# The Association of Inherited Thrombophilia and Recurrent Pregnancy Loss

**Magy Magdy Mohamed[1], Saad Sabry Alosh[1], Maher Borai Mohammad[1], and Amr Abdelmohsen Alnemr[2]**

1 Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt

2 Obstetrics and Gynecology Department, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt

**Corresponding author:** Magy Magdy Mohamed

**E-mail:** Dr.magymagdy27@gmail.com, mmagdy@medicine.zu.edu.eg

**Conflict of interest:** None declared

**Funding:** No funding sources

**Abstract**

Recurrent Pregnancy Loss (RPL) is diagnosed when two or more consecutive losses of pregnancy before twenty weeks of gestation occur, RPL represents about 5% of women of reproductive age**.** About 50%–65% of patients with unexplained RPL (URPL) were diagnosed with thrombophilia**.** Recent studies have found that inherited and acquired thrombophilia are important causes of URPL**.**

Thrombophilic disorders are in fact believed to exacerbate the state of hypercoagulability in pregnancy. Factor V Leiden (FVL) G1691A mutation, prothrombin (PT) G20210A mutation, methylene tetrahydrofolate reductase (MTHFR) C677T, protein c deficiency, antithrombin deficiency and protein s deficiency are considered major genetic risk factors for thrombophilia. testing for inherited thrombophilia is highly recommended to be included in premarital tests as RPL affects both the physical and psychological states of women. The RPL patients diagnosed with inherited thrombophilia and who were given LMWH (low molecular weight-heparin) with low-dose aspirin had higher live-birth rates and lower miscarriage rates than those patients with unexplained RPL.

**Keywords:** Antithrombin deficiency ; Factor V Leiden; Inherited thrombophilia; methylenetetrahydrofolate reductase C677T; protein C deficiency ;protein S deficiency Prothrombin G20210A; Recurrent miscarriage; pregnancy

**Tob Regul Sci. ™ 2022;8(1): 2722-2732**
**DOI: doi.org/10.18001/TRS.8.1.203**

## Introduction:

Recurrent pregnancy loss (RPL) is defined as two pregnancy losses prior to twenty weeks from the last menstrual period and it must be spontaneously and clinically confirmed, not

necessarily consecutive; it occurs in 1–2% of all couples trying to give birth **(American Society for Reproductive Medicine,2020; ESHRE Guideline Group *et al.*,2018)**.

**(ESHRE Guideline group ,2017)** used the term 'recurrent pregnancy loss'. however, **(RCOG,2011a)** in the UK used the term 'recurrent miscarriage' and defined it as three or more consecutive pregnancy losses.

Approximately 80% of all pregnancy losses occur in the first trimester **(Wang *et al.*,2003)**. The least risk of miscarriage was in women aged 25-29y old (10%), and increase rapidly after age 30y old, reaching 53% in women aged $\geq 45\ y\ old$ **(Magnus *et al.*,2019)**.

Causes of RPL:

*1) Chromosomal abnormalities:*

The majority of early pregnancy losses (50%–60%) are due to chromosomal abnormalities, which can be of parental origin, or arise de novo in the embryo from parents with normal chromosomes **(Werner *et al.*,2012)**.

*2)Antiphospholipid syndrome*

Antiphospholipid syndrome is characterized by the presence of antiphospholipid antibodies and has long been associated with RPL. The prevalence of APS in women with RPL is about 5%–20% **(Chighizola *et al.*,2015)**.

*3) Uterine factors*

They are considered a cause of RPL and can be classified as acquired or congenital **(Turocy and Rackow,2019)**. Acquired abnormalities of the uterus include intrauterine adhesions, myomas, and endometrial polyps that can cause RPL **(Deans and Abbott,2010)**. Congenital abnormalities are septate, bicornuate, unicornuate, didelphic, and arcuate uteri. They were found to be up to 10% of women with RPL **(Jaslow,2014)**.

