Immunological Risk Factors and Pathophysiology of Recurrent Pregnancy Loss

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Conflict of interest: None declared.

Funding: No funding sources

Abstract

Each pregnancy involves a newly regenerated endometrium and a genetically unique embryo. Certain pathological mechanisms probably converge to trigger pregnancy loss, although the underlying causes may differ between women.Several risk factors for recurrent pregnancy loss have been identified as Maternal age, previous number of miscarriages ,anti-phospholipid syndrome, congenital uterine malformation (uterine septum), acquired uterine malformations (uterine myomas, polyps or adhesions), chronic endometritis, overt hypothyroidism, abnormal parental karyotypes , obesity (BMI >30kg/m2), lifestyle factors (stress, smoking and excessive alcohol consumption).Since innumerable genes are involved at every physiological step to ensure successful mammalian reproduction, mutations could be the causative factors of the molecular aetiology of RPL.Numeral coding variants in 22 genes are potentially related to the phenotype resulting in RPL

Keywords: Recurrent Pregnancy Loss, Immunological Risk Factors

TobRegul Sci. ™ 2023; 9(1): 7614 – 7630

DOI: doi.org/10.18001 /TRS.9.1.540

Introduction

Unlike sporadic pregnancy loss, recurrent pregnancy loss (RPL) requires medical intervention encompassing access to specialists, investigations, and enhanced support and follow-up during future pregnancies [1].

Epidemiology

The incidence of early pregnancy loss depends on the method used to detect pregnancy. About 50% of all pregnancies are lost at preclinical stages due to biochemical loss or implantation failure [2, 3]. The incidence in clinically diagnosed pregnancies is about 9–20% [3, 4], occurring mainly during the first trimester (weeks 5–12 of gestation) [5]. Week-by-week miscarriage rates vary in early pregnancy, with studies reporting a sharp reduction after 12 weeks of gestation to an incidence of about 1% [5].

No geographical variation in the prevalence was found in one meta-analysis [6]; however, cultural and societal attitudes may cause women to not openly confess their pregnancy loss, leading to the underestimation of prevalence [7, 3].

Establishment of a Healthy Pregnancy

Implantation and Decidualization

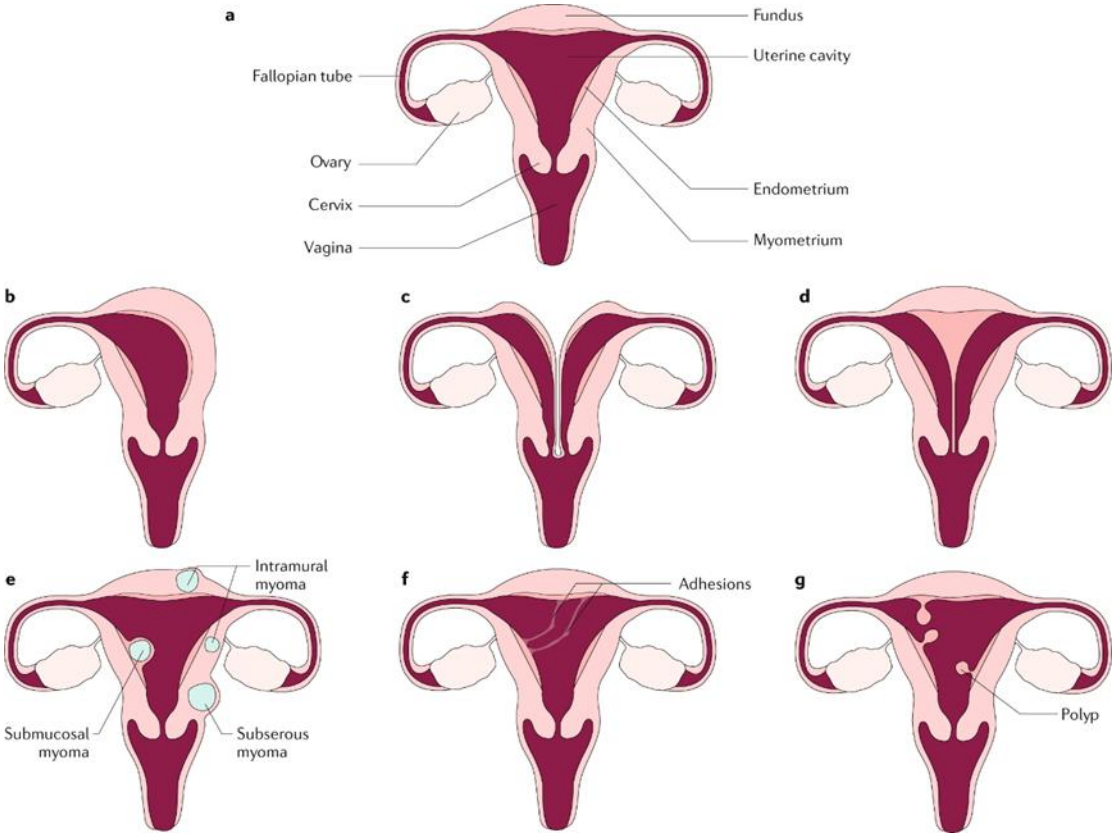
A successful pregnancy starts with the implantation of a competent embryo in the endometrium. To prepare for implantation, the endometrium undergoes remodeling, which is controlled by ovarian estradiol and progesterone during the menstrual cycle, culminating in a transient window during which an embryo can implant [8]. The implantation window is confined to the mid-secretory phase of the menstrual cycle, during which the endometrial luminal (surface) epithelium becomes permissive to adhesion of embryonic trophectoderm (the outer cells of the blastocyst) [9].

