasing trend throughout the pregnancy in both groups.

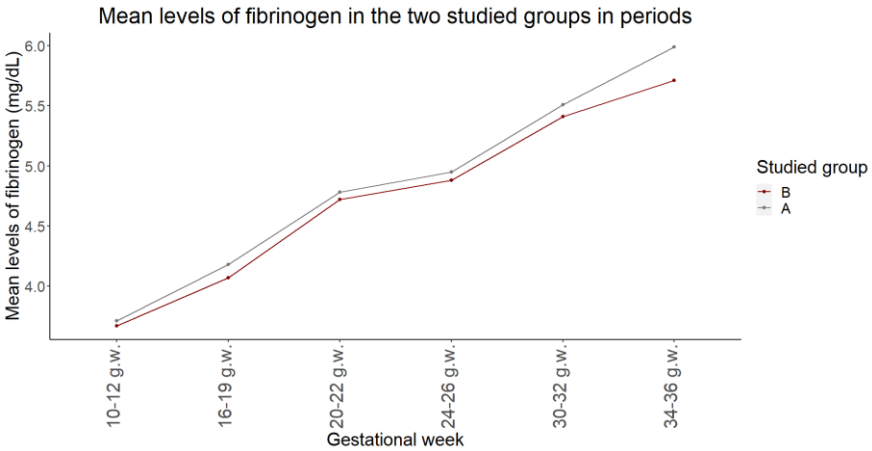

Figure 9. Mean levels of fibrinogen in two studied groups in periods

Tables 15 present the descriptive statistics for partial thromboplastin time (PTT) across different periods of study in pregnant women within the experimental and control groups—Table 15(a), the repeated measures analysis of variance—Table 15(b), and the Tukey post-hoc test for pairwise comparison of PTT across different periods—Table 15(c).

Table 15(a). Partial Thromboplastic Time Comparison at different study times in pregnancy with group analysis

PTT (per seconds)	A		B	
	M	S.D.	M	S.D.
10-12 weeks	23.27	0.78	24.28	0.63
16-19 weeks	23.95	0.49	24.89	0.14
20-22 weeks	24.51	0.67	25.10	0.07
24-26 weeks	23.00	0.22	24.39	0.47
30-32 weeks	24.69	0.34	26.04	1.14

34-36 weeks	23.53	0.76	25.23	0.79

Table 15(b). Partial thromboplastic time within group analysis – repeated measures ANOVA

	df	**F**	**P**
A	5,1842	383.85	0.001*
B	5,894	167.83	0.001*

Table 15(c). Partial thrombo plastic time comparison post Hoc Analysis

Fibrinogen	**A**		**B**	
	M.D.	**P**	**M.D.**	**P**
10-12 weeks				
16-19 weeks	0.68	0.001*	0.61	0.001*
20-22 weeks	1.23	0.001*	0.82	0.001*
24-26 weeks	0.24	0.001*	0.11	0.001*
30-32 weeks	1.40	0.001*	1.76	0.001*
34-36 weeks	0.27	0.001*	0.95	0.001*
16-19 weeks				
20-22 weeks	0.55	0.001*	0.20	0.003*
24-26 weeks	0.92	0.001*	0.50	0.001*
30-32 weeks	0.72	0.001*	1.15	0.001*
34-36 weeks	0.41	0.001*	0.34	0.001*

20-22 weeks				
24-26 weeks	1.47	0.001*	0.70	0.001*
30-32 weeks	0.16	0.001*	0.94	0.001*
34-36 weeks	0.96	0.001*	0.13	0.054*
24-26 weeks				
30-32 weeks	1.64	0.001*	1.65	0.001*
34-36 weeks	0.51	0.001*	0.84	0.001*
30-32 weeks				
34-36 weeks	1.13	0.001*	0.81	0.001*

The average values of partial thromboplastin time (PTT) are observed during 30-32 weeks of gestation in both groups: M = 24.69 ± 0.34 for Group A and M = 26.04 ± 1.14 for Group B. The lowest average PTT values, measured in seconds, are recorded during 10-12 weeks of gestation in Group B (M = 24.28 ± 0.63) and during 24-26 weeks of gestation in Group A (M = 23.00 ± 0.22). During 34-36 weeks, the average PTT values are 23.53 ± 0.76 in Group A and 25.23 ± 0.79 in Group B, with both groups showing a decrease compared to the 30-32 weeks period. Across all measurement periods, the average PTT values are higher in Group B compared to Group A.

According to the results from the repeated measures analysis of variance presented in Table 15(b), there is a statistically significant difference in the average PTT values across different measurement periods in both groups of women ($F(5,1842) = 383.85$, $p = 0.001 < 0.05$ for Group A and $F(5,894) = 167.83$, $p = 0.001 < 0.05$ for Group B). The subsequent Tukey post-hoc test confirms the presence of statistically significant differences in the average PTT values across all pairwise comparisons of periods in both groups. The largest differences in average PTT

values in Group A are observed between 24-26 weeks and 30-32 weeks, and in Group B between 10-12 weeks and 30-32 weeks.

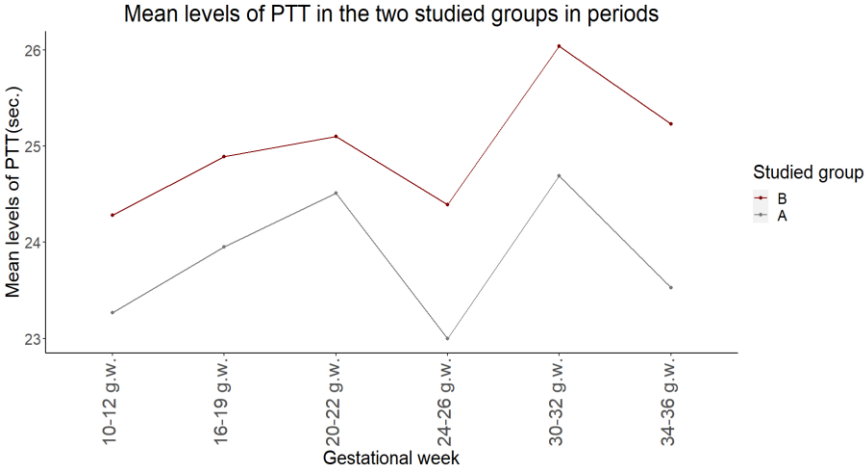

Figure 10. Mean levels of aPTT in two studied groups in periods

Table 16(a) D-Dimer comparison at different study times in pregnancy with group analysis

D-Dimer (mg/L)	A		B	
	M	**S.D.**	**M**	**S.D.**
10-12 weeks	0.38	0.07	0.15	0.05
16-19 weeks	0.68	0.07	0.38	0.11
20-22 weeks	0.78	0.07	0.43	0.12

24-26 weeks	1.41	0.17	0.90	1.14
30-32 weeks	1.90	0.26	0.94	0.24
34-36 weeks	2.28	0.54	1.12	0.29

Table 16(b). D-Dimer within group analysis repeated measures ANOVA

	Df	**F**	**P**
A	5,1842	2576.69	0.001*
B	5,894	640.42	0.001*

Table 16(c). Fibrinogen comparison Post-Hoc analysis

Fibrinogen	**A**		**B**	
	M.D.	**P**	**M.D.**	**P**
10-12 weeks				
16-19 weeks	0.30	0.001*	0.22	0.001*
20-22 weeks	0.41	0.001*	0.33	0.001*
24-26 weeks	1.03	0.001*	0.75	0.001*
30-32 weeks	1.52	0.001*	0.79	0.001*
34-36 weeks	1.91	0.001*	0.97	0.001*
16-19 weeks				

20-22 weeks	0.11	0.001*	0.10	0.001*
24-26 weeks	0.73	0.001*	0.53	0.001*
30-32 weeks	1.22	0.001*	0.56	0.001*
34-36 weeks	1.61	0.001*	0.74	0.001*
20-22 weeks				
24-26 weeks	0.62	0.001*	0.42	0.001*
30-32 weeks	1.11	0.001*	0.46	0.001*
34-36 weeks	1.50	0.001*	0.64	0.001*
24-26 weeks				
30-32 weeks	0.48	0.001*	0.04	0.068*
34-36 weeks	0.88	0.001*	0.21	0.001*
30-32 weeks				
34-36 weeks	0.38	0.001*	0.18	0.001*

The results from Table 16(a) show a consistently increasing trend in D-Dimer levels in both groups. The highest levels of D-Dimer are observed during 34-36 weeks of gestation in both groups: M = 2.28 ± 0.54 mg/L in Group A and M = 1.12 ± 0.29 mg/L in Group B. The lowest average D-Dimer levels are recorded during 10-12 weeks of gestation in both groups—M = 0.38 ± 0.07 mg/L in Group A and M = 0.15 ± 0.05 mg/L in Group B. Additionally, the average D-Dimer levels are approximately twice as high in Group A compared to Group B across all gestational weeks.

