

EVIDENCE BASED APPROACH TO RECURRENT MISCARNAGE

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SUMMARY

Recurrent miscarriage, defined as three or more consecutive misscarriages, affects approximately 0.5-1% of couples trying to have a child. Genetic and uterine abnormalities, thrombophilias, environmental, endocrinologic and immunologic factors have been proposed to play a role in the etiology of recurrent miscarriage. The underlying pathology remains unidentified in approximately half of the recurrent miscarriage patients. Couples' expectations for a treatment often causes physicians to employ empiric treatments. In this review we will discuss the prognosis and evidence-based approach to diagnosis and treatment of recurrent miscarriage.

Key words: early pregnancy loss, recurrent miscarriage, recurrent pregnancy loss, spontaneous abortion

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TEKRARLAYAN GEBELİK KAYIPLARINA KANITA DAYALI YAKLAŞIM

ÖZET

Gebelik planlayan kadınların yaklaşık % 0.5-1'i üç veya daha fazla ardışık gebelik kaybı yaşamaktadır. Etiyolojide genetik anormallikler, uterin patolojiler, trombofili, çevresel faktörler, endokrinolojik ve immünojik nedenler rol oynayabilir. Tüm bu faktörler araştırılsa da hastaların yaklaşık yarısında herhangi bir neden ortaya konulamamaktadır. Açıklanamayan tekrarlayan gebelik kayıpları olan hastalar tedavi beklentisi içinde olduğundan, hekimler ampirik tedavi alternatiflerine sık sık başvurmaktadır. Bu derlemede, tekrarlayan gebelik kayıplarının prognozu, tanı ve tedavi yöntemleri mevcut kanıtlar ışığında tartışılacaktır.

Anahtar kelimeler: düşük, tekrarlayan gebelik kaybı

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DESCRIPTION AND INCIDENCE

Recurrent pregnancy loss (RPL) is described as ≥ 3 consecutive pregnancies with the same partner, which end in spontaneous abortion before the twentieth gestational week^(1,2). Ten to twenty percent of women experience a miscarriage throughout their reproductive period, 2% have two consecutive abortions and 0.5-1% of them have three consecutive abortions⁽³⁻⁵⁾.

Primary RPL is described as ≥ 3 consecutive pregnancy losses occurring before the twentieth gestational week in the absence of a previous delivery. Secondary RPL is described as RPL following a pregnancy resulting in live birth, stillbirth or neonatal death after the twentieth gestational week.

The etiology of miscarriage and the prognosis are thought to vary with regard to the period when miscarriage occurred during pregnancy, it has been suggested to categorize pregnancy losses as: pre- embryonic for miscarriages before five weeks, embryonic for five to ten weeks, and fetal loss for ten weeks onwards⁽⁵⁾.

PROGNOSTIC FACTORS

1. Number of previous pregnancy losses

The number of previous pregnancy losses is one of the most important prognostic factors for RPL cases. The probability of the next pregnancy ending in a full-term live birth decreases as the number of previous miscarriages increases. The probability of a live birth subsequent to one miscarriage is 80%, and it is 70-80 %, 50-60%, 45%, 41% and 13% subsequent to 2, 3, 4, 5 and 6 consecutive pregnancy losses, respectively⁽⁶⁾.

2. Maternal age

When women with recurrent pregnancy loss are categorized by their ages, the probability of the next pregnancy ending in miscarriage is similar between the 30-34 years and the 35-39 years groups, and the risk rises dramatically to 70 % for the 40-44 years group⁽³⁾.

3. Prior live birth

It has been reported that the chances of having a live birth differ between women who had four consecutive pregnancy losses subsequent to one live birth in her first pregnancy and women who had a live birth in her second pregnancy following a miscarriage

in the first and then had three consecutive pregnancy losses⁽⁷⁾. It was reported that the patient group in the first scenario had a worse prognosis than the group in the second scenario. This study supports the concept that secondary RPL is not coincidental but some risk factors developed during the first pregnancy which results in a live birth. It is suggested that the gender of the first infant has an effect on the prognosis in patients with secondary RPL⁽⁸⁾. The probability of achieving a live birth following secondary RM was calculated to be 58%, when a boy was delivered in the first pregnancy and the same probability was found to be 76% for women who delivered a girl in the first pregnancy. Therefore; the firstborn infant being male is regarded as a negative prognostic factor. The development of an immune response against male specific minor histocompatibility antigens was suggested as a probable mechanism.

