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Recurrent Pregnancy Loss: A Comprehensive Review of Etiology, Pathophysiology, Diagnostic Approaches, and Therapeutic Strategies

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ABSTRACT

Recurrent pregnancy loss (RPL) is a significant reproductive health problem affecting about 2–3% of women of reproductive age. It is a multifactorial condition involving genetic, anatomical, endocrine, thrombophilic, immunological, and environmental factors, with nearly half of cases remaining unexplained. This review summarizes the current understanding of the causes, mechanisms, diagnosis, and treatment of RPL.

A systematic literature review was conducted using PubMed, Scopus, Web of Science, and the Cochrane Library for studies published between 2010 and 2024. The review focused on genetic and thrombophilic factors, diagnostic methods, and treatment approaches, with particular attention to findings from Bulgarian and Eastern European populations.

The results show that RPL is associated with chromosomal abnormalities, inherited thrombophilia (such as Factor V Leiden and MTHFR polymorphisms), uterine anomalies, endocrine disorders, antiphospholipid syndrome, immune dysfunction, and lifestyle factors. Genetic variations such as the M2/ANXA5 haplotype may contribute to placental dysfunction and pregnancy loss. Diagnosis requires a comprehensive evaluation including genetic testing, thrombophilia screening, uterine imaging, and hormonal assessment. Treatment depends on the underlying cause and may include surgical correction of uterine anomalies, anticoagulant therapy, progesterone supplementation, hormonal treatment, and genetic counseling.

Understanding the complex causes of RPL and integrating advanced diagnostic tools may improve patient management and reduce unexplained cases through more personalized treatment strategies.

1. INTRODUCTION

1.1 Definition and Epidemiology of Recurrent Pregnancy Loss

Recurrent pregnancy loss (RPL) represents one of the most clinically challenging and emotionally distressing conditions in reproductive medicine, affecting approximately 2-3% of couples attempting to conceive (ESHRE Guideline Group on RPL, 2023). The condition is defined by the loss of two or more pregnancies before 20-24 weeks of gestation, though definitions vary slightly across professional societies. The European Society of Human Reproduction and Embryology (ESHRE) defines RPL as the loss of two or more pregnancies, while the American Society for Reproductive Medicine (ASRM) historically required three or more losses, though recent guidelines have moved toward including two or more clinical pregnancies (Practice Committee of ASRM, 2020).

The psychological impact of RPL cannot be overstated. Each pregnancy loss represents not only the loss of a potential child but also the erosion of hope, self-esteem, and relationship stability. Couples experiencing RPL report levels of anxiety and depression comparable to those seen in patients with life-threatening illnesses, and the cumulative emotional burden increases with each subsequent loss (Kolte et al., 2015). The financial costs of RPL are also substantial, encompassing medical evaluations, treatments, and lost productivity.

The extreme fragility of early pregnancy renders it susceptible to a wide range of biological, hereditary, and environmental insults. While sporadic pregnancy loss is common—occurring in 15-20% of clinically recognized pregnancies—RPL represents a distinct pathological entity requiring systematic evaluation and management (Carp, 2020). Understanding the multifactorial nature of RPL is essential for developing effective diagnostic and therapeutic strategies.

1.2 Historical Perspective and Evolving Concepts

The recognition of RPL as a distinct clinical entity has evolved substantially over the past century. Early descriptions attributed recurrent losses to maternal constitutional factors or "habitual abortion," with little understanding of underlying mechanisms.

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The mid-20th century saw the identification of chromosomal abnormalities as a cause of pregnancy loss, fundamentally shifting understanding toward genetic factors (Capra et al., 2022).

The latter decades of the 20th century brought recognition of anatomical factors (uterine anomalies, cervical insufficiency), endocrine disorders (thyroid dysfunction, diabetes), and immunological mechanisms. The discovery of antiphospholipid syndrome in the 1980s established thrombophilia as a treatable cause of RPL and opened the door to anticoagulation therapy (Cozaru et al., 2019).

The 21st century has witnessed explosive growth in understanding genetic contributions to RPL. The identification of specific thrombophilic polymorphisms—Factor V Leiden, Factor II G20210A, MTHFR C677T, and PAI-1 4G/5G—has enabled more precise risk stratification (Levkova et al., 2020). More recently, the discovery of the M2/ANXA5 haplotype and its role in placental thrombosis has provided new insights into the pathophysiology of unexplained RPL (Ang et al., 2019; Tüttelmann et al., 2023).

1.3 The Burden of Unexplained RPL

Despite extensive investigation, approximately 50% of RPL cases remain unexplained (ESHRE Guideline Group on RPL, 2023). This "unexplained" category represents a significant clinical challenge and a source of frustration for both patients and providers. Couples with unexplained RPL often feel that their suffering is not validated and that they have been denied the opportunity for targeted treatment.

The high proportion of unexplained cases reflects several realities: (1) our incomplete understanding of all factors contributing to pregnancy maintenance; (2) limitations in current diagnostic technologies; (3) the probability that multiple subtle factors may interact to produce pregnancy loss; and (4) the role of chance in any reproductive outcome.

Emerging research suggests that some cases currently classified as unexplained may have identifiable causes using advanced techniques. Preimplantation genetic testing (PGT) can detect embryonic chromosomal abnormalities not present in parental karyotypes (Hadjidekova et al., 2022). Novel biomarkers, including those derived from platelet and erythrocyte morphology, may identify thrombophilic tendencies not captured by conventional testing (Andreeva et al., 2021; Langari et al., 2022). The continued evolution of diagnostic capabilities offers hope for reducing the unexplained category and enabling more targeted interventions.

1.4 Regional Perspectives: RPL Research in Bulgaria

Bulgarian researchers have made significant contributions to the understanding of RPL, particularly in the areas of thrombophilic genetic polymorphisms and their role in pregnancy loss. Levkova et al. (2020) investigated the frequency of thrombophilic gene polymorphisms (Factor V Leiden G1691A, Factor II G20210A, PAI-1 4G/5G, and MTHFR C677T) in Bulgarian women with RPL, identifying Factor V Leiden as the strongest thrombophilic etiological factor. Andreeva et al. (2021) provided novel insights into the structural and nanomechanical abnormalities of platelets in women with inherited thrombophilia, demonstrating increased expression of procoagulant surface markers and significant alteration of membrane Young modulus.

The role of the M2/ANXA5 haplotype in RPL has been extensively studied in Bulgarian populations. Ang et al. (2019) conducted a meta-analysis demonstrating that women with the M2/ANXA5 haplotype had 1.54 odds of RPL compared to those with normal haplotype, with male partners also contributing to risk. Tüttelmann et al. (2023) confirmed a strong association between M2/ANXA5 and early fetal losses at 10-15 weeks gestation in German and Bulgarian women.

Ivanov et al. (2020) identified polymorphism A1/A2 in the β3 subunit of integrins αIIb/β3 and αV/β3 as an etiological factor for implantation and placentation disturbances leading to RPL. Robeva et al. (2022) explored the role of melatonin receptor polymorphisms in reproductive failure, opening new avenues for understanding circadian and oxidative stress contributions to pregnancy loss.

This rich body of Bulgarian research informs the current review and highlights the importance of regional genetic backgrounds in shaping RPL risk.

1.5 Objectives of This Review

This comprehensive review aims to:

1. **Synthesize current knowledge** regarding the multifactorial etiology of recurrent pregnancy loss, with particular emphasis on genetic, thrombophilic, anatomical, endocrine, and immunological factors.
2. **Elucidate the pathophysiological mechanisms** linking identified etiological factors to pregnancy loss, including alterations in placental function, vascular integrity, cellular morphology, and immune regulation.
3. **Review diagnostic approaches** for RPL evaluation, including traditional and emerging techniques, and propose evidence-based algorithms for clinical practice.
4. **Examine therapeutic strategies** for RPL management, including surgical interventions, pharmacological treatments, and reproductive options.
5. **Identify knowledge gaps** and prioritize directions for future research in RPL pathophysiology, diagnosis, and treatment.
6. **Highlight regional contributions** to RPL research, particularly from Bulgarian investigators, and consider implications for personalized medicine based on population-specific genetic factors.

2. DEFINITIONS AND CLASSIFICATION

2.1 Clinical Definitions

The definition of recurrent pregnancy loss has evolved over time and varies among professional societies, creating challenges for research comparability and clinical standardization.

2.1.1 ESHRE Definition

The European Society of Human Reproduction and Embryology (ESHRE) defines recurrent pregnancy loss as the loss of two or more pregnancies before 24 weeks of gestation (ESHRE Guideline Group on RPL, 2023). This definition includes both consecutive and non-consecutive losses and encompasses biochemical pregnancies (confirmed by positive hCG) as well as clinical pregnancies. The inclusion of two

rather than three losses reflects recognition that even two losses warrant investigation given the cumulative emotional burden and the potential to identify treatable causes.

2.1.2 ASRM Definition

The American Society for Reproductive Medicine historically defined RPL as three or more pregnancy losses, based on epidemiological data showing that the risk of subsequent loss does not increase substantially until after three losses (Practice Committee of ASRM, 2020). However, recent ASRM guidelines have moved toward a more nuanced approach, recommending evaluation after two losses if they are first-trimester, and after one loss if it is second-trimester, given the higher likelihood of identifiable pathology in later losses.

2.1.3 Other Professional Societies

The Royal College of Obstetricians and Gynaecologists (RCOG) defines RPL as three or more consecutive pregnancy losses before 24 weeks. The International Federation of Gynecology and Obstetrics (FIGO) has endorsed a definition of two or more pregnancy failures, aligning more closely with ESHRE.

