Received: 18 December 2017 | Accepted: 4 February 2018

DOI: 10.1111/aji.12833

ORIGINAL ARTICLE

WILEY AJRI American Journal of Reproductive Immunology

Lymphocyte immunotherapy for recurrent miscarriages: Predictors of therapeutic success

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Problem: To evaluate the predictors of successful pregnancies in women with a history of recurrent miscarriages (RMs) having undergone lymphocyte immunotherapy (LIT).

Method of study: Retrospective, multicenter, observational study which involved 702 pregnant women with history of RM treated with LIT. Comparative analysis of women with a history of RM having undergone LIT and experienced treatment success vs those having experienced treatment failure along with the analysis of the association between the number of prior miscarriages and the efficacy of LIT.

Results: A total of 421 women were able to carry the pregnancy to term, with treatment success rate of 60%. The multivariate analysis showed that age, the association between autoantibodies and thrombophilia, and the number of previous miscarriages were factors associated with LIT failure. Secondary RMs alone were not found to be a factor predictive of LIT success or failure; however, secondary RMs among women with a history of 5 or more RM were found to be a predictor of LIT success (OR: 10.24; 95% CI: 1.9-55.8; $P = .007$).

Conclusion: Age, the number of previous miscarriages, and the association between autoantibodies and thrombophilia are associated with LIT failure. A higher number of previous miscarriages in cases of secondary RM resulted in better LIT outcomes.

KEYWORDS
lymphocyte immunotherapy, previous miscarriages, recurrent miscarriages

1 | INTRODUCTION

Recurrent miscarriage (RM) is defined by the World Health Organization (WHO) as the occurrence of 3 or more spontaneous and consecutive pregnancy losses at a gestational age of <20 weeks.[1] However, this definition has changed in recent years. The American Society for Reproductive Medicine has defined this condition as the occurrence of 2 or more miscarriages, irrespective of being consecutive or not, with pregnancy having been confirmed by histopathological or ultrasound examination.[2] This definition has also been adopted by the International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the WHO Revised Glossary on Assisted Reproductive Terminology, 2009.[3] Moreover, the European Society for Human Reproduction and Embryology and the Royal College of Obstetricians and Gynaecologists define recurrent miscarriage as the occurrence of 3 consecutive pregnancy (which may or may not be intrauterine) losses.[4,5] Recently, in an attempt to unify these concepts, the ICMART proposed that RPL be defined as the occurrence of 2 or more miscarriages at 22 weeks of gestation or less.[6]

Women with a history of RM are typically classified as having primary RM (when their previous pregnancies never lasted for

more than 20 weeks) or secondary RM (when pregnancy loss was preceded by a pregnancy lasting longer than 20 weeks or with an outcome of a live birth [most frequent], stillbirth, or neonatal death). Some authors have proposed a different classification according to the sequence of pregnancy losses and previous pregnancy with birth of a live fetus: (i) secondary RM if a live fetus is born in the first pregnancy and miscarriages follow and (ii) tertiary RM if the birth of a live fetus occurs in the middle of a sequence of miscarriages. RM affects approximately 2%-5% of couples of reproductive age, with a trend toward an increase in the incidence.[7]

Recurrent miscarriage is a gestational complication of multifactorial etiology, with well-established causes reported in the literature (defects in the uterine anatomy, hormonal diseases, obesity, and antiphospholipid syndrome). Some other possible causes still require more evidence (immune, autoimmune, and alloimmune disorders; hereditary thrombophilia; environmental factors; and partner-related causes).[7] The factors associated with RM are, most frequently, attributed to the woman. In approximately half the cases, at least 1 factor generally associated with RM is observed, although a definite cause remains to be established in a considerable number of cases.[7]

Maternal age, obstetric history, and the number of previous miscarriages are risk factors for future miscarriages. The risk of future miscarriage, compared with that among the general population (approximately 15%), increases with each miscarriage, particularly after 2 consecutive miscarriages (approximately 25%) and becomes higher after a third miscarriage (45%) and even more so after a fourth one (54%).[8] In addition, a higher number of previous miscarriages increase the risk of other obstetric complications such as preeclampsia, placental abruption, placenta previa, preterm prelabor amniorrhexis, congenital malformations, premature labor, and restricted intrauterine growth. Women with a history of secondary RM appear to have a better prognosis for future pregnancies than those with a history of primary recurrent miscarriage.[9]