*4) Inherited thrombophilias:*

These include factor V Leiden (FVL), prothrombin gene (PT G20210A) mutation, protein C and protein S deficiency, antithrombin III (ATIII) deficiency, and methylene tetrahydrofolate reductase (MTHFR) mutation **(Stevens *et al.*,2016)**.

Screening for inherited thrombophilias was recommended in pregnant women with a history of venous thromboembolism **(ACOG,2018)**.

*5)Endocrine factors*

- **Hyperprolactinemia** can cause infertility and miscarriage through anovulation or Luteal phase deficiency **(Hirahara *et al.*,1998)**.
- **Thyroid disorders:** Thyroid autoimmunity disease was associated with RPL, The most prevalent thyroid autoantibody is thyroid peroxidase(TPO) antibody. **(Godines-Enriquez *et al.*,2021)**.
- **PCOS** was also found to increase the risk of miscarriage. **(Glueck *et al.*,2003)**.
- **Uncontrolled diabetes** had been shown to increase the risk of miscarriage **(Jovanovic *et al.*,2005)**.
- **LPD or Luteal phase insufficiency:**

It had been proposed as a cause of early miscarriage and RPL **(Shah and Nagarajan,2013)**.

*6) Male Factors*

Sperm aneuploidy and DNA fragmentation had been studied in couples with recurrent pregnancy loss and it was found that there was a significant increase of sperm DNA fragmentation in husbands of women with RPL **(McQueen *et al.*,2019)**.

*7) Immunological causes*

**Autoantibodies** e.g., ANA, antithyroid antibodies, ACLA…. etc. had been associated with RPL in many studies, as they can cause breakage of auto tolerance **(Christiansen *et al.*,2008)**. It was found that women with HLA-DRB1*03 had a higher risk for the production of autoantibodies and RPL. **(Christiansen *et al.*,1998)**.

It was found that women with a history of RPL had **fewer Treg cells** in the first trimester and reduced fertility compared with the control group **(Winger and Reed,2011)**. **HLA-DRB1*04 and DRB1*15** were found to be significantly higher in RPL patients **(Meuleman *et al.*,2015)**.

*8) Environmental and psychological factors*

Several environmental factors have been associated with an increased risk of miscarriage e.g., obesity, Smoking, excessive alcohol intake, excessive caffeine consumption, and cocaine **(Pearson and Mahmood,2020) (Lassi *et al.*,2014)**.

*9)Unexplained RPL (URPL)*

URPL is considered if a complete genetic, anatomic, endocrine, and immune workup was performed and found to be normal **(Kutteh,2015)**.

Effect of inherited thrombophilia on pregnancy outcome

Thrombophilia is considered one of the causes of RPL, it was defined as a tendency to develop thrombosis, which was attributable to inherited and/or acquired disorders of blood coagulation or fibrinolysis **(Favaloro *et al.*,2009)**.

Clinicians usually apply the term thrombophilia only to a subset of patients with atypical thrombosis (1) early age of onset and (2) frequent recurrence. (3) strong family history (4) unusual migration or widespread locations, and (5) severity out of proportion to any recognized Stimulus **(Lane *et al.*,1996)**.

Thrombophilia is either inherited or acquired. Inherited thrombophilia can be divided into gain-of-function and loss-of-function disorders. The loss-of-function disorders include deficiencies or dysfunction of the endogenous anticoagulants (Antithrombin (AT), protein C, and protein S). The gain-of-function disorders usually represent weaker risk factors for VTE and include factor (F) V Leiden, the prothrombin 20210A variant, MTHFR, and genetically increased concentrations of procoagulant factors such as FVIII, von Willebrand factor (VWF), FV, FVII, FIX and FXI. The inherited type is less common but more potent to cause thrombosis **(Crowther and Kelton,2003).**

FV Leiden mutation varies from 0.6% to 7.0%, with the lowest frequency observed in Africa (0% 0.6%) and the highest in Southern Europe (7%). The mean prevalence in Northern Europe is 4%. The prevalence of Prothrombin G20210A varies from 0.2% to 3%, being lowest in Africa

(0% 0.3%) and highest in Southern Europe (3%). The mean value in Northern Europe is 2%. Protein C, protein S, and AT deficiencies are extremely rare (0.2% 0.4%, 0.03% 0.1%, and 0.02% 0.2%, respectively) **(Benedetto *et al.*,2010)**.