2.1.4 Implications of Definitional Variation

Variation in definitions affects:

- Prevalence estimates (higher with two-loss definition)
- Timing of investigation initiation
- Comparability of research studies
- Patient access to evaluation and treatment

Clinicians should be aware of guideline differences and consider individual patient factors (age, infertility, previous live births, patient anxiety) when deciding when to initiate evaluation.

2.2 Classification by Gestational Age

Pregnancy losses can be classified by gestational age, with different etiological profiles at different stages.

2.2.1 Biochemical Pregnancy Loss

Loss occurring before ultrasound visualization of a gestational sac, typically <5-6 weeks. Etiology is predominantly chromosomal abnormalities of the embryo, though endometrial receptivity factors may contribute.

2.2.2 Early Pregnancy Loss (First Trimester)

Loss occurring between 6-12 weeks. Chromosomal abnormalities account for approximately 50-60% of sporadic losses, but the proportion in RPL may be lower. Anatomical, endocrine, and thrombophilic factors become increasingly relevant.

2.2.3 Late Pregnancy Loss (Second Trimester)

Loss occurring between 12-24 weeks. Anatomical factors (cervical insufficiency, uterine anomalies), thrombophilia, and antiphospholipid syndrome are more prominent etiologies, while chromosomal abnormalities play a lesser role.

2.3 Classification by Etiology

RPL can be classified based on underlying etiological factors:

- **Genetic RPL**: Chromosomal abnormalities in parents or embryo; single-gene disorders
- **Anatomical RPL**: Congenital or acquired uterine anomalies
- **Endocrine RPL**: Thyroid dysfunction, diabetes, PCOS, luteal phase deficiency
- **Thrombophilic RPL**: Inherited thrombophilia, antiphospholipid syndrome
- **Immunological RPL**: Autoimmune disorders, alloimmune factors
- **Infectious RPL**: Chronic endometritis, specific infections
- **Environmental RPL**: Smoking, alcohol, obesity, toxins
- **Unexplained RPL**: No identifiable cause after comprehensive evaluation

This classification guides diagnostic evaluation and treatment selection but recognizes that many patients have multiple contributing factors.

3. EPIDEMIOLOGY AND RISK FACTORS

3.1 Prevalence and Incidence

The prevalence of RPL depends on the definition used and population characteristics:

- **Using two-loss definition**: Affects approximately 2-3% of reproductive-aged women
- **Using three-loss definition**: Affects approximately 1-2% of reproductive-aged women
- **Recurrence risk**: After one loss, risk of subsequent loss is approximately 15-20% (similar to baseline); after two losses, risk increases to 25-30%; after three losses, risk reaches 40-45%

3.2 Maternal Age

Maternal age is one of the strongest risk factors for both sporadic and recurrent pregnancy loss:

- **Age <35 years**: RPL risk approximately 2-3%
- **Age 35-40 years**: RPL risk increases to 5-8%
- **Age 40-45 years**: RPL risk increases to 15-20%
- **Age >45 years**: RPL risk exceeds 30-40%

The age-related increase in RPL risk reflects primarily the increasing incidence of embryonic chromosomal abnormalities due to oocyte aneuploidy, though endometrial factors may also contribute.

3.3 Paternal Age

Advanced paternal age (>40-45 years) has been associated with increased risk of pregnancy loss in some studies, though the effect is smaller than for maternal age. Proposed mechanisms include increased sperm DNA fragmentation, de novo mutations, and epigenetic alterations.

3.4 Number of Previous Losses

The number of previous losses is a powerful predictor of future loss risk:

- **0 previous losses**: Baseline risk 15-20%
- **1 previous loss**: Risk 15-20% (no increase)
- **2 previous losses**: Risk 25-30%
- **3 previous losses**: Risk 40-45%
- **≥4 previous losses**: Risk >50%

This increasing risk likely reflects the cumulative probability of underlying pathology being present and perhaps a "kindling" effect whereby repeated losses alter endometrial receptivity or immune responses.

3.5 Previous Live Birth

Parity influences RPL risk in complex ways. Primary RPL (no prior live births) and secondary RPL (prior live birth followed by losses) may have different etiological profiles. Secondary RPL is more likely to be associated with acquired uterine

pathology (Asherman's syndrome, leiomyomas), antiphospholipid syndrome, or new-onset endocrine disorders.

3.6 Family History

A family history of RPL suggests possible inherited factors, including:

- Thrombophilic mutations (autosomal dominant with variable penetrance)
- Balanced translocations (may run in families)
- Autoimmune disorders (familial aggregation)

3.7 Obesity

Obesity (BMI >30 kg/m²) is an independent risk factor for RPL, with odds ratios of 1.5-2.0 compared to normal-weight women. Mechanisms include:

- Insulin resistance and hyperinsulinemia
- Altered adipokine profiles (leptin, adiponectin)
- Chronic low-grade inflammation
- Endometrial dysfunction
- Increased risk of aneuploidy

3.8 Smoking and Alcohol

Cigarette smoking doubles the risk of pregnancy loss and contributes to RPL through:

- Direct embryotoxicity
- Impaired placental blood flow
- Oxidative stress
- Accelerated oocyte depletion

Alcohol consumption, even at moderate levels, increases pregnancy loss risk, with effects likely mediated by direct embryotoxicity and impaired implantation.

3.9 Geographic and Ethnic Variations

RPL risk varies across populations due to differences in:

- Prevalence of thrombophilic mutations (Factor V Leiden is more common in Caucasian populations, rare in Asian populations)
- Consanguinity rates (increased risk of recessive disorders)
- Environmental exposures
- Healthcare access and diagnostic practices

Bulgarian populations have been extensively studied for thrombophilic polymorphisms, with prevalence rates comparable to other European populations but with some unique features in haplotype distribution (Levkova et al., 2020; Ang et al., 2019).

4. ETIOLOGY OF RECURRENT PREGNANCY LOSS

4.1 Genetic Factors

4.1.1 Parental Chromosomal Abnormalities

Approximately 2-5% of couples with RPL carry a chromosomal abnormality, compared to 0.2% in the general population (Capra et al., 2022). The most common abnormalities include:

Balanced Reciprocal Translocations: Occur when two chromosomes exchange segments without loss of genetic material. Carriers are phenotypically normal but produce unbalanced gametes with a high probability of embryonic aneuploidy. Translocation carriers have a 50-70% risk of pregnancy loss depending on the specific chromosomes involved.

Robertsonian Translocations: Involve fusion of two acrocentric chromosomes (13, 14, 15, 21, 22) at the centromere. Carriers have 45 chromosomes but are phenotypically normal. Risk of unbalanced offspring and pregnancy loss varies by specific translocation.

Inversions: Occur when a chromosome segment is reversed. Paracentric inversions (not involving the centromere) and pericentric inversions (involving the centromere) can both lead to unbalanced gametes.

Sex Chromosome Abnormalities: Including 45,X mosaicism and 47,XXY, may be associated with RPL, particularly when present in the female partner.

Chromosomal Polymorphisms: Variants such as increased heterochromatin or satellite polymorphisms have been controversially associated with RPL. Angelova et al. (2021) reported on chromosomal polymorphisms in Bulgarian patients with reproductive problems, suggesting potential relevance.

4.1.2 Embryonic Chromosomal Abnormalities

The majority of sporadic pregnancy losses (50-60%) are due to embryonic chromosomal abnormalities, primarily aneuploidy. In RPL, the proportion of aneuploid losses is lower (30-50%), suggesting that recurrent losses in a given couple may not be due to chance aneuploidy but rather to other factors (Carp, 2020).

Preimplantation genetic testing (PGT) can identify embryonic chromosomal abnormalities and select euploid embryos for transfer, though the utility of PGT for improving live birth rates in RPL remains debated (Hadjidekova et al., 2022).

4.1.3 Single Gene Disorders

Rare single-gene disorders may present as RPL, particularly when the condition is lethal in utero. Examples include:

- Inherited thrombophilias (see Section 4.4)
- Metabolic disorders affecting fetal development
- Disorders of placental development

4.1.4 Thrombophilic Gene Polymorphisms

Inherited thrombophilia results from genetic polymorphisms that increase the risk of venous thromboembolism and, through placental thrombosis, pregnancy loss. Key polymorphisms include:

Factor V Leiden (G1691A) : The most common inherited thrombophilia in Caucasian populations. Factor V Leiden renders Factor V resistant to cleavage by activated protein C, creating a hypercoagulable state. Levkova et al. (2020) identified Factor V Leiden as the strongest thrombophilic etiological factor associated with RPL in Bulgarian women, with higher concentrations of homozygotes in RPL patients compared to controls. Odds ratios for RPL range from 2-4 for heterozygotes and higher for homozygotes.

Factor II (Prothrombin) G20210A: Increases prothrombin levels, promoting thrombin generation. Associated with a 2-3 fold increased risk of RPL. Levkova et al. (2020) reported increased frequency in Bulgarian RPL patients.

Methylenetetrahydrofolate Reductase (MTHFR) Polymorphisms: The C677T and A1298C variants reduce MTHFR enzyme activity, leading to elevated homocysteine levels. Hyperhomocysteinemia is associated with increased thrombosis risk and may directly impair embryonic development. However, the role of MTHFR polymorphisms in RPL remains controversial, with some studies showing

association and others not (Sowmya et al., 2022). Mihaila (2020) noted that in Bulgaria, the role of MTHFR C677T in RPL is still debated.

