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Review Article

Recurrent Miscarriage: A Review

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Abstract

Human reproduction is characterized by its inefficiency. The loss of pregnancy at any stage can be a devastating experience and particular sensitivity is required in assessing and counseling couples with Recurrent Miscarriage (RM). Recurrent Miscarriage (RM) represents a clinical challenge for physicians not only because there are multiple possible etiologies, but also because the diagnostic testing is costly and time consuming. Despite several well-known etiologic factors, the cause of RM cannot be determined in almost 50% of cases. Multiple potential etiologies for RM have been described and, as a consequence, several recommendations with very different levels of evidence have been published regarding the evaluation and management of this condition.

This comprehensive review addresses factors related to age, genetics, anti-phospholipid syndrome, uterine anomalies, thrombophilias, hormonal or metabolic disorders, infection, autoimmunity, sperm quality, and life-style issues involved in the etiology of RM.

Introduction

Recurrent Miscarriage (RM) is a highly frustrating problem not only for patients, but also for the physicians who must diagnose and treat it being a heterogeneous reproductive problem, with multiple etiologies and contributing factors [1].

Definition

Pregnancy losses have been traditionally defined as spontaneous abortions or miscarriages if they occur before the fetus reaches viability at 24 weeks of gestation and stillbirths if they occur after 24 weeks [2].

Recently, the European Society of Human Reproduction and Embryology (ESHRE) have updated the terminology regarding early pregnancy events [2]. Clinical miscarriages may be subdivided into early clinical pregnancy losses (before gestational week 12) and late clinical pregnancy losses (gestational weeks 12 to 21). The term miscarriage is preferred rather than abortion both by patients and physicians, although "spontaneous abortion" is still used in scientific literature [3,4].

For more than 30 years, controversy has existed on the number of miscarriages required to define RM and when diagnostic testing is warranted [5]. The definition ranges from two clinical miscarriages, not necessarily consecutive, according to the American Society for Reproductive Medicine (ASRM) [6] and a joint International Committee for Monitoring Assisted Reproductive Technology and World Health Organization glossary [7], affecting more than 3% of couples desiring a baby to three consecutive pregnancy losses (not necessarily intra-uterine) as defined by both the European Society for Human Reproduction and Embryology and the Royal College of Obstetricians and Gynecologists [4] and affecting 1% of couples trying to conceive [8].

Epidemiology

Early pregnancy loss is perhaps the most common obstetric complication, occurring in over two-thirds of human conceptions. Approximately 15 - 20% of all clinically recognized pregnancies will end in a miscarriage [4,9,10]. However, prospective cohort studies using sensitive and specific daily urinary hCG assays in women trying to conceive have demonstrated that only around one-third of conceptions progress to a live birth. Most of the losses occur before the clinical recognition of pregnancy as implantation failures. An estimated 30% of human conceptions are lost prior to implantation and a further 30% following implantation but before the missed menstrual period, that is in the third or fourth week of gestation. These are often termed preclinical losses [11].

The risk of sporadic miscarriage between 6 and 12 weeks of gestation in women less than 35 years of age is 9% to 12%. The risk increases with age. In women older than 40 years of age, the sporadic miscarriage rate approaches 50%. Late losses between 12 and 22 weeks occur less frequently and constitute around 4% of pregnancy outcomes [4,9].

Compared to sporadic miscarriage the prevalence of RM is considerably lower irrespective of whether biochemical losses are included or not. Approximately 1% of all women trying to conceive have recurrent miscarriage, defined as three previous miscarriages; when recurrent miscarriage is defined as two previous miscarriages, the proportion rises to 3% [12,13].

Etiology

Multiple potential etiologies for RM have been described (Table 1). As a consequence, several recommendations have been published regarding the evaluation and management of RM [3].

The potential etiologies of RM can be divided into embryologically driven causes (mainly due to an abnormal embryonic karyotype) and maternally driven causes which affect the endometrium and/or placental development [14,15]. Thus, studies that focus on RM have

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Etiology		Screening	Management	Controversial evidence	Not recommended
Genetic abnormalities	Embryonic chromosomal abnormalities	Genetic analysis of products of conception	Preimplantational genetic screening		
	Parental balanced reciprocal translocations	Parental karyotype	Preimplantational genetic screening		
	Sperm DNA fragmentation				
Thrombotic disorders	Hereditary thrombophilia	Thrombotic tests		Heparin, LDA	
	Antiphospholipid syndrome		LAC, ACA IgG & IgM antibody		
Alloimmunity			None	Uterine NK cells, cytokine profiles	Circulating NK cells HLA typing
Uterine anatomic abnormalities	Congenital uterine malformations	MRI 3D-ultrasound	Hysteroscopy: septal resection		
	Acquired anatomic disorders	MRI 3D-ultrasound	Hysteroscopy	Treatment of myomas	Cervical incompetence
Hormonal/metabolic etiology	Hypotidoidism, hyperprolactinemia, diabetes mellitus, polycystic ovarian syndrome	TSH, PRL, Hb A1C	Levothyroxine	Insulin resistance	Luteal phase progesterone
Environnmental, occupational, personal habits		Anamnesis	Alcohol cessation	Weight loss	

Table 1: Possible causes of Recurrent Miscarriage (RM).