***Inherited thrombophilia causes***

1) ***Antithrombin Deficiency***

AT deficiency is an autosomal-dominant disease and is associated with a 5- to 50-fold increased risk of venous thromboembolism, but does not apparently increase the risk of arterial thrombosis. AT deficiency represents one of the rarest but most severe forms of inherited risk factors for thrombosis. Its severity explains why AT deficiency was the first inherited disorder to be clinically defined **(Egeberg,1965)**.

Hereditary AT deficiency is caused by mutations in the SERPINC1 gene **(Lane *et al.*,1997).** Most of these SERPINC1 gene defects (72%) are single nucleotide variations (SNVs), while small gene variations (insertions, deletions or in/del), or gross gene defects represent 11% and 10% of SERPINC1 gene defects, respectively**.** However, there are no SERPINC1 gene defects in up to 20% of cases with antithrombin deficiency **(Corral *et al.*,2018).**

AT deficiency phenotypes are classified into Type I (quantitative) and Type II (qualitative). Type I AT deficiency is characterized by equally low functional and antigenic AT levels (about 50% of normal), while type II deficiency is characterized by normal antigen levels but impaired activity **(Patnaik and Moll,2008)** .

The immunological assay is used to measure the quantity of protein regardless of its impaired function, the functional assay represents the test of choice for initial screening, and it represents the most common method done by diagnostic laboratories **(Favaloro *et al.*,2005)**.

It was found that antithrombin deficiency was associated with poor pregnancy outcome and venous thromboembolism **(Sabadell etal.,2010).**

2) ***Protein C Deficiency***

Heterozygous protein C deficiency occurs in 0.14–1.5% of the general population **(Koster *et al.*,1995)**.

It occurs in 5–9% of patients with a history of VTE **(Melissari *et al.*,1992)**.

Most individuals with an inherited protein C deficiency are heterozygotes for a mutation in the *PROC* gene and present with a mild deficiency inherited in an autosomal-dominant manner **(Finazzi and Barbui,1994)**.

Similar to AT deficiency, protein C deficiency can be divided into two major subtypes. Type I deficiency results from a quantitative deficiency of protein C, while Type II deficiencies result from qualitatively abnormal protein C **(Khor and Van Cott,2010)**

Type I deficiency is the most common type of protein C deficiency **(Kottke-Marchant and Comp,2002)**. A functional assay should be used as the initial screening test, as type I deficiencies have decreased protein C in both functional and antigenic assays, whereas Type II deficiencies have normal antigenic protein C levels, with decreased functional protein C **(Bertina *et al.*,1984)**.

3) ***Protein S Deficiency***:

Protein S is a glycoprotein encoded by the gene *PROS1* on Chromosome 3. Many mutations impairing protein synthesis and/or function have been identified, which are found along the entire gene **(De Frutos *et al.*,2007)**. Hereditary protein S deficiency usually occurs as a partial (heterozygous) deficiency and is inherited in an autosomal-dominant manner. The prevalence of protein S deficiency is 0.1% in the general population **(Beauchamp *et al.*,2004)**.

Protein S deficiency is classified into three types:

1. type I (low total and free protein S antigen, reduced protein S activity).
2. type II (normal total and free antigen, reduced activity)
3. type III (normal total antigen level, and reduced free antigen level and activity).

Quantitative (type I/III) deficiencies account for up to 95% of all cases of protein S deficiency **(Castoldi *et al.*,2010)**.