Plasminogen Activator Inhibitor-1 (PAI-1) 4G/5G Polymorphism: The 4G allele increases PAI-1 expression, reducing fibrinolysis and promoting thrombosis. Sosic et al. (2020) reported that the combination of PAI-1 4G/5G and MTHFR C677T predicts RPL risk in Eastern European women.

4.1.5 M2/ANXA5 Haplotype

The M2/ANXA5 haplotype represents one of the most significant recent discoveries in RPL genetics. Annexin A5 (ANXA5) is a protein with potent anticoagulant activity, forming a protective shield over phospholipid surfaces to prevent excessive coagulation. The M2 haplotype in the ANXA5 promoter reduces gene expression, impairing this protective function (Ang et al., 2019).

Ang et al. (2019) conducted a meta-analysis demonstrating that women with the M2/ANXA5 haplotype had 1.54 odds of RPL (95% CI 1.27-1.87) compared to women without the haplotype. Notably, male partners with the M2 haplotype also contributed to RPL risk, suggesting that paternal haplotype affects placental function through expression in trophoblast cells derived from the fetal genome.

Tüttelmann et al. (2023) confirmed a strong association between M2/ANXA5 and "early" fetal losses at 10-15 weeks gestation in German and Bulgarian women. The haplotype appears particularly relevant for losses occurring after the first trimester, when placental function becomes critical for pregnancy maintenance.

4.1.6 Integrin Polymorphisms

Ivanov et al. (2020) identified polymorphism A1/A2 in the β3 subunit of integrins αIIb/β3 and αV/β3 as an etiological factor for RPL. Integrins mediate trophoblast-endometrial interactions during implantation, and polymorphisms affecting their function may impair implantation and placentation.

4.1.7 Melatonin Receptor Polymorphisms

Robeva et al. (2022) explored melatonin receptor 1B polymorphisms in Bulgarian women with reproductive failure. Melatonin regulates circadian rhythms and has antioxidant properties; polymorphisms affecting receptor function may impact oocyte quality and endometrial receptivity.

4.2 Anatomical Factors

4.2.1 Congenital Uterine Anomalies

Congenital uterine anomalies result from abnormal fusion or resorption of the Müllerian ducts during embryonic development. They affect approximately 5-10% of women with RPL, compared to 1-2% of the general population (Carbonnel et al., 2021).

Septate Uterus: The most common anomaly in RPL patients (accounting for 40-50% of congenital anomalies). A fibromuscular septum divides the uterine cavity, impairing implantation and placentation due to poor vascularization and altered endometrial receptivity. Associated with first-trimester losses and preterm birth. Surgical resection (hysteroscopic metroplasty) improves pregnancy outcomes.

Bicornuate Uterus: Two uterine horns with a single cervix, resulting from incomplete fusion. Associated with second-trimester losses and preterm labor due to reduced intrauterine volume and altered myometrial function.

Unicornuate Uterus: Single uterine horn with a rudimentary horn, resulting from arrested development of one Müllerian duct. Associated with increased RPL risk due to reduced cavity size and potential implantation in the rudimentary horn.

Didelphic Uterus: Complete duplication of uterus and cervix, resulting from complete failure of Müllerian fusion. Associated with RPL and preterm birth, though some women achieve successful pregnancies.

Arcuate Uterus: Mild indentation of the fundus, often considered a normal variant but possibly associated with slightly increased RPL risk.

4.2.2 Acquired Uterine Anomalies

Uterine Leiomyomas (Fibroids) : Benign smooth muscle tumors affecting 20-40% of reproductive-aged women. Submucosal leiomyomas distort the endometrial cavity and impair implantation, increasing RPL risk. Intramural leiomyomas may also affect pregnancy outcomes through altered uterine blood flow and contractility. Myomectomy improves pregnancy outcomes in women with submucosal or large intramural fibroids (Carbonnel et al., 2021).

Endometrial Polyps: Benign endometrial overgrowths that can interfere with implantation through mechanical disruption and altered endometrial receptivity. Hysteroscopic polypectomy is recommended before attempting pregnancy.

Intrauterine Adhesions (Asherman's Syndrome) : Result from endometrial trauma (typically from curettage), leading to synechiae that obliterate the uterine cavity. Implantation failure and pregnancy loss result from reduced endometrial surface area and altered receptivity. Hysteroscopic adhesiolysis restores cavity architecture and improves outcomes.

Adenomyosis: Endometrial tissue within the myometrium, associated with altered uterine contractility and implantation dysfunction. Increasingly recognized as a contributor to RPL, though optimal treatment remains undefined.

Cervical Insufficiency: Painless cervical dilation in the second trimester leading to pregnancy loss. May result from congenital cervical weakness, prior cervical trauma, or surgical procedures (cone biopsy, LEEP). Cervical cerclage reduces loss risk in selected patients.

4.3 Endocrine Factors

4.3.1 Thyroid Disorders

Overt Hypothyroidism: Clearly associated with increased pregnancy loss risk through multiple mechanisms including impaired folliculogenesis, reduced fertilization rates, and altered endometrial receptivity. Thyroid hormone replacement (levothyroxine) normalizes risk when instituted before pregnancy.

Subclinical Hypothyroidism (SCH) : Defined as elevated TSH with normal free T4. Associated with increased RPL risk, with odds ratios of 1.5-2.5. Treatment with levothyroxine is recommended by most guidelines for women with RPL and SCH, though evidence from randomized trials is limited.

Thyroid Autoimmunity: Presence of thyroid peroxidase (TPO) or thyroglobulin antibodies, even with normal thyroid function, increases RPL risk approximately 2-3 fold. Mechanisms may include direct effects on oocyte and embryo quality, impaired implantation, and associated immune dysfunction.

Levothyroxine treatment in euthyroid antibody-positive women remains controversial but is often considered in RPL patients.

4.3.2 Diabetes Mellitus

Overt Diabetes: Poorly controlled diabetes (elevated HbA1c) is strongly associated with increased pregnancy loss risk due to hyperglycemia-induced embryopathy and placental dysfunction. Preconception glycemic control reduces risk.

Gestational Diabetes: Does not cause early pregnancy loss but is associated with later complications.

Insulin Resistance: Even without overt diabetes, insulin resistance (common in PCOS) may contribute to RPL through effects on endometrial receptivity and oocyte quality.

4.3.3 Polycystic Ovary Syndrome (PCOS)

PCOS affects 5-10% of reproductive-aged women and is associated with increased RPL risk (odds ratios 2-3). Contributing mechanisms include:

- Insulin resistance and hyperinsulinemia
- Hyperandrogenism
- Altered endometrial receptivity
- Increased LH levels
- Obesity
- Associated thrombophilia

Treatment strategies include lifestyle modification, insulin sensitizers (metformin), and ovulation induction, though evidence for improved live birth rates in RPL is limited.

4.3.4 Luteal Phase Deficiency (LPD)

LPD refers to inadequate progesterone production by the corpus luteum, leading to impaired endometrial receptivity. Diagnosis is controversial due to lack of standardized criteria. Progesterone supplementation in early pregnancy may benefit a subset of women with RPL, particularly those with a history of bleeding in early pregnancy (ESHRE Guideline Group on RPL, 2023).

4.3.5 Hyperprolactinemia

Elevated prolactin inhibits gonadotropin secretion and may impair folliculogenesis and luteal function. Dopamine agonists (bromocriptine, cabergoline) normalize prolactin and may improve pregnancy outcomes.

4.3.6 Vitamin D Deficiency

Vitamin D receptors are present in endometrium and placenta, and vitamin D regulates genes involved in implantation and immune function. Deficiency has been associated with increased RPL risk, though evidence for supplementation improving outcomes is limited.

4.4 Thrombophilic Factors

4.4.1 Inherited Thrombophilia

As discussed in Section 4.1.4, inherited thrombophilia increases RPL risk through placental thrombosis. Key conditions include:

- Factor V Leiden (G1691A)
- Factor II (Prothrombin) G20210A
- Protein C deficiency
- Protein S deficiency
- Antithrombin deficiency
- MTHFR polymorphisms (controversial)
- PAI-1 polymorphisms

The risk of RPL is highest for women with combined thrombophilic defects or homozygosity for high-risk mutations.

4.4.2 Antiphospholipid Syndrome (APS)

APS is an acquired autoimmune thrombophilia characterized by antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies, anti-β2-glycoprotein I antibodies). APS is present in 5-15% of women with RPL, compared to 1-5% of the general population (Dugalic et al., 2019).

Diagnostic Criteria (Revised Sapporo Criteria) require at least one clinical criterion (pregnancy morbidity including RPL, thrombosis) and one laboratory criterion (positive antiphospholipid antibodies on two occasions ≥12 weeks apart).

Pathophysiology: Antiphospholipid antibodies activate endothelial cells, platelets, and complement, promoting thrombosis. They also directly impair trophoblast function and placentation.

Treatment: Combination of low-dose aspirin and heparin (unfractionated or low-molecular-weight) improves live birth rates from approximately 10-40% to 70-80%.

4.4.3 Structural and Nanomechanical Platelet Abnormalities

Andreeva et al. (2021) provided novel insights into thrombophilic mechanisms in RPL, demonstrating that inherited thrombophilia triggers structural and nanomechanical alterations in platelets. Using atomic force microscopy, they showed:

- Prominent cytoskeletal arrangement
- Reduced membrane roughness
- Increased expression of procoagulant surface markers (CD62P, CD63)
- Significant alteration of membrane Young modulus (stiffness)

These changes render platelets more susceptible to activation and aggregation, promoting thrombosis in the placental microvasculature. The findings suggest that platelet morphology could serve as a biomarker for thrombophilic risk in RPL.