LDA: Low Dose Aspirin

LAC: Lupus Antiocoagulant

ACA: Anticardiolipin Antibodies

Possible etiologies of recurrent miscarriages

I. Genetic abnormalities
   - Embryonic chromosomal abnormalities
   - Parental balanced reciprocal translocations

II. Thrombotic disorders
   - Thrombophilia
   - Acquired thrombophilic conditions: antiphospholipid syndrome

III. Alloimmunity

IV. Uterine anatomic abnormalities
   - Congenital uterine malformations
   - Acquired anatomic disorders

V. Hormonal/metabolic etiology
   - Hypothyroidism
   - Diabetes mellitus
   - Hyper-prolactinemia
   - PCOS
   - Luteal phased effect
   - Obesity
   - Environmental, occupational, personal habits

examined factors related to age, genetics, antiphospholipid syndrome, thrombophilias, uterine anomalies, hormonal or metabolic disorders, infection, autoimmunity, sperm quality and life-style issues. Most of sporadic losses before 10 weeks' gestation (approximately 50% of clinical pregnancies) result from random numeric chromosome errors, specifically, trisomy, monosomy and polyploidy in the embryos [16]. The parental factors most directly linked to an abnormal karyotype include chromosomal translocations [5]. In addition, endocrine dysfunction and metabolic disorders, auto-immune diseases, infectious diseases, environmental toxins and congenital or structural uterine anomalies have been implicated [17]. Of these likely etiologies, uterine anomalies and antiphospholipid syndrome are the most prevalent [5,9].

Despite thorough examinations to exclude several well-known etiologic factors, the cause for recurrent spontaneous abortion can often not be found in almost 50% of cases [18]. These are termed unexplained recurrent miscarriages. In recent years, progress in the fields of cytogenetics and immunogenetics and a greater understanding of implantation and maternal-embryo interactions has offered new insights into the possible causes of this condition, and opened up new avenues for research into its prevention and treatment [4].

Genetic abnormalities

Embryonic chromosomal abnormalities:

It is a generally accepted assumption that most of spontaneous miscarriages are due to chromosomal abnormalities in the embryo or fetus [17,19]. Overall, cytogenetic abnormalities (including fetal aneuploidy or polyploidy) are found in 50% to 70% of spontaneous abortion specimens arising from natural conceptions [20,21]. Indeed, some authors explain spontaneous miscarriages as a 'physiological' phenomenon, which prevents conceptions affected by chromosomal aberrations incompatible with life from progressing to viability [4,11].

Aneuploidy, including a missing (monosomy) or extra (trisomy) chromosome, is the most common type of chromosome abnormality and the leading cause of implantation failure, miscarriage and congenital abnormalities in humans [7,22]. The proportion of karyotypically abnormal abortus specimens is highest earlier in gestation, and drops with increasing gestational age. Studies using comparative genomic hybridization to assess the chromosomal complement of all blastomeres in preimplantation human embryos show that more than 90% embryos have at least one chromosomal abnormality in one or more cells. The reported rates of chromosomal abnormalities are up to 90% in an embryonic specimens, approximately 50% at 8 to 11 weeks' gestation and around 30% at 16 to 19 weeks' gestation [23].

Among natural conceptions, trisomy of chromosome 16 (accounting for 20 to 30% of all trisomies seen in abortus specimens) and monosomy X (45,X) are the most frequently observed abnormalities, followed by trisomy 2, 13-15, 18, 21 and 22 [8]. The most commonly encountered chromosomal abnormality among preimplantation human embryos is trisomy 21. The autosomal trisomies typically arise *de novo* owing to meiotic nondisjunction during gametogenesis. The parental karyotypes are normal in most of these cases conferring a minimal recurrence risk [24].

The risk of miscarriage resulting from chromosomal abnormalities of the embryo increases with advancing maternal age. Approximately 70% of fetal trisomies are of maternal origin and caused by a mal-segregation event in the first meiotic division. In contrast, sex chromosomal aneuploidies can more frequently be traced back to the father (50% of 47, XXY, 100% of 47, XYY and 70%-80% of 45, X) [3,8,25].

In general, as the number of miscarriages increases, the risk of euploid pregnancy loss increases [26]. Thus, the rate of chromosomal abnormalities among RM is lower than in sporadic miscarriages. However, different studies have reported that the risk of fetal aneuploidy increases in couples with previous spontaneous abortions or aneuploid conceptions due to both autosomes and sex chromosomes independently of the origin of the previous pregnancy either Natural Conception (NC) or Assisted Reproductive Treatment (ART) [27]. Furthermore, the incidence was increased when previous aneuploidies were in autosomes. Women who had a previous trisomic pregnancy, particularly those younger than 35 years old, appear to be at increased risk for subsequent trisomic pregnancies [9]. Aneuploidy for chromosomes 16 and 22 were more common in patients with previous autosomal aneuploidy in NC; an increase in aneuploidy for all chromosomes was detected in previous aneuploid pregnancies derived through ART.

