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REVIEW ARTICLE

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Lymphocyte immunotherapy in recurrent miscarriage and recurrent implantation failure

Marcelo Borges Cavalcante[1,2] | Manoel Sarno[3] | Ricardo Barini[4]

[1]Postgraduate Program in Medical Sciences, Fortaleza University (UNIFOR, Fortaleza, Brazil

[2]CONCEPTUS – Reproductive Medicine, Fortaleza, