Chapter

Recurrent Pregnancy Loss: Investigations and Interventions

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Abstract

Recurrent pregnancy loss (RPL) affects 0.8–1.4% of couples, and this prevalence increases with aging. However, etiology is commonly unknown, and most therapies are not supported by strong evidence. There are many examinations that investigate causes of RPL: hormonal status, spermatozoa morphology and DNA fragmentation, immunologic status, uterine assessment, thrombophilia, and others. Recently different types of treatment have emerged, most lacking good evidence. As for example, we may mention the use of anticoagulants, aspirin, corticosteroids, progesterone, and antioxidants and psychological support. It is argued that some procedures such as preimplantation genetic testing for aneuploidy and intracytoplasmic morphologically selected sperm injection would impact on the outcomes and help RPL management. This chapter will discuss the current evidence concerning examinations and treatments that would improve the outcomes in patients with RPL, with recommended practice.

Keywords: recurrent pregnancy loss, recurrent miscarriage, in vitro fertilization, infertility, preimplantation *genetic* testing for aneuploidy, thrombophilia, sperm DNA fragmentation, natural killer cells, reproductive techniques, maternal KIR, paternal HLA-C

Keypoints

The practice of physical activity, healthy eating, quitting smoking, and reduction of alcohol consumption are factors that interfere in the reproductive outcomes. Medical understanding and ability to listen to patients about their obstetric past are fundamentally important for the treatment.

The genetic investigation is controversial and consists of chromosomal evaluation of the conception products and the couple's karyotype. The goal is to identify the etiology of the loss and may be useful for future guidance of the couple. There is no consensus on performing IVF-PGT, and this option should be discussed case by case. In extreme cases IVF using donated gametes may be the last option.,

Patients with RPL without other risk factors for thrombosis should not be screened for inherited thrombophilias, and those with positive screening have no benefit from available treatment. The only thrombophilia that should be routinely investigated for early miscarriage is APS. The recommended treatment

is the use of low-dose AAS preconception and LMWH in a prophylactic dose initiated when diagnosing pregnancy.,

Screening immunological factors for patients with RPL is not recommended. There is also no recommendation to use venous immunoglobulin or corticosteroids empirically. Only antinuclear antibody can be ordered for prognostic purposes, according to ESHRE.,

Screening for congenital uterine anomalies is part of the investigation of women with a history of RPL. Nuclear magnetic resonance is the gold standard for diagnosis. The only finding that can be surgically corrected and prognosis improved is the septate uterus.,

The diagnosis of cervical incompetence is based on clinical history. The classic treatment is transvaginal cerclage between 12 and 16 weeks after first trimester morphological ultrasound.,

Patients with RPL should undergo through endometrial cavity evaluation. The gold standard is hysteroscopy. Although there is limited evidence linking submucosal fibroids, endometrial polyps, and synechiae with RPL, surgical correction in patients with RPL without other identifiable factors is suggested.,

There are no research and treatment benefits for PCOS patients and their associated endocrine disorders. Thyroid evaluation should be performed with serum TSH and anti-TPO, and clinical hypothyroidism should be treated. For prolactin, the test is not indicated in the absence of signs of hyperprolactinemia, but if this condition is diagnosed, treatment is indicated. Vitamin D test is not routinely recommended, but the preconception counseling in women with RPL may include prophylactic vitamin D supplementation due to the high prevalence of hypovitaminosis D in this population.,

The relationship of chronic endometritis with RPL is unclear. The current gold standard for the diagnosis of chronic endometritis is the pathological anatomy of immunohistochemically endometrial biopsy for the CD138 marker. A therapeutic option would be the use of doxycycline alone or in combination with other antibiotics.,

For male factor, measurement of spermatic DNA fragmentation index and Kruger morphology would be indicated. The use of antioxidants is a clinical treatment that can improve DNA fragmentation. In the presence of ICSI indication associated with increased spermatic DNA fragmentation, the use of testicular, IMSI, or PICSI sperm can be considered.

1. Introduction

Recurrent pregnancy loss (RPL) is defined by two or more losses with gestational age less than 20–24 weeks [1, 2]. Its prevalence varies between 0.8 and 1.4% considering only patients who have had a clinical pregnancy [2]. The pathogenesis is multifactorial, and in only 50% of the cases, the causal factor can be identified: immunological, endocrine, genetic, metabolic, and anatomical, among others [3]. The identification of etiology is not always possible, and recurrence of miscarriage seems to influence negatively the couple's psychological profile [2]. Thus, the understanding of diagnostic methods that can identify etiological factors and treatments that can improve the outcome is fundamental for the follow-up of couples with RPL.

2. Risk factors

Some personal factors such as lifestyle and even environmental exposure may be associated with obstetric complications and gestational loss. Advanced maternal age is one of the best-established risk factors in the literature for RPL [2]. Approximately 50–70% of early gestational losses are associated with chromosomal abnormalities, and their incidence increases with maternal age, reaching 50% in women over 40 years [3]. The European Society of Human Reproduction and Embryology (ESHRE) recommends that women should be informed of the highest risk of miscarriage after age 40 [2].

Obesity also has a major impact on women's reproductive health. High body mass index (BMI) is associated with worse outcomes in infertility treatments and a higher incidence of gestational loss [4]. One study with obese women showed a higher frequency of euploid miscarriages than nonobese women (58% vs. 37%) [5].

This is probably due to the association of obesity with several endocrine disorders, such as diabetes, hypothyroidism, and polycystic ovary syndrome and possibly endometrial changes [5]. The Royal College of Obstetricians and Gynecologists (RCOG) recommends prepregnancy weight loss due to the associated increased risk of miscarriage, stillbirth, preeclampsia, diabetes, and postpartum hemorrhages [6]. The practice of regular physical activity presents improvement in the obstetric outcome; however, there are no studies investigating the impact of exercise in patients with RPL [2].

Smoking seems to be related to defects in trophoblastic function, thus increasing the risk of gestational loss, in addition to poor obstetric prognosis [2]. Assisted reproduction societies recommend quitting smoking because of the negative impact on the chances of a live birth [2, 3]. Several studies have shown that drinking alcohol during pregnancy also increases the risk of gestational loss [2]. Although further studies are needed to establish if there is any safe dose for drinking in pregnancy, there are recommendations for couples with RPL not to drink alcohol.

Caffeine abuse can also affect fertility as well as be a risk factor for gestational losses. Ingestion of high caffeine levels (500 mg per day or > 5 cups per day) is associated with decreased fertility [7]. During pregnancy, drinking between 200 and 300 mg/day (2–3 cups) may increase the risk of miscarriage [3]. Thus, it seems sensible to guide this population to reduce caffeine consumption.

Few studies assess environmental exposure as a risk factor for RPL, one of which suggests that exposure to heavy metals and lack of micronutrients may cause gestational loss [8]. Another study suggests that ingestion of high concentrations of organochlorine pesticides may be associated with RPL [9].

It has been suggested in the past that stress could be associated with worsening the reproductive outcomes. There is a higher prevalence of depression in patients with RPL [10], but it is not known if this picture is not the cause or effect of RPL [2]. The American Society for Reproductive Medicine (ASRM) advises psychological support for these women who are more prone to feelings of grief, sadness, depression, anxiety, and guilt [3].

3. Genetic factors

The human conception is a vulnerable event—a large proportion of all conceptions are cytogenetically abnormal, and most of such pregnancies evolve to abortion. In couples with RPL, research can be divided into two main categories: genetic analysis of products of conception and parental genetic analysis.

3.1 Genetic analysis of products of conception

Studies in which products of conception were analyzed showed that genetic alterations, mainly aneuploidies, contribute to a significant portion of the causes of gestational losses, accounting for 50% of recurrent losses [11]. Despite the importance of genetic alterations as causes of miscarriage, there is still no consensus as to whether routine evaluation of pregnancy tissue should be performed. ASRM does not recommend genetic evaluation of conception products [3]. ESHRE, in turn, suggests that this analysis should not be done routinely but that it may be promoted for the purpose of clarifying the etiological factor and to assist in deciding whether further investigation or treatment is needed [2]. Other studies and guidelines, however, have proposed new algorithms in which the assessment of gestational repetition losses should be initiated with chromosome testing in conception products [12].