4. The timing of previous pregnancy losses

It has been reported that women with recurrent pregnancy loss during pre-embryonic or embryonic period have a better prognosis than women with recurrent fetal losses^(9,10). While the chance of a live birth is about 70% following early RM, pregnancy loss between 16-27th gestational weeks increase the risk of recurrence by 20 fold and the risk of fetal death after 28th gestational week by five fold^(10,11).

5. The chromosomal constitution of products of conception

An aneuploid miscarriage is considered to be a positive prognostic factor. The presence of aneuploidy suggests that the miscarriage has occurred due to a fetal cause and prognosis can be favorable because the probability of having a euploid embryo in the next pregnancy is high. The probability of a live birth is reported to be 41% for a pregnancy subsequent to a euploid miscarriage and while it is 68% subsequent to an aneuploid miscarriage⁽²⁾.

FACTORS WHICH ARE CONSIDERED TO PLAY A ROLE IN THE ETIOLOGY

1. The coagulation system

Thrombophilia

Hypercoagulability occurs in pregnancy as a result of physiological changes such as decreased protein-C

levels, the formation of resistance against the activated protein-C, increased clotting factors, and deteriorated fibrinolysis^(13,14). Thrombophilia is a group of conditions in which the risk of venous thrombosis is increased, and can be classified as hereditary and acquired thrombophilia. Thrombophilia is thought to play a role in RPL, as obstetric complications such as uteroplacental insufficiency and fetal losses are more frequent in patients with thrombophilia due to the development of placental infarcts subsequent to the thrombosis in decidual blood vessels⁽¹⁵⁻¹⁸⁾. Factor V Leiden mutation, prothrombin G20210A mutation and MTHFR gene mutations (677 and 1298 mutations), and protein C, protein S and antithrombin deficiencies can be included in hereditary thrombophilia group. The first trimester losses are not expected theoretically in patients with inherited thrombophilia, as the placental circulation does not develop exactly during the first trimester. However, some suggest that there is a relationship between hereditary thrombophilia and early pregnancy losses and RPL⁽¹⁹⁻²³⁾. In some studies, early pregnancy losses have been reported to be less frequent in carriers of thrombophilic mutation⁽²⁴⁻²⁷⁾. This discrepancy may be coincidental as well as due to the differences in the study designs. It should be noted that even the definitions of RPL used differ between studies. While RPL is defined as two or more consecutive pregnancy losses in some studies, the pregnancy losses up to 28th week of the pregnancy are also included in the definition of RPL in some studies. Inclusion and exclusion criteria are highly variable between the studies. Thrombophilia screening in clinical practice is still a controversial practice due to the conflicting results of studies showing the relationship between inherited thrombophilia and recurrent pregnancy loss. Moreover there is no evidence to recommend anti-thrombotic therapies due to lack of placebo-controlled randomized trials.

Antiphospholipid syndrome

There is a consensus regarding that antiphospholipid syndrome (APS), which is a typical example of acquired thrombophilia, causes RPL. All pregnant women, who have a history of three or more pregnancy losses prior to 10 weeks' gestation, one or more morphologically normal pregnancy losses after the 10th week of gestation or pre-eclampsia prior to 34 weeks' gestation during one or more pregnancy, and

premature delivery due to the placental insufficiency, should be investigated for the AFS⁽²⁸⁾. Miscarriage is considered to be deterioration of the fetoplacental circulation due to thrombosis formation and affected trophoblast functions in maternal decidua and intervillous space. Prostacyclin-thromboxane imbalance, increased expression of tissue factor, protein C activation, inhibition of activated protein C function are other possible mechanisms⁽²⁹⁻³²⁾. Antiphospholipid antibodies increase the aggregation of platelets and the interaction of platelets with endothelium. The expression of Annexin V that creates a protective antithrombotic shield on trophoblasts was found to be decreased in patients with APS^(33,34).

