

**UNIVERSITY OF MEDICINE AND PHARMACY  
"VICTOR BABEȘ" IN TIMISOARA  
FACULTY OF GENERAL MEDICINE  
DEPARTMENT XII - OBSTETRICS AND GYNAECOLOGY**

**IORDACHE OLIVERA**



# **DOCTORAL THESIS**

**CLINICAL AND PSYCHOSOCIAL IMPLICATIONS OF  
THROMBOPHILIA DURING PREGNANCY**

PhD supervisor

**PROF. UNIV. DR. ANASTASIU DORU**

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# **CHAPTER 1: A RETROSPECTIVE EVALUATION OF THROMBOPHILIA IN PREGNANT WOMEN WITH FIRST AND SECOND TRIMESTER PREGNANCY LOSS.**

## **CONTEXT**

Despite extensive research, there is not yet sufficient evidence to establish a definitive link between a2-GPI and pregnancy loss, as well as other mutations. Recurrent pregnancy loss remains a significant concern, with several unanswered questions. In response to this knowledge gap, the present study aimed to investigate and compare pregnant women who experienced first trimester pregnancy loss with those who experienced recurrent pregnancy loss in the second trimester. The main objective of the study was to compare the demographic and clinical characteristics of the two groups to identify any potential differences that may contribute to recurrent pregnancy loss.

As well as identifying any differences between the two groups, the study also aimed to identify the most important factors contributing to pregnancy loss. Previous research has suggested that various factors such as maternal age, uterine abnormalities, thrombophilia, hormonal imbalances and lifestyle factors may contribute to recurrent pregnancy loss. However, it remains unclear which of these factors play the most important role in miscarriage. Therefore, a secondary objective of the study was to identify the most common factors contributing to pregnancy loss and their relative contribution.

To achieve these objectives, the study adopted a retrospective observational design. The study sample included pregnant women who experienced either first trimester pregnancy loss or recurrent second trimester pregnancy loss. Clinical and demographic characteristics of the participants were collected and analysed using statistical methods, such as logistic regression, to determine any significant differences between the two groups. In addition, the study used multivariate analysis to determine the most common factors contributing to pregnancy loss and their relative contribution.

By comparing the clinical and demographic characteristics of women who experienced first trimester pregnancy loss with those who experienced recurrent second trimester pregnancy loss, this study aims to identify any potential risk factors that may contribute to recurrent pregnancy loss. Furthermore, by identifying the most common factors contributing to pregnancy loss and their relative contribution, this study aims to provide a better understanding of the etiology of recurrent pregnancy loss, which can inform the development of effective prevention and management strategies.

## **RESULTS**

The majority of patients were under 35 in both study groups, with only 21.7% overweight and obese in the first trimester group and 16.7% in the second trimester group, adjusted for gestational age. Substance use behavior identified 4.5% chronic alcohol users and 14.0% smokers among first trimester miscarriage patients, and 5.6% chronic alcohol users and 16.7% smokers among second trimester miscarriage patients. The most commonly observed comorbidity in the entire cohort was depression, which was seen in about 8% of all patients, followed by cardiovascular and metabolic disorders in about 4% of cases. A total of 16 (10.2%) patients had COVID-19 in the first trimester group, compared with 9 (16.7%) patients in the second trimester group, with no significant differences.

Obstetric characteristics of the study participants show that 54.1% of first trimester patients had three or more pregnancies, although only 10.2% of all delivered. In the other study group, a total of 68.5% of women had three or more pregnancies and only 9.3% gave birth. The difference in proportions was not statistically significant. The patients studied had a total of 496 miscarriages, with a statistically significant difference in proportions when comparing types. Thus, 18.5% of missed miscarriages occurred in the first trimester, compared with only 6.2% in the second trimester ( $p$ -value = 0.003). The most common type

was complete miscarriage, which occurred in 39.1% of miscarriages in the first trimester and 49.1% in the second trimester. History of induced abortion was not statistically significant between study groups. However, high obstetric risk was a major finding in patients who experienced second trimester pregnancy loss (38.9% vs. 21.0% in the first trimester, p-value = 0.009). Among pregnancy-related complications, only the proportion of maternal infections was statistically significantly different between study groups (27.8% in the second trimester vs. 15.3% in the first trimester, p-value = 0.041).