It was found that inherited protein c, and protein s could be also considered causes of RPL **(Mekaj et al., 2015).**

The currently recommended test for diagnosing protein S deficiency is measuring free protein S **(Marlar and Gausman,2011)**.

**Table. I:** Recommendations for clinical laboratory testing for protein c, protein s ,and Antithrombin III

| Component to assay | First screening assay Used | Done if abnormal |
|---|---|---|
| Antithrombin | AT activity – factor Xa-based | AT antigen |
| Protein C | PC activity – chromogenic or clotting-based | PC antigen |
| Protein S | Free PS antigen | PS activity (total PS antigen) |

***4)Factor V Leiden***:

The gene encoding FV is located on the first chromosome(1q23), spans 70 kb, and consists of 25 exons.

The most common mutation on the FV gene causing FV Leiden is a missense mutation at position 506, where an arginine is replaced by glutamine Arg506 Gln, thus rendering FV resistant to degradation that result in a mild hypercoagulable state**(Dahlbäck,2008)**. FV Leiden is inherited in an autosomal-dominant manner and is present almost exclusively among white individuals, with a prevalence comprised between 3-5% for heterozygous FV Leiden in the general population and approximately 20% among patients with VTE **(Meyer *et al.*,2015)**.

It was found that there was a statistically significant association between FV Leiden and preeclampsia **(Elzein *et al.*,2020)**. There was an association between FV Leiden and RPL **(Liu *et al.*,2021)** . A recent meta-analysis done in Middle Eastern countries revealed that the prevalence of FVL mutation was 12.6% in patients with RPL loss compared to 4.9% in controls, with an odds ratio of 2.37 **(Hamedi *et al.*,2020)**.

FVL mutation had a significant association with recurrent first-trimester fetal losses **(Sergi *et al.*,2015)**. It was found that there was a 40% increase in the risk of IUGR in FVL mutation carriers **(Hemsworth *et al.*,2016)**

5) ***Prothrombin G20210A Polymorphism:***

Prothrombin is encoded by a gene that is located on chromosome 11p11-q1212, it affects approximately 1 in 2,000,000 individuals in the general population so, inherited prothrombin deficiency is considered one of the rarest bleeding disorders, this mutation is found in approximately 3–17% in patients with VTE and 1–8% of healthy controls **(Jadaon,2011)**.

Such congenital defects are almost due to a single missense mutation (guanine to adenine; G > A) at nucleotide position20210, which is present in the 3′ untranslated region of the prothrombin gene.

This mutation does not affect the structure of the prothrombin molecule and it does not affect its function but it increases prothrombin values approximately one-third above the normal range **(Soria *et al.*,2000)**. DNA analysis is the test of choice for diagnosing the prothrombin G20210A mutation **(Cooper *et al.*,2012)**.

It was found that prothrombin G20210A mutation increases the risk of RPL, particularly in Europeans and women older than 29 years **(Gao and Tao,2015)**.

PT G20210A mutation was twice as likely to experience RPL (odds ratio = 2.08) in comparison with those with wild genotypes **(Liu *et al.*,2021)**.

**Table.II:** Inherited thrombophilia and preferred approach to the diagnostic workup

| Inherited thrombophilia | Preferred test |
|---|---|
| **FVL** | APC resistance, genetic testing |
| **Prothrombin *G20210A*** | Genetic testing |
| **AT deficiency** | Functional assay |
| **PC deficiency** | Functional assay |
| **PS deficiency** | Free PS antigen |

***6)MTHFR polymorphism :***

A MTHFR mutant is one of the most widely reported thrombophilic mutants when evaluating patients experiencing RPL.**(Govindaiah *et al.*,2009)**.

The human MTHFR gene contains 11 exons, located on chromosome 1p36.3, and encodes methylene tetrahydrofolate reductase (MTHFR) a key enzyme in folate and homocysteine metabolism. MTHFR catalyzes the biologically irreversible reduction of 5,10-

methylenetetrahydrofolate to 5-methyltetrahydrofolate, which provides the methyl group for the remethylating of homocysteine to methionine **(Bailey and Gregory III,1999)**.