Antiphospholipid antibodies (aPL) are divided into two main groups as aPL antibodies prolonging and not prolonging phospholipid-dependent clotting assays. The levels of the anti-cardiolipin (aCL), anti-phosphatidylethanolamine (aPE), anti-phosphatidylserine (aPS), anti-phosphatidylcholine (aPC), anti-phosphatidylglycerol (aPG), anti-phosphatidylinositol (aPI), anti-phosphatidic acid (aPA) antibodies and B2-glycoprotein I (a B2-GPI), which are APL antibodies those do not prolong phospholipid-dependent coagulation tests are measured quantitatively or semi-quantitatively. The results are expressed in aPL units, and one unit is equivalent to the binding capacity of 1mg/ml pure phospholipid. The measurement is carried out in GPL (IgG), MPL (Ig M) and APL (IgA) units depending on the immunoglobulin isotype⁽³⁵⁾. At least two of dilute Russell's viper venom time (dRVVT), kaolin clotting time, dilute prothrombin time, textarin time or taipan time tests should be used for the detection of lupus anticoagulants (LA) that prolongs phospholipid-dependent coagulation tests⁽³⁶⁾. The test result is positive or negative for LA⁽³⁷⁾. Classically, positive LA test result and/or aCL IgG level of >20 GPL u/ml and/or aCL IgM level of >20 MPL u/ml aPL is considered to be sufficient for the diagnosis of miscarriage due to aPLs. As APLs may be transiently detected in normal individuals, the test is recommended to be repeated at six-week intervals⁽³⁸⁻⁴¹⁾. APL tests should be done during pregnancy or at a time close to the pregnancy loss, because these tests tend to fall after the termination of pathologic pregnancies⁽³⁷⁾.

While aCL, APE and aPS cause thrombosis in placental blood vessels by targeting phospholipids in endothelial

cells, it has been suggested that aPE and aPS also cause trophoblast dysfunction by targeting phospholipids in trophoblasts. Furthermore aPC, aPG, and the aPI affects the embryogenesis phase by targeting phospholipids in the pre-embryonic tissue⁽³⁸⁾. LA and aCL are most frequently used in clinical practice. When the histological examination of the placenta demonstrates thrombosis it is recommended to test for aPLs that affect the endothelium, and to test for aPLs that affect trophoblasts and implantation for women who had pre-embryonic and early pregnancy losses⁽³⁵⁾.

Antibody titers and the number of positive aPL test results were found to be associated with clinical results^(42,43). While aCL and/or other aPL levels of >40 GPL u/ml indicate that pharmacological treatment can be beneficial for the subsequent pregnancy, aCL levels of >80 GPL u/ml indicate a high risk of fetal death for the subsequent pregnancy⁽⁴²⁾. More than one positive test result for aPL is a more reliable variable to predict pregnancy loss than one positive test result for aPL⁽⁴³⁾. A positive test result for aCL before pregnancy or an increase in the titration level during pregnancy is one of the poor prognostic factors⁽⁴⁴⁾. The chance of the live birth is reduced by 36 to 48% in patients with aCL in the absence of LA compared with women who are negative for both aCL and LA⁽⁴⁵⁾.

Low-dose aspirin, heparin, intravenous immunoglobulin (IVIG), glucocorticoids, and their combinations are proposed for the prevention of pregnancy losses in patients with APS. Aspirin is thought to reduce pregnancy losses by blocking cyclo-oxygenase and thromboxane synthesis in platelets, by providing anticoagulation, and stimulating the low levels of IL-3 in patients with APS⁽⁴⁶⁻⁴⁸⁾. In addition to the anti-inflammatory effects of heparin, it acts by binding antiphospholipid antibodies and protecting trophoblast phospholipids, by preventing adhesion of leukocytes to the endothelium, and by inhibiting the coagulation. Trials in RPL patients with APS, comparing aspirin with placebo showed no significant effect on livebirth rate⁽⁴⁹⁻⁵¹⁾, while the combination of aspirin and heparin was found to reduce pregnancy loss by 54%⁽⁵²⁾. Although there is a consensus about the concomitant use of heparin and aspirin in the treatment of APS, in our opinion, it is still unclear whether the aspirin component of the treatment is beneficial.

It has been reported that, the use of glucocorticoid alone or its combined use with other therapeutic agents

has no effect on the treatment of RPL, as well as it has possible side effects such as gestational diabetes, hypertension, pre-eclampsia, infection and osteoporosis^(53,54).

In the treatment of APS secondary to systemic lupus erythematosus (SLE), it is recommended to use the lowest dose of glucocorticoid that controls SLE⁽³⁵⁾.

IVIG is thought to be useful in RPL cases with APS by inhibiting aCL and LA, increasing antibody clearance and providing a reduction in the production of aCL via B-cell antigen receptors. However, IVIG has been reported to increase the risk of pregnancy losses and preterm birth in the meta-analysis of studies on IVIG use in women with APS⁽⁵²⁾.

The management of patients with positive antiphospholipid antibodies, and with pregnancy loss without a history of thrombosis is controversial. The treatment with the low-dose aspirin (75 mg/day) and prophylactic doses of unfractionated heparin or low molecular weight heparin (enoxaparin 1mg/kg/day) has been reported to be effective on patients with three or more pregnancy losses before 10th week of pregnancy^(55>56). Low-dose aspirin and full therapeutic dose of unfractionated heparin or low molecular weight heparin (enoxaparin 1mg/kg every 12 hours) have been found to be useful in the management of APS cases with a history of thrombosis or one or more fetal loss after 10th gestational week^(56,57). Low-molecular-weight heparin is more preferable than unfractionated heparin because of both its side-effect profile and its lesser injection volumes providing the same effectiveness.