Immunological theories of embryo acceptance (wherein the embryo is considered as an allograft in the maternal immune system) date back to 1966, when Clark and Kirby suggested that the antigenic disparity between the embryo and the mother is beneficial for gestation.[10] Since then, several immunological mechanisms involved in embryo implantation have been proposed. The first alloimmune mechanism proposed as cause of RM suggested that the compatibility of human leukocyte antigens (HLA) between father and mother would cause failure in the production of antipaternal cytotoxic antibodies, anti-idiotypic antibodies (Ab2), and mixed lymphocyte reaction blocking antibodies (MLR-Bf), thus leading to pregnancy loss. Later, other alloimmune mechanisms have been described as being responsible for RM, including (i) natural killer cells (NK) hyperactivity, (ii) imbalance of the T helper 1 (Th1) and Th2 immune response with predominance of Th1 response, and (iii) low concentration of regulatory T cells (Treg cells), CD4+ CD25+ FoxP3+.[11]

In the early 1980s, Taylor and Faulk considered kidney transplant immunology studies and described successful pregnancies in 3 patients with a history of recurrent miscarriages treated with leukocyte-rich plasma from an unrelated donor.[12] Later, lymphocyte immunization therapy (LIT) was used for the treatment of couples with a history of idiopathic recurrent miscarriages, sparking debates in the literature regarding the efficacy and safety of this practice.[13] The following factors hinder the emergence of strong evidence with respect to a subject: (i) studies with an inappropriate number of subjects (research costs are high and prevalence of RM is low); (ii) a lack of consensus on the selection criteria for treatment with LIT; (iii) different treatment protocols with different methods for preparing lymphocyte concentrates, different routes of administration (intravenous, intramuscular, subcutaneous, or intradermal), different concentrations of lymphocytes per dose, and lack of standardization regarding the ideal time for immunotherapy (only before, before and during, or only during pregnancy); and (iv) lack of standardization of pre-pregnancy immunotherapy control.[11] Women classified as having secondary RM with a history of multiple miscarriages and with autoantibodies appear to experience worse outcomes with LIT.[13-15]

Despite the controversy on the subject, several treatment centers routinely offer LIT as a therapeutic option for couples with idiopathic recurrent miscarriages. The objective of the current study was to evaluate the factors associated with LIT failure in an attempt to determine which group of couples with a history of recurrent miscarriage experiences the most success with the therapy.

2 | MATERIALS AND METHODS

2.1 | Patients

This was a retrospective observational study conducted from January 2006 to December 2016 at 6 Brazilian centers of reproductive immunology located in the cities of Campinas, Rio de Janeiro, Salvador, Porto Alegre, Recife, and Fortaleza.

This study reviewed medical records of patients with the following inclusion criteria: (i) women >18 years old and reproductive capacity, with history of 2 or more consecutive miscarriages in the first trimester, with or without previous pregnancies >20 weeks and (ii) absence of paternal antilymphocyte antibodies (negative crossmatch) during the investigation, situation defined as the presence of an alloimmune factor. Patients who did not have positive crossmatch after being submitted to LIT were excluded.

All patients included in the study underwent LIT using their partner's lymphocytes. The patients who underwent LIT had no previous history of infertility. All the evaluated pregnancies were the result of conception without the aid of assisted reproduction techniques. The patients underwent an investigation and treatment of other causes of RM according to the protocol described below, which was standardized among the sites involved in the study.

To investigate the variables associated with a high risk of LIT failure (analysis 1), the patients were first divided into 2 groups according to gestational outcome (LIT success = live birth; LIT failure = miscarriage) after the immunological treatment. Subsequently, to assess the association between the number of previous miscarriages, etiological factors, and pregnancy outcome, the patients

were divided into 4 groups based on the number of previous miscarriages (analysis 2). The first group consisted of couples that had 2 previous consecutive miscarriages (2 previous miscarriages = 2 PM). The second group consisted of couples that had 3 previous consecutive miscarriages (3 PM). The third group consisted of couples that had 4 previous consecutive miscarriages (4 PM), and the fourth group consisted of couples that had 5 or more consecutive previous miscarriages (≥5 PM). Details are shown in Figure 1.

