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Gestational and perinatal outcomes in recurrent miscarriages couples treated with lymphocyte immunotherapy

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ABSTRACT

Objective: This study aims to elucidate which types of recurrent miscarriage (RM) patients experienced a livebirth after paternal lymphocyte immunotherapy (LIT) and to evaluate the perinatal outcome.
Study design: Retrospective analysis of a multicenter, observational study which enrolled 1096 couples with a history of two or more spontaneous miscarriages without any intercalated delivery. We conducted an intention-to-treat analysis of couples with RM treated with or without LIT regarding to gestational and perinatal outcomes. We compared groups by using the Student's *t*-test or Kruskal–Wallis test, Fisher's exact-test and χ^2 test when appropriate.
Results: The success of gestation was significantly higher in the LIT group (60.1% vs. 33.1%; $p < 0.001$). A sub-analysis of four different immune disorder groups revealed a significantly higher success in the LIT group in all immune categories, except in patients who had autoantibodies positive. We observed no significant differences in perinatal outcomes such as gestational age at birth, preterm and extreme preterm birth, and birth weight in successful pregnancy in both groups. The success rate was significantly higher when LIT was administrated before and during pregnancy and only during pregnancy compared to only before pregnancy ($p < 0.01$).
Conclusions: Careful laboratory test phenotyping of RM patients may identify subgroups most likely to benefit and exclude those with little likelihood of benefit, and LIT during a pregnancy may significantly improve success rates.

Introduction

Historically, recurrent miscarriage (RM) was defined by World Health Organization (WHO) as the occurrence of three or more consecutive and spontaneous abortion [1]. The international definitions of RM differ with regard to the number of abortions and the sequence of previous pregnancies. The European Society of Human Reproduction and Embryology (ESHRE, 2006), the Royal College of Obstetricians and Gynaecologists (RCOG, 2011), and the Brazilian Federation of Gynecology and Obstetrics Associations (FEBRASGO, 2010) define RM as three or more consecutive miscarriages. By contrast, the American College of Obstetrics and Gynecology defines RM as two or more consecutive abortions (ACOG, 2002). The American Society for Reproductive Medicine (ASRM, 2013) and the Dutch Society of Obstetrics and Gynaecology (NVOG, 2007) define RM similarly but without using the word "consecutive" [2–4]. To unify the various concepts related to RM, the International Committee Monitoring Assisted Reproductive Technologies (ICMART) proposed that RM should be defined as the occurrence of 2 or more spontaneous abortions at less than 22-weeks of gestation [5]. This latter classification was used in our study.

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The incidence of RM is variable range from 0.5% to 2.3% according the number of previous miscarriages is considered and the characteristics of population. Recently, Rasmark Roepke et al. [6] noted an increase number of new RM patients in Sweden. Genetic factor, anatomical anomalies, antiphospholipid syndrome and hormonal dysfunctions are recognized causes of RM [6]. Current protocols do not recommend the investigation of hereditary thrombophilia and immunological causes. According to the guidelines of the American Society for Reproductive Medicine (ASRM) and European Society of Human Reproduction and Embryology (ESHRE), the cause of RM is diagnosed in only half of patients. Therefore, reproductive immunology can help to uncover a considerable number of idiopathic RM [7,8].

Immunology may play a vital role in embryo adaptation starting at implantation, but there is a lack of robust scientific evidence to support the use of immune therapies in case of reproductive failures. Although options have been proposed in the literature to obtain better outcomes for couples suffering from RM, including acetylsalicylic acid, progesterone, tumor necrosis factor alpha antagonists (anti-TNFα), corticosteroids, granulocyte-colony stimulating factor (G-CSF), hydroxychloroquine, intravenous immunoglobulin (IVIG), paternal lymphocyte immunotherapy (LIT), Intralipid® (lipid emulsions), these approaches are all considered controversial [9–13].

The first alloimmune mechanism proposed as the cause of RM hypothesized that in some couples similarities in human leukocyte antigens between father and mother (increased frequency of sharing HLA antigens at the A, B, and D/DR loci) could result in the failed production of blocking antibodies, thus leading to pregnancy termination. [14] RM was also attributed to other immunological mechanisms such as the hyperactivity of natural killer cells and the imbalance of T-helper (Th) 1 and Th2 responses consisting of a predominant Th1 response. Low concentrations of $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T cells have also been considered an RM risk factor [15–18].