The window of implantation also heralds the start of intense remodeling of the endometrial stromal compartment encompassing the spontaneous differentiation of stromal fibroblasts into decidual cells and the accumulation of innate immune cells in the endometrium (primarily uterine natural killer (NK) cells and, to a lesser extent, macrophages) [10].

Decidualization of endometrial stromal cells is caused by the secretion of prostaglandin E2 and relaxin [10]. Decidualizing endometrial stromal cells encapsulate the implanting embryo [11, 12] and form a tight immune-protective matrix around the conceptus [13].

Placenta Formation

Implantation is the first step in the formation of a deep haemochorial placenta. Trophectoderm cells of the blastocyst differentiate into the two major cell lineages of the placenta: the villous trophoblast (comprising cytotrophoblast and syncytiotrophoblast) and the extravillous trophoblast [14]. Extravillous trophoblast cells migrate to the decidua and the inner third of the uterine myometrium to remodel maternal spiral arteries, thereby creating the low-resistance, high-flow vessels required for the placental blood supply to accommodate the increasing demands for placental perfusion in the later stages of pregnancy (Figure 2) [15].



Hormonal Control

The integrity of the decidual–placental interface during pregnancy is dependent upon continuous progesterone signaling [15]. Soon after implantation, hCG concentrations increase exponentially, with doubling every ~1.5–2 days. hCG maintains ovarian progesterone and estrogen production, with the placenta taking over progesterone production at about 8 weeks of pregnancy, in a process termed luteoplacental shift [16].

Pathogenetic Factors Involved in RPL

Embryos express paternal antigens that are foreign to the mother and may therefore be viewed as allografts. Hence, a tolerogenic immune environment must prevail to suppress inflammation, prevent immunity toward paternal/fetal antigens, and ensure the survival of the semi-allogenic fetus [17]. The exact mechanisms that contribute to the establishment and maintenance of tolerance are not completely understood. However, studies show that the female genital tract environment during the peri-conception period plays a critical role in the establishment of an appropriate immune response [18].

Role of Human Leukocyte Allele HLA-G

The HLA system is the most important immune factor in pregnancy maintenance and might play a crucial role in the incidence of RPL [19]. HLA-G is an important factor in the maternal acceptance of the fetus, playing a central role in the maintenance of an immunosuppressive state

and in spiral artery remodeling [20, 21]. Thus, HLA-G has been extensively studied in placentation disorders such as pre-eclampsia and fetal growth restriction [22].