New chromosomal tests such as the chromosomal microarray analysis (CMA) have the potential to reduce costs since, in the presence of altered examination, costly and unnecessary evaluations will not be employed [13]. In addition, when a cause is identified, the tendency is to reduce the use of empirical treatments that have no scientific evidence [13]. Research has shown that in couples with previous embryonic aneuploidy, the likelihood of a child's birth during subsequent pregnancies was higher than patients with prior normal karyotype of conception products (71% vs. 44%) [14].

The suffering that the couple goes through experiencing abortion episodes without knowing the etiological factor can by itself justify the investigation of the existence of genetic alterations as a cause of the events.

3.2 Parental genetic analysis

In about 5% of all couples suffering two or more fetal losses, one partner carries a balanced chromosomal rearrangement, which represents approximately eightfold increase compared to the general population [15]. Guiding this couple for genetic counseling is important, as the likelihood of a healthy child born will depend on the type of rearrangement found and the chromosomes involved—for example, gestational losses are more present in carriers of balanced translocations and inversions than in carriers of Robertsonian translocations [16]. Even with one spouse carrying a chromosomal rearrangement, the cumulative rate of live birth, even in natural conception, is significant—63.4% despite the increased risk for miscarriage [17].

As for the existing guidelines regarding parental cytogenetic investigation, ESHRE determines that such assessment should not be performed routinely, but in specific cases after individual risk assessment [2]. ASRM, however, recommends routine parental karyotyping as information obtained may assist in counseling on the prognosis of future pregnancies, including guidance for performing preimplantation genetic testing (PGT), amniocentesis, or chorionic villus analysis [3].

Couples with structural cytogenetic changes have an increased number of gametes with chromosomal imbalances, so it would be expected that the implantation of embryos selected by PGT increases the rate of live births. However, in spouses carrying chromosomal rearrangement with RPL, the rate of live births, time to subsequent conception, and miscarriage rates were similar in both naturally conceived and in vitro fertilization associated with preimplantation diagnosis (IVF-PGT) [18]. Other papers showed discordant results. Similar live birth rate and time to new pregnancy were reported; however, the miscarriage rate was significantly lower in the IVF-PGT group [19]. Thus, there is no consensus showing the benefit of such strategy in this population, and no randomized controlled trials have been conducted to this date to validate possible benefits.

4. Thrombophilias

Thrombophilias are inherited and/or acquired conditions that predispose individuals to thrombosis, with varied prevalence in the general population [20]. The most common hereditary thrombophilias are methylenetetrahydrofolate reductase (MTHFR) gene polymorphism 4–16%, factor V Leiden mutation 1691G → A (heterozygote, 1–15%; homozygote, <1%), prothrombin mutation 20210G → A (heterozygote, 2–5%; homozygote, <1%), antithrombin deficiency (0.02%), protein C deficiency (0.2–0.4%), protein S deficiency (0.03–0.13%) [21], and serpin gene polymorphism. On the other hand, acquired thrombophilia is mainly represented

by the antiphospholipid antibody syndrome (APS) 2% [20]. Successful pregnancy requires an adequate endovascular implantation and remodeling measured by trophoblast, and these prothrombotic conditions would be the target of investigation and intervention with anticoagulant therapy to prevent miscarriage [21].

4.1 Inherited thrombophilias

The screening of inherited thrombophilias even in patients with a thrombosis context is still questioned [2]. The factor V Leiden mutation (1691G→ A) and the prothrombin mutation (20210G→ A) were related to recurrent miscarriage [22]; however, the lack of evidence that the treatment changes the gestational outcome leads to questioning the relevance of investigating such mutations. Other thrombophilias, such as protein C deficiency, protein S deficiency, and antithrombin deficiency, although associated with thromboembolic event, were not associated with RPL [2, 3, 20, 22]. MTHFR gene polymorphisms are no longer considered risk factors for thrombophilias [2].

The association between RPL and inherited thrombophilias is weak or absent [2]. Thus, thrombophilic screening should be restricted to patients with family history of thrombophilias or previous thrombotic event [1, 2]. There is no recommendation to screen inherited thrombophilias in patients with RPL without other risk factors [1, 2, 21, 23]. Screening tests may be influenced by physiological/pathophysiological changes in the pregnancy-puerperal period, thrombotic event, or use of anticoagulants [21]. It should be performed within 6 weeks or more after delivery, miscarriage, or thrombotic event or early if necessary [2, 21].