4.4.4 Erythrocyte Abnormalities

Langari et al. (2022) investigated erythrocyte morphology in women with early pregnancy loss, finding accelerated erythrocyte aging characterized by:

- Faster transformation of morphological shape
- Reduced membrane roughness
- Altered nanomechanical properties

These changes may reflect systemic hypercoagulability and oxidative stress, contributing to impaired placental blood flow.

4.5 Immunological Factors

4.5.1 Autoimmune Disorders

Systemic Lupus Erythematosus (SLE) : Associated with increased RPL risk through multiple mechanisms including antiphospholipid antibodies, placental inflammation, and thrombotic complications. Disease activity should be optimized before pregnancy.

Other Autoimmune Conditions: Rheumatoid arthritis, scleroderma, and other autoimmune diseases may increase RPL risk, particularly if associated with antiphospholipid antibodies.

4.5.2 Natural Killer (NK) Cells

Uterine NK cells play critical roles in implantation and placentation, regulating trophoblast invasion and spiral artery

remodeling. Abnormalities in NK cell number or activity have been associated with RPL, though assessment and treatment remain controversial.

Peripheral NK Cells: Elevated peripheral NK cell numbers or cytotoxicity have been reported in some RPL patients. However, correlation with uterine NK cells is poor, and clinical utility is uncertain.

Uterine NK Cells: Assessment requires endometrial biopsy; increased uterine NK cell density has been associated with RPL. Immunomodulatory treatments (corticosteroids, intralipid, IVIG) have been used but lack strong evidence support.

4.5.3 Human Leukocyte Antigen (HLA) Compatibility

Excessive HLA similarity between partners may impair maternal immune recognition of the embryo as "foreign," preventing appropriate immune modulation. HLA-G polymorphisms affecting expression have been associated with RPL.

4.5.4 Cytokine Imbalances

Imbalances between pro-inflammatory (Th1) and anti-inflammatory (Th2) cytokines may affect implantation and pregnancy maintenance. Elevated Th1 cytokines (IFN-γ, TNF-α) have been associated with RPL, while Th2 cytokines (IL-4, IL-10) promote pregnancy success.

4.6 Infectious Factors

4.6.1 Chronic Endometritis

Chronic endometritis is a persistent inflammation of the endometrial lining, typically caused by bacterial infection. Organisms implicated include *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and various streptococci and staphylococci (McQueen et al., 2021).

Diagnosis: Hysteroscopy with endometrial biopsy and culture or PCR. Hysteroscopic findings include focal or diffuse endometrial hyperemia, micropolyps, and stromal edema.

Treatment: Antibiotic therapy based on culture results improves subsequent pregnancy outcomes in women with RPL.

4.6.2 Other Infections

While acute infections can cause sporadic pregnancy loss, chronic infections are rarely implicated in RPL. *Toxoplasma gondii*, *Cytomegalovirus*, *Herpes simplex*, and other pathogens have been studied but lack consistent association with RPL.

4.7 Environmental and Lifestyle Factors

4.7.1 Smoking

Active smoking doubles RPL risk. Effects are dose-dependent and persist even after cessation for several years. Mechanisms include direct embryotoxicity, placental insufficiency, oxidative stress, and accelerated oocyte depletion.

4.7.2 Alcohol

Alcohol consumption increases RPL risk, with effects at moderate intake levels (>2 drinks/week). Complete abstinence during pregnancy and preconception is recommended.

4.7.3 Caffeine

High caffeine intake (>200-300 mg/day) has been associated with increased pregnancy loss risk in some studies. Moderate consumption appears safe.

4.7.4 Occupational and Environmental Exposures

Exposures to heavy metals (lead, mercury), organic solvents, pesticides, and radiation have been associated with increased RPL risk in some studies. Occupational history should be assessed.

4.7.5 Stress

Psychological stress may contribute to RPL through neuroendocrine effects on the HPA axis and immune function. However, stress is difficult to quantify, and causal relationships remain unclear.

4.8 Unexplained RPL

Despite comprehensive evaluation, approximately 50% of RPL cases remain unexplained (ESHRE Guideline Group on RPL, 2023). This "unexplained" category likely represents a heterogeneous group with:

- Undetected genetic abnormalities
- Multiple subtle factors interacting to cause loss
- Embryonic factors not captured by parental testing
- Limitations in current diagnostic capabilities
- Chance clustering of losses

Management of unexplained RPL focuses on supportive care, lifestyle optimization, and often empirical treatments with varying evidence support (progesterone, low-dose aspirin). The high rate of successful pregnancy even without treatment (50-70%) should be emphasized to provide hope.

5. PATHOPHYSIOLOGY OF RECURRENT PREGNANCY LOSS

5.1 Overview of Pathophysiological Mechanisms

The pathophysiological processes leading to RPL are diverse and directly related to the underlying etiological factors. These mechanisms can be broadly categorized as affecting:

1. **Implantation and early embryonic development** (genetic abnormalities, endometrial dysfunction, anatomical factors)
2. **Placental development and function** (thrombophilia, immunological factors, uterine anomalies)
3. **Fetal development and survival** (genetic abnormalities, endocrine disorders)
4. **Uteroplacental circulation** (thrombophilia, antiphospholipid syndrome, anatomical factors)
5. **Maternal-fetal immune interaction** (immunological factors, HLA compatibility)

5.2 Genetic Mechanisms

5.2.1 Chromosomal Abnormalities

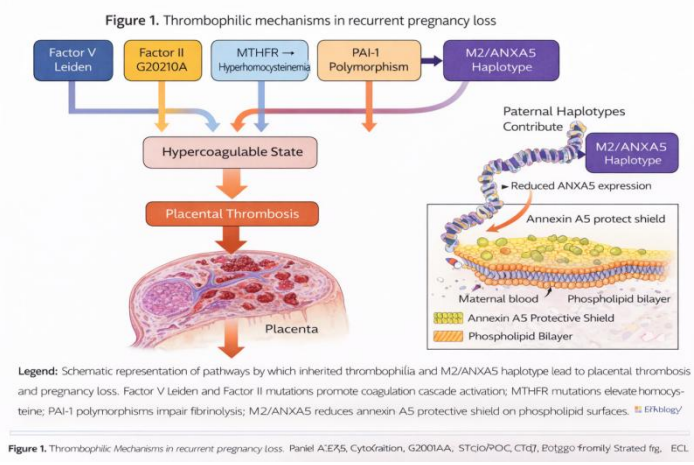
Embryonic chromosomal abnormalities prevent normal development through:

- **Gain or loss of critical genes**: Aneuploidy disrupts dosage-sensitive gene expression, leading to developmental failure
- **Impaired cell division**: Abnormal chromosome numbers disrupt mitosis and tissue organization
- **Activation of apoptotic pathways**: Cells with abnormal genetic content undergo programmed cell death
- **Placental dysfunction**: Trophoblast cells with abnormal chromosomes fail to form functional placenta

5.2.2 Thrombophilic Mechanisms

Inherited thrombophilia promotes pregnancy loss through placental thrombosis (Figure 1). Key steps include:

Figure 1. Thrombophilic Mechanisms in Recurrent Pregnancy Loss



Legend: Schematic representation of pathways by which inherited thrombophilia and M2/ANXA5 haplotype lead to placental thrombosis and pregnancy loss. Factor V Leiden and Factor II mutations promote coagulation cascade activation; MTHFR mutations elevate homocysteine; PAI-1 polymorphisms impair fibrinolysis; M2/ANXA5 reduces annexin A5 protective shield on phospholipid surfaces.

Hypercoagulable State: Thrombophilic mutations shift hemostatic balance toward thrombosis through:

- Factor V Leiden: Resistance to activated protein C
- Factor II G20210A: Increased prothrombin levels
- Protein C/S/antithrombin deficiencies: Reduced natural anticoagulants
- MTHFR polymorphisms: Hyperhomocysteinemia (endothelial injury and procoagulant effects)
- PAI-1 4G/5G: Impaired fibrinolysis

Placental Thrombosis: The placental vasculature is particularly vulnerable to thrombosis due to:

- Low-flow, high-resistance circulation
- Extensive surface area for thrombus formation
- Unique hemostatic requirements during development

Thrombosis of spiral arteries, intervillous spaces, or fetal vessels impairs maternal-fetal exchange, leading to hypoxia, infarction, and pregnancy loss.

ANXA5 Protective Shield Disruption: Annexin A5 forms a two-dimensional crystal over phospholipid surfaces, preventing access of coagulation factors. The M2/ANXA5 haplotype reduces ANXA5 expression, thinning this protective shield and allowing thrombosis (Ang et al., 2019; Tüttelmann et al., 2023). In trophoblast cells (derived from fetal tissue), paternal M2 haplotype contributes to risk, explaining the role of male partners.

5.2.3 Structural and Nanomechanical Cellular Abnormalities

Andreeva et al. (2021) elucidated novel pathophysiological mechanisms linking thrombophilia to RPL:

- **Platelet activation**: Inherited thrombophilia triggers increased expression of procoagulant surface markers (CD62P, CD63)
- **Cytoskeletal reorganization**: Prominent cytoskeletal arrangement alters platelet morphology
- **Reduced membrane roughness**: Atomic force microscopy demonstrates smoother platelet surfaces, potentially enhancing platelet-platelet interactions
- **Altered Young modulus**: Changes in membrane stiffness affect platelet deformability and activation

These abnormalities create a population of "primed" platelets more susceptible to activation and aggregation, promoting thrombosis.