Informed consent to administer immunotherapy was obtained from all participants, and the study was approved by the Local Ethics Committee of Federal University of Bahia (UFBA).

2.2 | Evaluation and treatment protocol

The standardized protocol at the study sites investigated the following causes of RM: genetic, anatomical, and hormonal causes; antiphospholipid syndrome; hereditary thrombophilia; autoimmune factors; and alloimmune factors. Genetic causes were assessed by determining the karyotype of the patient and of her partner in peripheral blood. Hysterosalpingography and/or hysteroscopy were used to evaluate uterine abnormalities. Thyroid function was assessed through the measurement of T4 and free thyroid-stimulating hormone, being this function corrected if necessary and fasting glucose levels were used to determine the presence of diabetes mellitus. The diagnosis of antiphospholipid syndrome (APS) was made based on international consensus.[16] Hereditary thrombophilia for which tests were performed were C-protein deficiency, S-protein deficiency, antithrombin deficiency, methylenetetrahydrofolate reductase (MTHFR) C667T and A1298C mutations, Leiden V gene mutation, and G20210A prothrombin gene mutation. Autoimmune factors were assessed by means of antinuclear antibody (ANA), anti-DNA, antithyroperoxidase (TPOAb), and antithyroglobulin (TgAb) tests. All patients had an alloimmune factor (negative cross-match), a condition for which LIT is indicated. Patients and their partners underwent ABO and Rh blood typing.

The cross-match test was determined by microcytotoxicity protocol in the initial evaluation. Briefly, partner peripheral blood lymphocytes were isolated from fresh defibrinated blood by Ficoll-Hypaque solution (GE Healthcare®, Zipf, Austria) density centrifugation and incubated for 30 minutes with patient serum. After this, rabbit complement was added and incubated for another 1 hour. If antibodies were directed against the lymphocytes of the partner, cell lysis would allow the incorporation of Trypan blue (vital dye). The reaction was observed under an optical microscope (score 0-8).