The use of anticoagulant therapy with low-molecular-weight heparin and/or aspirin has no benefit in preventing early (<10 weeks) or late (≥10 weeks) RPL [24]. Thus, ineffectiveness of the treatment, the risk exposure, and the increased cost do not justify treatment with anticoagulants in patients with inherited thrombophilias and RPL without other risk factors for thrombosis [2, 20].

4.2 Acquired thrombophilias

APS is indicated in patients with RPL, as well as in patients with adverse gestational outcome or episode of thrombosis without apparent cause [25]. The diagnosis of APS is based on the combination of at least one clinical criterion, which includes thrombotic events and/or gestational morbidity, and a laboratory criterion, which includes three antibodies: lupus anticoagulant, anticardiolipin, and anti-β2 glycoprotein 1 (anti-β2GP1) [25].

In the cases of late gestational loss, lupus anticoagulant was more closely related to RPL than any of the other antibodies [26, 27]. Anticardiolipin (IgG and IgM) has been associated with early and late gestational loss [26, 27]. The relationship between anti-β2GP1 and late gestational loss seems to be controversial [26, 27]. ESHRE recommends for patients with two losses, consecutive or not, to conduct a research for lupus anticoagulant antibodies and anticardiolipin, and the research should consider anti-β2GP1.

The use of combined therapy, low-molecular-weight heparin at prophylactic dose, and low aspirin dose (75–100 mg/day) increases the live birth rate in patients with APS and RPL from 10% to 70–80% [28]. In treatment failure, the use of heparin in therapeutic dose may be used, although there is no benefit evidence [28]. Other treatment regimens with limited evidence are the use of hydroxychloroquine or low dose of prednisolone in the first trimester [28]. The use of immunoglobulin is questioned because studies are limited and show no increase in live birth rate [28].

5. Immunological factors

To be successful in pregnancy, the maternal organism needs to undergo immunological changes that allow and assist in the trophoblastic invasion of the embryo. During pregnancy, the maternal immune system faces a dilemma: it needs to protect the mother against infection while accepting the semi-allogeneic fetus [29]. Leukocytes are important components of the endometrium, and their concentration increases in the middle of the secretory phase in which embryonic implantation is expected and continues to increase during early pregnancy [30]. The progesterone plays a key role in this balance by creating an appropriate environment for embryonic implantation and development [28]. This change in maternal endometrial immunology becomes essential for early pregnancy implantation and success. Changes in this phase can lead to implantation failure, miscarriage, and other unfavorable obstetric outcomes such as preeclampsia.

5.1 Natural killer (NK) cell

The uterine natural killer (uNK) cells are the most commonly found leukocytes in the maternal endometrium. Two phenotypes are observed—CD56bright and CD16dim—unlike peripheral blood where CD56dim and CD16+ are the largest population [31]. There is a variability of their own concentration during the menstrual cycle. There are a significant increase of NK cells in the endometrium 6 to 7 days after the peak of luteinizing hormone (LH), which persists throughout the early pregnancy. This increase suggests an important role of these cells in embryonic implantation, but the exact function is still unknown [30].

5.1.1 Killer immunoglobulin-like receptors (KIR)

The placental formation is regulated by the interaction between the killer immunoglobulin-like receptors (KIR) and the surface human leukocyte antigens on the embryo trophoblastic cells (HLA-C). The embryo presents maternal and paternal HLA-C, and both haplotypes are presented to NK cells that, in turn, will recognize the human leukocyte antigen (HLA) foreign to their organism. There are two types of HLA-C: C1 and C2 which are a strong ligand to the receptor. On the other hand, there are two KIR haplotypes: A, which is inhibitory, and B, which is stimulating. The receptors can then be AA, AB, or BB. The presence of haplotype B confers pregnancy protection, and its absence (in the cases of KIR AA) increases the risk of gestational complications.

Studies have shown that when maternal KIR is homozygous for haplotype A (KIR AA), there is an increased risk of gestational complications if the embryo carries paternal HLA-C2 [32, 33]. In the future, these studies may be applicable to couples who will undergo IVF. Further studies on the subject are still needed, and these tests are not quoted to be traced by societal guidelines.