This study aimed to evaluate the effectiveness of partner LIT in different groups of RM patients and to evaluate the perinatal outcome.

Materials and methods

Patients

Retrospective analysis was performed on a multicenter, observational study conducted in six Brazilian reproductive immunology centers (São Paulo, Rio de Janeiro, Salvador, Porto Alegre, Recife, and Fortaleza) from January 2006 to December 2016. We reviewed 1096 medical records of patients by using the following inclusion criteria: (i) women $\geq$ 18 years old with reproductive capacity and a history of 2 or more consecutive miscarriages (<20 weeks), with the same partner, with or without previous pregnancies $\geq$20 weeks; (ii) absence of anti-paternal HLA antibodies (negative crossmatch) during the investigation and with the situation defined as absence of evidence of a spontaneous pregnancy-induced immune response.

We offered immunotherapy with partner lymphocytes to all patients. Although LIT was provided to 752 patients (LIT group), 344 patients did not receive therapy for various reasons (no LIT group). All evaluated pregnancies were conceived naturally without the aid of assisted reproductive techniques. All patients were subject to investigation and treatment for other causes of RM according to the protocol (described below), which was standardized in all six centers involved in the study. The patients became pregnant within a year. After one year the patients were referred for infertility protocol. Patients who became pregnant by assisted reproductive techniques were not included in this study.

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

Protocol for evaluation and treatment

The standardized protocol at the centers involved the investigation of the following RM causes: genetic, anatomical, hormonal, antiphospholipid syndrome (APS), hereditary thrombophilic, autoimmunity, and anti-paternal HLA antibodies. Genetic causes were assessed by karyotyping the peripheral blood of patients and their partners. Furthermore, hysterosalpingography and/or hysteroscopy were used to evaluate uterine abnormalities. Thyroid function was assessed by evaluating thyroxine and free thyroid-stimulating hormone, and fasting glucose levels were used to assess the possibility of diabetes mellitus. For the diagnosis of APS, the patients had to fulfill the revised laboratory criteria of the Sydney classification. Tests were performed for hereditary thrombophilias such as protein C deficiency, protein S deficiency, antithrombin deficiency, methylenetetrahydrofolate reductase mutations C667 T and A1298C, Leiden V gene mutation, and G20210A prothrombin gene mutation. Autoimmune factors were assessed by antinuclear antibody (ANA), anti-DNA, antithyroperoxidase, and anti-thyroglobulin tests.

The identification of anti-paternal HLA antibodies was investigated by microlymphocytotoxicity assay (crossmatch), a cross reaction between maternal serum and paternal peripheral blood leukocytes. Crossmatching was carried out at room temperature against total mononuclear cells, T cells and B cells. A positive result was recorded when 50% or more cell death was observed at a serum dilution of 1:16 or greater [19]. A negative crossmatch was present in all patients, defined here as alloimmune factor and indicating LIT. Standardized maternal blood T cell-cytokine assays, natural killer (NK) cell assays, and Treg cell assays were not available. The crossmatch is easier to run and less expensive. Patients and partners were all subjected to ABO and Rh blood typing.

Progesterone was vaginally supplemented during the first trimester in all patients in the standard treatment protocol for both groups. Uterine malformations (e.g., uterine septum) that could be corrected were surgically repaired prior to a new pregnancy. Furthermore, couples with abnormal karyotypes received genetic counseling. On the basis of the diagnosis (alloimmune, autoimmune, or thrombophilia causes) and the proposed treatment, we placed patients into four groups, defined as four immune categories as summarized in Table 1: 1) Category 1 comprised patients with only a positive alloimmune factor (negative crossmatch); 2) Category 2 comprised patients with an alloimmune factor and at least one positive test for thrombophilia (APS and/or other hereditary thrombophilias with positive heterozygotic or homozygotic status); 3) Category 3 included patients with an alloimmune factor and at least one positive autoantibody (except patients with APS who were allocated to category 2); and 4) Category 4 comprised patients with an alloimmune factor associated with at least one thrombophilia and at least one autoantibody.