The two most important single nucleotide polymorphisms were C677T and A1298C, which can affect folate and total homocysteine (tHcy) status **(Frosst *et al.*,1995)**.

The MTHFR C677T polymorphism could be considered a cause of URPL **((Nguyen et al , 2022)**. It was found that women with MTHFR 677TT (homozygous mutation, TT) genotype have significantly lower vitamin D levels, higher homocysteine and natural killer (NK) cell cytotoxicities than those of women with MTHFR 677CC (wild type, CC) and 677CT (heterozygous mutation, CT) genotypes **(Ota *et al.*,2020)**.

It was found also that there was a significant association between hypermethylation of MTHFR promoter region in RPL patients compared to healthy controls**(Shaker,2021)**.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is considered the most widely used technique to detect MTHFR C677T mutation **(Ota *et al.*,2007)**.

## Conclusion

We concluded that inherited thrombophilia is considered a cause of RPL in unexplained cases and we recommend testing for inherited thrombophilia in women with recurrent miscarriages especially those history of venous thromboembolism and performing of clinical trials regarding the effectiveness of anticoagulants in treatment of women with recurrent miscarriages and inherited thrombophilia.

## References:

1. **Practice Committee of the American Society for Reproductive Medicine,(2020)**. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertility and Sterility* 113(3), 533-535**.**
2. **ESHRE Guideline Group, Bender Atik R, Christiansen OB, *et al.*,(2018)**. ESHRE guideline: recurrent pregnancy loss. *Human Reproduction Open* 2, hoy004 .doi.org/10.1093/hropen/hoy004**.**
3. **ESHRE Guideline Group,( 2017 )**.Early Pregnancy Guidline Development Group . *Guideline of the management of Recurrent pregnancy loss 2* ,1-153**.**
4. **Royal College of Obstetricians and Gynaecologists,(2011)**. Green top guide line number 17.The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage. *RCOG: London, UK***.**
5. **Wang X, Chen C, Wang L, *et al.*,(2003)**. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. *Fertility and Sterility* 79, 577-584**.**
6. **Magnus MC, Wilcox AJ, Morken NH, *et al.*,(2019)**. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. *bmj* 364,1869 doi:10.1136/bmj.l869.
7. **Werner M, Reh A, Grifo J, *et al.*,(2012)**. Characteristics of chromosomal abnormalities diagnosed after spontaneous abortions in an infertile population. *J Assist Reprod Genet* 29, 817-820.