Parental chromosomal abnormalities may represent an important etiology of recurrent miscarriage and an increased prevalence of balanced rearrangements has been observed in the couples with RM. The most commonly encountered abnormalities include balanced translocations and inversions that do not have any consequences for the phenotype of the carrier, but in pregnancy there is a 50% risk of fetus with an unbalanced chromosomal abnormality that can result in a miscarriage [17,28,29].

A chromosomal abnormality in one partner is found in 3% [17], 5% [28] to 21% of RM couples [8,30,31]. The abnormality is about twice as likely to occur in the mother as well as father. In one study of couples with RM and balanced parental chromosomal abnormalities, approximately 50% of chromosome abnormalities detected were balanced reciprocal translocations, 24% were Robertsonian translocations, and 12% were sex chromosomal mosaicisms in females. The remainders were chromosomal inversions and other sporadic abnormalities. The risk of miscarriage is influenced by the size and the genetic content of the rearranged chromosomal segments [30].

There is much controversy in the literature concerning the role of inv(9), and its clinical consequences remain unclear. Interestingly, the adjusted odds ratio of subsequent miscarriage in the couples with inv(9) in either partner was significantly higher in a recent study, although inv(9) generally is thought to have no adverse effect on reproduction as a normal variant [28].

During the last two decades, numerous Fluorescence *In Situ* Hybridization (FISH) studies to interphase nuclei of human spermatozoa ("sperm-FISH") have elucidated nondisjunction mechanisms and frequencies in male germ cells [25]. However, few data were available regarding aneuploidy in spermatozoa from men in couples with RM or the associated risk of spontaneous miscarriage, because only a small number men affected of RM had been analyzed; most of these analyses had been limited to chromosomes 13, 18, 21, X and Y. Initial sperm-FISH data among RM has indicated that these patients may have an elevated gonosomal disomy rate [32]. It is important to realize that sperm aneuploidy rates can be high even in men with normal sperm morphology [33]. It is noteworthy that although the overall mean aneuploidy seems to be small (0.18%–1.04%), it is up to four times higher than the aneuploidy observed in controls (0.03%-0.38%). Regrettably, there are no universally accepted standards for abnormal FISH results compared with those that exist for strict morphology and DNA fragmentation [33].

Regarding specific potentially RM-causing genetic mutations, no association has been found with NLRP2, NLRP7 and KHDC3L among these patients [34]. Furthermore, the study of genes involved in immune response (IFNG, IL10, KIR2DS2, KIR2DS3, KIR2DS4, MBL, TNF), coagulation (F2, F5, PAI-1, PROZ), metabolism (GSTT1, MTHFR) and angiogenesis (NOS3, VEGFA) have been thoughtfully assessed without finding a clear association with RM [35,36].

Management:

- Genetic causes of RM should be evaluated. In the evaluation of RM, parents should undergo peripheral karyotyping in order to detect any balanced structural chromosomal abnormalities
- Parental karyotyping is specially recommended. If maternal age is low at the second miscarriage, or if there is a history of two or more miscarriages in first degree relatives
- It is noteworthy that CGH is not useful for the detection of balanced translocations. Thus, traditional karyotype should be performed when testing parents with RM to exclude balanced chromosomal abnormalities
- Routine testing for sperm ploidy (e.g., Fluorescence *In Situ* Hybridization (FISH)) or DNA fragmentation is not recommended

- Since there is a high frequency of karyotypic abnormalities in products of conception while the incidence of karyotypic abnormalities in the parents is low, miscarriage chromosome testing is useful to determine which miscarriages are random, and which may be due to other factors associated with RM [26].
- Cell-free fetal DNA can be isolated from the maternal circulation from 7 weeks of gestation, and next generation sequencing techniques have been applied to detect fetal aneuploidies in cell-free fetal DNA. Since it will soon be possible to sequence the entire fetal genome from free fetal DNA in the maternal circulation, new insights will be achieved in relation to both chromosomal abnormalities and single gene disorders as a cause of sporadic and recurrent miscarriage [37]
- Once a structural genetic factor is identified genetic counseling is to be offered
- When one of the partners has a structural genetic abnormality, Preimplantation Genetic Screening (PGS) represents a therapeutic option. The transfer of embryos without chromosome abnormalities by means of PGS, would improve the reproductive performance of couples with RM due to karyotyping abnormalities. Furthermore, PGS also being increasingly used for patients with a history of RM, even in the absence of parental translocations [38,39]

Thrombosis and RM

The histologic findings of placental infarction, necrosis and vascular thrombosis in some cases of pregnancy loss led to the hypothesis that thrombosis in the utero-placental circulation may lead to placental infarction and ultimately, pregnancy loss, included miscarriages. Many studies have examined the association between thrombophilia and pregnancy complications, often with differing results [40].

Although the relationship between vascular thrombosis and obstetric complications was first recognized in women with Antiphospholipid Syndrome (APS), both inherited and acquired thrombophilia have been associated with recurrent pregnancy loss and pregnancy complications, such as severe pre-eclampsia, fetal growth restriction and stillbirth [41].