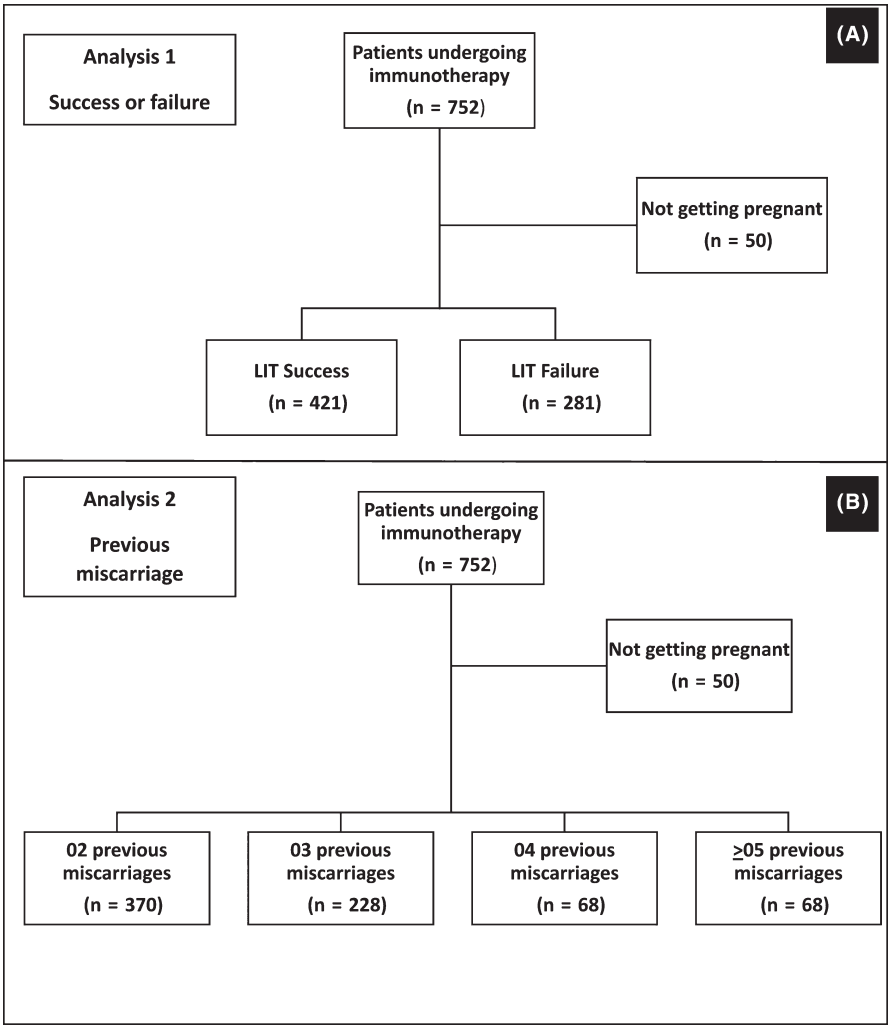

FIGURE 1 Flowchart of patients' analysis. LIT, lymphocyte immunotherapy; LIT success, live birth; LIT failure, miscarriage

Positive cross-match was defined when the score was greater than 4. Potentiated cross-match with human antiglobulin was also performed in order to detect low titers of antibodies.

In the standard treatment protocol, progesterone was vaginally supplemented during the first trimester in all patients. The uterine malformations that could be corrected were surgically repaired before a new pregnancy. Couples with abnormal karyotypes received genetic counseling. The patients were classified into 4 categories according to the diagnosis (alloimmune-, autoimmune-, or thrombophilia-related causes), and a treatment was proposed. Category 1 included patients who had only 1 positive alloimmune factor (negative cross-match). Category 2 included patients with 1 alloimmune factor and at least 1 positive test for thrombophilia (antiphospholipid syndrome and/or other hereditary thrombophilia; heterozygous and homozygous statuses were considered positive). Category 3 included patients with 1 alloimmune factor and at least 1 positive autoantibody (patients with antiphospholipid antibodies were assigned to category. 2). Finally, category 4 included patients with 1 alloimmune factor associated with at least 1 thrombophilia and at least 1 autoantibody. Category 1 patients received LIT according to the protocol described below. In addition to LIT, category 2 patients received a low dose of aspirin (80-100 mg once daily) starting on the first day of the last menstrual cycle and low-molecular-weight heparin (enoxaparin, 40 mg once daily) after a positive pregnancy test, which was maintained throughout the pregnancy. Category 3 patients received LIT and prednisone (20 mg once daily) after a positive pregnancy test until 12 weeks of gestation. Category 4 patients received all of the abovementioned therapies (LIT, aspirin, low-molecular-weight heparin, and corticosteroid).

2.3 | Lymphocyte immunotherapy protocol

The protocol for LIT used in this study has been published previously.[17] Fresh blood (80 mL) was obtained from participants' partners by peripheral venepuncture and drawn directly into heparinized Vacutainer vials (Becton Dickinson & Co., Franklin Lakes, NJ, USA). Peripheral mononuclear white blood cells (WBCs) were separated aseptically in laminar flow using Ficoll-Hypaque gradient centrifugation. WBCs were then washed in saline and resuspended in 1 mL of saline solution. Subsequently, 80-100 million cells were induced in the forearm of the woman by intradermal injection at 3 locations. Immunizations were performed on 3 different days, following the same routine, with a 3-week interval between them. Three weeks after the last immunization, a cross-match by complement-dependent cytotoxicity assay was performed to confirm antipaternal antibody production. To continue in the study, patients had to have a positive cross-match after the initial 3 doses. Patients underwent booster immunization every 3 months while attempting pregnancy tests and once every 4 weeks after a positive pregnancy test was observed. All Rh-D-negative patients received intramuscular antiRh D globulin (150 mg) immediately prior to the administration of paternal cells.

2.4 | Statistical analysis

The characteristics of the study population were described as means and standard deviations for the continuous variables. The categorical variables were described as numbers and percentages. Comparisons between the groups were performed using the Student's *t* test, the Kruskal-Wallis test, analysis of variance, Fisher's exact test, and/or the chi-squared test (χ^2) when appropriate. Adjusted multivariate analysis was performed using binary logistic regression and included the following independent variables: age, primary recurrent miscarriages, diagnosis/treatment category, number of previous miscarriages, and relationship between primary recurrent miscarriages and number of previous miscarriages. The interactions between variables were also calculated and included in the model when significant.