8. **Chighizola C, Andreoli L, De Jesus GR, *et al.*,(2015).** The association between antiphospholipid antibodies and pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature. *Lupus* 24, 980-984**.**
9. **Turocy JM, and Rackow BW, (2019).** Uterine factor in recurrent pregnancy loss, Seminars in Perinatology. Elsevier, pp. 74-79**.**
10. **Deans R, and Abbott J,(2010).** Review of intrauterine adhesions. *Journal of Minimally Invasive Gynecology* 17, 555-569.
11. **Jaslow CR,(2014).** Uterine factors. *Obstetrics and Gynecology Clinics* 41, 57-86**.**
12. **Stevens SM, Woller SC, Bauer KA, *et al.*,(2016).** Guidance for the evaluation and treatment of hereditary and acquired thrombophilia. *J Thromb Thrombolysis* 41, 154-164**.**
13. **American College of Obstetricians and Gynecologists' Committee on Practice Bulletins–Obstetrics.ACOG Practice Bulletin No. 197,(2018).** Inherited Thrombophilias in Pregnancy. *Obstetrics and gynecology* 132(1), e18-e34**.**
14. **Hirahara F, Andoh N, Sawai K, *et al.*,(1998).** Hyperprolactinemic recurrent miscarriage and results of randomized bromocriptine treatment trials. *Fertility and Sterility* 70, 246-252**.**
15. **Godines-Enriquez MS, Miranda-Velásquez S, Enríquez-Pérez MM, *et al.*,(2021).** Prevalence of thyroid autoimmunity in women with recurrent pregnancy loss. *Medicina* 57(2), 96 doi.org/10.3390/medicina57020096.
16. **Glueck CJ, Wang P, Bornovali S, *et al.*,(2003).** Polycystic ovary syndrome, the G1691A factor V Leiden mutation, and plasminogen activator inhibitor activity: associations with recurrent pregnancy loss. *Metabolism* 52, 1627-1632**.**
17. **Jovanovic L, Knopp RH, Kim H, *et al.*,(2005).** Elevated pregnancy losses at high and low extremes of maternal glucose in early normal and diabetic pregnancy: evidence for a protective adaptation in diabetes .*Diabetes Care* 28, 1113-1117**.**
18. **Shah D, and Nagarajan N,(2013).** Luteal insufficiency in first trimester. *Indian journal of endocrinology and metabolism* 17, 44**.**
19. **McQueen DB, Zhang J, Robins JC,(2019).** Sperm DNA fragmentation and recurrent pregnancy loss: a systematic review and meta-analysis. *Fertility and Sterility* 112, 54-60.
20. **Christiansen OB, Steffensen R, Nielsen HS, *et al.*,(2008).** Multifactorial etiology of recurrent miscarriage and its scientific and clinical implications. *Gynecologic and obstetric investigation* 66, 257-267**.**
21. **Christiansen OB, Ulcova-Gallova Z, Mohapeloa H, *et al.*,(1998).** Studies on associations between human leukocyte antigen (HLA) class II alleles and antiphospholipid antibodies in Danish and Czech women with recurrent miscarriages. *Human reproduction (Oxford, England)* 13, 3326-3331**.**
22. **Winger EE, and Reed JL,(2011).** Low circulating CD4+ CD25+ Foxp3+ T regulatory cell levels predict miscarriage risk in newly pregnant women with a history of failure. *American Journal of Reproductive Immunology* 66, 320-328.

23. **Meuleman T, Lashley LE, Dekkers OM, *et al.*,(2015).** HLA associations and HLA sharing in recurrent miscarriage: a systematic review and meta-analysis. *Human immunology* 76, 362-373
24. **Pearson AC, and Mahmood TA, (2020).** Obesity and recurrent miscarriage, Obesity and Gynecology. Elsevier, pp. 91-96.
25. **Lassi ZS, Imam AM, Dean SV, *et al.*,(2014).** Preconception care: caffeine, smoking, alcohol, drugs and other environmental chemical/radiation exposure. *Reproduction Health* 11 Suppl 3,S6. doi.org/10.1186/1742-4755-11-S3-S6.
26. **Kutteh WH, (2015).** Novel strategies for the management of recurrent pregnancy loss, Seminars in reproductive medicine.p 161-168.
27. **Favaloro EJ, McDonald D, Lippi G, (2009).** Laboratory investigation of thrombophilia: the good, the bad, and the ugly, Seminars in thrombosis and hemostasis, pp. 695-710.
28. **Lane DA, Mannucci PM, Bauer KA, *et al.*,(1996).** Inherited thrombophilia: part 1. *Thrombosis and haemostasis* 76, 651-662.
29. **Crowther MA, and Kelton JG,(2003).** Congenital thrombophilic states associated with venous thrombosis: a qualitative overview and proposed classification system. *Annals of internal medicine* 138, 128-134.
30. **Benedetto C, Marozio L, Tavella AM, *et al.*,(2010).** Coagulation disorders in pregnancy: acquired and inherited thrombophilias. *Annals of the New York Academy of Sciences* 1205, 106-117.
31. **Egeberg O,(1965).** Inherited antithrombin deficiency causing thrombophilia. *Thrombosis and Haemostasis* 13, 516-530.
32. **Lane D, Bayston T, Olds R, *et al.*,(1997).** Antithrombin mutation database: 2nd (1997) update. *Thrombosis and Haemostasis* 77, 197-211.
33. **Corral J, de la Morena-Barrio ME, Vicente V,(2018).** The genetics of antithrombin. *Thrombosis Research* 169, 23-29.
34. **Patnaik MM, and Moll S,(2008).** Inherited antithrombin deficiency: a review. *Haemophilia* 14, 1229-1239.
35. **Favaloro EJ, Bonar R, Sioufi J, *et al.*, (2005).** Multilaboratory testing of thrombophilia: current and past practice in Australasia as assessed through the Royal College of Pathologists of Australasia Quality Assurance Program for Hematology, Seminars in thrombosis and hemostasis 1, pp. 49-58.
36. **Sabadell, J., Casellas, M., Alijotas-Reig, J., et al. , (2010).** Inherited antithrombin deficiency and pregnancy: maternal and fetal outcomes. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *149*(1), pp.47-51.
37. **Koster T, Vandenbroucke J, Rosendaal F, *et al.* ,(1995).** Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. *The Lancet* 345, 152-155.
38. **Melissari E, Monte G, Lindo V, *et al.*,(1992).** Congenital thrombophilia among patients with venous thromboembolism. *Blood coagulation & fibrinolysis: an international journal in haemostasis and thrombosis* 3, 749-758.