The treatment performed in each category is summarized in Table 1. Category 1 patients of the LIT group received LIT according to the protocol described below, whereas the remainder did not (No LIT group). Patients in category 2 received low-dose aspirin (80–100 mg once daily) from the first day of the last menstrual cycle and low-molecular-weight heparin (40 mg enoxaparin once daily) from the beginning of a positive pregnancy test and throughout the pregnancy regardless of whether they received LIT. Category 3 patients received prednisone (20 mg once daily) after a positive pregnancy test and until 12 weeks of gestation

Table 1
Summary of patient's categories according to laboratory investigation and treatment.

Categories according the test results				
Category	Category 1	Category 2	Category 3	Category 4
Alloimmune factor	Positive	Positive	Positive	Positive
Thrombophilic factor	Negative	Positive	Negative	Positive
Autoimmune factor	Negative	Negative	Positive	Positive

Categories according the treatment				
Category	Category 1	Category 2	Category 3	Category 4
LIT	LIT Group – Yes No LIT Group - No	LIT Group – Yes No LIT Group – No	LIT Group – Yes No LIT Group - No	LIT Group – Yes No LIT Group - No
Aspirin and heparin	No	Yes	No	Yes
Prednisone	No	No	Yes	Yes

Alloimmune factor positive: absence of anti-paternal HLA antibodies (negative crossmatch); Thrombophilic factor: at least one positive test for thrombophilia (antiphospholipid syndrome and/or other hereditary thrombophilias with positive heterozygotic or homozygotic status); Autoimmune factor: at least one positive autoantibody (except patients with APS who were allocated to category 2). LIT: lymphocyte immunotherapy.

regardless of whether they received LIT. Finally, patients in category 4 received all previous therapies (aspirin, enoxaparin, and prednisone) regardless of whether they received LIT.

LIT protocol

In this study, we used a previously published LIT protocol and collected fresh blood (80 mL) from participants' partners by peripheral venipuncture directly into heparinized Vacutainer vials (Becton Dickinson & Co., Franklin Lakes, NJ). Immediately after blood collection, peripheral mononuclear white blood cells (WBCs) were then separated aseptically under laminar flow by using Ficoll–Hypaque gradient centrifugation. WBCs were washed in saline and resuspended in 1.0 mL saline solution. We administered 80–100 million WBCs into the forearm of females intradermally (dividing this 1 ml into three injections, side by side) and repeated such immunizations on three different days with the same routine and with a 3-week interval between each procedure. Three weeks after the last immunization, we conducted a crossmatch assessment by using a complement-dependent cytotoxicity assay to confirm antipaternal antibody production. Only patients who exhibited a positive crossmatch after the initial three doses were retained in the study. Patients underwent booster immunization every three months while attempting pregnancy and once every four weeks after a positive pregnancy test was obtained. All RhD-negative patients received intramuscular anti-RhD globulin (150 mg) immediately before the administration of paternal cells. One group of patients did not receive LIT prior to pregnancy but received it only after conception (patients who had an unexpected pregnancy before the start of the pre-conception immunization).

Statistical analysis

The outcomes in each patient subgroup were determined on an intention-to-treat basis and not by analysis of only those achieving pregnancy. The characteristics of the study population are described as mean ± standard deviation or medians and interquartile ranges for continuous variables on the basis of sample distribution. Categorical variables are described as numbers and percentages. We compared groups by using the Student's *t*-test or Kruskal–Wallis test for numerical variables and Fisher's exact-test or χ^2 test when appropriate for categorical variables. The collected data were transferred to an Excel 2007 worksheet (Microsoft Corp., Redmond, WA), and SPSS 20.0 software (SPSS Inc., Chicago, IL) was used for statistical analysis. We considered $p < 0.05$ statistically significant.

Results

Table 2 presents the distribution of demographic characteristics and clinical history between two groups (LIT group and No LIT group). No significant differences were found among variables such as age, number of miscarriages, genetic and anatomic factors, and the number of patients in category 4 in each group (LIT vs. No LIT). By contrast, we identified significant differences in the population proportions when comparing the number of previous gestations, deliveries, primary RM, and the number of patients in the categories 1, 2 and 3.