Endocrinological factors

Luteal phase defect

The human embryo reaches the uterine cavity 4 days after ovulation, and implantation occurs approximately 7 days after ovulation, or 19-24 days after the last menstrual period⁽⁵⁸⁾. The rate of early pregnancy loss has been reported to be increased with increasing interval between ovulation and implantation⁽⁵⁹⁾. 17.4 to 28% of cases of recurrent pregnancy loss have been reported to have luteal phase defect (LPD)^(60,61). The delay in endometrial growth and maturation due to insufficient secretion of progesterone in the corpus luteum is thought to be associated with early pregnancy loss. LPD is assessed by histological evaluation of endometrium or by measuring

progesterone level in luteal phase. However, inter/intra-observer and inter-cycle variability of pathological examination of the endometrium is high. For example, in a study in which the same sample was evaluated by the same pathologist at two different times, exact agreement occurred in 24%. In a separate part of the study, the patients underwent endometrial biopsy two times in different cycles, and exact agreement in the diagnoses was found to be only 4%⁽⁶²⁾. 26.7% of the of regularly menstruating women of proven fertility was reported to have LFD diagnosed by endometrial biopsy⁽⁶³⁾. In conclusion, the reliability of the diagnosis of LPD based on the histological evaluation of the endometrium is poor. Mid-luteal progesterone levels of <10ng/ml is considered as the threshold for the diagnosis of LPD. Progesterone levels of all women were found to be in normal limits in a study measuring progesterone levels of the patients diagnosed with LPD by endometrial biopsy⁽⁶¹⁾. In another study comparing patients with and without LPD by their progesterone levels, luteal phase progesterone levels were found to have no predictive value for future pregnancy losses⁽⁶⁴⁾. LPD, in our opinion, should be treated as a separate clinical entity due to both the controversies in the diagnostic criteria for LPD and the lack of strong evidence about its role in the etiology of RPL. Invasive and expensive tests should be avoided for the diagnosis of LPD.

Hypothyroidism

Although hypothyroidism is not a common condition in patients with RPL, it is recommended to include the TSH testing in routine investigation. Maternal hypothyroidism has negative effects on fetal development and TSH measurement is an easy test providing accurate results⁽⁶⁵⁻⁶⁷⁾.

Diabetes Mellitus

The diabetic patients with good glycemic control are not at risk for RPL (66). Pre-pregnancy hemoglobin A1c levels of 7.5% or less substantially reduces the risk of pregnancy loss⁽⁶⁸⁾. It is obvious that pre-pregnancy optimization of the glycemic control will be beneficial for the health of the mother and fetus, even though it is considered to not make a difference for RPL.

Polycystic ovary syndrome

Patients with polycystic ovary syndrome (PCOS) have an increased risk of pregnancy loss⁽⁶⁹⁾. This increase is thought to be due to higher levels of LH

and testosterone, and insulin resistance. Although its mechanism could not be fully elucidated, obesity has been reported to increase the incidence of first trimester pregnancy losses and RPL⁽⁷⁰⁾. A negative correlation has been reported between body mass index (BMI) and Leukemia Inhibitory Factor (LIF), which is thought to have an important role in the implantation⁽⁷¹⁾. As about half of patients with PCOS are overweight or obese, increased incidence of miscarriage in this patient group may be associated with obesity. The weight loss should be presented as an option to these patients.

Progesterone

Progesterone is secreted in the corpus luteum under the influence of hCG and creates the secretory changes that are required for the continuation of implantation and pregnancy in endometrium⁽⁷²⁾. Csapo demonstrated in his classical study that luteoectomy performed before the 8th gestational week had resulted in the miscarriage⁽⁷³⁾.

Progesterone is widely used in threatened abortion cases because of its immunomodulatory and anti-abortive effects. However, in meta-analysis of studies on progesterone use in pregnant women with threatened abortion, no positive effect of progesterone was found irrespective of the dosage and administration, however in the subgroup analysis of patients with RP, it has been reported to be effective in reducing the incidence of pregnancy losses⁽⁷⁴⁾. But the power and reliability of the studies are poor due to small sample size, variation in treatment protocols and definitions of RPL, inclusion of older age groups. Moreover, the vast majority of studies were not placebo-controlled. Well-designed, large-sampled, randomized controlled trials are needed to recommend progesterone therapy routinely to these patients.