39. **Finazzi G, and Barbui T,(1994).** Different incidence of venous thrombosis in patients with inherited deficiencies of antithrombin III, protein C and protein S. *Thrombosis and Haemostasis* 71, 015-018**.**
40. **Khor B, and Van Cott EM,(2010).** Laboratory tests for protein C deficiency. *American journal of hematology* 85, 440-442**.**
41. **Kottke-Marchant K , and Comp P,(2002).** Laboratory issues in diagnosing abnormalities of protein C, thrombomodulin, and endothelial cell protein C receptor. *Archives of pathology & laboratory medicine* 126, 1337-1348**.**
42. **Bertina R, Broekmans A ,Krommenhoek-van Es, *et al.*,(1984).** The use of a functional and immunologic assay for plasma protein C in the study of the heterogeneity of congenital protein C deficiency. *Thrombosis and haemostasis* 51, 001-005**.**
43. **De Frutos PG, Fuentes-Prior P, Hurtado B, *et al.*,(2007).** Molecular basis of protein S deficiency. *Thrombosis and Haemostasis* 98, 543-556**.**
44. **Beauchamp NJ, Dykes AC, Parikh N, *et al.*,(2004).** The prevalence of, and molecular defects underlying, inherited protein S deficiency in the general population .*British journal of hematology* 125, 647-654**.**
45. **Castoldi E, Maurissen LF, Tormene D, *et al.*,(2010).** Similar hypercoagulable state and thrombosis risk in type I and type III protein S-deficient individuals from families with mixed type I/III protein S deficiency. *haematologica* 95(9), 1563-1571.
46. **Mekaj, Y., Lulaj, S., Daci, F., et al. (2015).** Prevalence and role of antithrombin III, protein C and protein S deficiencies and activated protein C resistance in Kosovo women with recurrent pregnancy loss during the first trimester of pregnancy. *Journal of human reproductive sciences*, *8*(4), p.224
47. **Marlar R, Gausman J,(2014).** Laboratory testing issues for protein C ,protein S, and antithrombin. *International Journal of Laboratory Hematology* 36, 289-295**.**
48. **Dahlbäck B,(2008).**Advances in understanding pathogenic mechanisms of thrombophilic disorders. *Blood, The Journal of the American Society of Hematology* 112, 19-27**.**
49. **Meyer MR, Witt DM, Delate T, *et al.*,(2015).** Thrombophilia testing patterns amongst patients with acute venous thromboembolism. *Thrombosis Research* 136, 1160-1164.
50. **Elzein HO, Saad AA, Yousif AA, *et al.*,(2020).** Evaluation of Factor V Leiden and prothrombin G20210A mutations in Sudanese women with severe preeclampsia. *Current research in translational medicine* 68, 77-80**.**
51. **Liu X, Chen Y, Ye C, *et al.*,(2021).** Hereditary thrombophilia and recurrent pregnancy loss: a systematic review and meta-analysis. *Human reproduction* 36, 1213-1229**.**
52. **Hamedi B, Feulefack J, Khan A, *et al.*,(2020).** Association between factor V Leiden mutation and recurrent pregnancy loss in the middle east countries: a Newcastle–Ottawa meta-analysis. *Archives of Gynecology and Obstetrics* 302, 345-354**.**