Role of T Helper 1 and T Helper 2 Cells

Type 1 T helper (Th1) and type 2 T helper (Th2) cells play important roles in immune responses, particularly in immune rejection and tolerance [23, 24]. Based on a previous report, the normal range of the Th1/Th2 cell ratio should be less than 10.3. Moreover, Th2 cell dominance is important for the maintenance of normal pregnancy [25].

Role of Natural Killer Cells and Cytokine Release

Signaling by the T cell immunoglobulin (type I membrane protein) and mucin-containing protein 3 (Tim-3) in natural NK cells has an essential protective role. The number of Tim-3+ pNK cells (peripheral NK cells) transiently increased during the first trimester of pregnancy, as compared with Tim-3– NK cells [26]. Tim-3+ NK cells display immunosuppressive activity during early pregnancy by producing increased levels of anti-inflammatory cytokines, including transforming growth factor (TGF)-β1, IL 10, IL-4, and decreased pro-inflammatory cytokines, including TNF-α [26].

Role of Human Amniotic Epithelial Cells

Human amniotic epithelial cells play a potent regulatory role in the immune response by reducing TGF-β production and regulating the secretion of interferon (IFN) and IL-17 by T cells [27].

Role of Tumor Necrosis Factor-α Alpha

Tumor necrosis factor-α alpha (TNF-α) is a proinflammatory cytokine encoded on chromosome 6 in Th1 [28]. Macrophages are the primary producers of TNF-α in pregnant women, and TNF-α is involved in the signal transduction pathway in the placenta [29]. In pregnancy, the immunological system plays critical roles both in the development of complications and in ensuring normal pregnancy development. A pregnancy is successful when the balance of Th1, Th2, Th17 cytokines, and T-reg cells works appropriately [30]. It is reported that TNF-α upregulates the level of programmed death-1 (PD-1) in monocytes, which binds with PD-L to promote the production of IL-10 and relies on IL-10 to inhibit the expansion of CD4 T cells [31].

Increased levels of TNF-α and reduced IL-10 levels increase throughout pregnancy and have been associated with preterm birth, miscarriages, pre-eclampsia, and fetal losses [32].

TNF-α-targeted therapies therefore constitute a promising approach in improving or curing these disorders [33]. A recent study revealed neither any maternal nor fetal major adverse

reactions (including fetal malformations) to TNF-α blockers. In fact, the incidence of fetal malformations was 3% lower than expected [34].

Role of Colony Stimulating Factor

Colony stimulating factor (CSF)-1/M-CSF plays an important role in pregnancy. The expression of CSF-1/M-CSF significantly increases during pregnancy by 2-fold and 1,000-fold in serum and the uterus, respectively [35]. The CSF family members were detected in human cytotrophoblasts and syncytiotrophoblasts of the decidual stromal cells, endometrial glands, and epithelium of the placental and maternal cells, in addition to being located on local NK cells. CSFs are involved in numerous reproductive functions, including ovulation, embryo implantation, placental growth, and embryo development [36].

During ovulation, CSF-3/G-CSF promotes ovulation through leukocyte attraction and activation [37]. Local uterine production of CSF-3/G-CSF may contribute to modulating the cytotoxicity of uterine NK cells by IFNγ and IL-18 production [38]. Uterine dendritic cells (DCs) are critical for activating the T cell response, thereby mediating maternal immune tolerance of the semiallogeneic fetus. Absence of GM-CSF, a known regulator of DCs, may impair the generation of T cell mediated immune tolerance at the outset of pregnancy. This may contribute to the altered DC profile and dysregulated T cell tolerance evident in infertility and miscarriage [39].

Role of DNA Methylation and Transcription Factors

DNA methylation is one of the major epigenetic modifications that plays important roles in embryonic implantation and development [40].