The results from Table 16(b) indicate that the repeated measures analysis of variance within each group shows a statistically significant difference in the average D-Dimer levels across different measurement periods ($F(5, 2576.69) = 5.1842$, $p = 0.001 < 0.05$ for Group A and $F(5, 640.42) = 5.894$, $p = 0.001 < 0.05$ for Group B). Table 16(c) presents the results of the Tukey post-hoc test for pairwise comparisons, which confirms statistically significant differences in D-Dimer levels across the studied periods of pregnancy in both groups. The largest difference in mean D-Dimer values in both groups is observed between 10-12 weeks and 34-36 weeks. The only period where there is no statistically significant difference at the 95% confidence level is between 24-26 weeks and 30-32 weeks in Group B, which is expected, as the average difference between these two periods is -0.04 mg/L, which is negligible.

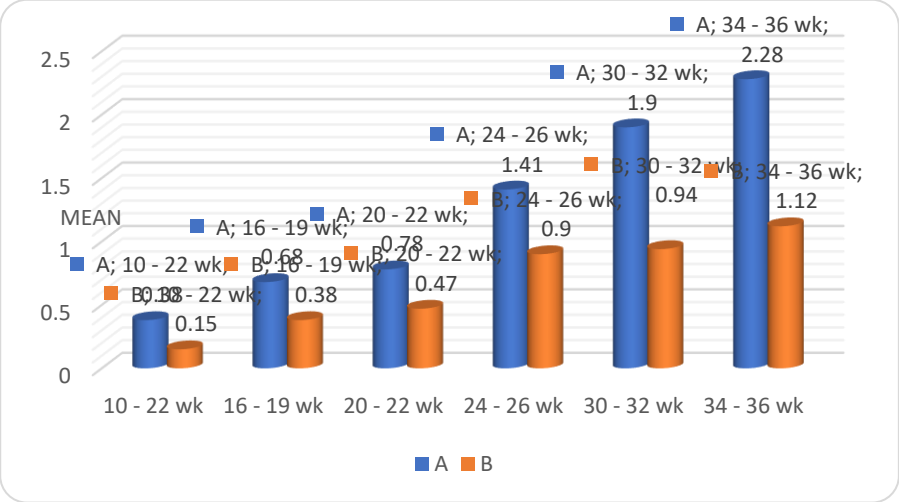

Figure11. Mean levels of D-dimer in two studied groups in periods

Table 17. Presents the dynamic changes in arterial pressure across the different patient groups. (MS. D)

Period	**10-12 g.w.**	**16-18 g.w.**	**20-23 g.w.**	**26-28 g.w.**	**30-33 g.w.**	**35-37 g.w.**	**N**
Blood pressute	**mm Hg**	**mm Hg**	**mm Hg**	**mm Hg**	**mm Hg**	**mm Hg**	
TF+ PAI I systolic diastolic	100 ±8 70±4	107 ±5 75 ±4	110 ±7 80±5	120 ±4 83±4	120 ±6 84 ±3	135 ±10 87±5	**225**
TT + PAI I + ACE (D/D, I/D) systolic diastolic	128± 5 82±3	135 ±4 90±5	145 ±7 92±4	148 ±6 95±3	155 ±8 105 ±4	163 ±5 112 ±3	**84**
Control group systolic diastolic	95±9 70 ±10	100 ±6 75 ±6	105 ±7 78±5	107± 5 80±4	112 ±3 81 ±4	130± 5 85±2	**150**

According to the results in Table 17, several trends are observed in systolic and diastolic arterial pressure among the different patient groups. Firstly, in all three studied groups, there is an increase in both systolic and diastolic blood pressure with the progression of pregnancy. The highest blood pressure values are recorded during 35-37 weeks of gestation across all groups. In the control group, there is a more gradual increase in systolic and diastolic pressure compared to the group with thrombophilic factors + PAI I, which shows higher values than the control group at all examined periods for both systolic and diastolic arterial pressure. The highest values for both diastolic and systolic blood pressure across all studied periods are observed in the group with thrombophilic factors, PAI I, and ACE DD,I/D. Notably, after 26-28 weeks of gestation, the difference in diastolic arterial pressure between this group and the other two groups increases. It is also important to note that the differences in systolic and diastolic blood pressure between the control group and the group with thrombophilic factors and PAI I are not substantial, with values remaining relatively close, especially for diastolic arterial pressure.

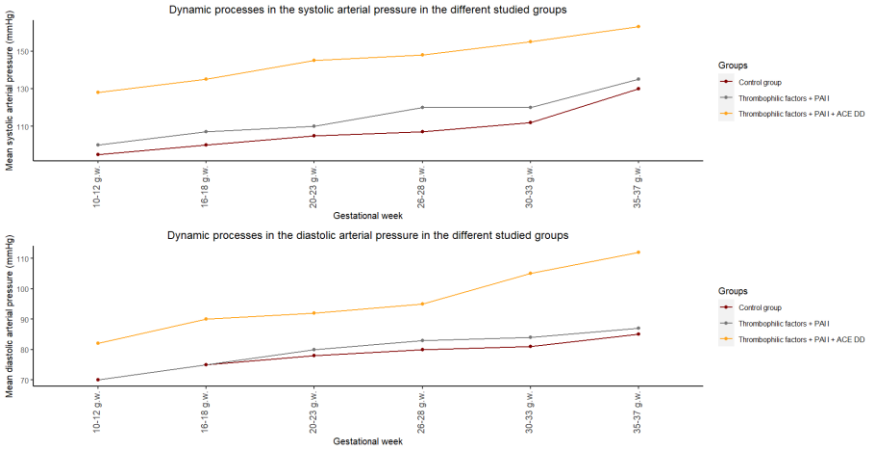

Figure 12 and 13. Dynamic changes in the systolic and diastolic arterial pressure in diferent studied groups

Table 18. Distribution of pregnant women in the three studied groups based on their method of delivery.