Analysis of thrombophilia factors was mostly insignificant when comparing first and second trimester pregnancy losses, although four factors were identified as more prevalent in one of the groups. Thus, factor V Leiden homozygosity was statistically significantly more common in pregnant women who experienced first trimester pregnancy loss compared to those who experienced second trimester miscarriages (10.8% vs. 1.9%, p-value = 0.041). In the first trimester group, the presence of antiphospholipid syndrome antibodies was also a significantly more common finding compared to second trimester pregnancy loss (17.8% vs. 33.3% in the first trimester, p-value = 0.034; 19.7% in the second trimester, p-value = 0.041).

Multivariate risk factor analysis determined a number of significant risk factors from the panel of mutations and thrombophilic deficiencies for pregnancy loss in both the first trimester and second trimester. The strongest independent risk factors for first trimester pregnancy loss were FVL and PT compound mutations (OR = 3.11), followed by FVL homozygous mutation (OR = 3.66) and APS antibodies (OR = 4.47). In terms of risk factors for second trimester pregnancy loss, the strongest were FVL and PT compound (OR = 3.24), followed by glycoprotein Ia polymorphism (OR = 3.61) and APS antibodies (OR = 3.85).

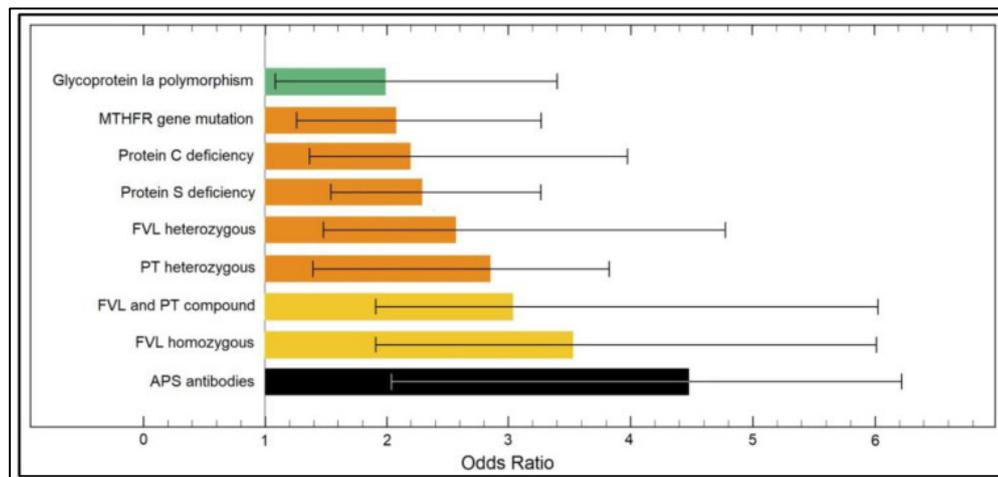


Figure 1. Analysis of risk factors for first trimester pregnancy loss.

Table 4. Analysis of risk factors for pregnancy loss in the first and second trimester.

Risk factors	First quarter	P	Second quarter	P
FVL (heterozygote)	2.54 (1.33-4.96)	0.036	1.82 (1.24-3.25)	0.049
FVL (homozygous)	3.66 (1.85-6.11)	0.001	2.27 (1.51-3.88)	0.007
PT (heterozygous)	2.79 (1.27-3.82)	0.022	2.81 (1.58-4.33)	0.001
FVL and PT mutation	3.11 (1.89-6.18)	0.001	3.24 (1.80-5.76)	0.001
Protein C deficiency	2.15 (1.32-3.93)	0.009	2.98 (1.75-5.04)	0.001
Protein S deficiency	2.25 (1.46-3.23)	0.001	1.93 (1.16-2.83)	0.012
Antiphospholipid Antibodies	4.47 (2.03-6.32)	0.001	3.85 (1.83-5.41)	0.001
MTHFR mutation	2.02 (1.24-3.32)	0.017	2.48 (1.37-4.29)	0.001
Glycoprotein Ia (polymorphism)	1.97 (1.08-3.40)	0.033	3.61 (1.22-4.94)	0.001