We examined 1096 couples with a previous history of two or more miscarriages by comparing the LIT and No LIT groups (Fig. 1). Overall, successful gestation was significantly higher in the LIT group (60.1% vs. 33.1%; $p < 0.001$, OR 0.55, CI 95% 0.47–0.65). A subanalysis of the four different immune groups revealed a higher prevalence of immune category 1 in both groups. Success was significantly higher in the LIT group in immune category 1 (62.9% vs. 32.6%, p<0.001, OR 0.51, CI 95% 0.42-0.63), category 2 (62.2% vs. 34.6%, p<0.001, OR 0.54, CI 95% 0.36-0.81), and category 4 (37.3% vs. 10%, p=0.04, OR 0.29, CI 95% 0.07–1.10), whereas no differences were observed in category 3 (56.5% vs. 53.3%, p<0.76, OR 0.94, CI 95% 0.65–1.37) (LIT vs. No LIT in all groups; χ^2 test, all patients, categories 1–3; Fisher's exact-test, category 4; Fig. 2). Categories 1 and 2 revealed a significantly higher success in the LIT group. By contrast, category 4 demonstrated poor prognosis compare to categories 1 and 2, but significantly higher success compare to No LIT group. Although category 3 did not exhibit any differences between the LIT and No LIT groups, it exhibited a higher rate of successful pregnancy in both groups simultaneously. Statistical analysis of the patients according to the obstetric history (primary or secondary RM) showed that LIT had a beneficial effect in categories 1 and 2 (Table 3).

The number of APS patients in category 2 was similar in both groups, 9.6% (72/752) versus 6.1 (21/344), p = 0.05, LIT and No LIT respectively. The prevalence of APS and other autoantibodies in category 4 were similar in LIT and No LIT groups. The number of APS cases in category 4 was 41.2% (21/51) in LIT group and 55% (11/20) in No LIT group, p = 0.211. The prevalence of ANA in category 4 was 39.2% (20/51) versus 55% (11/20), p = 0.22, LIT and No LIT respectively. The prevalence of anti-TPO in category 4 was 58.8% (30/51) versus 45% (9/20), p = 0.29, LIT and No LIT respectively.

Despite the superior pregnancy maintenance of the LIT group, no significant difference was observed in perinatal outcomes such as gestational age at birth, preterm or extreme preterm birth, and birth weight (Table 4). Among the 1096 couples examined in this study, we obtained newborn data from only 566 couples (51.6%).

Table 2
Demographic characteristics and clinical history among two groups, the LIT and No LIT groups.

Variables	All (n = 1096)	LIT Group (n = 752)	No LIT Group (n = 344)	*P*
Age (years, mean ± SD)	34.22 ± 4.9	34.06 ± 4.9	34.55 ± 4.9	0.14
Gestations (number, mean ± SD)	2.97 ± 1.2	2.89 ± 1.1	3.14 ± 1.3	0.001
Deliveries (number, mean ± SD)	0.22 ± 0.4	0.17 ± 0.4	0.41 ± 0.6	<0.001
Miscarriages (number, mean ± SD)	2.71 ± 1.0	2.71 ± 0.9	2.72 ± 1.1	0.99
Primary RM, *n* (%)	857 (78.2)	636 (84.6)	221 (64.2)	<0.001
Genetic factor, *n* (%)	68 (6.2)	50 (6.6)	18 (5.2)	0.37
Primary RM, *n*	53	39	14	
Anatomic factor, *n* (%)	27 (2.5)	16 (2.1)	11 (3.2)	0.29
Primary RM, *n*	21	13	8	
Immune category 1, *n* (%)	668 (60.9)	426 (56.6)	242 (70.3)	<0.001
Primary RM, *n*	527	370	157	
Immune category 2, *n* (%)	219 (20.0)	167 (22.2)	52 (15.5)	0.006
Primary RM, *n*	169	139	30	
Immune category 3, *n* (%)	138 (12.6)	108 (14.,4)	30 (8.7)	0.009
Primary RM, *n*	105	87	18	
Immune category 4, *n* (%)	71 (6.5)	51 (6.8)	20 (5.8)	0.54
Primary RM, *n*	56	40	16	

P < 0.05 was considered statistically significant, using Student's *t*-test or Kruskal–Wallis test for numerical variables and Fisher's exact-test or χ^2 test when appropriate for categorical variables. LIT: lymphocyte immunotherapy. RM: recurrent miscarriage. SD, Standard deviation; *n*, number. Category 1: alloimmune factor positive; Category 2: alloimmune factor and thrombophilic factor: at least one positive test for thrombophilia (antiphospholipid syndrome and/or other hereditary thrombophilias with positive heterozygotic or homozygotic status); Category 3: alloimmune factor and autoimmune factor (at least one positive autoantibody, except patients with APS who were allocated to category 2. Category 4: alloimmune factor associated with at least one thrombophilia and at least one autoantibody.