Langari et al. (2022) demonstrated similar abnormalities in erythrocytes from women with early pregnancy loss:

- Accelerated morphological transformation
- Reduced membrane roughness
- Impaired deformability affecting microcirculatory flow

5.2.4 Integrin-Mediated Implantation Failure

Integrins αIIb/β3 and αV/β3 mediate trophoblast-endometrial interactions during implantation. The A1/A2 polymorphism identified by Ivanov et al. (2020) affects integrin function, potentially impairing:

- Trophoblast adhesion to endometrium
- Trophoblast migration and invasion
- Signaling for decidualization
- Placental anchoring

5.3 Anatomical Mechanisms

5.3.1 Impaired Implantation

Uterine structural abnormalities disrupt implantation through:

- **Reduced surface area**: Septa and adhesions reduce available endometrial surface for implantation
- **Poor vascularization**: Septa are relatively avascular, unable to support developing pregnancy
- **Altered endometrial receptivity**: Distorted cavity alters gene expression and receptivity
- **Mechanical interference**: Leiomyomas and polyps physically impede implantation

5.3.2 Placental Insufficiency

Anomalies affecting cavity shape (bicornuate, didelphic uterus) reduce intrauterine volume and distort placental development, leading to:

- Placenta previa or accreta
- Placental insufficiency
- Preterm labor
- Fetal growth restriction

5.3.3 Cervical Insufficiency

Cervical insufficiency results from structural weakness of the cervical stroma, leading to painless dilation and second-trimester loss. Mechanisms include:

- Congenital short cervix or poor collagen content
- Prior cervical trauma disrupting structural integrity
- Incompetent internal os

5.4 Endocrine Mechanisms

5.4.1 Thyroid Dysfunction

Thyroid hormones regulate multiple aspects of reproduction:

- **Oocyte quality**: Thyroid hormone receptors in granulosa cells; hypothyroidism impairs folliculogenesis

- **Endometrial receptivity**: Thyroid hormones regulate endometrial gene expression and decidualization
- **Placental development**: Trophoblast expresses thyroid hormone receptors; hormone deficiency impairs placentation
- **Fetal neurodevelopment**: Severe hypothyroidism directly affects fetal brain development

Thyroid autoimmunity may cause pregnancy loss through:

- Direct effects of antibodies on trophoblast
- Associated immune dysfunction
- Subtle thyroid dysfunction not detected by TSH alone

5.4.2 Hyperhomocysteinemia

MTHFR polymorphisms and folate deficiency elevate homocysteine, which causes:

- Endothelial dysfunction and injury
- Procoagulant effects (platelet activation, factor V activation)
- Direct embryotoxicity
- Impaired DNA methylation (epigenetic effects)

5.4.3 Insulin Resistance and Hyperinsulinemia

Insulin resistance (common in PCOS and obesity) affects:

- Endometrial receptivity through altered gene expression
- Oocyte quality via metabolic effects on granulosa cells
- Plasminogen activator inhibitor levels (increasing thrombosis risk)

5.4.4 Progesterone Deficiency

Progesterone is essential for:

- Endometrial decidualization
- Maintaining endometrial receptivity
- Suppressing uterine contractility
- Modulating maternal immune response

Luteal phase deficiency impairs these functions, compromising implantation and early pregnancy maintenance.

5.5 Immunological Mechanisms

5.5.1 Antiphospholipid Antibody Effects

Antiphospholipid antibodies cause pregnancy loss through multiple mechanisms:

- **Thrombotic**: Activate endothelial cells, platelets, and complement; promote thrombosis
- **Non-thrombotic**: Directly impair trophoblast function, inhibiting proliferation, migration, and invasion
- **Inflammatory**: Activate complement, causing placental inflammation
- **Impaired placentation**: Disrupt spiral artery remodeling

5.5.2 NK Cell Dysfunction

Uterine NK cells normally promote successful pregnancy by:

- Regulating trophoblast invasion
- Promoting spiral artery remodeling
- Secreting angiogenic factors
- Modulating local immune responses

Excessive NK cell numbers or cytotoxicity can:

- Damage trophoblast cells
- Promote apoptosis
- Secrete inflammatory cytokines
- Impair vascular remodeling

5.5.3 Cytokine Imbalance

Successful pregnancy requires a shift from pro-inflammatory (Th1) to anti-inflammatory (Th2) immune responses. Th1 cytokines (IFN-γ, TNF-α) promote:

- Trophoblast apoptosis
- Activation of macrophages and NK cells
- Thrombosis through endothelial activation

Th2 cytokines (IL-4, IL-10) support:

- Trophoblast growth and function
- Immune tolerance
- Placental development

5.6 Multifactorial Interactions

In many RPL patients, multiple pathophysiological mechanisms interact:

- Thrombophilia and anatomical factors: Uterine anomaly increases thrombosis risk
- Genetic and endocrine factors: MTHFR mutation and thyroid dysfunction both elevate homocysteine
- Immunological and thrombophilic factors: Antiphospholipid antibodies and inherited thrombophilia synergize

Understanding these interactions is essential for comprehensive patient evaluation and treatment.

6. DIAGNOSTIC APPROACH TO RECURRENT PREGNANCY LOSS

6.1 Principles of RPL Evaluation

The diagnostic evaluation of couples with RPL should be:

- **Comprehensive**: Addressing all potential etiological categories
- **Systematic**: Following a logical sequence from least to most invasive
- **Individualized**: Tailored to patient characteristics and history
- **Evidence-based**: Utilizing tests with proven clinical utility
- **Patient-centered**: Considering emotional impact and cost

6.2 Initial Evaluation: History and Physical Examination

6.2.1 Medical History

Detailed history should include:

Pregnancy History:

- Number, timing, and gestational age of each loss
- Method of pregnancy confirmation (hCG, ultrasound)
- Presence of fetal cardiac activity before loss
- Symptoms associated with losses (pain, bleeding)
- Prior live births and their outcomes
- Prior pregnancies with same or different partners

Menstrual History:

- Cycle regularity and length
- Symptoms of ovulatory dysfunction
- Previous fertility treatment

Medical History:

- Thrombotic events (deep vein thrombosis, pulmonary embolism)

- Autoimmune disorders (SLE, antiphospholipid syndrome)
- Endocrine disorders (thyroid disease, diabetes)
- Infections
- Surgical history (particularly uterine or cervical procedures)

Family History:

- Pregnancy losses in siblings, parents
- Thrombotic events
- Autoimmune diseases
- Genetic disorders

Lifestyle Factors:

- Smoking, alcohol, caffeine
- Occupational exposures
- Medications and supplements

6.2.2 Physical Examination

Physical examination may identify:

- Signs of endocrine disorders (goiter, hirsutism, acanthosis nigricans)
- Uterine enlargement or irregularity
- Cervical abnormalities
- Signs of thrombophilia (varicose veins, prior thrombotic scars)

6.3 Genetic Evaluation

6.3.1 Parental Karyotyping

Karyotyping of both partners is recommended for couples with RPL (ESHRE Guideline Group on RPL, 2023). Indications include:

- Two or more pregnancy losses
- One loss if structural abnormality suspected
- Abnormal offspring or family history of genetic disorders

Karyotyping identifies balanced translocations, inversions, and other structural abnormalities in 2-5% of couples. Findings guide genetic counseling and reproductive options.

6.3.2 Analysis of Products of Conception (POC)

Chromosomal analysis of POC from a loss provides valuable information:

- Determines if loss was aneuploid (reassuring if caused by chance abnormality)
- Identifies recurrent abnormalities suggesting parental translocation
- May prevent unnecessary testing if loss was aneuploid

Techniques include conventional karyotyping, chromosomal microarray (CMA), and quantitative fluorescence PCR (QF-PCR). CMA offers higher resolution and detects submicroscopic abnormalities.

6.3.3 Preimplantation Genetic Testing (PGT)

PGT involves biopsy of embryos created through in vitro fertilization (IVF) and testing for chromosomal abnormalities or specific genetic disorders (Hadjidekova et al., 2022).

PGT-A (for aneuploidy) : Screens embryos for chromosome number abnormalities. May reduce miscarriage rate in couples with RPL by selecting euploid embryos, though randomized trials have not consistently shown improved live birth rates.

PGT-SR (for structural rearrangements) : Identifies embryos with unbalanced translocations in couples where one partner carries a balanced translocation.

PGT-M (for monogenic disorders) : Tests for specific single-gene disorders when indicated.

PGT should be offered with genetic counseling to discuss benefits, limitations, and alternatives.

6.3.4 Thrombophilia Genetic Testing

Testing for inherited thrombophilia is recommended in RPL evaluation (ESHRE Guideline Group on RPL, 2023). Panel typically includes:

- Factor V Leiden (G1691A)
- Factor II (Prothrombin) G20210A
- MTHFR C677T and A1298C (controversial)
- PAI-1 4G/5G

Testing for protein C, protein S, and antithrombin deficiencies requires functional assays rather than genetic testing due to multiple possible mutations.

6.3.5 M2/ANXA5 Haplotype Testing

Given accumulating evidence of association with RPL (Ang et al., 2019; Tüttelmann et al., 2023), testing for M2/ANXA5 haplotype may be considered in unexplained RPL, particularly in European populations where prevalence is higher.

