

Review Article

Recurrent Pregnancy Loss: Proposal for a Novel Diagnostic Protocol with New Molecular Genetics Insight

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Introduction

Recurrent Pregnancy Loss (RPL) is defined as three consecutive Pregnancy Loss (PL) prior to 20 weeks of gestation, about 1-2% of women will be affected [1,2]. Recently, the American Society of Reproductive Medicine (ASRM) has redefined RPL as two or more pregnancy losses [3]. Using this definition, the incidence of RPL is up to 5% of women [1-3]. Different risk factors are associated with RPL, including advanced maternal age, and number of previous miscarriages [1]. Although RPL remains unexplained in about 50% of the cases, gynecological, endocrinological, immuno-hematological and genetical factors have been described.

Gynecologic Factors

The most common gynecologic factors associated with RPL are: (I) uterine malformations, (II) uterine fibroids, (III) uterine synechiae, (IV) infections and (V) progesterone deficiency.

I. Uterine malformations (i.e., Mullerian anomalies), such as septate uterus, can be up to three times more frequent in women with

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history of RPL [2,3]. However, surgical treatment of malformations does not seem to be associated with an improved reproductive outcome [4].

II. The intramural and submucosal fibroids could increase the risk of miscarriage and decrease live birth rate. However, there are still limited evidence on benefits of surgical treatment of uterine fibroids on reproductive outcomes [5].

III. There is some evidence that the uterine synechiae (Asherman's syndrome) may cause infertility and the RPL [6].

IV. Given the factors described above, it may be reasonable to look for uterine factors in young women with RPL, including ultrasound scan also with the three-dimensional approach or with sonohysterography, according to the local resource.

V. Regarding the potential role of local infections, the role of Ureaplasma, Chlamydia and Mycoplasma, or other agents, is still a subject of debate. Therefore, detection and therapy for these infections are not routinely recommended [2,3].

VI. The role of progesterone in RPL is controversial. Evidence showed that progesterone supports the pregnancy. For example, leutectomy prior to 7 weeks causes miscarriage, low progesterone levels have been linked to increase risk of first trimester miscarriage, and progesterone antagonist (mifepristone) have been successfully used in induction of abortion. Therefore, the central role of progesterone in early pregnancy led clinicians and researchers to hypothesize that progesterone deficiency could be a cause of some miscarriages. This hypothesis has results in numerous clinical trials of progesterone supplementation in early pregnancy bleeding, as well as in women with history of recurrent miscarriage. However, when studied in well designed placebo-controlled randomized trials, supplementation with progesterone did not result in improved reproductive outcome in women with RPL [7].

Endocrinological Causes

Endocrinological factors involved in RPL include (I) thyroid function, (II) glucose metabolism, (III) Polycystic Ovary Syndrome (PCOS) and (IV) hyperprolactinemia.

I. Because some data suggests that clinical or subclinical hypothyroidism is associated with RPL [3], many guidelines recommended testing for Thyroid-Stimulating Hormone (TSH) levels in RPL patients and treating only those with overt hypothyroidism [8]. Our proposal is to test thyroids hormone levels in RPL patients and refer to endocrinologist in case of abnormal results.

II. Regarding glucose metabolism, different guidelines report that well controlled diabetes is not a risk factor for RPL [1] so we suggest testing glucose and glycosylated hemoglobin only in case of presence of clinical suspect.

III. A lot of studies were performed on the possible influence of PCOS on the pregnancy and RPL [1,9]: however strong evidence of this

hypothesis is still not present so routine screening for PCOS is not recommended for treatment or investigation of RPL [1,10].

IV. Elevated prolactin may cause ovulatory dysfunction and infertility but its role in RPL is still controversial and unclear. The European Society of Human Reproduction and Embryology guidelines do not recommend testing prolactin in the absence of clinical suspicion, while the American Society for Reproductive Medicine states that testing can be considered [1]. There is some weak evidence to suggest that normalising hyperprolactinemia can improve live births in RPL [1]. Our suggestion is to test prolactin level only in case of signs or symptoms and eventually refer to a specialist.

Immuno-Hematologic Causes

Two main immuno-hematological issues are been involved in RPL: (I) a thrombophilic status with particular attention to antiphospholipid syndrome and (II) an autoimmune dysregulation.