## CONCLUSIONS

Several thrombophilia risk factors for early and late pregnancy loss have been identified, including several mutations that appear to affect fetal development, particularly during the first or second trimester. However, it is not currently suggested that women who have not previously experienced difficulties during pregnancy should be regularly tested for thrombophilic abnormalities. Despite this, the avoidance of miscarriage, early and late onset fetal growth restriction and stillbirth continues to be a significant and current public health concern. With regard to hereditary thrombophilia associated with early or late pregnancy loss and other pregnancy-related problems, it is currently unclear whether the process itself, as well as the natural history of the condition, is fully understood. Because of the rarity of hereditary thrombophilia in the general population, previous research on this topic has often been underpowered to identify significant findings, including the results of the current investigation. Even if screening for thrombophilia is risk-free and effective, there is no intervention that has been shown to be effective after screening to reduce the rate of recurrent pregnancy loss.

## **CHAPTER 2: MACROSCOPIC EXAMINATION OF PLACENTAL VASCULATURE WITH A CORROSIVE AGENT IN PREGNANT WOMEN DIAGNOSED WITH THROMBOPHILIA**

### **CONTEXT**

Thrombophilias, whether inherited or acquired, are haematological disorders that predispose individuals to thrombo-occlusive events or thromboembolic disease due to molecular abnormalities in the haemostatic system. These disorders are often associated with an increased risk of fetal loss, particularly during pregnancy, and the risk may be even higher in the second and third trimesters. Thrombophilias are relatively common, affecting about 5-10% of the European population, and can be congenital or acquired, characterised by an increased tendency for blood to clot and a consequent risk of thromboembolism.

Placental development is a crucial factor in fetal growth and development, and maternal-fetal placental vasculature plays an essential role in this process. Placentation involves a complex interplay of angiogenesis and vasculogenesis, as evidenced by several studies that have highlighted the essential roles of various angiogenic factors in this process. Other studies have highlighted the roles of different classes of factors in vascular morphogenesis that are non-specific to the placenta. Angiogenesis in maternal and fetal placental tissues is extensive and is accompanied by a marked increase in uterine and umbilical blood flows.

In the context of thrombophilia, abnormal haemostatic mechanisms can affect the normal process of placental development, leading to fetal growth restriction, placental detachment and other complications that increase the risk of fetal loss. For example, thrombophilia can disrupt angiogenesis and vasculogenesis, leading to abnormal placental development, inadequate blood supply to the fetus and subsequent fetal loss. This mechanism may be particularly relevant in the second and third trimesters, when the placenta's demand for oxygen and nutrients increases significantly and the risk of fetal loss is highest.

The objectives of this study are to investigate the prevalence of thrombophilias in pregnant women in a specific population, to identify the most common types of thrombophilias associated with an increased risk of fetal loss. In addition, this study aims to assess the impact of thrombophilias on fetal growth and development during different trimesters of pregnancy, including the second and third trimesters. Furthermore, this study will examine potential mechanisms underlying the association between thrombophilia and fetal loss, including the effects of thrombophilia on placental development and maternal-fetal placental vasculature. The study also aims to identify the most effective strategies for managing thrombophilia during pregnancy to reduce the risk of fetal loss, such as anticoagulant therapy or lifestyle changes. Finally, this study aims to investigate the potential role of angiogenic factors in the development of placental abnormalities in pregnant women with thrombophilia, using corrosive substances to sample the placenta vasculature, and their impact on fetal growth and development. The findings of this study may help improve the diagnosis, management and treatment of thrombophilia during pregnancy, ultimately improving fetal outcomes.

### **RESULTS**

This follow-up study was conducted over a two-year period, during which time data were collected from 30 patients in 2015 and 42 patients in 2016, all of whom were diagnosed with thrombophilia during routine check-ups. Examination of placental parameters such as shape, weight and thickness was performed as a preliminary step to study placental architecture. Examination of placental surfaces provided data on possible pathologies that could affect placental function and fetal development. Our analysis revealed no evidence of abnormal placental shape in the study population, indicating normal placental development. The lack of shape abnormalities in the placenta is an important finding as it suggests that thrombophilia may not be associated with changes in placenta shape. However, it should be

noted that the study had a relatively small sample size and larger studies may be needed to confirm these findings. Overall, this study contributes to our understanding of placental development and function in people with thrombophilia and provides information that can guide the management of thrombophilia during pregnancy to improve fetal outcomes.