Abnormal DNA methylation is associated with miscarriage, preeclampsia, abnormal embryonic development, and birth abnormalities [41, 42]. In RPL, CAMP-responsive element binding protein 5 (CREB5) expression levels were increased due to CREB5 hypomethylation [43]. As a result, its over expression leads to increased levels of tumor necrosis factor (TNF)-α and decreased levels of interleukin (IL)-10, and enhances the expression of nuclear factor kabba B (NF-Kb) in monocytes [44].

Role of Forkhead Box P3 (FOXP3)

Forkhead Box P3 (FOXP3), specifically expressed in CD4+ CD25+ T cells, is a human transcription regulator [45] and high expression levels of the FOXP3 gene are key to the development and function of T reg cells [46]. Methylation levels of the FOXP3 promoter were reported to be higher in patients with RPL than in the control group, and conversely, FOXP3 protein levels were found to be lower in the RPL group than in the control group. The effect of FOXP3 on the differentiation of T-reg cells is the probable cause of immune tolerance failure and subsequent RPL [47].

Genes and Mutations in Recurrent Pregnancy Loss

Since innumerable genes are involved at every physiological step to ensure successful mammalian reproduction, mutations could be the causative factors of the molecular etiology of RPL [8, 48]. Numerous coding variants in 22 genes are potentially related to the phenotype resulting in RPL [49]. The coding variants are associated with biological processes involved in cell adhesion trophoblast endometrium interaction (TRO, CDH11, and CDH1), coagulation (THBD, F5, and FGA), extracellular matrix remodeling ((MMP10 and MMP9), angiogenesis (FLT1 and EPAS1), cell proliferation, differentiation, migration, and apoptosis (LIFR, FGFR2, and BMP7), metabolism (AMN), immunological function modulation (IDO2, CR1, TLR3, and TRAF3IP1), and steroidal nuclear receptor activation (NCOA1) [49].

Risk Factors of Recurrent Pregnancy Loss

Each pregnancy involves a newly regenerated endometrium and a genetically unique embryo. Certain pathological mechanisms probably converge to trigger pregnancy loss, although the underlying causes may differ between women [3].

Several risk factors (Figure 1) for RPL have been identified, including: maternal age, previous number of miscarriages, anti-phospholipid syndrome, congenital uterine malformation (uterine septum), acquired uterine malformations (uterine myomas, polyps, or adhesions), chronic endometritis, overt hypothyroidism, abnormal parental karyotypes, obesity (BMI >30kg/m2), and lifestyle factors (stress, smoking, and excessive alcohol consumption) [50]. However, no risk factors are identified in 50–70% of couples with RPL [51, 52].

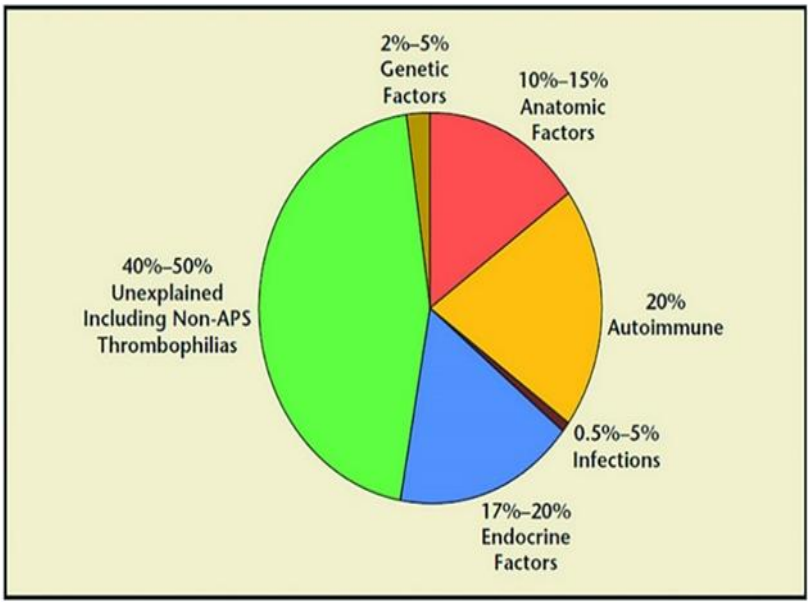


Figure (1) Risk factors of recurrent pregnancy loss. (Please provide details about Figure 1 here, such as a description of what it depicts or a link to the image.) APS, Antiphospholipid syndrome [53].