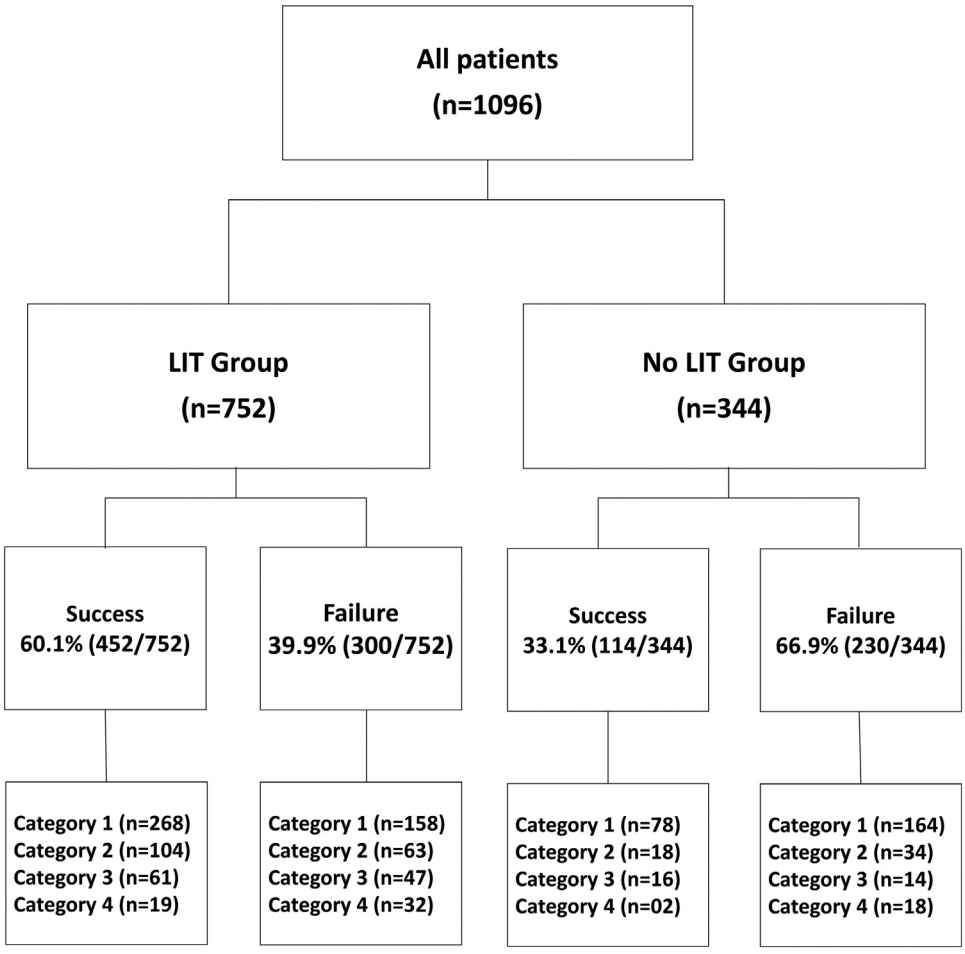

Fig. 1. Sample distribution according to the LIT and No LIT groups, gestation success, and subgroups in different immune categories.
*LIT: lymphocyte immunotherapy. Category 1: alloimmune factor positive; Category 2: alloimmune factor and thrombophilic factor: at least one positive test for thrombophilia (antiphospholipid syndrome and/or other hereditary thrombophilias with positive heterozygotic or homozygotic status); Category 3: alloimmune factor and autoimmune factor (at least one positive autoantibody, except patients with APS who were allocated to category 2. Category 4: alloimmune factor associated with at least one thrombophilia and at least one autoantibody.