Hereditary thrombophilias include deficiencies of antithrombin, protein C and protein S, and abnormalities of pro-coagulant factors, particularly, Factor V Leiden (FVL), the prothrombin G20210A mutation, and the thermo-labile variant of the Methylene Tetrahydrofolate Reductase (MTHFR) gene. Other relatively common thrombophilias with a combination of heritable and acquired components include elevated plasma factor VIIIc, hyperhomocysteinaemia and acquired activated protein C resistance [42].

The association among hereditary thrombophilia, Recurrent Miscarriage (RM) and obstetric complications yet uncertain was nicely summarized in a meta-analysis of 31 retrospective studies by Rey and coworkers. This group showed that thrombophilic defects are more prevalent in women with recurrent first trimester pregnancy loss, although thrombophilia and late pregnancy loss are more consistently associated [43].

Factor V Leiden has been associated with recurrent first-trimester fetal loss (OR 2.01,95% CI 1.13–3.58), recurrent fetal loss after 22 weeks (OR 7.83,95% CI 2.83-21.67) and non-recurrent fetal loss after 19 weeks (OR 3.26, 95% CI 1.82-5.83). A recently published meta-analysis of 16 case-control studies reported that carriers of factor V Leiden or prothrombin gene mutation have double the risk of experiencing RM compared with women without these mutations. Activated protein C resistance has been associated with recurrent first-trimester fetal loss (OR 3.48, 95% CI 1.58-7.69). Prothrombin gene mutation has been associated with recurrent first-trimester fetal loss (OR 2.32, 95%CI 1.12-4.79), recurrent fetal loss before 25 weeks (OR 2.56, 95% CI 1.04-6.29) and late non-recurrent fetal loss (OR 2.3, 95%CI 1.09-4.87). Protein S deficiency has been associated with recurrent fetal loss (OR 14, 95%CI 0.99-218) and non-recurrent fetal loss after 22 weeks (OR 7.39, 95%CI 1.28-42.83). On the other hand, MTHFR polymorphism, 677 TT homozygosity, deficiencies of PC, ATIII and the MTHFR mutation associated with hyperhomocysteinemia were not found to increase the risk for recurrent early pregnancy loss [43].

In contrast to the positive relationships suggested in multiple case-control and retrospective cohort-control studies, large prospective studies have not demonstrated a relationship between hereditary thrombophilias and obstetric complications. Furthermore, a meta-analysis of prospective cohort studies failed to find a causal relationship between the prothrombin mutation and RM [44,45].

Acquired thrombophilic conditions

Up to 15% of the patients with RM have been found to be positive for antiphospholipid antibody syndrome (APS) [46]. Antiphospholipid Syndrome (APS) is an acquired and autoimmune thrombophilic condition that is marked by the presence in blood of antiphospholipid antibodies (aPL), lupus anticoagulant, anti-cardiolipin antibodies or anti-B2 glycoprotein-I, identified in repeated samples taken 3 months apart and prior to pregnancy [4,47] and adverse pregnancy outcome or vascular thrombosis [48,49]. Classification for this syndrome needs at least one clinical manifestation together with positive tests for circulating antiphospholipid antibodies, including lupus anti-coagulant or anticardiolipin, or both, at medium-high values, detected at least twice in 6 weeks. The APS is the most important treatable cause of recurrent miscarriage [9]. In women with RM associated with antiphospholipid antibodies, the live birth rate in pregnancies with no pharmacological intervention has been reported to be as low as 10% [50].

Fetal loss (≥10 weeks of gestation) is more strongly associated with aPL than are earlier pregnancy losses [50]. These patients have up to 80% risk of current pregnancy loss (110). Both IgG and IgM anticardiolipins are associated with an increased risk of miscarriage, albeit to a lesser degree than lupus anticoagulant. The most widely accepted tests are for Lupus Anticoagulant (LA), anticardiolipin antibody (aCL) and anti- B2 glycoprotein I [51]. Antiphospholipid antibodies (aPL) can be broadly categorized into those antibodies that prolong phospholipid-dependent coagulation assays, known as Lupus Anticoagulants (LA), or anticardiolipin antibodies (aCL), which target a molecular congener of cardiolipin (a bovine cardiac protein). They are present in 15% of women with recurrent miscarriage [50]. By comparison, the prevalence of aCL and LAC in normal healthy populations with a low-risk obstetric history has been reported to range between 1.0% and 5.6% and between 1.0% and 3.6%, respectively. A positive LA appears to be more specific for APS than an elevated aCL [47,52].

In the detection of lupus anticoagulant, the dilute Russell's viper venom time test together with a platelet neutralization procedure is more sensitive and specific than either the activated partial thromboplastin time test or the kaolin clotting time test. Anticardiolipin antibodies are detected using a standardized enzyme-linked immunosorbent assay [3,50]. The detection of antiphospholipid antibodies

is subject to considerable inter-laboratory variation. This is a result of temporal fluctuation of antiphospholipid antibody titles in individual women, transient positivity secondary to infections, suboptimal sample collection and preparation and lack of standardization of laboratory tests for their detection.