Method of delivery	**TF + PAI I + ACX I/I**		**TF + PAI I + ACE D/D, I/I**		**Control goup**	
	n=216	%	n=71	%	n=143	%
Vaginal birth	**141**	**65.28%**	**18**	**25.35%**	**107**	**74.83%**
Spontaneous	119	84.40%	13	72.22%	99	92.52%
Induced	22	15.60%	5	27.78%	8	7.48%
Cesarean delivery	**74**	**34.26%**	**53**	**74.65%**	**36**	**25.17%**
Elective	59	79.73%	45	84.91%	12	33.33%
Urgent	15	20.27%	8	15.09%	24	66.67%

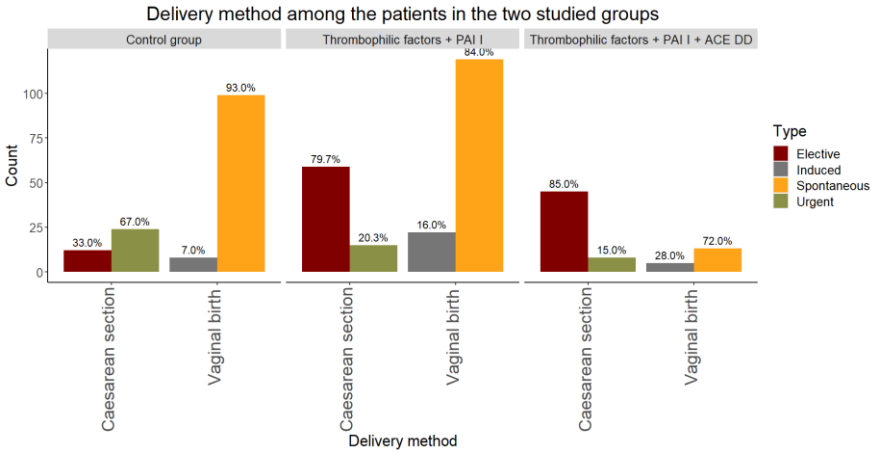

Figure 14. Delivery method amog the patients in the two studied groups

In Table 18 and the subsequent figure, it is observed that in the Control group and the group of women with thrombophilic factors and PAI I, the majority of vaginal deliveries were spontaneous (93% in the Control group and 84% in the thrombophilic factors + PAI I group). Additionally, in the thrombophilic factors + PAI I group, 65.28% of women had a normal vaginal delivery, with 16% having an induced delivery. In contrast, the Control group had 74.83% vaginal deliveries, with only 7.48% being induced.

For the group with thrombophilic gene mutations + PAI I + ACE (D/D,I/D) different trends are observed: only 25.35% of women had a vaginal delivery, with 72% being spontaneous and 28% induced, which is a higher proportion of induced deliveries compared to the other two groups.

In terms of cesarean sections, the group with thrombophilic gene mutations + PAI I + ACE (D/D,I/D) shows the highest percentage—74.65%, with 85% being elective and only 15% emergency. In the thrombophilic factors + PAI I group, 34.26%

of deliveries were cesarean sections, with 20.27% being emergency and 79.73% elective. The Control group had only 25.17% cesarean sections, with 66.67% emergency and 33.37% elective.

Table 19 presents the descriptive statistics for postpartum blood loss in the three studied groups, along with the analysis of variance for this indicator and the Tukey post-hoc test.

Table 19. Descriptive statistics for postpartum blood loss in the three studied groups

Blood loss	**TF + PAI I + ACE I/I n=216**	**TF + PAI I + ACE D/D, I/I n=71**	**Control goup n=143**
Blood loss, ml.	382.2+/-31.6*	664.28+/-73.20*	220,.3+/-12,9

Table 19(a) shows that the average postpartum blood loss differs among the groups. In the group with thrombophilic gene mutations and PAI I, the average blood loss is 382 ml with a standard deviation of 31.6 ml. In contrast, the group with thrombophilic gene mutations + PAI I + ACE (D/D, I/D) exhibits approximately twice the average blood loss, with a mean of 644.28 ml and a standard deviation of 73.20 ml. The Control group has the lowest average postpartum blood loss, with a mean of 220.31 ml and a standard deviation of 12.9 ml.

Table 19(b). One–way analysis of variance of postpartum blood loss in the three studied groups

Blood loss	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Group	2	9290706	4645353	3185.8	2.20E-16 ***
Residual component	427	622623	1458		

According to the results in Table 19(a), there is a statistically significant difference in postpartum blood loss among the three studied groups, with an F-value of 3185.8 and a p-value of 2.20E-16.

Table 19(c). Post-Hoc Tukey test results of Bloob loss in the three studied groups.

Rairs of group	***Difference in mean***	***min***	***max***	***p adj***
Group 1 - Control group	165.3429	155.661	175.025	0
Group 2 - Control group	441.6128	428.574	454.651	0
Group 2 - Group 1	276.27	263.984	288.556	0

The Tukey post-hoc test results presented in Table 19(c) confirm the findings of the analysis of variance described in Table 19(b). Statistically significant differences are observed between all pairs of groups (p-value < 0.05). Group 1 refers to the group with thrombophilic factors and PAI I + ACE I/I, while Group 2 consists of individuals with thrombophilic gene mutations + PAI I + ACE (D/D, I/D).

The largest difference in mean postpartum blood loss is observed between Group 2 and the Control group, with an additional 441.61 ml of blood loss in the group with thrombophilic gene mutations + PAI I + ACE (D/D,I/D) compared to the Control group. Additionally, the average difference in blood loss between the two thrombophilic gene mutation groups is 276.27 ml, favoring the second group.

Table 20 presents anthropometric measurements and APGAR scores of the newborns in the studied groups, along with the

analysis of variance for APGAR scores at one and five minutes after birth, and the corresponding post-hoc tests for repeated measures.

Table 20(a). Anthropometric measurements and APGAR scores of the newborns in the studied groups

Indicator	**TF + PAI I + ACE I/I n= 216**	**TF + PAI I + ACE D/D, I/D n= 71**	**Control group n= 143**
Weight of the baby, gr.	3541±320.87	**2135.8±135.3**	3451,2±122.3
Lenght, sm.	51.2±0.85	**47.6±0.41**	52.3±0.75
APGAR score first min.	7±1.5	**5±2. 4**	8 ± 0.5
Apgar score fifth min.	8±0.75	**7±1. 4**	9 ± 0.7

According to the results in Table 20(a), the group of pregnant women with thrombophilic gene mutations + PAI I + ACE (D/D, I/D) exhibits the lowest average values for fetal weight (M = 2135.8 ± 135.3 g), length (M = 47.6 ± 0.41 cm), and APGAR scores at both one minute and five minutes (M = 5 at one minute and M = 7 at five minutes).

The other two groups show similar values with minor differences in the indicators. The Control group has the highest APGAR scores at one minute (M = 8) and five minutes (M = 9), followed by the group with thrombophilic gene mutations + PAI I (M = 7 at one minute and M = 8 at five minutes).

The most significant increase in APGAR score from one minute to five minutes after birth is observed in the second studied group—thrombophilic gene mutations + PAI I + ACE (D/D, I/D). This group shows an increase in the average APGAR score from 5 to 7, with a corresponding reduction in variance, decreasing by one standard deviation (from 2.4 to 1.4).

Table 20(b): One-way analysis of variance (ANOVA) for the APGAR score between the studied groups

APGAR score first min.	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Group	2	432.8	216.4	80.696	2.20E-16***
Residual component	427	1145.1	2.682		

The one-way analysis of variance (ANOVA) for the APGAR score at one minute reveals a statistically significant difference among the three groups. This indicates that the expected APGAR scores vary between the groups of pregnant women. Table 20(c) also reports statistically significant differences for all pairwise comparisons of the APGAR score at first minute. The most notable difference in means is observed between the second group of women (thrombophilic gene mutations + PAI I + ACE D/D) and the Control group.

Table 20(c). Post-Hoc Tukey test results for the APGAR score on first minute between the studied groups couples

Paired comparation	Difference in mean	min	max	p adj
Group 1 - Control group	-0.9451	-1.3603	-0.5299	4E-07
Group 2 - control group	-3.0191	-3.5783	-2.46	0
Group 1 - Group 2	-2.074	-2.6009	-1.5471	0

The next one-way analysis of variance (ANOVA) focuses on the APGAR score at five minutes post-delivery across the three studied groups, as presented in Table 20(d).

Table 20(d). One-way analysis of variance (ANOVA) for the APGAR score on fifth minute between the studied groups couples

APGAR score fifth min.	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Group	2	185.16	92.578	111.08	2.20E-16***
Residual component	427	355.88	0.833		

The results show a statistically significant difference in the APGAR score at five minutes after birth among the three studied groups. The post-hoc Tukey test results, presented in Table

20(e), confirm the findings from Table 19(d), demonstrating statistically significant differences in the APGAR score at five minutes between all pairs of groups.