Data were transferred to an Excel 2007 spreadsheet (Microsoft Corp., Redmond, WA, USA), and the SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA), was used for statistical analysis. The differences were considered significant when $P < .05$.

3 | RESULTS

This study reviewed 752 medical records from patients with a history of 2 or more consecutive miscarriages who then underwent LIT. Of the 752 treated patients, 702 (93.3%) became pregnant, a percentage of spontaneous pregnancy that was similar to that of the previously assessed groups. However, there was a tendency toward a lower rate of pregnancy with increasing number of previous miscarriages.

The analyses were performed in the first pregnancy of these 702 women after the standard investigation and treatment protocol were instituted (Figure 1). Of the total number of pregnancies studied, 597 patients (85%) had a history of primary recurrent miscarriages and 105 patients (15%) had a history of at least 1 (maximum 3) previous pregnancy lasting longer than 20 weeks. The mean age of the patients at the time of the first consultation was 34.16 ± 4.9 years (ranging from 18 to 43 years). Among all the patients studied, the mean number of gestations, the median number of pregnancies carried to term, and the mean number of previous miscarriages were as follows: 2.87 ± 1.1 (ranging from 2 to 8 pregnancies), 0.17 ± 0.4 (ranging from 0 to 3 pregnancies carried to term), and 2.70 ± 0.9 (ranging from 2 to 7 miscarriages), respectively (Table 1).

Cytogenetic abnormalities were observed in 41 couples (5.8%), and 23 patients (3.3%) exhibited some type of anatomical abnormality. The frequencies of these 2 variables were similar between the groups. The presence of at least 1 inherited or acquired thrombophilia (category 2) was observed in 156 of the 702 patients (22.2%). Ninety-nine patients (14.1%) had at least 1 positive autoantibody (category 3). As shown in Table 1, 51 patients (7.3%) exhibited an association of at least 1 thrombophilia and at least 1 positive autoantibody (category 4).

When all pregnancies were considered, 421 patients were able to carry a pregnancy to term, an outcome which reflects a treatment

success rate of 60%. There was a significant reduction in treatment success rate between the groups with 2, 3, 4, or 5 or more prior miscarriages (64.9%, 57%, 50%, and 47.2%, respectively). The comparison of the variables between the groups according to the pregnancy outcome revealed that women who experienced LIT failure (another miscarriage) tended to be older and to have a higher number of pregnancies and previous miscarriages. The groups were similar in terms of causes of pregnancy loss diagnosed during the investigation, but the association of thrombophilic and autoimmune factors (category 4) was statistically more frequent in the group that experienced LIT failure (Table 1).

Among the pregnant women, 370 (52.7%) had a history of 2 consecutive previous miscarriages, 228 (32.4%) had 3 previous consecutive miscarriages, 68 (9.7%) had 4 previous consecutive miscarriages, and 36 (5.2%) had 5 or more previous consecutive miscarriages. The patients' mean age and the causes of recurrent miscarriage were similar among the 4 groups. The number of previous miscarriages did

TABLE 1 Demographic characteristic, obstetric history, and etiology in the group of patients treated based on their gestational outcomes

Variable	All patients (n = 702)	Success (n = 421)	Failure (n = 281)	*P*
Age: mean ± SD	34.16 ± 4.9	33.02 ± 4.6	35.87 ± 5.0	**<.001**
Number of pregnancies: mean ± SD	2.87 ± 1.1	2.76 ± 1.0	3.05 ± 1.4	**.001**
Number of pregnancies carried to term: mean ± SD	0.17 ± 0.4	0.14 ± 0.4	0.20 ± 0.4	**.112**
Number of miscarriages: mean ± SD	2.70 ± 0.9	2.61 ± 0.8	2.84 ± 1.0	**.001**
Primary RM, n (%)	597 (85)	366 (86.9)	231 (82.2)	**.054**
Anatomical defect, n (%)	23 (3.3)	13 (3.1)	10 (3.6)	**.731**
Abnormal karyotype, n (%)	41 (5.8)	26 (6.2)	15 (5.3)	**.642**
Category 1, n (%)	396 (56.4)	249 (59.1)	147 (52.3)	**.073**
Category 2, n (%)	156 (22.2)	97 (23)	59 (21)	**.052**
Category 3, n (%)	99 (14.1)	56 (13.3)	43 (15.3)	**.445**
Category 4, n (%)	51 (7.3)	19 (4.5)	32 (11.4)	**<.001**

RM, recurrent miscarriage; SD, standard deviation; success, treated patients who had a live birth; failure, patients who had another miscarriage after LIT; category 1, alloimmune factor (negative cross-match); category 2, alloimmune factor and at least 1 positive test for thrombophilia; category 3, alloimmune factor and at least 1 positive autoantibody; category 4, alloimmune factor associated with at least 1 thrombophilia and at least 1 autoantibody.