There are studies reporting that the use of progesterone increases the risk of hypospadias^(75,76). Therefore, the potential teratogenic effects of maternal progesterone therapy should not be ignored.

Chorionic Gonadotropin

Human chorionic gonadotropin (hCG), allows the survival of the corpus luteum in early pregnancy. hCG levels and consequently progesterone levels decrease in pregnancies that could not continue its normal development. The meta-analysis of the trials of the use of hCG in the management of RPL showed a significant reduction in

the risk of the miscarriage (Odds Ratio: 0.26, 95% confidence interval: 0.14 to 0.52)⁽⁷⁷⁾. Some of these studies were not placebo controlled and no information was given about randomization. In the conclusion section of meta-analysis, it was emphasized that the data was insufficient and not convincing for the routine use of hCG in clinical practice due to the methodological weaknesses of the studies. In a randomized placebo-controlled, double-blind study, which was included in meta-analysis, hCG was reported to be beneficial in only a subgroup of patients with history of oligomenorrhea (Odds Ratio: 0.15, 95% confidence interval: (0.03-0.83).

Immunological factors

The rejection of the embryo as a result of a defect in the maternal immune tolerance against semi-allogenic fetus is thought to be another mechanism that can play a role in the etiology of RPL. The role of the number/activity of NK cells has also been studied as well as the role of the increased sharing of human leukocyte antigens (HLA) between the couples, and molecular immunosuppressive factors such as cytokines and growth factors that are secreted at the level of local decidual trophoblasts⁽⁷⁸⁻⁸¹⁾.

The relationship between the increase of HLA sharing between the couples and the prognosis of subsequent pregnancies could not be detected⁽⁸²⁻⁸⁵⁾. Classical HLA class I and II antigens other than HLA C antigen were not expressed in early embryos and trophoblasts^(86,87). HLA-G is the HLA type existing abundantly in maternal-fetal interface⁽⁸⁸⁾. NK cells has the potential to destroy cytrophoblasts by recognizing the cells that are not classical major histocompatibility (MHC) I molecules. HLA G has been suggested to have inhibitory effects on NK cells⁽⁸⁹⁾. Although HLA-G polymorphism in RPL cases was investigated and some of the HLA-G alleles have been reported to be associated with RPL, this relationship could not been confirmed in later studies⁽⁹⁰⁻⁹²⁾. It has been suggested that there may be a relationship between maternal HLA DR1 and DR3 antigens and RPL⁽⁹³⁾. TNF- α gene is associated with HLA-DR, and RPL is thought to occur due to a predisposition to hypersecretion of TNF- α ⁽⁹⁴⁾.

The expression of the Th2-dependent anti-inflammatory cytokines (IL-3, IL-4, IL-5, IL-6, IL-10, IL-13) is increased compared to that of the Th1 dependent pro-inflammatory cytokines (TNF α , IFN γ , IL-2, IL-12, IL-18)⁽⁹⁵⁾ during pregnancy. While Th2-dependent cytokine

production is more dominant in normal pregnancy, the risk of abnormal development of placenta and embryo and fetal loss has been suggested to be increased under the influence of TH1-dependent cytokines preponderance⁽⁹⁶⁻⁹⁸⁾. Th1 cytokine response was increased in patients with RPL and recurrent implantation failure⁽⁹⁹⁾. Th1-dependent cytokines are thought to harm the pregnancy by inducing hypercoagulability.

Exposure to semiallogenic embryogenic antigens increases the number of intracellular progesterone receptors (PR) in the endometrium⁽¹⁰⁰⁾. Activated maternal lymphocytes produce a protein called progesterone-induced blocking factor (PIBF) in the presence of progesterone⁽¹⁰¹⁻¹⁰²⁾. PIBF increases the production of IL-4, IL-6 and IL-10 by changing Th1/Th2 ratio in favor of Th2⁽¹⁰³⁻¹⁰⁵⁾. A positive feed-back mechanism is created by inducing the release of hCG by IL-4 and IL-6 in trophoblasts, followed by the stimulation production of progesterone by hCG and secretion of Th2 cytokines by progesterone. Type of the cytokine predominance during antigen presentation in fetomaternal microenvironment is thought to be important for the determination of the dominance of Th1 or Th2 and therefore for a successful pregnancy . Decidual natural killer (NK) cells have been suggested to control trophoblast invasion via the immunomodulatory cytokines and angiogenic factors⁽¹⁰⁶⁾. NK cells have been reported to be increased in endometrium of patients, who had recurrent pregnancy loss⁽¹⁰⁷⁻¹⁰⁹⁾. It is suggested that increased number and activity of NK cells will cause the stimulation of the secretion of inflammatory cytokines in Th1 cells, the production of TNF- α and nitric oxide with decidual macrophage activation via IFN- γ , and therefore a damage to conceptus via apoptosis^(96,110-112). However, studies investigating the effect of the number of NK cells on RPL cases have conflicting results^(107,108,113-117).