The largest difference in mean APGAR scores is again observed between the second group of women (those with thrombophilic factors + PAI I + ACE D/D, I/D) and the Control group.

Table 20(e). Post-Hoc Tukey test results for the APGAR score on fifth minute between the studied groups couples

Paired comparation	**Difference in mean**	**min**	**max**	**p adj**
Group 1-Control group	-0.9029	-1.1344	-0.6714	0
Group 2-Control group	-1.9362	-2.2479	-1.6245	0
Gropu 1-Group 2	-1.0333	-1.327	-0.7396	0

Tables 20(f) and 20(g) contain the results from the repeated dispersion analysis conducted on the APGAR scores at one minute and five minutes after birth across all groups.

According to the results in Table 20(f), there is a statistically significant difference in the average APGAR scores at one minute and at five minutes after birth across all groups. The Bonferroni test for comparison between the two time points, as presented in Table 20(f), confirms the findings from the dispersion analysis.

Table 20(f). Repeated dispersion analysis conducted on the APGAR scores at one minute and five minutes after birth across all groups

Effects	DFn	DFd	F	p p<.05	ges
Period	1	429	146.055	3.84e-29 *	0.108

Table 20(g). Bonferoni test for group for comparisons forAPGAR score in first and fith minute after birth

Dependent variable	Period 1	Period 2	n1	n2	Statis-tic	df	p	p.adj
APGAR score	First minute	Fifth minute	430	430	-12.1	429	3.84 E-29	3.84e-29 ****

In the clinical group, depending on the identified genetic factors for thrombophilia, screening for preeclampsia, and monitoring of coagulation status, after a positive pregnancy test, the appropriate anticoagulant and antiplatelet therapy was determined and administered. This included: low molecular weight heparin (LMWH) in prophylactic doses of 0.3/0.4 mg/kg and/or Aspirin 100/150 mg and/or Nataspin H. The therapy was monitored and adjusted continuously through tracking of coagulation status and Doppler examination of the pulsatility index of the uterine arteries.

Table 21. Pregnancy outcome in the examined groups of women

Pregnancy outcome	Groups					
	TF + PAI I + ACE I/I		TF + PAI I + ACE D/D,I/D		Control Group	
	n=225	%	n=84	%	n=150	%
Miscarriage <10 g.w.	9	4.00%	13	**15.48%**	0	0.00%
Stillbirth	1	0.44%	3	**3.57%**	0	0.00%
Pre-eclampsia	28	**12.44%**	51	**60.71%**	7	4.66%
IUGR	7	3.11%	32	**38.10%**	0	0.00%
Pre therm birth < 37 g.w. (spontaneous)	14	6.22%	22	**26.19%**	3	2.00%
Pre therm birth < 37 g.w. (iatrogenic)	2	0.89%	7	**8.33%**	0	0.00%
Gestational diabetes	83	**36.89%**	29	**34.52%**	12	8.00%
Macrosomic foetus	15	**6.67%**	0	**0.00%**	7	4.67%
Normal oucome of the pregnancy	81	**36.00%**	5	**5.95%**	133	**88.66%**

Table 21 presents the outcomes of pregnancy in the studied groups of women, showing that patients with mutations in the angiotensin-converting enzyme (ACE) gene, combined with a mutation in PAI-1 (4G/4G), experience significantly more adverse pregnancy outcomes compared to patients in the control group. Comparing the groups with the two different genetic variants of the angiotensin-converting enzyme—ACE I/I and ACE D/D—reveals a markedly higher prevalence of pregnancy pathology among patients with the ACE D/D,I/D genotype. The most significant difference is observed in intrauterine growth restriction, which is 12 times more common in women with the ACE D/D genotype compared to those with the normal ACE gene. Stillbirths are 7 times more frequent, and pre-eclampsia (PE) is 5 times more common. The rates of all other adverse pregnancy outcomes are also elevated. Only approximately 6% of women with the ACE D/D genotype experience normal pregnancies, compared to 88.66% in the control group.

Table22. *Mean gestational period of delivery in the three studied groups*

Gestational period	TF + PAI I + ACE I/I n= 216	TF+ PAI I + ACE D/D, I/D n= 71	Control group n= 143
Mean Gestationa period of delivery	**38.06 ± 0.67**	**35.43 ± 1.76**	**39.28 ± 0.34**

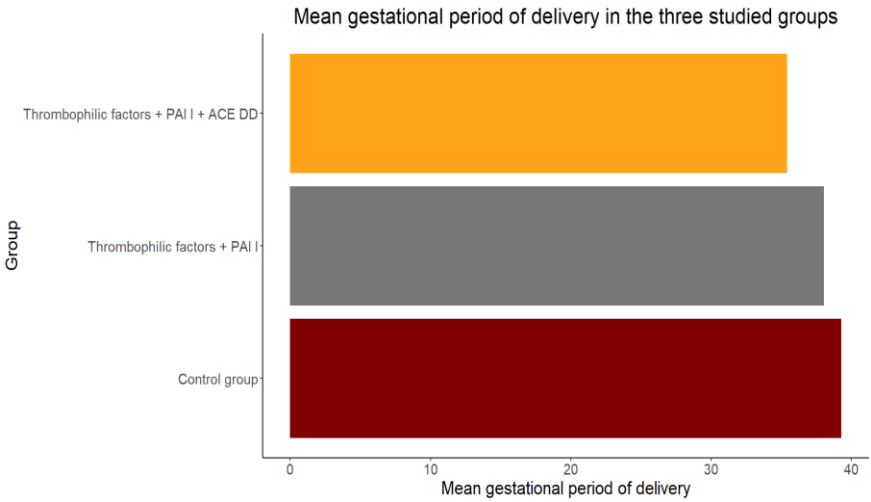

Figure 15. *Mean gestational period of delivery in the three studied groups*

From Table 22 and the subsequent figure, it can be observed that women in the group with thrombophilic factors + PAI I + ACE D/D,I/D have the lowest average gestational age at delivery (M = 35.43 ± 1.76 weeks), while the difference between the other two groups is minimal.

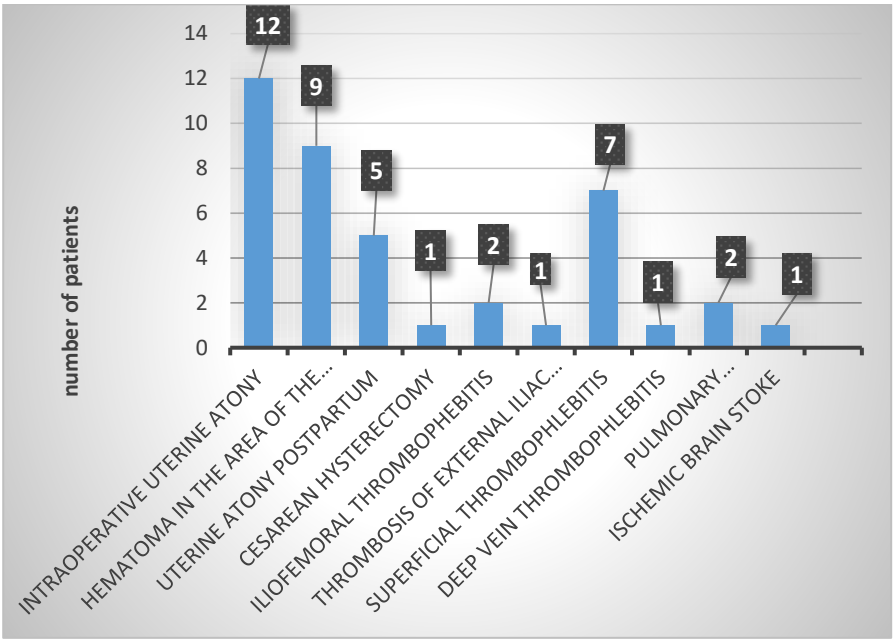

Figure. 16: *Complications during the postpartum period in the clinical group*

Figure 16 lists the complications experienced by patients in the clinical group during the postpartum period. It is evident that the most common complication is intraoperative uterine atony, observed in 12 women (3.88%), followed by hematoma at the site of the surgical wound in 9 patients (2.91%), and uterine atony after normal delivery in 5 women (1.61%). Caesarean hysterectomy was performed in 1 patient (0.32%), ileo-femoral thrombophlebitis in 2 women (0.64%), and external iliac artery thrombosis in 1 woman (0.32%). Additionally, superficial thrombophlebitis was noted in 7 women (2.26%), deep vein thrombophlebitis in 3 women (0.97%), pulmonary microthromboembolism in 2 women (0.64%), and ischemic cerebral stroke in 1 woman (0.32%).