The results of our study showed a strong positive correlation between different pregnancy parameters in women with thrombophilia, as well as in previous research. We observed a significant association between gestational period (shown in weeks) and the APGAR score the baby received at birth, as well as between fetal weight and APGAR score. There was also a significant association between placenta weight and thickness, fetal weight and gestational period. However, we found no significant association between maternal age and gestational period.

In the second part of our study, we investigated the impact of smoking on fetal weight in women with thrombophilia. Our analysis showed that fetuses from smoking mothers were significantly smaller than those from non-smoking mothers ( $p < 0.001$ ). This finding suggests that smoking during pregnancy may adversely affect fetal growth and development. In addition, smoking is associated with an increased risk of complications during pregnancy, such as placenta previa and preterm labor, which can have significant repercussions on fetal health.

The data show that gestational age was significantly associated with fetal weight as well as placental weight and thickness. Fetal weight was also significantly associated with placental weight. In addition, there was a significant positive correlation between gestational period and fetal length and weight. However, we found no significant association between maternal age and gestational period. Overall, our study provides valuable information about the course of pregnancy in women with thrombophilia, highlighting the importance of monitoring various pregnancy parameters and managing risk factors, such as smoking, to improve fetal outcomes.

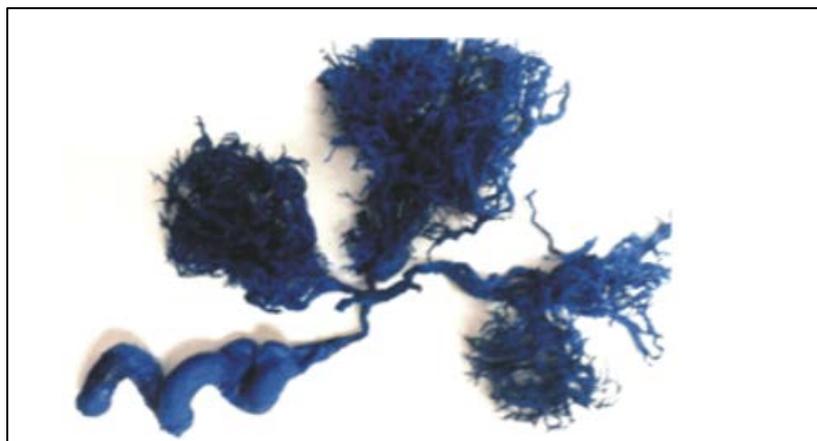


Figure 2. Placenta - fetal face - corrosive agent.



Figure 3. Placenta at 32 weeks - fetal face - corrosive agent.

## CONCLUSIONS

In conclusion, our study highlights the importance of normal placental development and function in fetal growth and development and emphasizes the essential role of maternal-fetal placental vasculature in this process. The complex process of placental development involves extensive angiogenesis, and any dysfunction of the villous vasculature can lead to placental insufficiency and fetal loss. While our preliminary data suggest that anticoagulant-based prophylaxis may improve the chances of carrying a pregnancy to term in thrombophilic women with unexplained recurrent fetal loss, it is important to remember that treatment during pregnancy must be individualized.

In conclusion, our study provides valuable insights into the pathophysiology of thrombophilia during pregnancy and its impact on fetal outcomes. Identification of various pregnancy parameters that are significantly associated with thrombophilia may help in the management of this condition during pregnancy, such as the use of anticoagulant-based prophylaxis. Further research is needed to confirm these findings and to determine the most effective strategies for managing thrombophilia in pregnancy, taking into account individual patient factors.

# **CHAPTER 3: A CROSS-SECTIONAL ANALYSIS OF INTIMACY ISSUES, STRESS LEVELS AND COUPLE SATISFACTION AMONG WOMEN WITH THROMBOPHILIA AFFECTED BY RECURRENT PREGNANCY LOSS**

## **CONTEXT**

Recurrent pregnancy loss (RPL) is one of the most difficult and challenging areas of fertility medicine because of the enormous emotional burden placed on families with RPL. Because the pathogenesis is not fully understood, there are few diagnostic and therapeutic options related to management. Chromosomal and uterine abnormalities, endometrial diseases, endocrine abnormalities, antiphospholipid syndrome, hereditary thrombophilia, immune disorders, genetic causes, environmental determinants, socioeconomic status and psychosocial stressors have been suggested as causes of RPL.