5.2 Macrophages

The macrophages represent 20–30% of leukocytes in the maternal endometrium and are the second largest group behind only NK cells. Macrophages differ in specific phenotypes to perform different biological functions and can be divided into two subgroups: M1 and M2. M1 macrophages are pro-inflammatory and antimicrobial, whereas M2 have anti-inflammatory function [34]. For maternal and fetal tolerance to occur, more macrophages are polarized into the M2 subtype with immunosuppressive properties necessary for normal pregnancy

to occur [35]. When polarization of these cells does not occur correctly favoring the M1 subgroup, improper remodeling of the arteries and trophoblastic invasion occurs, leading to a higher incidence of miscarriage, preeclampsia, and premature birth [35].

5.3 Regulatory T cells

Regulatory T cells (Treg) are a subpopulation of T cells that play an essential role in maintaining maternal immune tolerance. These cells are activated by the presented antigens and from that moment secrete cytokines that will determine the differentiation of T cell subtypes, thus modulating the immune response. Depending on the released cytokines, T cells may differentiate into Treg cells expressing interleukin 10 and transforming growth factor β (TGFβ) responsible for immune tolerance to the conceptus or Th17 expressing interleukins 17, 21, and 22 responsible for autoimmunity and gestational loss. Treg cells will then regulate the response to foreign antigens when an aggressive response is not appropriate, having the ability to inhibit type 1 helper (Th1) cells. There is evidence in the cases of recurrent gestational loss of unknown cause to increase Th17 and to decrease Treg cells, leading to an inadequate immune response [29].

6. Anatomical factors

Uterine anatomical abnormalities, both acquired and congenital, are associated with RPL. It is estimated that uterine factors may account for 10–50% of RPL [36].

6.1 Congenital uterine anomalies

6.1.1 Congenital Müllerian duct anomalies

Congenital uterine anomalies (CUA) arise from defects along any stage of the Müller duct development process during embryonic development, whether in formation, fusion, or reabsorption. The frequency of CUA has been reported between 1.8 and 37.6% in women with a history of RLP. This variation is due to the different diagnostic methods and criteria [37]. Septate uterus is the most common anomaly in patients with a history of abortion. Arched, septate, and bicornuate uterus account for up to 85% of anomalies [38].

In a meta-analysis it was observed that patients with septate or bicornuate uterus had a higher rate of miscarriage in the first and second trimester than a control group [39]. In another meta-analysis, the evaluation of uterine abnormality subtypes resulting from fusion defect showed that women with unicornuate and bicornuate uterus were more likely to have first-trimester abortion compared to those with normal uterus [40].

ASRM's original classification system for congenital uterine anomalies has been modified and adapted and is still the most widely used today [41]. In 2012, ESHRE/ESGE published a classification system aiming to replace the subjective criteria of ASRM's classification by the absolute morphometric criteria [42]. Based on this classification, up to 58% of women previously diagnosed with ASRM arched uterus would be reclassified as having a partial septate uterus. There would be a potential increase in the number of surgical corrections for uterine anomaly, without any evidence showing that such a practice would be beneficial [43]. Therefore, caution is needed in using this new classification until further prospective, randomized,

controlled, long-term studies are available to associate the severity of uterine cavity distortion with reproductive results.

Given the suspicion, it is necessary to use diagnostic methods that can clearly visualize the external contour of the uterus and endometrial cavity. Both 3D ultrasound with inversion mode (3D US) and magnetic resonance imaging (MRI) can be used for this purpose, with good correlation between them [44]. The disadvantages of MRI are that it is a more expensive and less available method than ultrasound.

In a comparative study of different diagnostic modalities, higher accuracy of 3D hysterosonography compared with 3D US and 2D hysterosonography was observed, although the differences between these imaging techniques did not reach statistical significance in the diagnosis of arched, bicornuate, and septate uterus [45].