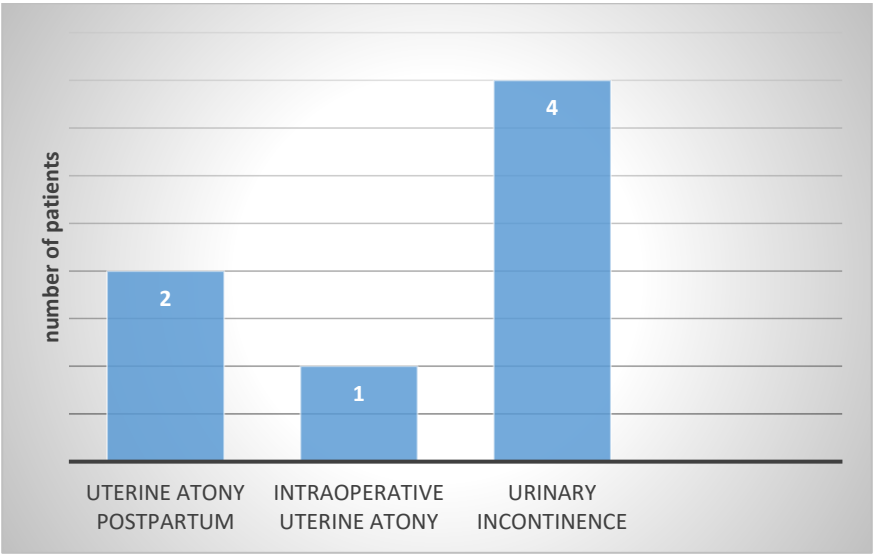

Figure. 17. *Complications during the postpartum period in the control group*

Figure 17 presents the complications observed during the puerperal period in the control group. In this group, 4 women (3.80%) experienced urinary incontinence, 2 women (1.90%) had postpartum hemorrhage, and 1 woman (0.95%) had intraoperative uterine atony. No cases of vascular incidents or hematomas at the site of the surgical scar were recorded in this group.

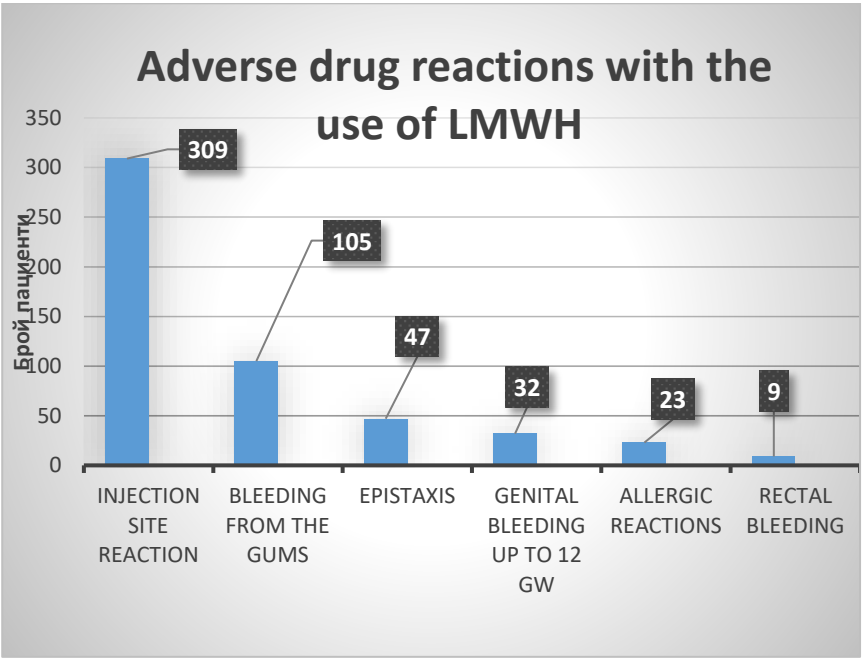

Figure. 18. *Adverse drug reactions with the use of LMWH*

Figure 18 presents the adverse drug reactions reported by women on low molecular weight heparin (LMWH) based on survey data. Analysis of the data revealed that 309 women (100%) reported reactions at the injection site, including swelling, burning, pain, and redness. The most common complaints following injection site reactions were:

- Gum bleeding in 105 women (33.98%)
- Epistaxis (nosebleeds) in 47 women (15.21%)
- Bleeding from the genitals up to 12 weeks of gestation in 32 women (11.32%)
- Allergic reactions (rash, itching) in 23 women (7.44%)
- Rectal bleeding in 9 women (2.91%)

V. DISCUSSION

The results of this study are highly relevant to obstetric practice and contribute significant data to the existing literature on the subject. Although the study was conducted independently and without any borrowed conclusions from existing literature, the results align with those of most existing studies on the topic, which demonstrate the effects of inherited thrombophilia on various pregnancy parameters. The findings of this study confirm the conclusions drawn from the literature review regarding the role of management during pregnancy and the implementation of preconception therapy in patients with recurrent pregnancy loss and hereditary thrombophilia.

Women carrying genetic mutations associated with thrombophilia are at higher risk for developing complications during pregnancy, such as early and late pregnancy loss, preeclampsia, intrauterine fetal growth restriction, placental abruption, and stillbirth. These pregnancy complications are linked to genetic thrombophilia through the mechanism of thrombosis in microvascular structures.

According to the results of this study, various thrombophilia factors are responsible for earliest and late pregnancy losses. The data show that 98% of women with thrombophilia factors report having experienced pregnancy losses, while only 2% do not report a miscarriage. Additionally, among those participants who report a spontaneous abortion, 66% report early pregnancy loss before 10 weeks of gestation, while 29% report pregnancy loss after 10 weeks of gestation. The results further indicate that participants with thrombophilia report pregnancy losses up to 24 weeks of gestation. On the other hand, only 15% of participants who do not carry thrombophilia factors report having had a miscarriage. These results demonstrate a direct correlation between pregnancy loss, particularly in early stages, and inherited thrombophilia.

These findings are supported by other authors, confirming that thrombophilic risk factors are responsible for early and late pregnancy losses and related complications, which can impact fetal development, especially during the first or second trimester. Additionally, pregnancy loss, early and late intrauterine growth restriction, and stillbirths are the most common adverse pregnancy outcomes associated with thrombophilic factors. Based on the results of both studies, it can be concluded that thrombophilic factors are responsible for earliest and late pregnancy losses, as well as complications such as early and late intrauterine growth restriction and stillbirth.

The results highlight the degree of risk associated with adverse outcomes linked to congenital thrombophilia, based on five factors, including Factor V Leiden, the G20210A mutation in the prothrombin gene, the *C677T* genetic variant in the methylenetetrahydrofolate reductase gene, the genetic variant in the plasminogen activator inhibitor 1 (PAI-1) gene (*4G/4G* genotype), and the ACE D/D genotype of the angiotensin-converting enzyme. According to the findings, the *C677T* genetic variant in the methylenetetrahydrofolate reductase gene and the plasminogen activator inhibitor 1 (PAI–I) *4G/4G* genotype are the most prevalent genetic factors associated with thrombophilia. Additionally, the results indicate that the Factor V Leiden genetic factor is the least commonly encountered. Furthermore, participants with thrombophilic factors, particularly PAI I and ACE D/D, most frequently experience adverse pregnancy outcomes compared to carriers of other thrombophilic factors. Despite differences in outcomes among carriers of various factors, a general conclusion can be drawn that patients with inherited thrombophilia have a higher likelihood of developing adverse pregnancy outcomes compared to those without these factors. These results align with findings reported by Han et al. [153], who state that inherited thrombophilic factors, including Factor V Leiden mutations, prothrombin mutations, and MTHFR, as well as protein S

deficiency, are associated with reproductive failures and/or complications in late pregnancy. Based on the current study's results and those of Han et al. [153], we can confirm that inherited thrombophilia is linked to adverse pregnancy outcomes and an increased risk of early pregnancy loss.