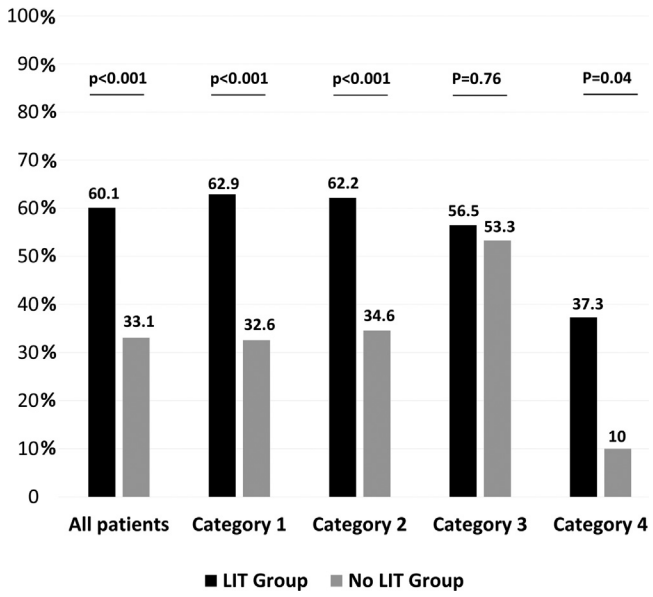

Fig. 2. Gestational success according to immune categories.
*Category 1: alloimmune factor positive; Category 2: alloimmune factor and thrombophilic factor: at least one positive test for thrombophilia (antiphospholipid syndrome and/or other hereditary thrombophilias with positive heterozygotic or homozygotic status); Category 3: alloimmune factor and autoimmune factor (at least one positive autoantibody, except patients with APS who were allocated to category 2. Category 4: alloimmune factor associated with at least one thrombophilia and at least one autoantibody.

Table 3
Success rate between categories according obstetric history (Primary or Secondary RM).

Category Category 1	LIT Group Patients (n = 426)	No LIT Group) Patients (n = 242)	*P*
Primary RM, n (%)	236/370 (63.8)	51/157 (32.5)	<0.001
Secondary RM, n (%)	32/56 (57)	28/85(32.9)	0.008
Category 2	**Patients (n = 167)**	**Patients (n = 52)**	
Primary RM, n (%)	90/139 (64.7)	13/30 (43.3)	0.018
Secondary RM, n (%)	14/28 (50)	5/22(22.7)	0.049
Category 3 (n = 138)	**Patients (n = 108)**	**Patients (n = 30)**	
Primary RM, n (%)	49/87 (56.8)	9/18 (50)	0.395
Secondary RM, n (%)	12/21 (57.1)	7/12 (58.3)	0.590
Category 4 (n = 71)	**Patients (n = 51)**	**Patients (n = 20)**	
Primary RM, n (%)	15/40 (37.5)	2/16 (12.5)	0.060
Secondary RM, n (%)	4/11 (36.3)	0/4 (0)	0.330

P < 0.05 was considered statistically significant, using Fisher's exact-test or χ^2 test when appropriate for categorical variables. LIT: lymphocyte immunotherapy. RM: recurrent miscarriage. *n*, number. Category 1: alloimmune factor positive; Category 2: alloimmune factor and thrombophilic factor: at least one positive test for thrombophilia (antiphospholipid syndrome and/or other hereditary thrombophilias with positive heterozygotic or homozygotic status); Category 3: alloimmune factor and autoimmune factor (at least one positive autoantibody, except patients with APS who were allocated to category 2. Category 4: alloimmune factor associated with at least one thrombophilia and at least one autoantibody.

Fig. 3 shows a comparison between three different types of LIT intervention: (a) before and during pregnancy, (b) only before pregnancy, and (c) only during pregnancy. The success rate was significantly higher when LIT was administered before and during pregnancy or only during pregnancy compared with when LIT was administered only before pregnancy. LIT result before and during pregnancy was similar when was performed only during pregnancy, 78.3% vs. 74.2%, p = 0.68. However, success rate was higher when LIT was performed before and during pregnancy compare to only before pregnancy, 78.3% vs. 34.1%, p < 0.001.

Discussion

Allogenic embryo recognition is well established, but immunomodulation remains controversial [10,20]. The limitations of this study include its retrospective nature, differences between the intervention and nonintervention groups, and the loss of approximately 49% of newborn outcome data. Nevertheless, the substantial sample size of 1096 couples should provide some interesting conclusions. However, factors that could result in poor treatment prognosis (thrombophilia and presence of autoantibodies) were more prevalent in the group that underwent immunotherapy, which could contribute to superior immunotherapy results.

The LIT group contained a higher proportion of primary RM. The literature discusses the influence of obstetric history on future gestational outcome, which could explain some of the beneficial outcomes in the treated group because primary RM benefits the most from LIT treatment according to some studies [21,22]. However, Shapira et al. [23] observed similar live birth rates among patients with primary and secondary RMs, but observed that women with primary RM were more prone to adverse obstetric and neonatal outcomes.