The uterine septum is the most common abnormality related to RPL [36] and the only remediable one. Despite the lack of randomized and controlled prospective studies comparing surgery to expectant treatment, limited studies indicate that hysteroscopy septal resection is associated with a reduction in subsequent abortion rates and an improvement in live birth rates in patients with RPL [41]. After hysteroscopic resection of the septum, an interval of at least 2 months should be expected for complete healing of the endometrial cavity before a new pregnancy [41].

In general, CUA may be associated with renal abnormalities in approximately 11–30% of individuals [41]; for this reason there is a need for urinary tract investigation in these cases.

6.1.2 Cervical incompetence

Cervical incompetence (CI) is the inability of the cervix to keep the intrauterine fetus in the absence of uterine contractions or labor (painless cervical dilatation) due to a functional or structural defect. It is a recognized cause of RPL in the second trimester, but the true incidence is unknown, as the diagnosis is essentially clinical [2].

The CI can be congenital or acquired. The most common congenital cause is a defect in the embryological development of the Müllerian ducts. The most common acquired cause is cervical trauma, such as cervical lacerations during childbirth, cervical conization, or forced cervical dilation during uterine procedures [46].

The diagnosis is usually based on a history of miscarriage in the second trimester, preceded by spontaneous rupture of membranes or painless cervical dilation. There are currently no objective tests capable of identifying women with cervical weakness in the nonpregnant state [2].

Transvaginal ultrasound may be used in at-risk patients during pregnancy. CI might be suspected when there is a short cervical length, less than or equal to 25 mm, or funneling, protrusion of the membrane into a dilated internal orifice but with closed external orifice [46].

Many surgical and nonsurgical modalities have been proposed to treat cervical incompetence. Among nonsurgical activities, restriction of activities and bed rest were not effective in the treatment of cervical incompetence. Its isolated use is discouraged. The use of vaginal pessary is another option, but the evidence is still limited. Surgical approaches include transvaginal and transabdominal cervical cerclage [46].

6.2 Acquired anatomical factors

Acquired anatomical factors commonly associated with RPL include uterine fibroids, endometrial polyps, and uterine synechiae. They usually develop after puberty due to physical or hormonal stimuli and are present in about 12% of patients with RPL [47].

6.2.1 Uterine fibroids

Fibroid is reported in 8,2% of women with RPL [48]. Submucosal fibroids deform the endometrial cavity, thus affecting implantation and embryonic development [47]. Hysteroscopy is considered the gold standard for the diagnosis of submucosal fibroids, but this pathology can be identified through other imaging exams, such as ultrasound mapping [2]. The evaluation of the uterine cavity is strongly recommended for all women with RPL, since the removal of submucosal fibroids in infertile patients seems to reduce the chance of miscarriage [2, 49]. Regarding fibroids that do not distort the uterine cavity, there is no evidence indicating that myomectomy may reduce the chances of an abortion [2, 49].

6.2.2 Uterine polyps

There seems to be a higher prevalence of endometrial polyps in women with gestational loss (2.4%), but with no well-defined clinical importance [2, 47]. Hysteroscopy is considered the gold standard exam for the diagnosis and treatment of endometrial polyps but can also be identified through other imaging exams, such as ultrasound with color Doppler [2]. Although there is no evidence of the benefit of polypectomy in women with RPL, hysteroscopic removal should be considered when the polyp is larger than 1 cm when no other known etiology is found [2, 47]. ASRM reports that research for uterine polyps in women with gestational loss is controversial as there is no conclusive evidence that surgical treatment reduces the risk of gestational loss [49].

6.2.3 Uterine synechiae/Asherman syndrome

The prevalence of uterine synechiae ranges from 0.5 to 28% in patients with RPL [47]. Women with RPL are more likely to have uterine synechiae as they often undergo curettage or manual vacuum aspiration. The probable pathophysiology of abortion occurs due to a reduction in the amount of functional endometrium which may interfere with the invasion and normal development of the placenta [47]. The gold standard exam for the diagnosis of synechiae is hysteroscopy and should be the exam of choice in the cases of suspicion [2]. ESHRE concludes that there is insufficient evidence to recommend adhesiolysis in women with RPL as there are only small observational studies. ESHRE reinforces that treatment should focus on preventing recurrence of adhesions [2, 3]. However, ASRM points out that surgical correction of significant uterine cavity defects should be considered [3]. Nonsurgical experimental techniques for the treatment of uterine synechiae and endometrial fibrosis, such as stem cell therapy, should be further studied before being indicated in clinical practice [2].