It is therefore emphasised that adequate screening and consistency is needed for those affected by RPL, in particular to assess possible dangers and appropriate treatment options. The mutation generated by a change in the position of a single base pair increases prothrombin levels, thereby increasing the risk of thromboembolism. Due to the inefficiency of the MTHFR enzyme, two forms of polymorphism have been found. In homozygous individuals, the level of efficiency is significantly lower than average. Consequently, the amount of homocysteine increases.

In other women with placental circulatory disorders, thrombophilic risk factors are more prevalent. In pregnancies associated with slow intrauterine growth, such as in pre-eclampsia, late fetal death and placental abruption may occur. Despite conflicting results, many doctors prefer to test women for thrombophilia because there are studies linking thrombophilia to poorer pregnancy outcomes. However, women with a history of RPL experienced greater psychosocial and emotional problems during subsequent pregnancies; couples also had to deal with the cumulative effects of RPL, including increasing fatigue and stress, as well as marital problems caused by miscarriages following pregnancy.

The study aims to explore the psychological impact of a diagnosis of thrombophilia on women with recurrent pregnancy loss and its correlation with stress levels and marital dissatisfaction. The study hypothesizes that couples experiencing recurrent pregnancy loss are more likely to have relationship problems, increased stress levels and anxiety problems due to their inability to achieve their goal of having a family with children.

The current study also aims to clarify the specific impact of thrombophilia diagnosis on women's psychological well-being in the context of recurrent pregnancy loss. Thrombophilia is a condition in which there is a tendency to develop blood clots, which can lead to pregnancy complications such as recurrent pregnancy loss. The study aims to identify how a diagnosis of thrombophilia affects women's mental health, specifically their stress levels and anxiety. In addition, the study aims to investigate how recurrent pregnancy loss is associated with marital dissatisfaction. Couples who experience recurrent pregnancy loss may face challenges in their relationship, including feelings of loss, grief and disappointment. The study aims to clarify how the experience of recurrent pregnancy loss impacts couples' relationships and whether it is associated with increased marital dissatisfaction.

## **RESULTS**

A total of 238 patients were analysed in the current cross-sectional study. There were 157 questionnaires completed by women with thrombophilia and recurrent pregnancy loss and 81 questionnaires completed by women with thrombophilia and recurrent pregnancy loss who achieved a normal pregnancy and a preterm or term birth. Among these patients, it was observed that relationship status was statistically significant, as only 86.6% of patients in the case group were married compared to 95.1% in the control group ( $p$ -value = 0.044). Also, the income level was significantly higher among patients who achieved pregnancy and gave birth

(62.4% versus 76.5%,  $p$ -value = 0.027). There was no significant difference in age, body mass index and a number of comorbidities of the participants. However, self-reported marital dissatisfaction was found to be significantly higher among patients with thrombophilia with recurrent pregnancy loss compared to those who achieved a single birth (15.9% vs. 6.2%,  $p$ -value = 0.031).

The first group consists of cases, which are women with thrombophilia and recurrent pregnancy loss, while the second group is the reference or control group, which includes women with thrombophilia and recurrent pregnancy loss who achieved a normal pregnancy and a preterm or term birth.

The results show that the reference group had a significantly higher proportion of women who had tried to get pregnant three or more times, with 54.1% compared to 39.5% in the case group. Similarly, the proportion of women who experienced three or more miscarriages was significantly higher in the reference group, at 68.8% compared to 55.6% in the case group. In addition, the number of associated complications was significantly higher among women with thrombophilia who did not give birth.

However, the study found that pregnant women with thrombophilia affected by RPL, but who managed to give birth after several attempts, accessed significantly more assisted reproductive techniques (64.2% versus 24.8%). This suggests that assisted reproductive techniques may be effective for women with thrombophilia who have had recurrent pregnancy losses. The reference group was also found to be affected by significantly more thrombophilic mutations, with the proportion of women with three or more mutations being 55.4% compared to only 19.8% in the control group. This indicates that multiple thrombophilic mutations may be associated with successful pregnancy outcomes in women with recurrent pregnancy loss and thrombophilia.