This study demonstrates the high efficacy of LIT for RMs, mainly in women with no other immune disorder and when administered before and during pregnancy. The results significantly corroborate several previous findings [15,18,24–26]. To date, only one clinical trial in 1999 has demonstrated adverse results from LIT intervention. Ober et al. [27] used purified paternal mononuclear cells stored at 4 °C overnight and studies in a murine allogeneic recurrent abortion model where immunotherapy is effective, storing cells at 4 °C abrogated the protective effect of immunization [28]. Our LIT protocol used fresh mononuclear cells.

Since Ober et al. publication, all trials have reported the beneficial effect of LIT on pregnancy success after RMs [29–32]. In 2016, we presented a meta-analysis and systematic review of the main clinical trials and compared LIT with no intervention in RM. The intention-to-treat analysis in this type of research should be highlighted to obtain realistic results for clinical applications [15].

This study not only focused on pregnancy success but also subanalyzed an association of different immune disorders for the first time to produce interesting results. Despite the poor pregnancy success rate, category 4 presented significantly better results in LIT group than the No LIT group (37.3% vs. 10%; p = 0.041). The association of different etiological factors could explain these

Table 4
Comparison between the LIT and No LIT groups according to the success of pregnancy, gestational age at birth, preterm and extreme preterm birth, and weight at birth.

	All couples	LIT patients	No LIT patients	*P*
Success, *n* (%)	566 (51.6)	452 (60.1)	114 (33.1)	<0.001
Birth gestational age (weeks, SD)	37.0 (25–41 weeks; ±2.6)	37.1 (25–41 weeks; ±2.5)	3,69 (26–41 weeks; ±3.1)	0.54
Preterm birth, *n* (%)	144 (25.,4)	110 (24.3)	34 (29.9)	0.23
Extreme preterm birth, *n* (%)	7 (1.,2)	4 (0.,9)	3 (2.6)	0.13
Birth weight (g, SD)	2.897 (510–4500; ± 608)	2.896 (760–4500; ±579)	2,898 (510–4100; ±699)	0.98

Preterm birth, deliveries less than 37 weeks; extreme preterm birth, deliveries less than 28 weeks. LIT: lymphocyte immunotherapy. SD, Standard deviation; *n*, number.