In a study on rodents, it was detected that the subjects that had no NK cells developed placental hypertrophy and consequently fetal death occurred, and therefore the positive role of NK cells for the continuation of pregnancy has been proven⁽¹¹⁸⁾. However, a strong correlation between the number and activity of NK cells and pregnancy outcome could not be demonstrated. On the other hand, progesterone has been suggested to suppress the activity of NK cells via PIBF and prevent rejection of the embryo by increasing the production of asymmetric antibodies in B lymphocytes⁽¹¹⁹⁻¹²¹⁾.

Although the alloimmune causes of recurrent pregnancy

loss have not been fully elucidated and proved, a variety of immunological treatments are being tested. Immunomodulatory therapies are being tested especially in patients with unexplained RPL, by considering that this condition is due to immunological problems, which disrupt normal placentation but have not been identified yet. The most commonly applied therapies are paternal leukocyte immunization and intravenous immunoglobulin (IVIG) therapy.

Paternal leukocyte immunization has been suggested to act by induction of paternally derived fetal antigens to reinforce maternal T cells to stimulate anti-T cell receptor idiopathic antibody (anti-TCR) formation thus regulate the maternal response to the fetus⁽¹²²⁻¹²⁴⁾. It is also suggested that paternal leukocyte immunization suppress Th1 cytokines and reduce the activity of NK cells by enhancing asymmetric antibodies against R80 K antigens, which are recognized by NK cells on the surface of trophoblast (L25). All of these theories comply very poorly with the clinical outcome. In meta-analysis of 12 randomized controlled trials about paternal leukocytes iso-immunization and RPL, this treatment has been shown to have no clinical impact⁽¹²⁶⁾.

IVIG therapy has been reported to suppress in vitro production of pro-inflammatory cytokines and decrease the number/activity of NK cells^(127,128). It has been suggested that IVIG neutralizes pathologic antibodies by interacting with their Fc portions or passively via anti-idiotypic antibodies^(129,130). In addition, IVIG has been shown to create anti-inflammatory effects by inhibiting the complement system in animal studies^(131,132). IVIG as a treatment in RPL patients has not been shown to be of benefit in terms of live birth rates in meta-analysis of 8 randomized controlled trials⁽¹²⁶⁾. Nephrotoxicity, alopecia, aseptic meningitis, retinal necrosis, thrombosis, ischemic heart disease and cerebrovascular events have been reported after IVIG treatment in the literature⁽¹³³⁾. In the view of all of these information, we believe that immunotherapy remain experimental and should not be offered as a treatment for RPL cases, outside the research context.

GENETIC FACTORS

Parental karyotype

Balanced translocation has been identified in 3 to 5% of couples, who experience RPL^(134,135). Although

these individuals have no chromosomal material loss or duplication in their own somatic cells and are phenotypically normal, meiotic segregation in haploid gonadal cells results in duplication or lack of genetic material. 60% of balanced translocations are reciprocal translocations that are formed by exchange of segments between two non-homologous chromosomes, and 40% are Robertsonian translocations that are formed by the fusion of two acrocentric chromosomes at centromere with the loss of short arms. Boue and Gallano carried out a collaborative study involving 71 European prenatal diagnosis centres on the karyotype of fetuses of 1356 couples in which a parent was detected to have balanced structural chromosomal abnormalities. It has been reported that abnormal chromosomes were transferred to 40% of fetuses⁽¹³⁶⁾. In other words, 60% of fetuses whose parents have balanced translocation have euploid chromosomal constitution. The live birth rates in untreated RPL cases are about 60%. Carp et al evaluated chromosomal structure of 1108 couples, who had RPL, and chromosomal structure of subsequent abortion materials of these couples.

The most common chromosomal abnormality detected was balanced translocation, followed by inversions in RPL couples.