Lupus Anticoagulants (LA) reduces the coagulant potential of the plasma and prolongs the clotting time in coagulation tests based on the activated partial thromboplastin time. Consensus guidelines recommend screening for LA with 2 or more phospholipid-dependent coagulation tests. Anticoagulant therapy may interfere with the detection of LA. Anticardiolipin antibodies (aCL) share a common *in vitro* binding affinity for cardiolipin and can be detected using enzyme-linked immunosorbent assays. Enzyme-linked immunosorbent assay tests for aCL are poorly standardized and aCL testing has shown poor concordance between laboratories [53].

It was initially suggested that the association between the presence of aPL and miscarriages was caused by an increased risk of thrombus formation in the nascent placental vessels resulting in placenta infarctions. However, it is noteworthy that women with the presence of aPL and no evidence of placental thrombosis also experience pregnancy loss [41,54]. Thus, pathophysiology other than placental thrombosis may influence pregnancy outcome. Antiphospholipid antibodies have a variety of effects on the trophoblast, including inhibition of trophoblastic function and differentiation, induction of syncytiotrophoblast apoptosis, and activation of complement pathways at the maternal-fetal interface resulting in a local inflammatory response [55].

In vitro studies have shown that the effect of antiphospholipid antibodies on trophoblast function and complement activation [9,55] may be reversed by heparin and both low-dose aspirin and low molecular weight heparin have been recommended for the cases of obstetric APS. Unfortunately, 30% of the cases continue to experience pregnancy loss in spite of treatment with no obvious cause and no effective treatment [46].

Catastrophic Antiphospholipid Syndrome (CAPS) is a rare variant that accounts for 1% of patients with APS. Despite its low frequency, the mortality-related is very high ranging from 50% of patients in the first series to 37% in the most recent data. The current knowledge of this potential devastating entity comes from the International Registry of patients with CAPS, named CAPS Registry [56]. Treatment is based on the combination of anticoagulation, glucocorticoids, plasma exchange and/or intravenous immunoglobulins, the so-called triple therapy. In refractory cases or in those with initial life-threatening situation, rituximab may be an effective option. Some cases of CAPS have been effectively treated with the addition of eculizumab to the triple therapy [56].

Management:

- Routine testing of women with RM for inherited thrombophilias is not currently recommended. Screening may be clinically justified only when a patient has a personal history of venous thromboembolism in the setting of a non-recurrent risk factor (such as surgery) or a first-degree relative with a known or suspected high-risk thrombophilia
- The efficacy of thromboprophylaxis during pregnancy in women with recurrent first-trimester miscarriage who have inherited thrombophilias, but who are otherwise asymptomatic, has not been assessed in prospective randomized controlled trials
- Cohort studies have suggested that heparin therapy may improve the live birth rate for these women
- Regarding RM associated with antiphospholipid antibodies (aPL), testing for aPLs is indicated in the setting of three or more unexplained spontaneous abortions before the 10th week of gestation when maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes have been excluded
- A single unexplained loss of a morphologically normal fetus at or beyond 10 weeks of gestation also is considered to warrant testing for aPLs
- Women with RM and antiphospholipid syndrome should be given a combination of either low-dose heparin or low-molecular weight heparin and low-dose aspirin [52,54]. Aspirin (81 mg/d) should be started with attempted conception
- Treatment of refractory or catastrophic APS syndrome is based on the triple therapy (combination of anticoagulation, glucocorticoids, plasma exchange and/or intravenous immunoglobulins) but in some cases additional therapies should be assayed
- Other therapeutic options such as prednisone or intravenous immunoglobulins do not improve results compared with heparin and low-dose aspirin and are associated to maternal morbidity
- On the basis of presumed similarities in pathogenesis between RM associated with the antiphospholipid syndrome and unexplained recurrent miscarriage [57,58], aspirin and low-molecular-weight heparin are frequently prescribed for women with unexplained RM. The benefits of aspirin or heparin treatment among these patients are unproven, whereas the risks, although low, are real. Therefore, it should be concluded that, at present, there is no evidence to suggest aspirin or heparin treatment in unexplained RM patients [12]

Alloinmunity

Successful pregnancy is the result of a fine balance of immune reactions. Indeed, it has been an enigma how the implanting embryo and trophoblast escape maternal immunological rejection in the uterus in spite of carrying allogeneic proteins encoded by paternal genes. An adequate immune response is considered a key factor in the control of endometrial receptivity and fertility in women. The implantation success requires an adequate maternal immune tolerogenic microenvironment that protects the semiallogenic fetus from maternal immune rejection [4,15,36,59].

The mechanisms by which the fetus is protected from the maternal immune system during normal pregnancy are not fully understood. The immune system of the mother is tightly controlled to defend against microbial infections, but to accept the embryo, which expresses semiallogenic paternal antigens through its development. It is likely that mechanisms have developed to prevent immune rejection of the embryo including local and systemic immune responses involving immunoglobulins, cytokines, hormonal and other endometrial factors, and only when several mechanisms fail in a woman RM will occur [13,15,60].

It has been postulated that a proportion of RM may be due to immune causes, i.e., a sort of immunological impairment at the feto-maternal unit. Considerable evidence has associated adverse immune responses with infertility problems, and proinflammation molecules have been reported to be involved in compromised endometrial receptivity and fetus implantation [14,61].