Patients carrying multiple thrombophilic factors (genetic mutations and biological changes) give birth to infants at a younger gestational age and with lower neonatal birth weights. Multifactorial thrombophilia manifests as thrombotic lesions in the placenta, which compromises uteroplacental circulation and can later lead to restricted fetal growth, increased blood pressure, placental abruption, and stillbirth.

The objective of the present study was to develop a prevention algorithm for prenatal and postnatal complications associated with genetic thrombophilia factors. According to the results from both studies, there is a direct correlation between acquired and inherited thrombophilia, and a significant portion of adverse pregnancy outcomes occur in the early stages of pregnancy. Additionally, it is reported that inherited thrombophilia has a direct impact on blood parameters during pregnancy and after delivery. Various thrombophilic genotypes are directly linked to the development of spontaneous abortion and increase the risk of early pregnancy loss.

Studies show that individuals with thrombophilic factors more frequently develop conditions such as varicose veins, thrombotic incidents, hypertension, insulin resistance, diabetes, and anemia. A significant family history and evidence of chronic infection are other factors that can guide the diagnosis of thrombophilia. The most common comorbid pathology in both the clinical and control groups is the presence of varicose veins.

The characteristics of hemostatic balance in women who experience spontaneous abortion due to inherited thrombophilia

are marked by platelet and endothelial activation and activation of the coagulation cascade, with intact fibrinolysis. The results from the two studies underscore the necessity for an individualized approach to each case of pregnancy loss.

The results from the study by Brenner et al. [61] align with those reported in the present study, primarily concerning the combinations of thrombophilia factor types. Brenner et al. [61] additionally link inherited thrombophilia factors with a high risk of thrombosis and the direct impact of the duration of anticoagulant therapy on the development of venous thromboembolism (VTE) or other thrombotic events. Thus, the results from both studies confirm that different thrombophilia factors have varying effects on pregnancy outcomes depending on the type of carrier status, whether homozygous or heterozygous. It is concluded that the most prevalent thrombophilia factor is heterozygous carriage of the plasminogen activator inhibitor 1 (PAI-1) 4G/5G.

According to the results of the present study, there is a correlation between fetal growth restriction (FGR) and preeclampsia in the late stages of pregnancy, as well as a correlation between high uterine pulsatility index (PI-Ut) and early pregnancy loss. A higher frequency of recurrent pregnancy loss (RPL) or recurrent early pregnancy loss (REPL) within the first 6–12 weeks after conception is found in homozygous individuals for certain thrombophilia mutations. The ACE D/D genotype and the PAI *4G/4G* genotype (plasminogen activator inhibitor-1 4G/4G) are associated with an increased risk of these conditions, as well as the development of high blood pressure, preeclampsia, and intrauterine fetal restriction (IUGR) [7].

The study also focuses on analyzing antenatal complications associated with the presence of genetic thrombophilia factors in women with a history of reproductive failures. Although numerous studies have been conducted on this topic, very few

concentrate on analyzing these complications with the aim of developing a prevention and treatment algorithm. The results show that female patients with existing inherited thrombophilia report a higher body mass index (BMI) compared to women without inherited thrombophilia. Additionally, the results reveal a direct correlation between spontaneous miscarriages and existing inherited thrombophilia, with a significant number of miscarriages occurring in the early stages of pregnancy—before 12 gestational weeks. The majority of participants are carriers of the MTHFR *C677T* factor, while the smallest proportion are carriers of the Factor V Leiden factor. The genetic variant *C677T* in the MTHFR gene and the PAI-I *4G/4G* genetic factor are the most prevalent genetic factors associated with thrombophilia-related complications.

To optimize reproductive health in women with genetically determined thrombophilia, an individualized and evidence-based approach is essential. This approach includes genetic testing, risk assessment, careful monitoring, therapy adjustment, and patient education. To ensure the health and well-being of both mother and fetus, collaboration among clinicians (obstetricians-gynecologists, hematologists, immunologists, endocrinologists, and cardiologists) is crucial.

VI. DISCUSSION OF RESULTS

1. 309 pregnant patients with congenital thrombophilia who were carriers of genetic thrombophilic factors and reproductive failures in the past were studied.

2. An analysis of the genetic spectrum of thrombophilic mutation carriers, anamnestic data on personal thrombotic incidents, family history, accompanying pathology, obstetric history, course of past pregnancies, course of the current pregnancy, childbirth and puerperal period was performed in the diagnosed patients.

3. To examine the course of pregnancy, childbirth and the postpartum period, the examined group was divided into two subgroups: women with thrombophilic factors + mutation in the PAI I gene and normal ACE I/I genotype (n=225) and women with thrombophilic factors + PAI I gene mutation + ACE D/D gene mutation (n=84).

5. The demographic characteristics, anamnestic data and exclusion factors for the characterization of the clinical group of studied women with reproductive failures and carriers of one or more of the following 5 genetic factors associated with thrombophilia led to the conclusion that in both groups the pregnant women are about 31 annual with a mean deviation of ± 5.5-6 years, i.e. women in group A and group B were of similar mean age and BMI.

6. With descriptive statistics of sociodemographic factors - smoking, marital status, number of pregnancies, number of births and clinical conditions - varicose veins, anemia, pregnancy, family history of thrombosis and family history of diabetes, it is concluded that smokers in group A women are 39.5% compared to 60.5% non-smokers, and in group B there are 76% to 24% in favor of non-smokers, i.e. women in group B were more likely to not smoke than those in the other group. Regarding family status, married women in group A are more

than those in group B - 55.4% to 42.7%. Group A pregnant women showed a higher frequency of pregnancies as 34% and 32% had more than 3 and more than 2 pregnancies respectively, cumulatively 65% had more than one pregnancy, 28.5% of this group had only one pregnancy and only 5.5% did not were pregnant.

7. Accompanying diseases were observed in women in group A, with 33.01% having varicose disease of the lower limbs, 14.56% having it on the vulva and vagina, and 52.43% having no such pathology. Group B shows a completely different trend - 89.33% of women do not have varicose veins, 9.33% have them on the lower limbs and 1.3% have them on the vulva and vagina. For hypertensive disease in group A, 17.48% of women have such a disease and 82.52% do not, while in B only 8.67% have hypertensive disease and 91.33% do not. In insulin resistance, 25.24% of women in group A had no manifestation, 43.69% had such resistance, 1.29% had type I diabetes, 5.18% had type II diabetes and 24.60% had PCOS. In group B, no women with insulin resistance were observed. In both groups of women, it turns out that 73.3% of women in group A do not have thyroid disease, 21.68% have subclinical Hashimoto's thyroiditis and only 0.97% have Based's disease. In group B, there are no women with developed thyroid disease.

8. Family history of thrombosis, myocardial infarction and/or stroke shows that 13.9% of women in group A have such a family history of encumbrance and 86.1% do not, while in group B only 6.67% have a family history of thrombosis, myocardial infarction myocardium and/or stroke and 93.33% do not.

9. The data on the method of pregnancy during the current pregnancy in the two studied groups show that 68.28% of pregnant women in group A conceived spontaneously, 26.86% after ART and 4.854% after insemination. In group B, 92% were women who became pregnant spontaneously and only 8% after ART, and none became pregnant after insemination.