Genetic Factors

Embryonic aneuploidy is a frequent cause of pregnancy loss in women with RPL [54, 55]. Based on conventional genetic analysis, such as karyotyping, the frequency of chromosomal abnormalities is lower in women with RPL than in those with spontaneous pregnancy loss [56, 57].

The most common fetal chromosomal abnormalities are caused by meiotic non-disjunction (that is, the failure of chromosomes to separate, causing, for example, trisomy and monosomy), and structural chromosomal abnormalities (balanced translocations or inversions) [58].

Parental translocations, inversions, and copy number variations are more prevalent in couples with RPL (2–5%) than in the general population (0.7%) [59]. A genome-wide association study found four distinct susceptibility loci related to progesterone production, placentation, and gonadotropin regulation, which are associated with sporadic and recurrent pregnancy loss [60].

Maternal age is the main risk factor for embryonic aneuploidy; the proportion of aneuploid embryos rises from 25–35% in women under 35 years of age to 55–85% in women aged 40–45 years [61, 62].

Defective Sperm

Some studies have found epigenetic modifications of sperm DNA [63] and increased DNA fragmentation in sperm could contribute to RPL [64, 65].

Uterine Factors

Uterine structural abnormalities are often diagnosed during fertility investigations. Congenital uterine abnormalities, including arcuate, septate, unicornate, bicornate, and didelphis uteri, are more prevalent in women who have experienced pregnancy loss than in the general population [66]. Acquired uterine abnormalities include uterine myomas (fibroids), endometrial polyps, and intrauterine adhesions or Asherman syndrome. Submucosal myomas may be associated with an increased risk of pregnancy loss [67].

Chronic endometritis is localized inflammation of the endometrium that is caused by infection, usually asymptomatic [68] and commonly caused by Escherichia coli, Enterococcus faecalis, Streptococcus spp., Staphylococcus spp., Chlamydia, Mycoplasma, Ureaplasma, and yeast [69, 70]. Increased prevalence of RPL is increased in women having chronic endometritis [71].

Luteal phase defect. Owing to the critical role of sustained progesterone signaling for implantation and pregnancy, low progesterone production during the luteal phase of the menstrual cycle is widely perceived to be an important cause of pregnancy loss [72]. Vaginal administration of micronized progesterone improves outcomes in women presenting with vaginal bleeding in pregnancy and a history of one or more miscarriages [73].

Endocrine Factors

Overt hypothyroidism is a known risk factor for miscarriage and impaired fetal neurocognitive development [75]. Subclinical hypothyroidism may not be associated with an increased risk of RPL [76].

Polycystic ovarian syndrome (PCOS). The diagnosis of PCOS, as defined by the Rotterdam criteria, requires two of the following three perturbations to be present: oligoovulation (irregular ovulation) or anovulation, hyperandrogenism, and/or the presence of polycystic ovaries on ultrasonography [77]. PCOS has been associated with a spectrum of pregnancy complications, including fetal loss, gestational diabetes mellitus, and pre-eclampsia; however, these disorders may relate to the comorbidities of PCOS (obesity, metabolic syndrome, hyperinsulinemia, or hyperandrogenism) rather than to PCOS directly [78]. The prevalence of PCOS in women with RPL is the same as in the general population [79] and PCOS status does also not affect the prognosis of RPL [80].

Obesity

Obesity (BMI ≥30 kg/m2) is an independent risk factor for RPL [81]. Obesity is associated with many endocrine disorders, including PCOS, hypothyroidism, and diabetes mellitus; however, many of these comorbidities individually do not show a compelling association with RPL [80].

Emerging evidence indicates that obesity affects the endometrium (for example, impairment of decidualization and trophoblast invasion in mice with diet-induced obesity [82]. Women with obesity have increased endometrial accumulation of advanced glycosylation end products, which impairs decidualization and inhibits trophoblast invasion in vitro [83].