Various alternative approaches have been adopted to study the role of immune cells and molecules in the etiology of RM. These include

the analysis of immune cell populations and cytokines in: the peripheral blood of women who suffer RM and normal fertile women either before pregnancy or at the time of miscarriage; endometrial tissue obtained from women with RM and normal fertile women in the peri-implantation period in the non-pregnant state; and placental tissue obtained at the time of miscarriage from women with a history of RM, from women with a spontaneous, non-RM and from women requesting terminations of normal pregnancy [62].

The population of leukocytes in human endometrium has been extensively studied and consists mainly in uterine Natural Killer (uNK) cells, T cells and macrophages. The most abundant immune cells in the uterine decidua around the time of implantation and early placental development are the uNK cells. Altered numbers of uNK cells have been associated with several human reproductive disorders, including RM [14,46,60].

The numbers and proportions of each cell type vary through the menstrual cycle and in early pregnancy. T cells make up approximately 45% of leukocytes in the proliferative endometrium, and although their absolute numbers remain constant throughout the cycle and in early pregnancy their relative numbers decreases as the proportion of uNK cells increase. Antiphospholipid antibodies augment NK cell numbers and cytotoxicity, and result in an increased recruitment of decidual NK cells. Under these conditions, noncytotoxic decidual NK cells might change to cytotoxic $CD56^{+}/16^{+}$NK cells, which in turn act via several mechanisms such as the mediation of trophoblastic tissue apoptosis and the secretion of various proinflammatory cytokines causing decidual microvessel thrombosis and fetal loss [46].

Recognition of foreign cells occurs due to the expression of Major Histocompatibility Complex (MHC) molecules on the cell surface, and the maternal immune system should recognize fetal trophoblast cells as foreign if they express paternal MHC molecules. Fetal extra-villous cytotrophoblast cells do not express the classical MHC 1 molecules, HLA-A and HLA- B, and MHC class II molecules are also absent. Instead, they express the non-classical HLA-G and E molecules, together with low expression of HLA-C [63].

Human Leukocyte Antigen (HLA)-G is a non-classic class 1 protein that is expressed on the surface of invading cytotrophoblast and is thought to play a role in immunoprotection of the developing pregnancy. There have been several reports linking HLA-G deficiency to RM, and certain polymorphisms in this gene have been associated with increased miscarriage rates in selected populations. Unlike other HLA genes, HLA-G shows an almost complete lack of polymorphism in its nucleotide sequence, which means that the HLA-G protein is essentially invariant in the human population [64]. However, although HLA-G shows little polymorphism, its mRNA undergoes alternative splicing to produce five main forms of the molecule. HLA-G is also expressed by human embryos, and measurements of sHLA-G in culture supernatants of early embryos obtained by IVF before transfer have shown that its presence appears to be essential for successful pregnancy outcome [14].

Studies of Human Leukocyte Antigen (HLA) typing, embryotoxic factors, decidual cytokine profiles, blocking or anti-paternal antibody levels, HLA-G polymorphism, and other immunologic traits and factors have produced inconsistent data that generally have not been reproduced in more than one laboratory [9].

Management:

- There is no clear evidence to support the hypothesis of human leucocyte antigen histoincompatibility between couples, the absence of maternal leucocytotoxic antibodies or the absence of maternal blocking antibodies as the etiology of RM. Hence, they should not be offered routinely in the investigation of couples with RM [3]
- Proposed treatment options for RM where immunologic dysregulation is suggested to play a role, include prednisone, allogeneic lymphocyte immunization, intra-venous immunoglobulin infusion and injection of Tumor Necrosis Factor α (TNFα) antagonists or Granulocyte Colony-Stimulating Factor (G-CSF). Such immunomodulatory treatments for RM have not been proven effective. The use of immunotherapy should no longer be offered to women with unexplained recurrent miscarriage [65,66]

Anatomy

Several investigations have measured the prevalence of uterine anomalies among patients with RM.

Congenital uterine abnormalities are associated with second trimester pregnancy loss in addition to other complications, including preterm labor, fetal malpresentation, and increased rates of cesarean delivery. Although the role of uterine malformations in first-trimester RM is debatable, assessment of uterine anatomy is widely recommended. Potentially relevant congenital Mullerian tract anomalies include unicornuate, didelphic, bicornuate, septate or arcuate uteri. The presence of a uterine septum was not only the most prevalent congenital defect, but also the only congenital defect to be more common in patients with primary RM, occurring in this group at more than double the rate of septal defects among the women in the general population [5,13,21,29,36,67].

These findings support the contention that correction of septate defects in particular may have beneficial effects among women with primary RM [68] and should be considered in this setting of patients. Woelfer et al., found no correlation between septum length and adverse pregnancy outcomes, yet Salim et al., [69] reported that women with RM had a greater ratio of the septum length relative to the uterine cavity compared with a control group. Jaslow et al., [5] did not examine whether there were differences in the types of septal defects in different RM groups, but if proportionately larger defects do correlate with pregnancy loss, it is possible that septal defects in patients with primary RM may be more severe than those in patients with secondary RM. The primary limitation of these data is the lack of randomized, controlled therapeutic trials.