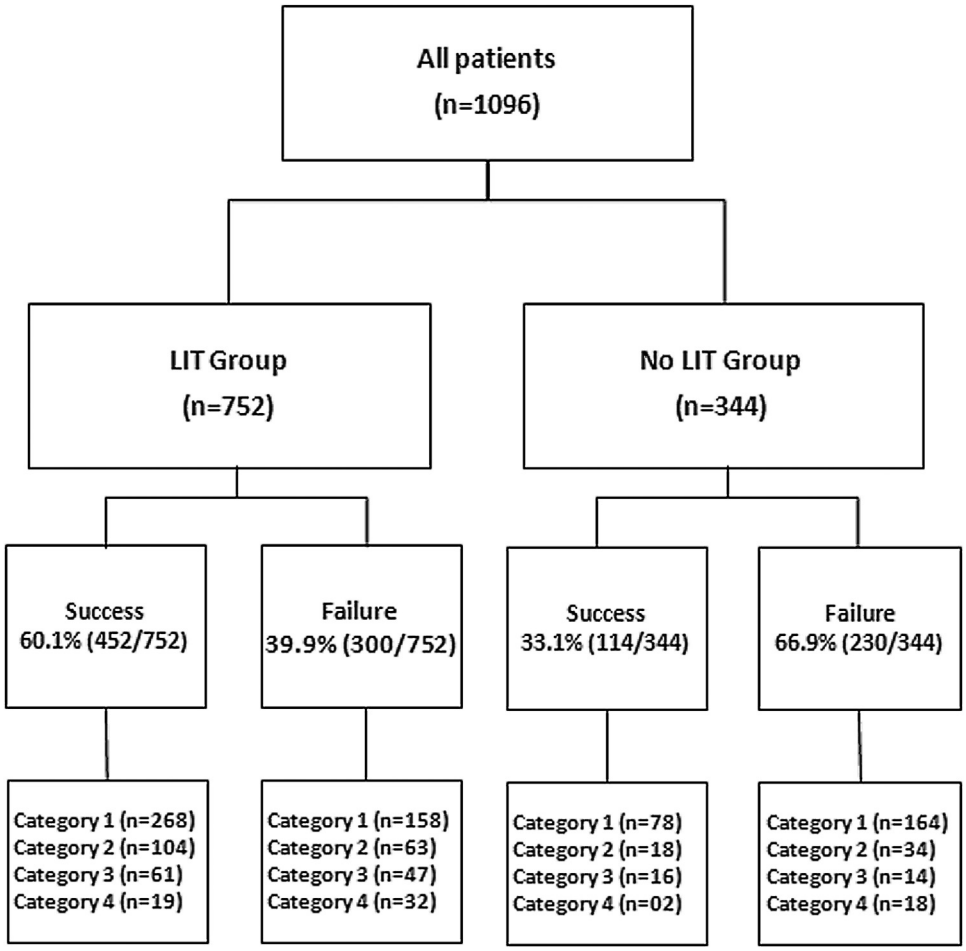

Fig. 3. Obstetric success according to three different moments of LIT: (a) before and during pregnancy; (b) only before pregnancy; and (c) only during pregnancy.
*Category 1: alloimmune factor positive; Category 2: alloimmune factor and thrombophilic factor: at least one positive test for thrombophilia (antiphospholipid syndrome and/or other hereditary thrombophilias with positive heterozygotic or homozygotic status); Category 3: alloimmune factor and autoimmune factor (at least one positive autoantibody, except patients with APS who were allocated to category 2. Category 4: alloimmune factor associated with at least one thrombophilia and at least one autoantibody.

worse results. Categories 1 and 2 both exhibited significant benefits, with 62.9% and 62.2% success rates in the LIT group, compared with 32.2% and 34.6% success rates in the No LIT group, respectively. No differences were observed in category 3 (56.0% vs. 53.0%).

The pathological mechanisms that promote gestational loss in patients with autoantibodies are still unknown, so it is not possible to understand the lack of efficacy of LIT in the autoimmune patients. Previous studies have shown that patients with positive ANA and antithyroid antibodies who undergo LIT have a higher risk of miscarriage [9,32–35]. These results suggest that patients with isolated autoantibodies (except antiphospholipids) have poor prognosis and do not benefit from LIT (category 3). Patients with an autoimmune association and some thrombophilic factors formed the group (category 4) with the worst gestational success rate; however, these patients may benefit from LIT. However, category 4 was the smaller and more heterogenous group.

Although we determined a good prognosis for a successful pregnancy, no difference was observed in obstetric outcome when comparing successful groups with or without the LIT intervention in parameters such as birth gestational age, preterm birth, extreme preterm birth, and birth weight. However, we found a high rate of preterm deliveries in both groups (LIT, 24.3%; no LIT, 29.9%), thus indicating that RM history is already a significant risk factor for prematurity; this result is similar to those in recently published literature [36].

A meta-analysis by Liu et al. [25] demonstrated the superiority of the immunological treatment with paternal lymphocytes compared with placebo (77.8% vs. 46.1%). Furthermore, they found an OR of 4.67 (CI: 3.70–5.90) for the group treated before and during gestation compared with an OR of 2.00 (CI: 1.39–2.88) for the group treated only before pregnancy. By comparing the LIT before and during treatments with only the LIT before treatments, our results produced similar results (78.3% vs. 34.1%; OR: 4.89; CI: 3.48–6.85). This result demonstrates the importance of the maintenance of the immune stimulus promoted by LIT at the beginning of pregnancy, even if the patient had positive cross-match prior to pregnancy.

The immune system plays a decisive role in placental adaptation, and an aggressive response to gestation is involved in the genesis of reproductive failures such as abortion, placental insufficiency, preeclampsia, or implantation failures in cycles of assisted reproduction. However, controversy exists regarding the best method for performing immunomodulation in this situation. This study provides crucial information on which groups could benefit from treatment with paternal lymphocytes.

Our results revealed that categories 1 and 2 benefit the most from LIT and confirmed worse prognosis for patients with autoantibodies positive. Nonetheless, further studies are needed,

preferably randomized clinical trials enrolling group of patients without autoantibodies, to determine the optimal immunotherapies and the immune disorder groups that are most likely to respond favorably.

Conflict of interest

The authors have no conflicts of interest to declare.

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