Vitamin D Deficiency

The exact role of vitamin D in pregnancy failure remains controversial. While some studies demonstrated an association of RPL with decreased vitamin D levels, lower expression of vitamin D receptors, or lower levels of 1α-hydroxylase [84, 85], others reported no association between vitamin D deficiency and pregnancy failure [86, 87].

Thrombophilia

Thrombophilia and the predisposition to improper coagulation can affect chorionic blood flow and cause vasculopathy leading to pregnancy loss [88]. The most prevalent types of thrombophilia associated with RPL are hereditary (single mutations, such as in F5 (encoding Factor V Leiden) or F2 (encoding Factor II), or multiple mutations that result in protein C, protein S, and anti-thrombin deficiencies) [89] or acquired as antiphospholipid syndrome (APS) [90].

APS is an autoimmune condition featured by antiphospholipid antibody formation and associated with thrombotic events and pregnancy complications, including RPL [19, 74]. The prevalence of antiphospholipid antibodies is estimated at 15–20% among women with RPL [91, 92]. APS causes an inflammatory response to antiphospholipid antibodies on vascular endothelium and chorionic/placental cells, which promotes thrombosis [74].

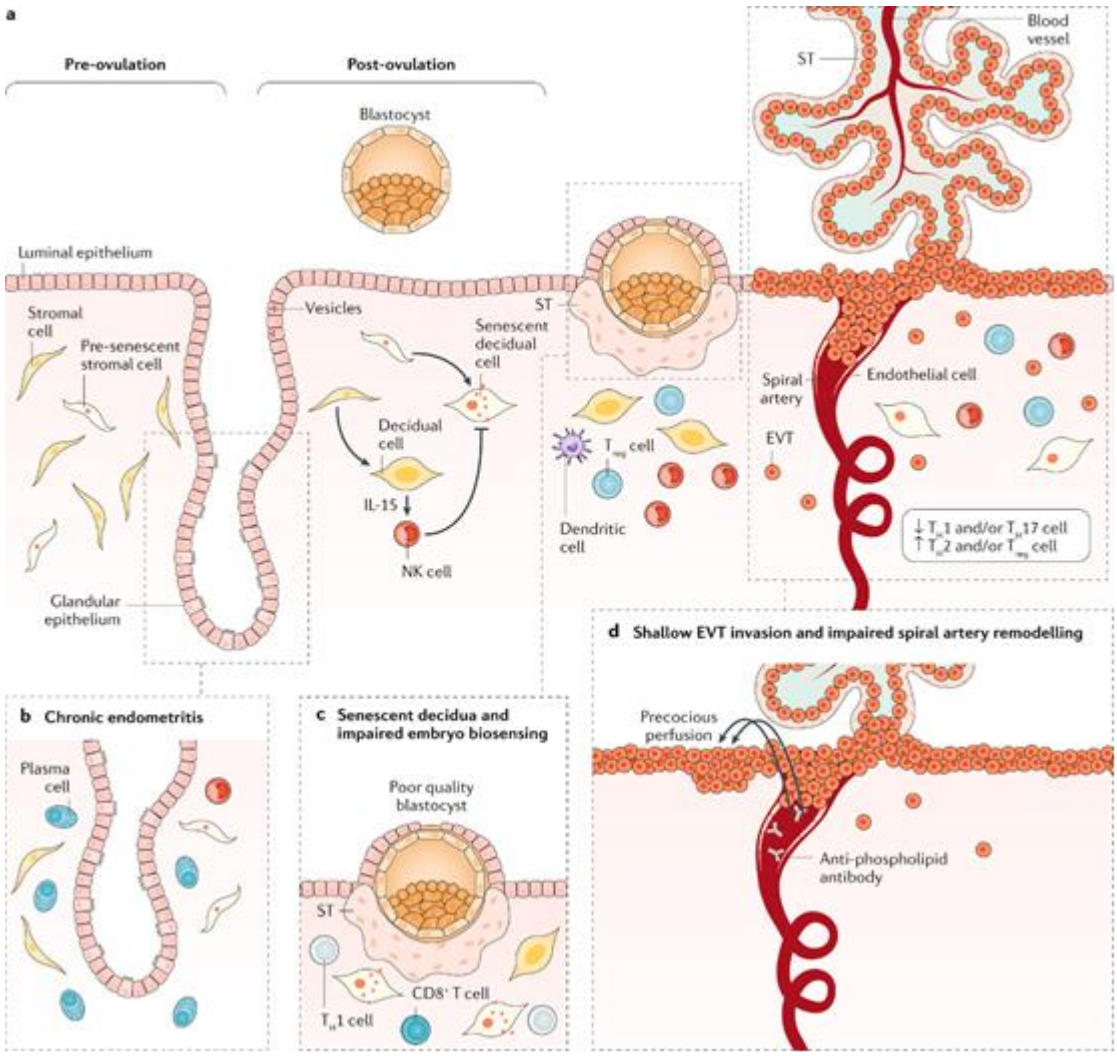


Figure (3): Impaired endometrial function and recurrent pregnancy loss. (Please provide details about Figure 3 here, such as a description of what it depicts or a link to the image.)

a | Healthy endometrial remodeling, implantation, and establishment of pregnancy.

b | Low-grade infection of the endometrium can cause chronic endometritis.

c | Women with fewer clonal stromal cell precursors during the implantation window are more susceptible to excessive decidual senescence.

d | Antiphospholipid syndrome. [74]

Diagnosis of RPL

For a proper diagnostic approach, a careful past medical history of patients with RPL and identification of etiological and risk factors should be performed. The evaluation strategy for women with a recurrent miscarriage should be focused on those etiological and risk factors that could be modified and, thus, the patient could be effectively treated [74, 93]; however, in about 50% of cases, termed "idiopathic", no risk factor is identified [94, 1]. The uncertainty regarding RPL pathogenesis has resulted in guidance and care variation, with multiple investigations and treatments being offered, despite the lack of sufficient supporting evidence [93, 95].