The Frequencies of acquired defects (fibroids, adhesions, polyps) are more difficult to determine. Acquired anomalies of all types have been reported in 11%–23% of patients with RM [9], yet estimates for the frequency of fibroids alone range from 2.7% in pregnant women to about 50% in women of reproductive age.

Intrauterine Adhesions (IUA) or synechiae were first described in 1894, by Heinrich Fritsch [70]. In 1988, the ASRM published a classification system that categorized intrauterine adhesions from filmy to dense [71]. Although adhesions generally are known to impair reproductive success, studies suggest that the likelihood of adverse pregnancy outcomes is greater among women with more severe (e.g., dense) adhesions. The category of possible fertility symptoms in patients with IUAs includes secondary RM.

Like adhesions, fibroids have been linked to negative reproductive outcomes with different degrees and types of impairment associated

with different categories of fibroids. As described by Bajekal and Li [72], fibroid categories include submucosal fibroids that distort the uterine cavity and subserosal fibroids and intramural fibroids that do not protrude into the cavity. Recent reviews suggest that submucosal fibroids have the greatest adverse impact on reproductive success, compared with the intramural and subserosal types [73].

Uterine adenomyosis is defined as the presence of endometrial glands and estroma surrounded by the hypertrophic and hyperplasic myometrium. Generally, adenomyosis is thought to be found most likely during the fourth and fifth decades of life and after childbearing activity. However, with the trend of delayed childbearing, adenomyosis has come to be diagnosed more frequently in fertility clinics [74]. The impact of adenomyosis on reproductive success is controversial. Regarding surgical removal of adenomyosis, a recent review concluded that uterus-sparing surgery for adenomyosis appears to be feasible and satisfactory although pointing out the need of prospective well designed studies [75]. At this stage, the true impact of various treatments on fertility outcomes of adenomyosis-associated subfertility has not been fully clarified.

Management:

- Uterine imaging (hysterosalpingography, MRI, 3-D ultrasound imaging) is recommended for patients with two consecutive miscarriages.There is little advantage to delaying uterine imaging until after she has suffered a third loss.
- Surgical correction of significant acquired uterine cavity defects should be considered. A septate uterus is amenable to hysteroscopic surgical correction.
- Early IUA detection is important because early treatment can prevent further complications. Treatment aims to restore the normal size and shape of the uterine cavity and normal endometrium function

Hormonal and Metabolic Etiology

Environmental, occupational or personal habits: It is generally agreed that maternal endocrine disorders should be evaluated and treated [76]. The prevalence of hypothyroidism with or without underlying thyroid autoimmunity is significant among women in fertile age. There is evidence that thyroid dysfunction and thyroid autoimmunity is associated with infertility and pregnancy loss both in the situation where the woman is euthyroid with thyroid antibodies and in a thyroid antibody negative woman with an elevated level of Thyroid Stimulating Hormone (TSH) [76].

According to a recent meta-analysis of 38 studies, the presence of antibodies against Thyroperoxidase (TPO-Ab) increased the risk of sporadic miscarriage with an odds ratio of 3.73 (95% CI 1.8 to 7.6) as well as RM (OR 2.3, 95% CI 1.5 to 3.5) [77]. However, this is problematic given the lack of consensus regarding the definition of a normal upper limit of TSH. Whereas TSH values of 4.0–5.0 mIU/L were once considered normal, a consensus is emerging that TSH values above 2.5 mIU/L are outside the normal range. In a large prospective study including pregnant thyroid antibody negative women, a TSH level within the normal range but higher than 2.5 mIU/L in the first trimester, nearly doubled the risk of a miscarriage. However, the true significance of thyroid dysfunction and the value of its correction in improving outcomes in RM remain unclear [78].

The prevalence of diabetes mellitus in women who suffer recurrent miscarriage is similar to that reported in the general population [79]. Current evidence shows that well-controlled diabetes is not a risk factor for RM. However, uncontrolled diabetes is associated with increased pregnancy loss thus; attention should first be given to optimal metabolic control of diabetic women during the preconceptional period [80].

Hyperprolactinemia may be associated with recurrent pregnancy loss through alterations in the hypothalamic-pituitary-ovarian axis, resulting in impaired folliculogenesis and oocyte maturation, and/or a short luteal phase. Normalization of prolactin levels with a dopamine agonist improved subsequent pregnancy outcomes in patients with recurrent pregnancy loss [81].

The role of other hormonal abnormalities remains controversial. Polycystic Ovarian Syndrome (PCOS) is a common endocrine disorder of reproductive-age women. PCOS may be associated with ovulatory disorder and miscarriage when fertility is desired. It has been estimated that 40% of pregnancies in women with PCOS will result in spontaneous loss [80]. However, using strict criteria the prevalence of PCOS among women with RM is estimated to be 8.3% to 10% [82]. The mechanisms behind an increased miscarriage risk in women with PCOS remains partly unclear. The current view is that the main cause may be the associated obesity, as well as insulin resistance, hyperinsulinaemia and hyperandrogenemia. Metformin treatment of PCOS patients decreases insulin resistance, thus improving ovulation cycles and, therefore, conception rates in infertile women but it is uncertain whether it decreases the rate of miscarriage in PCOS patients as no proper RCT has been conducted.