Aneuploidy was identified in 30.8% of fetal karyotypes in parents with parental chromosomal anomalies, and in 23.2% of fetal karyotypes of couples with no parental chromosomal aberrations, and the difference was not statistically significant⁽¹³⁷⁾. The same team conducted another study and evaluated the karyotypes of 916 couples who had RPL, the live birth rate was found to be decreased from 55.3% to 45.2% in the presence of a parental chromosomal anomaly. This difference was not also statistically significant⁽¹³⁸⁾. In the study of Clifford et al, 38% of couples with RPL and chromosomal abnormality was reported to have a successful pregnancy in the past⁽⁶⁶⁾. The number of previous pregnancy losses, maternal age and chromosomal structure of previous pregnancy product appear to be more effective than parental chromosomal structure for the prognosis of subsequent pregnancy in RPL patients.

Although it is difficult to assume that RPL is only due to the unbalanced transfer of chromosomal aberrations in couples with parental karyotype abnormality, assessment an existing genetic problem will be beneficial in terms of clarifying the etiology and informing the couples about which risks other than

pregnancy loss are associated with the genetic problem that they are carrying. American College of Obstetricians and Gynecologists of (ACOG), the Royal College of Obstetricians and Gynecologists (RCOG) and the European Society of Human Reproduction and Embryology recommend carrying out parental karyotyping routinely in the evaluation of RPL (65,139,140). Parental karyotyping is more affordable in our country compared to both North America and European countries in terms of cost and, offering parental karyotyping to RPL couples and consulting a clinical geneticist in case of detection of an abnormality seem to be the most appropriate approach. It should be kept in mind that that couples, who had a high number of pregnancy losses, even if not consecutive, may have genetic problems, because pregnancies of translocation carrier couples may also achieve successful live births.

Cytogenetic Analysis of Abortion Material

Although recurrent aneuploidies considered to be one of the causes of RPL, and 16% of pregnancy loss products following aneuploid abortion were detected to have aneuploidy, and this risk was not different from the initial risk of aneuploid abortion (15%) of all pregnant women(141).

Trisomies are the most frequently detected chromosomal abnormalities in abortion materials in recurrent pregnancy losses and aberrations of 21st, 16th and 18th chromosomes were the most prevalent anomalies(12).

Although the frequency of aneuploidy has been suggested to be increased in RPL cases(142,143), the frequency of aneuploidy was reported to be similar or even lower in patients with RPL compared to controls(144-146).

While RCOG has recommended routine karyotyping of abortus material for the evaluation of RPL, ACOG guideline states that a definitive recommendation for routinely obtaining abortus karyotypes cannot be made (147). As a result of the joint working party of RCOG (140) and the Royal College of Pathologists, they recommend to take the tissue sample with hysteroscopy for fetal karyotyping and to examine samples of placenta and full-thickness skin in order to overcome problems such as maternal cell contamination and lack of reproduction in cell culture(148).

In summary, detection of fetal aneuploidy indicates that abortion has a fetal origin and subsequent

pregnancies may have a higher likelihood of live birth. While live birth rates of subsequent pregnancy were found to range from 62 to 67% in the case of the detection of aneuploidy in abortion material, these rates were found to range from 37 to 38% in the case of the detection of euploidy(12,146). The role and the psychological benefit of the assessment of the chromosomal structure of abortus material in informing the family about prognosis are invaluable. To overcome the above-mentioned technical obstacles, it would be accurate to consult the genetic laboratory for the proper sampling and delivery to the laboratory.

Preimplantation genetic screening (PGS)

Chromosome abnormalities have been identified in 50-70% of spontaneous abortions(149-151). Considering that 10 to 15% of clinically detected pregnancies end with spontaneous abortions, 5 to 10% of pregnancies result in sporadic abortions due to chromosomal abnormalities. Recurrent pregnancy loss is seen in 1% of the population, and this rate is higher than the rate that can be associated with recurrent aneuploidy ($0.15 \times 0.15 \times 0.15 = 0.0034$).

In the studies of Ogasawara and Stern including patients, who had two or more pregnancy losses, the rate of aneuploidy was found to range from 50 to 60% (146,152).

Ferro et al demonstrated aneuploidy in 67% of biopsies of chorionic and embryonic tissues in abortus material which were obtained by hysteroembryoscopy. (153). Carp and colleagues have found the frequency of chromosomal abnormalities as 29% in abortion materials of 125 RPL cases of which had the classical definition of RPL(12). A negative correlation between the number of abortion and the incidence of chromosomal abnormalities has been reported(146). The frequency of karyotype abnormalities in subsequent abortion materials was found to be 59.4% in women with previous three abortions, 55.5% in women with previous four abortions, 39.6% in women with previous five abortions, and 28% in women with previous six abortions(146).