TABLE 2 Demographic characteristics, obstetric history, and etiology in the group of patients treated based on their gestational outcomes

Variable	All patients (n = 702)	02 PM (n = 370)	03 PM (n = 228)	04 PM (n = 68)	≥5 PM (n = 36)	*P*
Age: mean ± SD	34.16 ± 4.9	33.88 ± 4.9	34.11 ± 5.0	34.87 ± 4.7	36.03 ± 5.0	.061
Number of pregnancies: mean ± SD	2.87 ± 1.1	2.15 ± 0.4	3.18 ± 0.4	4.18 ± 0.4	5.94 ± 0.9	<.001
Number of pregnancies carried to term: mean ± SD	0.17 ± 0.4	0.13 ± 0.4	0.18 ± 0.4	0.18 ± 0.4	0.40 ± 0.6	<.001
Primary RM, n (%)	597 (85)	328 (88.6)	190 (83.3)	56 (82.4)	23 (63.9)	.001
Secondary RM, n (%)	105 (15)	42 (11.4)	38 (16.7)	12 (17.6)	13 (36.1)	.001
Category 1, n (%)	396 (56.4)	212 (57.3)	126 (55.3)	39 (57.4)	19 (52.8)	.839
Category 2, n (%)	156 (22.2)	79 (21.4)	49 (21.5)	18 (26.5)	10 (27.8)	.850
Category 3, n (%)	99 (14.1)	49 (13.2)	40 (17.5)	6 (8.8)	4 (11.1)	.176
Category 4, n (%)	51 (7.3)	30 (8.1)	13 (5.7)	5 (7.4)	3 (8.3)	.738

RM, recurrent miscarriage; SD, standard deviation; 2 PM, two consecutive prior miscarriages; 3 PM, three consecutive prior miscarriages; 4 PM, four consecutive prior miscarriages; 5 PM, five or more consecutive prior miscarriages; category 1, alloimmune factor (negative cross-match); category 2, alloimmune factor and at least 1 positive test for thrombophilia; category 3, alloimmune factor and at least 1 positive autoantibody; category 4, alloimmune factor associated with at least 1 thrombophilia and at least 1 autoantibody.

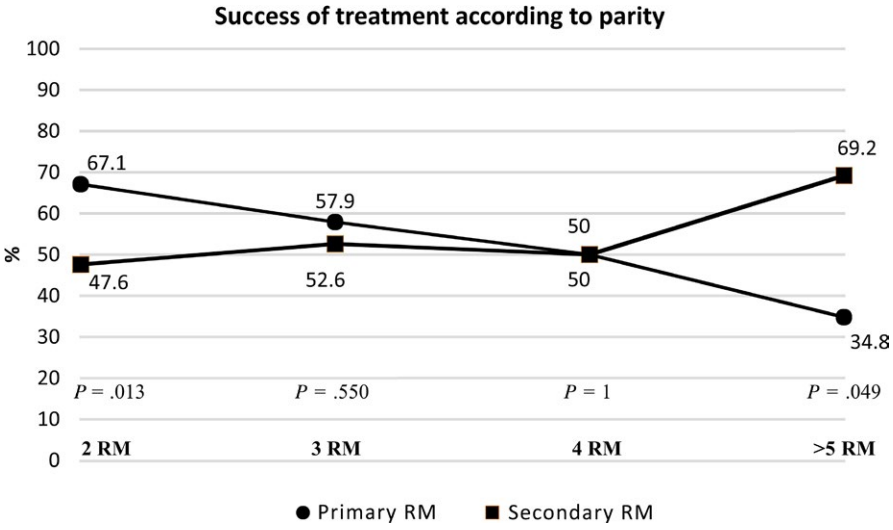

FIGURE 2 Immunotherapy outcomes in recurrent miscarriage (RM) subgroups (primary or secondary) according to the number of previous miscarriages (PM). 2 PM, two previous consecutive miscarriages; 3 PM, three previous consecutive miscarriages; 4 PM, four previous consecutive miscarriages; and 5 PM, five or more previous consecutive miscarriages. Comparison of cases of primary recurrent miscarriage according to the number of PM: $P = .002$. Comparison of cases of secondary RM according to the number of PM: $P = .0596$