6.4 Anatomical Evaluation

6.4.1 Ultrasound

Two-Dimensional (2D) Ultrasound:

- Initial screening for uterine anomalies, fibroids, polyps, adhesions
- Performed in follicular phase for optimal endometrial visualization
- Limited sensitivity for subtle anomalies

Three-Dimensional (3D) Ultrasound:

- Superior for diagnosing congenital uterine anomalies
- Enables coronal view of uterine cavity and fundal contour
- Distinguishes septate from bicornuate uterus
- Non-invasive alternative to hysteroscopy/laparoscopy

Saline Infusion Sonohysterography (SIS) :

- Saline infused into uterine cavity during ultrasound
- Excellent for detecting intracavitary lesions (polyps, fibroids, adhesions)
- Outperforms standard ultrasound for cavity assessment

6.4.2 Hysteroscopy

Hysteroscopy is the gold standard for evaluating uterine cavity (Carbonnel et al., 2021):

- Direct visualization of cavity, ostia, and endometrium
- Diagnoses subtle abnormalities missed by imaging
- Enables simultaneous treatment (polypectomy, adhesiolysis, septum resection)
- Can assess for chronic endometritis (endometrial biopsy)

Office hysteroscopy is minimally invasive and well-tolerated.

6.4.3 Magnetic Resonance Imaging (MRI)

MRI provides detailed assessment of:

- Complex congenital anomalies
- Adenomyosis
- Deep infiltrating endometriosis

- Myometrial involvement of fibroids

Useful when ultrasound findings are inconclusive or for surgical planning.

6.4.4 Laparoscopy

Laparoscopy is reserved for cases where:

- Ultrasound cannot distinguish septate from bicornuate uterus (combined with hysteroscopy)
- Suspected endometriosis or pelvic adhesions
- Concurrent tubal assessment needed

6.5 Endocrine Evaluation

6.5.1 Thyroid Function Tests

Recommended tests include:

- TSH (thyroid stimulating hormone)
- Free T4 (if TSH abnormal)
- TPO antibodies (thyroid peroxidase antibodies)
- Thyroglobulin antibodies (optional)

Abnormal TSH should be repeated and evaluated for treatment.

6.5.2 Diabetes Screening

- Fasting plasma glucose or HbA1c
- Oral glucose tolerance test if risk factors present

6.5.3 PCOS Evaluation

- Androgen levels (testosterone, androstenedione, DHEAS)
- LH/FSH ratio
- Pelvic ultrasound for polycystic ovarian morphology
- Assessment of ovulatory function

6.5.4 Progesterone Assessment

- Mid-luteal progesterone to assess ovulation and luteal function
- Endometrial biopsy for luteal phase dating (rarely performed)

6.5.5 Prolactin

- Serum prolactin (elevated in hyperprolactinemia)

6.5.6 Vitamin D

- 25-hydroxyvitamin D levels

6.6 Thrombophilia Evaluation

6.6.1 Inherited Thrombophilia

As described in 6.3.4, includes genetic testing and functional assays.

6.6.2 Antiphospholipid Syndrome

Testing requires (Dugalic et al., 2019):

- Lupus anticoagulant (functional assay)
- Anticardiolipin antibodies (IgG, IgM)
- Anti-β2-glycoprotein I antibodies (IgG, IgM)

Positive tests must be confirmed after ≥12 weeks per diagnostic criteria.

6.6.3 Novel Biomarkers

Emerging tests include:

- Platelet morphology assessment (atomic force microscopy) (Andreeva et al., 2021)
- Erythrocyte morphology (Langari et al., 2022)
- Thrombin generation assays
- Global coagulation assays

6.7 Immunological Evaluation

6.7.1 Autoimmune Screening

- Antinuclear antibodies (ANA)
- Extractable nuclear antigens (ENA)
- Rheumatoid factor
- Other autoantibodies based on symptoms

6.7.2 NK Cell Testing

- Peripheral NK cell numbers and cytotoxicity (flow cytometry)
- Uterine NK cell density (endometrial biopsy)

6.7.3 Cytokine Profiles

- Th1/Th2 cytokine ratios (research setting)

6.7.4 HLA Typing

- HLA class I and II typing of both partners (research setting)

6.8 Infectious Evaluation

- Endometrial biopsy for chronic endometritis (culture or PCR)
- Screening for specific infections based on risk factors

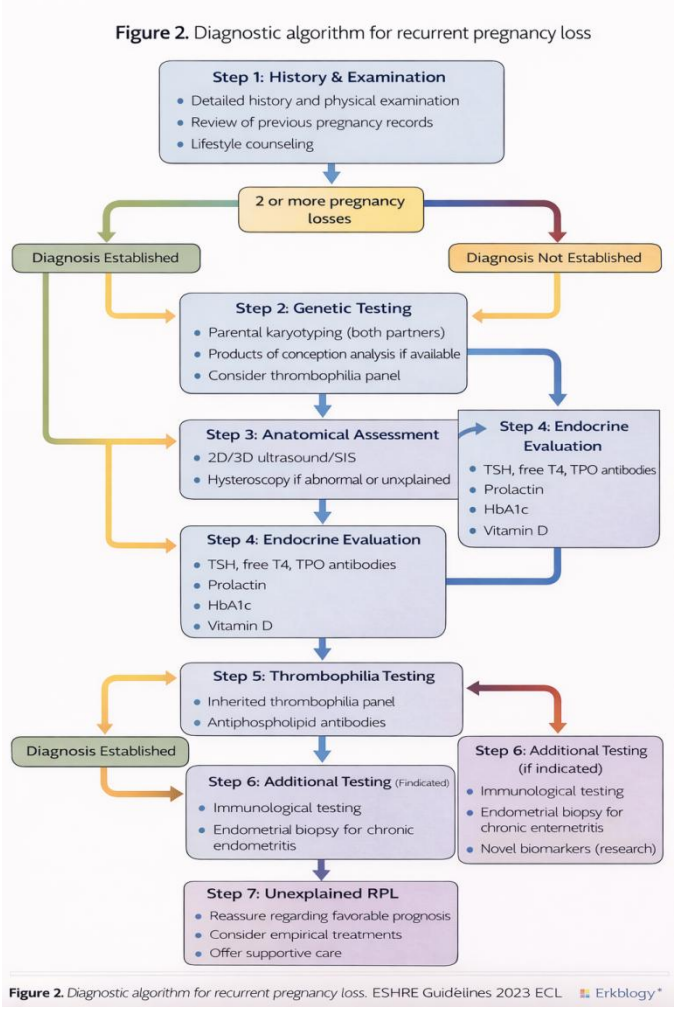
6.9 Psychosocial Assessment

- Screening for anxiety, depression, and relationship stress
- Referral to mental health professionals as indicated

6.10 Diagnostic Algorithms

Figure 2 presents a diagnostic algorithm for RPL evaluation based on ESHRE guidelines (2023) and current evidence.

Figure 2. Diagnostic Algorithm for Recurrent Pregnancy Loss



Legend: Flowchart illustrating stepwise evaluation of couples with RPL, beginning with history and physical examination,

proceeding through genetic, anatomical, endocrine, thrombophilic, and immunological testing, with treatment decisions based on findings.

Step 1: Initial Consultation
- Detailed history and physical examination
- Review of previous pregnancy records
- Lifestyle counseling

Step 2: Genetic Testing
- Parental karyotyping (both partners)
- POC analysis if available
- Consider thrombophilia panel

Step 3: Anatomical Assessment
- 2D/3D ultrasound or SIS
- Hysteroscopy if abnormal or unexplained

Step 4: Endocrine Evaluation
- TSH, free T4, TPO antibodies
- Prolactin
- HbA1c
- Vitamin D

Step 5: Thrombophilia Testing
- Inherited thrombophilia panel
- Antiphospholipid antibodies

Step 6: Additional Testing (if indicated)
- Immunological testing
- Endometrial biopsy for chronic endometritis
- Novel biomarkers (research)

Step 7: Unexplained RPL
- Reassure regarding favorable prognosis
- Consider empirical treatments
- Offer supportive care

7. TREATMENT AND MANAGEMENT OF RECURRENT PREGNANCY LOSS

7.1 Principles of RPL Management

Treatment of RPL should be:
- **Evidence-based**: Guided by results of diagnostic testing
- **Individualized**: Tailored to identified etiologies and patient preferences
- **Multidisciplinary**: Involving reproductive specialists, genetic counselors, mental health professionals
- **Supportive**: Addressing emotional needs alongside medical treatment
- **Realistic**: Providing accurate prognosis information

7.2 Genetic Counseling and Reproductive Options

7.2.1 Genetic Counseling

All couples with RPL should receive genetic counseling when:
- Abnormal parental karyotype identified
- Multiple aneuploid losses
- Family history of genetic disorders
- Considering PGT

Counseling addresses:
- Recurrence risks based on specific findings
- Reproductive options
- Prenatal testing options in future pregnancies
- Psychological impact

7.2.2 Natural Pregnancy

For couples with unexplained RPL or low-risk genetic findings, natural pregnancy with close monitoring is appropriate. Live birth rates in subsequent pregnancies are 50-70% even without treatment.

7.2.3 Preimplantation Genetic Testing (PGT)

PGT may be offered when:
- Parental balanced translocation/inversion
- Recurrent aneuploidy in POC
- Advanced maternal age with high aneuploidy risk
- Single-gene disorders

PGT requires IVF, with associated costs, risks, and emotional burden. Success depends on embryo number and quality.

7.2.4 Gamete Donation

Donor gametes (oocyte or sperm) eliminate genetic abnormalities from the affected partner. Consider when:
- Severe genetic abnormality with high recurrence risk
- Failed PGT cycles
- Advanced maternal age with poor oocyte quality

7.2.5 Adoption

Adoption provides option for family building without pregnancy. Should be discussed as one of several possibilities.