Retrospective evidence suggests that obesity increases the risk of miscarriage [79]. Obese women with RM have a higher frequency of euploid miscarriage compared with non-obese women. Obesity is associated with many endocrine disorders, such as diabetes, hypothyroidism, and PCOS, which theoretically could result in an increased risk of euploid miscarriage due to suboptimal implantation related to endocrine changes.

Boots and Stephenson [83] completed a systematic review evaluating whether obesity increases the rate of miscarriage in spontaneously conceived pregnancies. The odds of having RM were increased for obese women (odds ratio [OR] 1.31, 95% CI 1.18–1.46) and overweight women (OR 1.11, 95% CI 1.00– 1.24), when compared with women with normal BMI.

A shortened luteal phase has been associated with pregnancy loss but the assessment and interpretation of a putative luteal phase defect is problematic [86]. The use of histologic and biochemical end-points as diagnostic criteria for endometrial dating are unreliable and not reproducible utilizing the traditional histological criteria or other biochemical approaches [9,85].

The evidence on the effect of environmental risk factors is based mainly on data studying women with sporadic rather than RM. The results are conflicting and biased by difficulties in controlling for confounding factors and the inaccuracy of data on exposure and the measurement of toxin dose.

Maternal cigarette smoking and caffeine consumption have been associated with an increased risk of spontaneous miscarriage in a dose-dependent manner. Smoking-related complications in late pregnancy are substantial and well documented. However, current evidence is insufficient to confirm the association with miscarriage [4]. Nevertheless, cigarette smoking has been suggested to have an adverse effect on trophoblastic function and a link to an increased risk of

sporadic pregnancy loss has been proposed [86]. A recent review reports an increased risk of pregnancy loss among smokers whereas a large prospective study including 24.608 pregnancies could not demonstrate an association between smoking and miscarriage [87].

Other life-style habits such as cocaine use, alcohol consumption (3 to 5 drinks per week), and increased caffeine consumption (>3 cups of coffee), have been associated with risk of miscarriage. Heavy alcohol consumption is toxic to the embryo and the fetus. Even moderate consumption of five or more units per week may increase the risk of sporadic miscarriage [3,4,9].

Management:

- As long as Thyroid-Stimulating Hormone (TSH) levels are in the normal range, there is insufficient evidence to recommend routine thyroxine (T_4) testing or screening for anti-thyroid antibodies
- Prolactin levels should be determined and treated if they were abnormal
- Polycystic ovarian morphology, elevated serum luteinising hormone levels or elevated serum testosterone levels, although markers of PCOS, do not predict an increased risk of future pregnancy loss among ovulatory women with a history of RM who conceive spontaneously
- Endometrial biopsy for dating is not recommended, although continued research on the emerging molecular markers of endometrial development should be encouraged
- Administration of progesterone to women with sporadic miscarriages is ineffective
- Smoking, alcohol consumption and heavy caffeine consumption are discouraged although no prospective data on RM is available

Conclusion:

Recurrent Miscarriage (RM) is highly frustrating for both patients and physicians. The incidence of spontaneous abortion increases after miscarriage, from 13% in those with no previous miscarriage to 23% after 1 miscarriage, to 29% after 2 miscarriages, and to 33% after 4 miscarriages.

The most accepted definition of RM implies three pregnancy losses but full evaluations should be offered to women who have experienced at least two consecutive pregnancy losses. Both, diagnostic assessment and therapeutic proposals should be evidence based.

Parental karyotyping is recommended as part of the evaluation in RM couples with a high risk of carrier status. However, even among genetically abnormal products of conception the incidence of karyotypic abnormalities in the parents is low. Cytogenetic analysis of products of conception should be performed to determine which miscarriages are random, and which may be due to other factors associated with RM. In case of genetic miscarriages or when one of the partners has a balanced genetic abnormality, Preimplantation Genetic Screening (PGS) represents a therapeutic option.

The efficacy of thromboprophylaxis during pregnancy in women with recurrent first-trimester miscarriage who have inherited thrombophilias, but who are otherwise asymptomatic, has not been assessed in prospective randomised controlled trials. Pregnant women with antiphospholipid syndrome should be considered for treatment with low-dose aspirin plus heparin to prevent further miscarriage.

Regarding immunological aspects of pregnancy, there is not sufficient evidence to propose the measurement of peripheral Natural Killer cells (pNK) among patients suffering RM. Although uterine NK cells could play a role in trophoblastic invasion and angiogenesis, its density or activity are not predictive for pregnancy outcome. Thus, testing for uNK cells should not be offered routinely in the investigation of recurrent miscarriage.

Although congenital uterine abnormalities are associated with second trimester pregnancy loss rather than first trimester RM, assessment of uterine anatomy is widely recommended. The presence of a uterine septum is not only the most prevalent congenital defect, but also the only congenital defect to be more common in patients with primary RM than in general population. Correction of septate defects may have beneficial effects among women with primary RM.

Certain metabolic disorders environmental factors may be associated with RM and the diagnostic work-up of patients should include assessment of thyroid function, carbohydrate metabolism, obesity as well as a review of some toxic habits.

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