Table 1: Suggested Diagnostic Evaluation of Recurrent Pregnancy Loss Based on Etiology [53] (Please provide details about Table 1 here, such as a description of what it contains or a link to the table.)

Etiology	Suggested Diagnostic Evaluation
Genetic	Parental karyotype
Anatomic	HSG or office hysteroscopy 2D or 3D ultrasound Saline-infusion Sono hysterography
Endocrine	TSH
	Possible testing for insulin resistance, serum prolactin level, ovarian reserve testing, antithyroid antibodies
Infectious	No evaluation recommended unless patient has evidence of chronic endometritis/cervicitis on examination, or is immunocompromised
Autoimmune	Anticardiolipin antibody levels (IgG and IgM)
	Lupus anticoagulant
Non-APS thrombophilias	Homocysteine, factor V Leiden, prothrombin promoter mutation, activated protein C resistance

Table 2: Therapeutic Interventions for Recurrent Pregnancy Loss Based on Aetiology [53] (Please provide details about Table 2 here, such as a description of what it contains or a link to the table.)

Disorder	Therapy
Genetic	Genetic counselling

Disorder	Therapy
Anatomic	
Müllerian anomalies	Hysteroscopic resection of septa, adhesions, and submucosal fibroids
Asherman syndrome	
Leiomyomas	Myomectomy for those intramural and subserosal fibroids >5 cm
Endocrine	
PCOS	Metformin
Hypothyroidism	Thyroid hormone replacement
Luteal phase defect/unexplained	Progesterone supplementation
Diabetes mellitus	Appropriate management of diabetes, insulin if indicated
Infectious	Antibiotics for endometritis or underlying infection
Autoimmune	Low-dose aspirin plus prophylactic LMWH in women without a history of a systemic autoimmune disease such as SLE, or a history of thrombosis
APS	Combined thrombophilic defects—therapeutic anticoagulation
Other	
Non-APS thrombophilias	Isolated defect and no personal or strong family history of thrombotic complications—prophylactic anticoagulation
Environmental exposures	supplemental folic acid (0.4–1.0 mg/d), vitamin B_6 (6 mg/d), and possibly vitamin B_{12} (0.025 mg/d)
	Limit exposures that could be factors (eg, tobacco, alcohol, caffeine)