7.3 Surgical Treatment of Anatomical Factors

7.3.1 Hysteroscopic Septum Resection

Septate uterus is corrected by hysteroscopic metroplasty (septum resection) (Carbonnel et al., 2021):
- **Technique**: Hysteroscopic incision of septum using scissors, resectoscope, or laser
- **Timing**: Follicular phase to allow endometrial healing
- **Outcomes**: Improves live birth rates from approximately 5-30% to 50-80%
- **Risks**: Uterine perforation, fluid overload, intrauterine adhesions

7.3.2 Myomectomy

Submucosal and intramural fibroids affecting the cavity should be removed:
- **Hysteroscopic myomectomy**: For submucosal fibroids
- **Laparoscopic or abdominal myomectomy**: For intramural or subserosal fibroids
- **Outcomes**: Improves pregnancy rates, reduces miscarriage risk
- **Timing**: Allow 3-6 months for uterine healing before attempting pregnancy

7.3.3 Polypectomy

Endometrial polyps should be removed hysteroscopically. Simple polypectomy improves implantation and reduces miscarriage risk.

7.3.4 Adhesiolysis

Intrauterine adhesions (Asherman's syndrome) require hysteroscopic adhesiolysis:
- **Technique**: Division of adhesions with scissors or electrode
- **Post-operative**: Estrogen therapy to promote re-epithelialization
- **Follow-up**: Second-look hysteroscopy to confirm cavity restoration
- **Outcomes**: Restores menstruation and improves pregnancy rates

7.3.5 Cervical Cerclage

Cervical insufficiency is treated with cerclage:

- **Indications**: History-indicated (prior second-trimester losses), ultrasound-indicated (short cervix), or physical examination-indicated (dilated cervix)
- **Timing**: Usually placed at 12-14 weeks
- **Types**: McDonald (purse-string), Shirodkar (higher placement), transabdominal (for failed vaginal cerclage)
- **Outcomes**: Reduces preterm birth and pregnancy loss in appropriately selected patients

7.4 Endocrine Treatments

7.4.1 Thyroid Hormone Replacement

Levothyroxine for hypothyroidism:

- **Overt hypothyroidism**: Titrate to achieve TSH in pregnancy-specific range (<2.5 mIU/L)
- **Subclinical hypothyroidism**: Treatment recommended by most guidelines for RPL patients
- **Thyroid autoimmunity with euthyroidism**: Controversial; some guidelines recommend low-dose levothyroxine (25-50 μg/day)

Dosing should be optimized before pregnancy and monitored throughout gestation.

7.4.2 Progesterone Supplementation

Progesterone support is recommended for:

- **Luteal phase deficiency**: 200-400 mg vaginal progesterone daily from ovulation to 12 weeks
- **Early pregnancy bleeding in RPL patients**: ESHRE guidelines suggest vaginal progesterone (400 mg twice daily) may improve outcomes (ESHRE Guideline Group on RPL, 2023)
- **IVF cycles**: Standard luteal phase support

Evidence for progesterone in unexplained RPL without bleeding is limited.

7.4.3 Metformin

Metformin improves insulin sensitivity and may benefit RPL patients with:

- PCOS with insulin resistance
- Impaired glucose tolerance
- Obesity

Typical dose: 1500-2000 mg/day in divided doses. Continue through conception and first trimester.

7.4.4 Dopamine Agonists

For hyperprolactinemia:

- Bromocriptine (2.5-5 mg/day) or cabergoline (0.5-1 mg/week)
- Titrate to normalize prolactin
- Discontinue once pregnancy confirmed

7.4.5 Vitamin D Supplementation

For deficient patients: 1000-2000 IU/day vitamin D3. Optimal dosing for RPL prevention not established.

7.5 Thrombophilia Treatments

7.5.1 Anticoagulation for Inherited Thrombophilia

Treatment recommendations vary by thrombophilia type and history (ESHRE Guideline Group on RPL, 2023):

Low-risk thrombophilia (Factor V Leiden heterozygous, Factor II heterozygous):

- Antepartum surveillance with postpartum prophylaxis or
- Low-dose aspirin

High-risk thrombophilia (homozygous mutations, combined defects, protein C/S/antithrombin deficiency):

- Low-molecular-weight heparin (LMWH) throughout pregnancy
- Continue 6 weeks postpartum

Dosing: LMWH at prophylactic doses (e.g., enoxaparin 40 mg daily). Adjust for weight and renal function.

7.5.2 Antiphospholipid Syndrome (APS)

Standard treatment for obstetric APS (Dugalic et al., 2019):

- Low-dose aspirin (75-100 mg daily) preconception
- Add LMWH (prophylactic dose) once pregnancy confirmed
- Continue throughout pregnancy and 6 weeks postpartum

Treatment improves live birth rates from 10-40% to 70-80%.

7.5.3 M2/ANXA5 Haplotype

No established treatment, but low-dose aspirin or LMWH may be considered given thrombotic mechanism. Clinical trials are needed.

7.5.4 Folate Supplementation

For MTHFR polymorphisms or elevated homocysteine:

- High-dose folic acid (1-5 mg daily) preconception and through first trimester
- Consider adding vitamin B12 and B6

7.6 Immunological Treatments

7.6.1 Corticosteroids

Used in selected immunological indications:

- Prednisolone (10-20 mg/day) for some autoimmune disorders
- Not routinely recommended for unexplained RPL due to side effects

7.6.2 Intravenous Immunoglobulin (IVIG)

IVIG has been used for:

- Elevated NK cell activity
- Autoimmune conditions
- Unexplained RPL with immune dysfunction

Evidence is mixed, and treatment is expensive. Not routinely recommended.

7.6.3 Intralipid Infusions

Intralipid (soybean oil emulsion) may suppress NK cell activity. Used in some centers for elevated NK cells, but evidence limited.

7.6.4 TNF-α Inhibitors

Adalimumab and other TNF inhibitors have been used in RPL with elevated Th1 cytokines. Limited evidence, potential risks.

7.6.5 Hydroxychloroquine

Used in SLE and APS; may have immunomodulatory benefits in RPL with autoimmune features.

7.7 Treatment of Chronic Endometritis

- **Antibiotic therapy**: Based on culture results (doxycycline, metronidazole, ciprofloxacin, or combination)
- **Duration**: Typically 10-14 days
- **Follow-up**: Repeat endometrial biopsy to confirm eradication

- **Outcomes**: Improved pregnancy rates after treatment

7.8 Lifestyle Modifications

7.8.1 Smoking Cessation

- Strongly advise complete cessation
- Offer nicotine replacement therapy or other cessation aids
- Partner should also stop smoking

7.8.2 Alcohol Avoidance

- Complete abstinence during preconception and pregnancy
- Partner should moderate intake

7.8.3 Weight Management

- BMI optimization (18.5-24.9 kg/m²)
- Diet and exercise counseling
- Consider referral to weight management programs

7.8.4 Stress Reduction

- Counseling or support groups
- Mind-body interventions (yoga, meditation)
- Acupuncture (limited evidence)

7.8.5 Occupational Modifications

- Avoid exposure to toxins, radiation, excessive heat

7.9 Supportive Care

7.9.1 Early Pregnancy Monitoring

- Early ultrasound (6-7 weeks) to confirm viability
- Serial hCG monitoring in some cases
- Reassurance and support

7.9.2 Psychological Support

- Acknowledge emotional impact of RPL
- Offer referral to mental health professionals
- Support groups for couples with RPL
- Partner involvement in care

7.9.3 Multidisciplinary Care

- Coordination between reproductive specialists, obstetricians, maternal-fetal medicine
- Genetic counseling when indicated
- Social work support for practical concerns

7.10 Treatment Algorithms

Table 1 summarizes treatment approaches based on identified etiologies.

Table 1. Treatment Strategies for Recurrent Pregnancy Loss by Etiology

Etiology	Treatment	Evidence Level
Parental balanced translocation	Genetic counseling; PGT-SR; gamete donation	Moderate
Uterine septum	Hysteroscopic septum resection	Moderate-High
Submucosal fibroids	Hysteroscopic myomectomy	Moderate
Intrauterine adhesions	Hysteroscopic adhesiolysis	Moderate
Cervical insufficiency	Cervical cerclage	High
Overt hypothyroidism	Levothyroxine to normalize TSH	High
Subclinical hypothyroidism	Levothyroxine (TSH <2.5)	Moderate
Thyroid autoimmunity	Levothyroxine (controversial)	Low-Moderate
Luteal phase deficiency	Progesterone supplementation	Moderate
PCOS with insulin resistance	Metformin; lifestyle modification	Moderate
Hyperprolactinemia	Dopamine agonists	High
Antiphospholipid syndrome	Low-dose aspirin + LMWH	High
Inherited thrombophilia (high-risk)	LMWH prophylaxis	Moderate
Inherited thrombophilia (low-risk)	Aspirin or surveillance	Low-Moderate
M2/ANXA5 haplotype	Consider aspirin/LMWH (research)	Very Low
Chronic endometritis	Antibiotics based on culture	Moderate
Unexplained RPL	Supportive care; consider progesterone	Low-Moderate

8. PROGNOSIS AND OUTCOMES

8.1 Live Birth Rates in Subsequent Pregnancies

Prognosis for couples with RPL varies based on:

- **Number of previous losses**: Live birth rates decrease with increasing number of losses
  - After 2 losses: 70-75% live birth rate
  - After 3 losses: 60-65% live birth rate
  - After ≥4 losses: 50-55% live birth rate
- **Maternal age**: Strongly influences prognosis
  - Age <35: 65-75% live birth rate
  - Age 35-40: 55-65% live birth rate
  - Age >40: 35-45% live birth rate
- **Underlying etiology**: Treatable causes (APS, uterine anomalies) have good prognosis with treatment; unexplained RPL has 50-70% live birth rate even without treatment
- **Prior live birth**: Secondary RPL may have slightly better prognosis than primary RPL

8.2 Factors Predicting Successful Outcome

- Younger maternal age
- Fewer previous losses
- Identified and treatable etiology
- Normal embryonic karyotype in previous loss
- Achievement of pregnancy within 6 months of trying

8.3 Psychological Outcomes

RPL has significant psychological impact:

- **Anxiety**: 30-50% of women experience clinically significant anxiety in subsequent pregnancies
- **Depression**: 20-30% meet criteria for depression
- **PTSD**: 10-20% develop post-traumatic stress disorder symptoms
- **Relationship strain**: Communication difficulties, blame, sexual dysfunction

Psychological support should be integrated into RPL care.