10. Factor V Leiden mutation was present in 12.30% of the studied women in group A, with homozygous being 2.27% and heterozygous being 10.03%. A mutation in the prothrombin gene G20210A was detected in 12.62% of women divided into: 3.88% homozygous and 8.74% heterozygous. MTHFR C677T occurred in 85.43% of women in group A, with the homozygote present in 29.77% of women and the heterozygote in 55.66%.

VII CONCLUSIONS

1. The multigenic form of thrombophilia represents a pathophysiologically adverse background and a risk factor for triggering the most severe obstetric complications (recurrent early and late pregnancy losses, preeclampsia, gestational diabetes, as well as thrombosis and thromboembolism during the puerperium).

2. In patients with recurrent pregnancy loss, the most common adverse thrombophilic factors are: MTHFR C677T mutation in 85.43% of women; mutation in the gene for Plasminogen Activator Inhibitor 1 (PAI-I) 4G/4G and 5G/4G in 82.84% of women; and mutation in the angiotensin-converting enzyme in 27.18% of women.

3. Family history of thrombosis, myocardial infarction, and/or stroke is burdened in 13.9% of women with genetically determined thrombophilia.

4. The mean pulsatility index of the uterine arteries was determined using Doppler in patients with genetically determined thrombophilia and normal pregnancy. The mean pulsatility index of the uterine artery is significantly higher during pregnancy in women with thrombophilic mutations compared to women with a normally progressing pregnancy.

5. It was found that combined carrier status of the polymorphisms PAI-1 4G/4G and ACE D/D,I/D further increases the risk of reproductive failures, both early and late pregnancy loss, as well as complications during pregnancy and potential complications such as chronic hypertension, preeclampsia, intrauterine growth restriction, gestational diabetes, deep vein thrombosis, and pulmonary embolism in the puerperium.

6. For patients with multigenic thrombophilia and a complicated obstetric history, timely initiation of prophylaxis with low molecular weight heparin (LMWH), aspirin, and/or nattokinase allows for a reduction in the frequency and severity

of reproductive failures in 91.59% of cases, as well as an improvement in perinatal and postnatal outcomes. Safe and effective use of anticoagulants and antiplatelet agents in pregnant women requires not only indications for their use but also appropriate conditions for their administration. The main prerequisites for their safe use in obstetrics include:

- No signs of chorionic or placental abruption (confirmed by ultrasound)
- Absence of bleeding from the genital tract, gums, and nose
- Adequate surgical hemostasis during delivery
- No documented allergies to LMWH and aspirin

VIII CONTRIBUTIONS OF THE DISSERTATION WORK

1. Original Scientific Contributions

- For the first time in the Bulgarian population, the frequency of five genetic mutations associated with thrombophilia in women with reproductive failures has been investigated and analyzed.
- For the first time in Bulgaria, the impact of thrombophilic factors on pregnancy outcomes, delivery, and the puerperal period has been evaluated.
- An algorithm for managing patients with congenital thrombophilic factors and a history of reproductive failures (early and late spontaneous abortions, preeclampsia, intrauterine growth restriction, stillbirth) has been developed.

2. Confirmatory Scientific Contributions

- A substantial volume of literature on the subject has been analyzed, confirming the relevance of the problem and

demonstrating the need for management algorithms (therapy and prevention) for patients with congenital thrombophilia to reduce the frequency of reproductive failures.

- The role of combined carriage of the PAI-1 4G/4G and ACE D/D polymorphisms as a factor further increasing the risk of reproductive failures (chronic hypertension, early preeclampsia, and intrauterine growth restriction) has been confirmed.
- It has been found that the mean pulsatility index of the uterine arteries is significantly higher at 6-7 weeks of gestation in patients with congenital thrombophilic factors and the PAI-1 4G/4G and ACE D/D polymorphisms, as well as in cases of early preeclampsia and intrauterine fetal growth restriction, compared to normal pregnancy.
- The effectiveness of early initiation of low molecular weight heparin (LMWH) and aspirin prophylaxis and the monthly monitoring of coagulation factors D-dimer, aPTT, and anti-Xa has been confirmed.

3. Scientific contributions of practical value

- Genetic testing for thrombophilic factors and PAI-1 (4G/4G, 5G/4G) and ACE (D/D, I/D) polymorphisms can be used in clinical practice and genetic counseling to assess risk for both early and late pregnancy loss, as well as for the course of pregnancy and expected visible complications such as PE, IURP, GD, DVT and BTE in the post partum period.
- The importance of an individual approach for each pregnant woman, depending on the severity of the thrombophilic spectrum and accompanying risk factors, has been confirmed in the women's consultation.

- An algorithm for the prevention of reproductive failures and adverse pregnancy outcome has been developed.

IX. APPENDICES

1.Algorithm for preventing adverse pregnancy outcomes in patients with genetic thrombophilia.

- Pelvic exam and assessment of risk factors and outcomes of previous pregnancies.
- Blood tests: Hormonal status; TSH, anti-TG, anti-TPO antibodies; AFA, ANA, NK-cell phenotype; Vit. D and vit. B12.
- Screening for inherited thrombophilia.
- Partner karyotyping after more than 2 spontaneous miscarriages.

- Step one+
- Screening for aneuploidies, preeclampsia, gestational diabetes, and preterm birth - 11-13+6 weeks of gestation.
- Oral glucose tolerance test at 12 weeks and 24-28 weeks of gestation for women with obesity and a family history of diabetes in first-degree relatives.
- Monitoring of hemostasis every 3-4 weeks.
- Doppler of the uterine artery: 6-7 weeks, 11-13 weeks, 20-22 weeks, 30-33 weeks.
- Ultrasound assessment of the fetal-placental condition, ultrasound examination of cervical length at 16 and 20-22 weeks of gestation.
- Partner karyotyping after more than 2 spontaneous miscarriages.

- Complete blood count and haemostasis.
- Assessment of risk factors.

Before Conception

- Planing and preparation for pregnancy (6 months)
- Antioxidants;
- Omega-3 fatty acids;
- Low doses of Aspirin 75-100 mg (day 15 to 25 of the menstrual cycle);
- Infusions with Intralipid or Immunoglobulins (IVIG), before ovulation or embryo transfer, in cases of high peripheral NK cells;
- Folic acid at least 4 mg/day (in cases of MTHFR C677T mutation, at least 8 mg/day));

During Pregnancy

- Step one+
- Tocolysis + spasmolysis;
- Progesterone no more than 200 mg per day in high-risk thrombophilia;
- If needed: Treatment of anemia, correction of hemostasis.
- LMWH 0.3/0.4 according to D-dimer and aPTT values, Aspirin 100/150 mg and/or Nattokinase after a positive pregnancy test
- Corticosteroid prophylaxis for the fetus;
- (NST) monitoring of the fetus twice a week after 32 weeks of gestation;
- Hospitalization at 37-38 weeks of gestation;
- For patients with low-risk forms of thrombophilia, discontinue LMWH one week before EDD if D-dimer and aPTT are within normal values. For patients with high-risk forms of thrombophilia, discontinue LMWH 12 hours before cesarean section, or continue it until delivery if the delivery is vaginal;
- Aspirin and Nattaspin H are discontinued at 35 weeks of gestation

After giving birth

- Early mobilization after delivery Start LMWH 6 hours after giving birth.
- For patients with low-risk forms of thrombophilia, without additional risk factors and with normal D-dimer and aPTT values, we start nattokinase up to 4 weeks postpartum
- For patients with high-risk forms of thrombophilia and/or the presence of additional risk factors, we start LMWH (in prophylactic doses, and for patients with overweight, dosed per kg/mă) 6 hours after delivery and continue for 6 weeks postpartum.
- There are no contraindications for breastfeeding.

Table 1. First step. Preconception algorithm - 6 months before planned pregnancy.