It was considered that implantation rates can be increased and abortion rates can be decreased by enhanced embryo selection with normal karyotypes by PGS. Rubio et al have reported live birth rate of 83% with PGS in patients with two or more pregnancy losses (mean pregnancy loss = 2.9)(154). However, control group in this study included patients without a history of RPL and it is not appropriate to compare the results with this group. In addition, it is

possible that the results of the study to have been influenced by the number of previous pregnancy losses. As mentioned above the incidence of chromosomal abnormalities is lower in subsequent pregnancies of RPL cases with three or more pregnancy losses. Another study stated that pregnancy rate increases with PGS in RPL cases, however it was reported that, while PGS was beneficial in women over 35 years old, the results were disappointing in women aged 35 years and under⁽¹⁵⁵⁾. This study did not include a control group, and the birth rate was compared with hypothetical birth rates calculated according to a formula with the main determinants of maternal age and previous pregnancy loss. Considering the fact that RPL patients don't have infertility issues thus high pregnancy rates are expected following IVF treatment. In the study of Plateau et al, ongoing pregnancy rate was 29% in young RPL cases (less than 37 years old) undergoing PGS. Live birth rates of > 60% can be expected in this patient group just with expectant management. All of the studies in the literature regarding PGS in RPL patients are observational studies and there are no randomized controlled trials (L56).

It is not possible even for the most experienced hands to correctly perform blastomere biopsy, nuclear fixation and FISH analysis, which are the important stages of PGS. Accuracy rate of FISH analysis per probe ranges from 92 to 99%. Therefore, the use of multiple probes increases the possibility of misdiagnosis^(157,158). Mosaicism is a common condition in human pre-implantation embryos^(159,160). Aspiration of normal blastomere reduces the rate of diploid blastomeres in diploid-aneuploid mosaicism and lead to transfer of an embryo with an increased proportion of abnormal cells. On the other hand, aspiration of aneuploid blastomere results in the discarding the embryo by considering it as aneuploid, despite the fact that the procedure actually increased the proportion of normal blastomeres and the embryo has the viability potential.

When considering the cost and risks of treatment as well as the lack of sufficient scientific evidences, we believe that PGS should not be offered to RPL group on a routine basis.

ANATOMICAL FACTORS

Uterine anomalies

Anatomical defects of the uterus are responsible for 6 to 38% of cases of recurrent pregnancy loss^(66,161,162).

This wide range may be due to differences in diagnostic criteria and techniques. The most common congenital anomaly of the uterus in RPL cases is the subseptate uterus. The proposed mechanisms of the abortion are poor decidualization and placental development due to the avascular uterine septum as well as uncoordinated myometrial contractions caused by increased muscle tissue in the septum⁽¹⁶³⁾. The degree of distortion of uterine cavity in subseptate uterus seems to be more important than the length of the septum. The risk of implantation on the septum and thus the risk of miscarriage increase with the degree of uterine cavity distortion⁽¹⁶⁴⁾. A significant post-operative improvement was detected in the meta-analysis comparing pregnancy outcomes before and after hysteroscopic septoplasty⁽¹⁶³⁾.

The second most common congenital uterine anomaly is the arcuate uterus⁽¹⁶⁵⁾. However, the relationship between the arcuate uterus and the pregnancy loss is controversial. Arcuate uterus has been reported to be mostly associated with 2nd trimester losses and premature births⁽¹⁶⁶⁻¹⁶⁸⁾. Some suggest that the only therapeutic approach to prevent the second-trimester losses is cervical cerclage⁽¹⁶⁹⁾.

SUPPORTIVE TREATMENTS

Although most physicians recommend bed rest for threatened abortion cases, randomized controlled trials indicate that the incidence of pregnancy losses does not decrease with bed rest⁽¹⁷³⁾. Beyond this, long-term bed rest is associated with complications such as thromboembolism, weight loss and muscle atrophy^(174,175). Women on bed rest have shown to experience denial, anger, loneliness and depression. In addition to these negative emotions, guiltiness has been experienced in patients who had poor compliance with bed rest prior to the miscarriage⁽¹⁷⁶⁾. Although there is no biological explanation, psychological support and or close follow-up with ultrasound examinations have been seen to reduce the risk of miscarriage to 2-4 times compared with the control groups. The successful pregnancy rate was reported to range from 74 to 86% in patients who had received supportive treatment^(11, 177-180). RCOG and ACOG recommend supportive care for unexplained RPL cases and consult the patients regarding the potential for the successful outcome of the subsequent pregnancy without receiving any pharmacological treatment^(139,140).

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