Overall, these findings suggest that women with thrombophilia who have had recurrent miscarriages and have successfully achieved a normal pregnancy and a preterm or term birth may have different obstetrical characteristics than those who have not given birth, including a higher number of pregnancy attempts, fewer miscarriages, and a higher number of thrombophilic mutations. In addition, assisted reproductive techniques may be an effective treatment option for women with thrombophilia and recurrent pregnancy loss.

The standardized questionnaires showed multiple significant differences between the two study groups. When examining the results of the CISS questionnaire presented in Table 3 and Figure 1, it was observed that patients in the reference group were more likely to be emotional (42.7% vs. 27.2%,  $p$ -value = 0.019). Among the significant differences, it was observed that women in the reference group had higher levels of dissatisfaction and lower levels of self-acceptance, pleasure and marital quality scores, while perception accuracy was similar to that of the control group. The Sexual Interaction Instrument (SII) total score was statistically significantly lower in the reference group (71.6 vs. 75.8,  $p$ -value = 0.003). Similar to the SII score, the Dyadic Sexual Communication Total Score (DSCS) was significantly lower among those who failed to give birth (41.3 vs. 44.6,  $p$ -value = 0.002).

It was observed that women in the reference group had significantly higher intimacy problems and lower openness scores than women in the control group (36.5 vs. 33.1,  $p$ -value = 0.008), respectively (36.2 vs. 38.7,  $p$ -value = 0.019). Levels of consent, affect and commitment were similar in both study groups, according to the MIQ questionnaire. Prenatal psychosocial profile was analyzed between women with thrombophilia and recurrent pregnancy loss (cases), women with thrombophilia and recurrent pregnancy loss who achieved a normal pregnancy and preterm or term birth (controls). It was observed that social support from partners and others was equivalent in both groups, although stress levels were significantly higher in the control group (28.1 vs. 26.3,  $p$ -value = 0.004), respectively, self-esteem levels were significantly lower (23.6 vs. 25.2,  $p$ -value = 0.020), according to the PPP questionnaire.

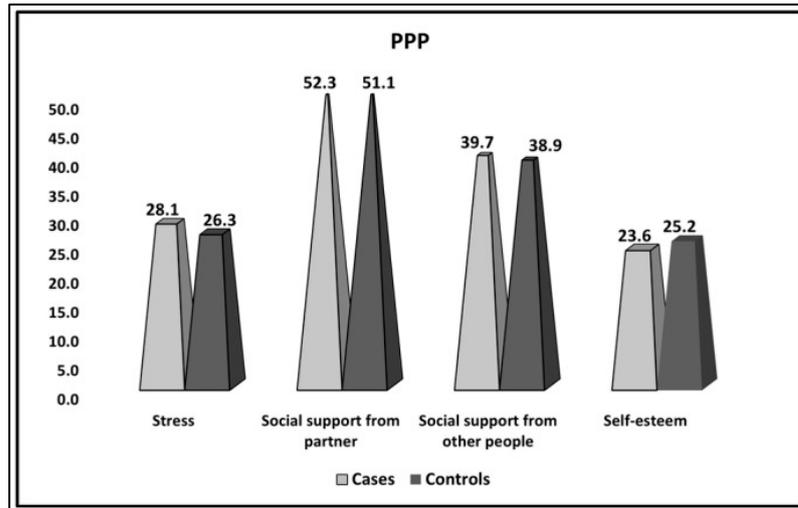


Figure 4. Comparison of PPP survey results.

## CONCLUSIONS

Marital satisfaction is likely to be significantly lower among women with thrombophilia with recurrent pregnancy loss compared to other women with thrombophilia and recurrent pregnancy loss but who eventually achieved a birth. Because the financial status of those who achieved a pregnancy and the rate of assisted reproductive techniques was higher in the latter group, it is advisable to target families affected by infertility due to thrombophilia and other causes to facilitate their access to ART interventions. By doing so, affected families will achieve their goals, which is likely to reduce the level of dissatisfaction, divorce rates and stress levels in these families.