Investigations	Preventive and curative measures
1. Gynecological examination 2. FBC + Coagulation 3. Microbiology of vaginal content, cervix and uterus (Microbiota). 4. Hormonal status (LH, FSH, Estradiol, Prolactin, 4-androsendion, Testosterone, Progesterone) 5. TSH, anti TG, anti TPO 6. AFA, ANA, 7. NK Cells phenotyping 9. Vitamins (D and B12) 10. Thrombophilia spectrum 11. Consultation with genetics and Hematologist if necessary	1. Treatment of accompanying diseases and disorders in the coagulation status 2. Treatment of genital microbiome 3. Hormonal treatment in case of disorders 4. TSH <2.5 microE/ml, negative ant TG and anti TPO. 5. Normal immunological status 6. Weight control ..... - Antioxidants - Omega 3 fatty acids - Low Dose Aspirin 75-100 mg (15-25 day in second phase of the menstrual period) - Intralipid Sol. i.v. infusion before ovulation/ embryo transfer, if NK peripheral cells are high or Immunoglobulins (IVIG). - Folic acid not les then 4 mg/Daly (in cases with MTHFR C677T not less than 8 mg/Daly) - in cases with high D-dimers – Nattokinase

Table 2: Step two. Gestational management during pregnancy

Investigations	**Preventive and curative measures**
1. First stage + 2. First trimester screening for aneuploidies, Pre-eclampsia, gestational diabetes and preterm birth $11\text{-}13^{+6}$ g.w. 3. Oral glucose tolerance test in 12 g.w. in obesity patients with anamnesis of diabetes mellitus in first line relatives and in 24-28 g.w. 4. Control of Hemostasis on every 3-4 weeks 5. Doppler of Uterine artery: 11-13 g.w., 20-22 g.w., 30-33 g.w. 6. Ultrasound of feto-placental unit Ultrasound of cervical lent at 16 and 20-22 g.w.	First stage + - Polyvitamines - Tocolytics + spasmolitics - Progesterone not more than 300 mg per day in case of high risk Thrombophilia if need - Treatment of anemia - Correction of hemostasis **LMWH 0.3/0.4 depend of the D-dimer and aPTT, anti Xa Aspirin 100/150 mg or Nattocinase** Corticosteroid prophylaxis in case of preterm birth CTG monitoring 2 times weekly after 32 g.w. Hospitalization on 37-38 g.w. In heterozygous patient we stop LMH one week before term for delivery, if d-dimer and aPTT is in normal rates. Stop LMH 12 hours before cesarean delivery in high risk Thrombophilia patients Stop Aspirin and Nattocinase in 35 g.w.

Table 3: Step Three. Prevention of complications in the puerperal period

Investigations	Preventive and curative measures
FBC + coagulation	- Early movement of the patient - Start with the LMH on the 6 hour after delivery - In patients with monogenic or heterozygous Thrombophilic gene mutations and normal D-dimers, fibrinogen, aPTT and anti X a, we start with Nattocinase 4 weeks pos partum - In Homozygous patients with family history for DVT, Pulmonary thrombosis, Myocardial infarction, Stroke, Personal history for thrombosis, obesity, We start with LMH at the 6 th hour. 6 weeks postpartum.

2. Risk factors leading to Adverse Pregnancy Outcome (RPL, PE, IUGR, GDM)

1. Mother's age >35 years.

2. Smoking > 10 cigarettes/day

3. BMI > 30 kg/m2

4. Family history (first relative up to 50 years of age)

4.1. Varicose veins of the lower limbs

4.2. Hypertensive disease

4.3. Myocardial infarction, Ischemic stroke, BTE, Deep venous thrombophlebitis

5. Personal history of the patient

5.1. Varicose disease

5.2. Myocardial infarction, Ischemic stroke

5.3. Pulmonary thromboembolisam

5.4. Deep venous thrombophlebitis

6. Obstetric history

6.1. Early fetal loss <10 g.w., 2 or more

6.2. Late fetal losses up to 24 g.w.

6.3. History of stillbirths

6.4. Preeclampsia moderate/severe

6.5. Abruption of the placenta

6.6. Fetoplacental insufficiency (Fetal growth restriction)

7. Somatic status

7.1. Dysmetabolic syndrome

7.2. Inflammatory diseases of the urinary tract

7.3. Chronic hypertensive disease

7.4. Diabetes mellitus with vascular damage

7.5. Diseases of the thyroid gland

8. Thrombophilia (gene mutations)

8.1. Factor V Leiden gene mutation

8.2. Mutation in the prothrombin gene G20210A

8.3. Antithrombin III, Protein C and Protein S deficiency

9. Thrombogenic polymorphisms

9.1. Genetic variant C677T in the Methylenetetrahydrofolate reductase gene

9.2. Genetic variant in the gene of Plasminogen activator inhibitor 1 (PAI-1) (carriage of genotype 4G/4G)

9.3. Angiotensin-converting enzyme - ACE D/D.

10. Function of the trophoblast

10.1. Low levels of PAPP - A

10.2. Low levels of PLGF

11. Immunological disorders

11.1. NK cells phenotype

11.2. LA

11.3 Antiphospholipid syndrome

12. Hypovitaminosis

12.1. Low level Vit. D

12.2. Low level Vit. B12

X. PUBLICATIONS AND SCIENTIFIC COMMUNICATIONS RELATED TO THE DISSERTATION WORK

X.1. Publications related to the dissertation work

1. **Kirovakov Zl.** *Inherited Thrombophilia and recurrent pregnancy* loss – *REVIEW OF LITERATURE*. „XXI National scientific session for students and teachers", Meical University – Pleven, 2023, pg. 105 -115; ISBN 978-954-756-325-4.
2. Kirovakov Z, Konova E, Hinkova N, Markova S, Penchev P. *Immunological Risk Factors in Recurrent Pregnancy Loss in Patients With Hereditary Thrombophilia*. Cureus Journal of Medical Science, 2024, 16(3):e56555. ISSN: 2168-8184; **Web of Science. IF – 1.2**
3. **Zlatko Kirovakov,** Nadezhda Hinkova, Emiliana Konova, Stefani Markova. *Frequency of Thrombophilic Factors in Patients with Recurrent Pregnancy Loss*. Journal of Medical and Pharmaceutical Sciences, 2024, 2(3): 27-35. DOI:org/10.5281/zenodo.10877053. e-ISSN: 2584-0150.
4. **Zlatko Kirovakov,** Emiliana Konova, Nadezhda Hinkova, Stefani Markova. *The Role of Gestational Management and use of LMWH and Aspirin in Patients with Inherited thrombophilia.* Journal of Medical and Pharmaceutical Sciences, 2024, 2(3): 7-14. DOI:org/10.5281/zenodo.10791430. e-ISSN(Online): 2584-0150.

X.2. Participation in scientific forums in Bulgaria:

1. **Kirovakov. Zl.,** E. Konova, N. Hinkova, Ant. Dushepeev, St. Markova. *Angiotensin-converting enzyme D/D polymorphisam and inheridet Trombophilic factors as a cause of Preeclampsia and IUGR*. „IV National conference on innovations in obstetrics and gynecology'', Sunny Beach, 25-28.05.2023.
2. Kirovakov. Zl., Pl. Penchev. *Maternal genotype and Preeclampsia, IUGR, Gestational diabetes*. „XIV National

conference on rare diseases and orphan drugs“, Plovdiv, 29-30.09.2023.

3. **Kirovakov. Zl.,** *The role of pre-gestational management and use of LMWH and Aspirin in patients with recurrent pregnancy loss and inherited thrombophilia*. XX International medical scientific conference for students and young doctors. Pleven, 16-20.10.2023.

4. **Kirovakov Zl.** *Inherited Thrombophilia and recurrent pregnancy – REVIEW OF LITERATURE. „XXI National scientific session for students and teachers“, Meical University – Pleven, 2023*“, Meical University – Pleven, 2023,

5. **Kirovakov Zl.,** St. Markova. *Inherited Thrombophilia and adverse pregnancy outcome*. Third Autumnal Medical Forum. Burgas, 03-04.11.2023.