7. Endocrine factors

Hormones play a key role in placentation, and their changes may result in the risk of miscarriage [2].

7.1 Luteal phase insufficiency

It is a condition of insufficient exposure to progesterone to maintain a secretory endometrium that will lead to normal embryo implantation and growth [50]. The

diagnostic criteria for luteal insufficiency are not well established which makes it difficult to conduct studies that can demonstrate the causal link between luteal phase insufficiency and RPL. Thus, luteal phase failure testing is not recommended for patients with RPL [2, 3]. The use of progesterone or human chorionic gonadotropin (hCG) for its treatment is divergent in the literature [2, 3].

7.2 Thyroid disorders

Studies relating subclinical hypothyroidism, defined as thyroid-stimulating hormone (TSH) > 2.5 mU/L and normal free thyroxine, and increased risk of RPL, have low levels of evidence [2]. The anti-thyroid peroxidase antibodies' (anti-TPO) presence in patients with RPL, even euthyroid, is an important gestational prognostic factor [51]. Thus, a TSH and anti-TPO dosage is recommended for women with RPL. And, in detecting abnormal levels of the above exams, it recommends that T4 levels should be evaluated [2].

Patients with clinical hypothyroidism should be treated with levothyroxine [2, 3]. In women with RPL and subclinical hypothyroidism, the benefit of treatment should be evaluated as the evidences are conflicting [2, 3]. In addition, euthyroid women with positive anti-TPO should not be treated with levothyroxine [2, 52].

7.3 Polycystic ovary syndrome and disorders of insulin metabolism

Several abnormalities observed in patients with polycystic ovary syndrome (PCOS) have been independently associated with RPL, including insulin resistance, hyperinsulinemia, hyperandrogenemia, hyperprolactinemia, and obesity.

There is a higher prevalence of insulin resistance among women with RPL than controls [53]. However, no study has confirmed the cause-effect relationship between insulin resistance and RPL. Thus, there is insufficient evidence to recommend assessment of PCOS, fast insulin and fast glucose, and insulin and glycemia nor the use of metformin in pregnancy to prevent gestational loss in women with RPL and defects in glucose metabolism [2].

The presence of an independent link between hyperandrogenemia and RPL remains controversial. Therefore, researching androgen levels is not recommended in women with RPL [2].

7.4 Prolactin disorders

Most studies fail to establish a direct link between RPL and serum prolactin concentration. Thus, prolactin test is not routinely recommended in the absence of clinical signs of hyperprolactinemia [2]. But if hyperprolactinemia is detected, treatment with dopaminergic agonists may be considered in women to increase live birth rates [2, 3].Since hyperprolactinemia is an easily treatable cause, most centers routinely test serum prolactin levels.

7.5 Vitamin D

There are few studies evaluating the association between vitamin D deficiency and RPL [2]. One of them showed increased prevalence of hypovitaminosis D in women with RPL, but it was unable to demonstrate cause-effect relationship [2, 54]. Thus, based on the significant prevalence of hypovitaminosis D in women with RPL and possible association with obstetric and fetal complications, the preconception counseling in these women may include prophylactic vitamin D supplementation [2].

8. Chronic endometritis

Chronic endometritis (CE) is defined as a persistent inflammation of the endometrial mucosa caused by the presence of bacterial pathogens in the uterine cavity [55]. Its prevalence in patients with RPL is approximately 12–13% [56]. The influence of CE on reproductive capacity is controversial, but many authors suggest that CE may negatively affect embryonic implantation [56]. Some studies suggest an infectious etiology with positive cultures in 75% of women with histologically confirmed CE, with the most common bacteria being *Escherichia coli*, *Enterococcus faecalis*, and *Streptococcus agalactiae* (77.5%) [57]. Most patients are asymptomatic, with pain on uterine or cervical mobilization being the most common clinical presentation [58, 59].