9. SPECIAL CONSIDERATIONS

9.1 Recurrent Pregnancy Loss in Bulgaria: Genetic Epidemiology

Bulgarian researchers have made significant contributions to understanding RPL genetics:

- **Factor V Leiden**: Prevalence 5-8% in Bulgarian population, strongly associated with RPL (Levkova et al., 2020)
- **Factor II G20210A**: Prevalence 2-3%, increased in RPL patients

- **MTHFR C677T**: High prevalence (40-50% heterozygous, 10-15% homozygous), but role in RPL debated (Mihaila, 2020)
- **M2/ANXA5**: Prevalence in Bulgarian RPL patients consistent with European populations (Ang et al., 2019; Tüttelmann et al., 2023)
- **Integrin polymorphisms**: Identified in Bulgarian RPL population (Ivanov et al., 2020)
- **Melatonin receptor polymorphisms**: Preliminary findings in Bulgarian women (Robeva et al., 2022)

These findings highlight importance of population-specific genetic factors in RPL and support inclusion of thrombophilia and M2/ANXA5 testing in Bulgarian RPL patients.

9.2 Male Factor in RPL

The male partner contributes to RPL through:

- **Sperm DNA fragmentation**: Associated with increased miscarriage risk; testing and treatment (antioxidants, varicocele repair) may improve outcomes
- **Genetic abnormalities**: Balanced translocations, Y chromosome microdeletions
- **M2/ANXA5 haplotype**: Paternal contribution to placental function (Ang et al., 2019)
- **Advanced paternal age**: Increased risk of de novo mutations

9.3 RPL After Assisted Reproductive Technology

RPL can occur in pregnancies conceived through ART. Considerations include:

- **Embryo factors**: Aneuploidy rates higher in some ART populations
- **Endometrial factors**: Altered receptivity from ovarian stimulation
- **Multiple pregnancy**: Increased loss risk with multiples
- **Underlying infertility**: May share etiologies with RPL

9.4 RPL and Recurrent Implantation Failure (RIF)

RIF refers to failure to achieve pregnancy after multiple embryo transfers. Overlap with RPL exists, with shared etiologies (uterine anomalies, thrombophilia, immune factors). Evaluation similar to RPL.

10. FUTURE DIRECTIONS AND RESEARCH PRIORITIES

10.1 Emerging Diagnostic Technologies

- **Next-generation sequencing**: Whole exome/genome sequencing to identify novel genetic causes
- **Chromosomal microarray**: Higher resolution than karyotyping for parental and POC analysis
- **Cell-free DNA testing**: Non-invasive screening of embryonic chromosomal status
- **Proteomics and metabolomics**: Identification of novel biomarkers
- **Artificial intelligence**: Integration of multiple data types for risk prediction
- **Advanced imaging**: 3D/4D ultrasound, MRI for subtle uterine anomalies

10.2 Novel Therapeutic Approaches

- **Targeted anticoagulation**: Direct oral anticoagulants in RPL (requires pregnancy safety data)
- **Immunomodulators**: More selective agents with fewer side effects
- **Growth factors**: Endometrial scratching, G-CSF infusion to improve receptivity
- **Stem cell therapy**: Endometrial regeneration for Asherman's syndrome
- **Gene therapy**: For single-gene disorders (future)

10.3 Personalized Medicine

- **Genetic profiling**: Individualized risk assessment based on multiple polymorphisms
- **Biomarker panels**: Integrated assessment of thrombotic, inflammatory, and endocrine status
- **Machine learning**: Algorithms to predict optimal treatment based on patient characteristics

10.4 Research Priorities

- **Prospective multicenter registries**: To track outcomes and refine treatment algorithms
- **Randomized controlled trials**: For controversial treatments (progesterone in unexplained RPL, aspirin in M2/ANXA5)
- **Basic science studies**: Mechanisms of M2/ANXA5 action, platelet abnormalities
- **Epigenetic studies**: Role of DNA methylation, histone modification in RPL
- **Long-term follow-up**: Health outcomes for children born after RPL pregnancies
- **Implementation science**: Translating evidence into clinical practice

11. CONCLUSIONS

11.1 Summary of Key Findings

This comprehensive review has synthesized current knowledge regarding recurrent pregnancy loss, with the following key conclusions:

1. **RPL is a multifactorial condition** affecting 2-3% of reproductive-aged women, with diverse etiologies including genetic, anatomical, endocrine, thrombophilic, immunological, and environmental factors.
2. **Genetic factors play a central role**, encompassing parental chromosomal abnormalities (2-5% of couples), embryonic aneuploidy, and specific polymorphisms affecting thrombophilia (Factor V Leiden, Factor II G20210A, MTHFR, PAI-1) and placental function (M2/ANXA5 haplotype).
3. **Thrombophilic mechanisms** are particularly important, with inherited thrombophilia and antiphospholipid syndrome accounting for a significant proportion of RPL cases. Novel insights into platelet and erythrocyte structural abnormalities (Andreeva et al., 2021; Langari et al., 2022) expand understanding of thrombotic pathways.
4. **The M2/ANXA5 haplotype** represents a significant recent discovery, with meta-analysis demonstrating 1.54-fold increased RPL risk through disruption of the annexin A5 protective shield (Ang et al., 2019; Tüttelmann et al., 2023).

5. **Anatomical factors** (congenital uterine anomalies, acquired defects) impair implantation and placentation and are correctable through hysteroscopic surgery.
6. **Endocrine disorders**—particularly thyroid dysfunction, diabetes, PCOS, and luteal phase deficiency—contribute to RPL and are amenable to medical treatment.
7. **Diagnostic evaluation** requires a systematic approach encompassing history, genetic testing (parental karyotype, POC analysis, thrombophilia panel), anatomical assessment (ultrasound, hysteroscopy), endocrine evaluation, and selected immunological testing.
8. **Treatment is etiology-specific**, with proven interventions including surgical correction of uterine anomalies, anticoagulation for APS and high-risk thrombophilia, levothyroxine for hypothyroidism, progesterone supplementation, and lifestyle modifications.
9. **Prognosis is favorable** for most couples, with 50-75% achieving live birth in subsequent pregnancies, though psychological support remains essential.
10. **Bulgarian researchers** have made important contributions to RPL understanding, particularly in thrombophilic genetic polymorphisms, M2/ANXA5 haplotype, and cellular abnormalities (Levkova et al., 2020; Andreeva et al., 2021; Ang et al., 2019; Ivanov et al., 2020; Robeva et al., 2022).

11.2 Clinical Implications

For clinicians managing couples with RPL, the following implications emerge:

- **Initiate evaluation after two losses** (or one if second-trimester) to identify treatable causes
- **Adopt a systematic diagnostic approach** covering all potential etiological categories
- **Provide genetic counseling** when abnormalities are identified or when considering PGT
- **Treat identified causes** with evidence-based interventions
- **Offer supportive care** to all couples, including early pregnancy monitoring and psychological support
- **Maintain hope** while providing realistic prognostic information

11.3 Unanswered Questions

Despite significant advances, important questions remain:

- What causes the 50% of RPL cases that remain unexplained?
- How do multiple subtle factors interact to cause pregnancy loss?
- What is the optimal treatment for M2/ANXA5-associated RPL?
- Can platelet and erythrocyte morphology serve as clinical biomarkers?
- What is the role of epigenetic modifications in RPL?
- How can we better predict which couples will achieve live birth?

11.4 Final Remarks

Recurrent pregnancy loss remains one of the most challenging conditions in reproductive medicine, combining complex pathophysiology with profound emotional impact. The past decade has witnessed remarkable advances in understanding genetic and thrombophilic contributions, with discoveries such as the M2/ANXA5 haplotype providing new insights into previously unexplained cases. Bulgarian researchers have contributed significantly to this progress, particularly in characterizing thrombophilic polymorphisms and cellular abnormalities in their population.

The integration of novel biomarkers, advanced imaging, and molecular diagnostics promises to further reduce the proportion of unexplained cases and enable more targeted, personalized treatment. However, even with optimal evaluation and treatment, some couples will continue to experience losses, underscoring the importance of compassionate, supportive care.

For the clinician caring for couples with RPL, the fundamental principles remain: thorough evaluation, evidence-based treatment when possible, acknowledgment of the emotional toll, and unwavering support through the journey. For the researcher, the many unanswered questions provide a rich agenda for future investigation. And for the couples themselves, the hope of a successful pregnancy—supported by advances in diagnosis and treatment—remains a powerful motivator to persevere.

As we continue to unravel the complexities of human reproduction, each discovery brings us closer to the goal of helping every couple achieve their dream of a healthy child. The journey is far from complete, but the progress to date offers reason for optimism.

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