not increase the prevalence of autoimmune (02 PM: 21.4%, 03 PM: 21.5%, 04 PM: 26.5%, ≥05 PM: 27.8%, $P = .85$) or thrombophilic factors (02 PM: 13.2%, 03 PM: 17.5%, 04 PM: 8.8%, ≥05 PM: 11.1%, $P = .176$). The percentage of patients with a history of primary and secondary recurrent miscarriages was similar between the groups, however, with the exception of the group that experienced 5 or more miscarriages (Table 2).

In the primary and secondary recurrent miscarriage subgroups, there was a significant reduction in the treatment success rate in cases of primary RM. This rate ranged from 67.1% among patients who had 2 previous miscarriages to 34.8% among patients who had 5 or more previous miscarriages ($P = .002$). In the group of patients with a history of secondary recurrent miscarriage, there was an insignificant improvement in the treatment success rate as the number of previous miscarriages increased ($P = .596$) (Figure 2).

The binary logistic regression showed that age (per year), association between autoantibodies and thrombophilia (category 4), and number of previous miscarriages were factors associated with LIT failure (Table 3). Obstetric history of a previous pregnancy lasting more than 20 weeks was not enough to predict the success or failure of immunotherapy. However, a history of secondary recurrent miscarriages among women with a history of 5 or more previous miscarriages was found to be a predictor of LIT success (odds ratio: 10.24; 95% confidence interval [CI]: 1.9-55.8; $P = .007$).

TABLE 3 Binary logistic regression-based factors predictive of lymphocyte immunotherapy success

Variable	Adjusted OR (95% CI)	*P*
Age, y	0.881 (0.851-0.913)	<.001
Primary RM	0.608 (0.305-1.211)	.157
Category 4	0.320 (0.169-0.605)	<.001
2 miscarriages	1	–
3 miscarriages	0.654 (0.443-0.963)	.032
4 miscarriages	0.524 (0.287-0.956)	.035
≥5 miscarriages	0.288 (0.114-0.728)	.009
2 miscarriages/primary or secondary RM	1	–
3 miscarriages/primary or secondary RM	1.801 (0.661-4.903)	.250
4 miscarriages/primary or secondary RM	2.306 (0.494-10.759)	.288
≥5 miscarriages/primary or secondary RM	10.346 (1.917-55.835)	.007

OR, odds ratio; CI, confidence interval; RM, recurrent miscarriage.

4 | DISCUSSION

Lymphocyte immunotherapy is a therapeutic option for couples with idiopathic RM. The proposed LIT mechanisms of action are production of antipaternal cytotoxic antibodies, anti-idiotypic antibodies (Ab2) and mixed lymphocyte reaction blocking antibodies (MLR-Bf), reduced NK cell activity, improved Th-1/Th-2 balance with Th-2 predominance, and improved Treg cell profile.[11]

It has been used since the 1980s and good success rates have been described in the vast majority of publications, ranging from 45% to 86%.[18] In 1999, however, Ober et al[19] suggested that the therapy was ineffective, which led some countries to recommend the use of this immunotherapy only for research protocols. Since then, studies have sought to identify the profiles of patients who benefit most from this therapy so that favorable robust evidence on LIT can be obtained.

Different factors predictive of LIT success have been studied. The best pre-gestational biomarker for the prediction of a live birth in post-treatment pregnancies appears to be the presence of the partner's antilymphocyte antibodies in the patient's serum. This was an inclusion criterion for the patients in our study.[20]

Maternal age is the main factor associated with failed pregnancies, including patients with a history of RM, both in women who undergo LIT and untreated patients.[21] The risk of miscarriage due to maternal age is associated with a higher incidence of embryonic

chromosomal abnormalities, a fact that was not considered in our sample because genetic studies of the aborted embryonic material were not performed.[22,23]