- I. Thrombotic events can cause abortion at any time of pregnancy. A thrombophilic status can be due to specific genetic polymorphisms (see Genetical causes) or to other acquired conditions that can alter coagulation status of the patient. The most frequent of these acquired conditions in RPL patients is the presence of anticardiolipin and/or lupus anticoagulant antibodies that, when associated with pregnancy loss or thrombotic events, is also known as Antiphospholipid Syndrome (APS). The APS, by the direct inhibition of placenta ion or the disruption of adhesion molecules or the thrombosis of placental vasculature, can play an important role in the RPL pathogenesis [1]. There are a lot of studies that have demonstrated the correlation between RPL and APS [11]. In fact, many guidelines recommend testing the APS in RPL woman and also suggest treating RPL patients with APS positivity with aspirin and low molecular weight heparin [1-3]. Consequently our suggestion is to check complete coagulation profile and APS antibodies in RPL patients and to refer to hemostasis specialist if there are abnormal values.
- II. There are a lot of studies that have investigated the potential role of immune system in the pathogenesis of RPL. This includes the study of human leukocyte antigen (HLA) typing, natural killer cells, proinflammatory interleukin polymorphisms and immunomodulation with, for example, intravenous immunoglobulin, corticosteroids, intralipid infusion, auto-transfusion of lymphocytes and platelet rich plasma [12-16]. Anyway there is limited evidence that immunodysregulation and/or immunomodulation has any effect on RPL so investigations for auto-immunity, outside of APS, are not recommended [1]. Thus we don't suggest any specific autoimmunity analyses, rather than those for APS.

Genetical Causes

To date, there are two principal genetics factors that can influence the pregnancy outcome: (I) the presence of chromosomal abnormalities and (II) the presence of genomic DNA mutations.

- I. It has been calculated that about 50-60% of abortions is due to a lethal chromosomal abnormalities: complete trisomies, monosomies or polyploidies [17-21]. These aneuploidies are sporadic and generally originate from a non-disjunction maternal event [19-21]. The diagnosis requests karyotype on the abortion specimens or, sometimes, Non Invasive Prenatal Test (NIPT) may be used.

Although these anomalies are frequent in spontaneous abortions, they are also isolated and couples experiencing this event do not present a significant risk of recurrence for future pregnancies. Unfortunately, in the majority of cases is not possible to perform the genetic tests on abortion specimens and the miscarriage remains idiopathic. An important part of these idiopathic abortions are due to a rearrangement of a chromosomal balanced anomaly present in one of the partners (translocation, inversion or insertion). It has been shown that in about 5% of couples with RPL, one of the partners carries a balanced asymptomatic chromosomal anomaly [1,2] that can be transmitted in unbalanced lethal form to the offsprings. Moreover, this chromosomal rearrangement presents a relatively high risk of recurrence. Options for these couples include Pre-implantation Genetic Diagnosis (PGD), spontaneous conception with invasive testing of subsequent pregnancies to know and avoid imbalance transmission (chorionic villus sampling or amniocentesis), gamete donation [1]. The preimplantation genetic diagnosis, using medically assisted procreation techniques, can exclude the possibility of transmitting unbalanced chromosomal anomalies by the genetic selection of embryos to transfer. Our suggestion is to perform peripheral standard karyotype for RPL couples that haven't diagnosis of miscarriage with completed aneuploidy. Our proposal is also to perform a high-resolution karyotyping, known as Comparative Genomic Hybridization Array, if they have normal standard karyotype but positive family history of RPL for the female partner. This new molecular technique can identify the woman with a rearrangement on X chromosome that could be lethal or pathogenetic for male's offspring.

- II. If the RPL patients present positive familial history of a specific mendelian disease, the causative genes could be screened in partners in order to identify the pathogenetic mutations and eventually use the preimplantation genetic diagnosis. This therapeutical option can be applied also to consanguineous partners with RPL if the molecular analyses identify the specific mutations that cause the recurrent abortions. A lot of genes were studied and potentially involved in the pathogenesis of RPL such DYNC2H1, KIF14, RYR1, GLE1, AMN, THBD, PROCN, VEGF, TP53, NOS3, JAK2 and so many others [22,23] but there is not a strong evidence of pathogenicity in miscarriage or recurrent gene mutations to test. So, except for rare cases, our opinion is that there isn't to date any specific gene or mutation related to RPL in general population that can be studied or proposed. However, many polymorphisms are under investigation, but the evidence is still limited. Between these, the polymorphisms related to thrombotic predisposition are been largely studied and often suggested [24-26]. A lot of papers have reported the association between V Leiden and Prothrombin polymorphisms and RPL [27-31]. Our proposal is to check complete coagulation biochemical screening with addition only of V Leiden and Prothrombin polymorphisms for all RPL patients. Finally, because it is now possible to investigate a lot of genes simultaneously using the next generation sequencing, we also suggest for all RPL woman that present a suggestive family history of male abortions to refer to genetic counsellor in order to screen X linked gene diseases that are lethal in male.

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