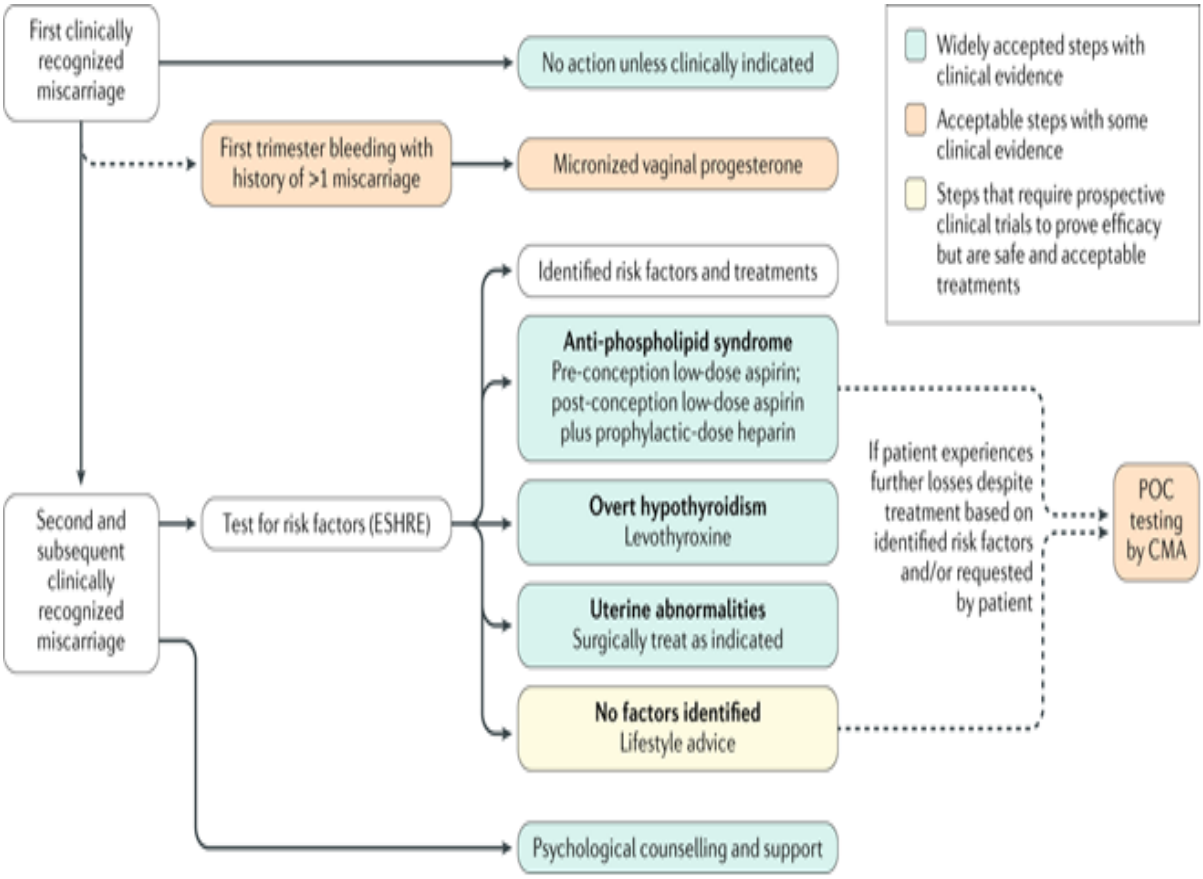


Figure 4: An example of a clinical protocol for the management of recurrent pregnancy loss. (Please provide details about Figure 4 here, such as a description of what it depicts or a link to the image.) CMA, chromosomal microarray; ESHRE, European Society of Human Reproduction and Embryology guidelines 2017; POC, products of conception. [74]

No Conflict of interest.

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