The number of previous miscarriages is another factor predictive of failed pregnancy irrespective of patients having undergone treatment or not. This has long been established in the literature and was also true for our sample when all the patients were considered as a whole regardless of their obstetric history. Daya et al[13] evaluated a group of patients with a history of primary RPL and found fewer successful pregnancies among patients with a higher number of previous miscarriages, including those who had undergone LIT; however, the rate of miscarriage among treated patients was much lower than that among untreated patients. These results were similar to those of other authors. This difference in therapeutic efficacy in the RM subgroups has also been observed in studies using intravenous human immunoglobulin.[13,24,25]

The presence of autoantibodies (in isolation or not) in patients who undergo LIT appears to be a predictor of future miscarriages. Protocols suggest that women with a history of pregnancy loss and positive ANA and antithyroid antibody tests should be warned of the high risk of failure if they choose to undergo immunotherapy.[15,26] In our series, the presence of at least 1 autoantibody in women who underwent LIT (alone) was not associated with a worse prognosis. However, when at least 1 autoantibody was associated with a thrombophilic factor, therapy was less effective.

In our sample, patients were divided into 4 categories according to the therapeutic protocol; patients with antiphospholipid syndrome were assigned to the same group as patients with hereditary thrombophilia, which may explain the insignificance of category 2 (positive autoantibodies, with the exception of antiphospholipids) as a poor predictor. However, patients belonging to category 4 (autoimmunity, antiphospholipid syndrome, and hereditary thrombophilia) experienced a higher rate of miscarriages.

Some authors suggest that the immune mechanism of pregnancy loss in patients with a history of primary RM differs from that in patients with secondary RM. Piosik et al[27] observed a higher concentration of TNF-α in patients with a history of secondary RM. This difference in immune response may be due to a longer time of exposure to fetal antigens during a pregnancy lasting until the third trimester, which may increase the risk of future autoimmune diseases and, consequently, the risk of additional pregnancy loss.[28] However, other authors believe that a history of a pregnancy carried to term should lead to greater maternal immunotolerance, that is, obstetric history may be associated with gestational prognosis and immunotherapy success. However, there is no consensus in the literature regarding which subgroup has a better obstetric outcome and whether patients should be investigated and receive the same treatment.[29-32]

Carp et al studied women who underwent LIT after experiencing 5 or more miscarriages and observed better outcomes following immunotherapy among women with a history of primary RM (3 consecutive losses with no gestation lasting longer than 20 weeks) and tertiary RM (1 live birth in the middle of a sequence of miscarriages) than those among patients with a history of secondary RM (1 live birth before the series of miscarriages). The relative risks were 2.04 (CI: 1.24-3.58) and 2.92 (CI: 1.37-7.04) for primary and secondary RM, respectively. Other authors divided the patients into primary and secondary RM groups according to the classic definition and observed better immunotherapy outcomes among patients with no history of pregnancy lasting more than 20 weeks.[33]

The result that stood out in our study was the distinct efficacy of LIT when the primary and secondary RM subgroups were evaluated in relation to the number of previous miscarriages. The increasing number of previous miscarriages negatively affected LIT success among patients with primary RM, but among patients with secondary RM, the opposite occurred: LIT outcomes tended to be better with increasing number of previous miscarriages.

One limitation of the study was the performing the genetic study of the product of gestational loss, since embryonic genetic aberrations account for most of the losses in the first trimester. This failure can be justified due to the technical difficulties to perform this investigation (pregnancies with few weeks of evolution, improper collection of material, and biological material not feasible). Another limitation was the lack of data on LIT side-effects in medical records. The most common side-effect of LIT is reaction at the site of intradermal injection.

In our sample of patients, immunotherapy using the partner's lymphocytes proved to be a possible treatment for cases of RM, but the success rates varied according to the patient's obstetric history and associated autoimmune and thrombophilic factors. Therefore, evidence of LIT efficacy may become clearer if studies using strict protocols to select couples assess different patient subgroups, focusing on the other etiological factors involved and on history of pregnancy and miscarriage.

CONFLICT OF INTEREST

None.

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How to cite this article: Cavalcante MB, Sarno M, Niag M, et al. Lymphocyte immunotherapy for recurrent miscarriages: Predictors of therapeutic success. *Am J Reprod Immunol*. 2018;e12833. https://doi.org/10.1111/aji.12833