CE is histopathologically diagnosed as a lymphoplasmacytic infiltrate in the endometrial stroma [58, 59]. Immunohistochemistry for the marker present in CD138 plasma cells is used to improve diagnostic accuracy [60]. A diagnostic video hysteroscopy can help identify CE, with direct visualization of the endometrial cavity, which usually presents with mucosal edema, focal or diffuse endometrial hyperemia, or micropolyps. The sensitivity, specificity, and positive and negative predictive values of hysteroscopy in diagnosing CE were 86.36, 87.30, 70.37, and 94.82%, respectively [61].

Up to a few years ago, the uterine cavity was thought of as a sterile environment. Recently, there has been discussed that an imbalance of the uterine microbiota might compromise embryonic implantation or induce an abortion. Endometrial biopsy for next-generation sequencing (NGS) microbiota evaluation and etiological agent research can now be done through commercial kits [55]. However, further studies are needed to evaluate diagnostic efficacy and therapy on the reproductive outcomes.

Some studies suggest that treatment is related to increased live birth rates and reduced abortion rates [62]. There are several therapeutic options; the main ones mentioned in the literature refer to the use of doxycycline alone (100 mg, 12/12 hours orally, for 14 days) or the combination of metronidazole (250 mg, 12/12 hours orally, for 14 days) and ciprofloxacin (250 mg orally 12/12 hours for 14 days) [59].

9. Male factors

There is a growing acceptance of male etiological factors for RPL. Its screening consists of detailed sperm analysis. Excessive sperm DNA fragmentation is an important constraint to conception. Two meta-analyses have shown the association of gestational losses with high rates of sperm DNA fragmentation [63, 64]. The available tests for sperm DNA fragmentation index are the sperm chromatin structure assay (SCSA), the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL), the Sperm Chromatin Dispersion test, and the comet assay.

Some clinical conditions are related to increased fragmentation of sperm DNA. High seminal plasma leukocyte concentration, systemic infections, varicocele, and smoking, among others, were related to spermatic DNA damage [65]. A Cochrane meta-analysis suggests that the use of antioxidants, including vitamins C and E, may have benefits for subfertile men with no apparent cause, improving sperm DNA fragmentation [66]. The generally recommended dose is 1 gram of vitamin C and 1000 IU of vitamin E per day for at least 2 months [67]. However, this effect is not yet established in patients with RPL. ESHRE determines that sperm DNA fragmentation research should be considered for explanatory purposes for RPL [2].

For intracytoplasmic sperm injection (ICSI)-indicated couples, laboratory techniques may be performed to select sperm with lower DNA fragmentation rate, such as physiological intracytoplasmic sperm injection (PICSI) and intracytoplasmic morphologically selected injection (IMSI). However, the use of testicular sperm

seems to improve fertilization, pregnancy, and live birth rates when compared to PICS and IMSI techniques [68]. Nevertheless, further studies are needed to identify the best method for selecting sperm to reduce abortion rates.

The morphological analysis of sperm is another point to consider in cases of RPL. The presence of spermatozoa with structural anomalies may be associated with aneuploidy, resulting in aneuploid embryos that usually do not implant or are aborted. This is especially true in cases of globozoospermia and macrospermia, forms of monomorphic teratospermia—when all sperms have the same anomaly [69]. Infertility is generally associated with these cases, and the prognosis of IVF is reserved. Thus, when associated with abortion, IVF followed by embryonic biopsy for preimplantation genetic testing for aneuploidies (PGT-A) may be an option.

10. Conclusion

Recurrent spontaneous abortion is an entity with a multifactorial etiology, and in approximately 50% of cases, we did not identify the cause of the loss. This explains the large number of controversies regarding the investigation and treatment of the pathologies that lead to repeated losses.

Despite so much controversy, there are some points on which experts agree. Psychological support for couples is essential and is associated with a better prognosis in subsequent pregnancy. Undergoing through periodic consultations and ultrasounds especially during the period of previous losses reduces the stress of these couples. The woman's age and number of previous losses are the most important factors in predicting the couple's chance of having a live baby in the next pregnancy.

There is a need for consensus among human reproduction societies on the tests that must be ordered and diagnostic criteria for all specialists to evaluate couples evenly. In this way, we will be able to evaluate the effectiveness of each available treatment, avoiding further financial burns, emotional disorders, and iatrogenesis for these couples.

Conflict of interest

The authors have no conflicts of interest that are relevant to this report.

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