53. **Sergi C, Al Jishi T, Walker M,(2015)**. Factor V Leiden mutation in women with early recurrent pregnancy loss: a meta-analysis and systematic review of the causal association. *Archives of Gynecology and Obstetrics* 291, 671-679**.**
54. **Hemsworth EM, O'Reilly AM, Allen VM, *et al.*,(2016)**. Association between factor v leiden mutation, small for gestational age, and preterm birth: a systematic review and meta-analysis. *Journal of obstetrics and gynaecology Canada* 38, 897-908**.**
55. **Jadaon MM,(2011)**. Epidemiology of prothrombin G20210A mutation in the Mediterranean region. *Mediterranean journal of hematology and infectious diseases* 3(1):e2011054. DOI: 10.4084/MJHID.2011.054**.**
56. **Soria JM, Almasy L, Souto JC, *et al.*,(2000)**. Linkage analysis demonstrates that the prothrombin G20210A mutation jointly influences plasma prothrombin levels and risk of thrombosis. *Blood, The Journal of the American Society of Hematology* 95, 2780-2785**.**
57. **Cooper PC, Goodeve AC, Beauchamp NJ, (2012)**. Quality in molecular biology testing for inherited thrombophilia disorders, Seminars in thrombosis and hemostasis. Thieme Medical Publishers, pp. 600-612**.**
58. **Gao H, and Tao Fb,(2015)**. Prothrombin G20210A mutation is associated with recurrent pregnancy loss: a systematic review and meta-analysis update. *Thrombosis Research* 135, 339-346**.**
59. **Govindaiah V, Naushad SM, Prabhakara K, *et al.*,(2009)**. Association of parental hyperhomocysteinemia and C677T Methylene tetrahydrofolate reductase (MTHFR) polymorphism with recurrent pregnancy loss. *Clinical biochemistry* 42, 380-386**.**
60. **Bailey LB, and Gregory III JF,(1999)**. Folate metabolism and requirements. *The Journal of nutrition* 129, 779-782**.**
61. **Frosst P, Blom H, Milos R, *et al.*,(1995)**. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature genetics* 10, 111-113**.**
62. **Nguyen NN, Tran TM, Trieu TS, et al.,(2022)**. Evaluating the Association Between Genetic Polymorphisms Related to Homocysteine Metabolism and Unexplained Recurrent Pregnancy Loss in Women. The Application of Clinical Genetics 15, 55-62**.**
63. **Ota K, Takahashi T, Han A, *et al.*,(2020)**. Effects of MTHFR C677T polymorphism on vitamin D, homocysteine and natural killer cell cytotoxicity in women with recurrent pregnancy losses. *Human reproduction* 35, 1276-1287**.**
64. **Shaker MM,(2021)**. Correlation of methylation status in MTHFR promoter region with recurrent pregnancy loss. *Journal of Genetic Engineering and Biotechnology* 19, 1-9**.**
65. **Ota M, Fukushima H, Kulski JK, *et al.*,(2007)**. Single nucleotide polymorphism detection by polymerase chain reaction-restriction fragment length polymorphism. *Nature protocols* 2, 2857-2864**.**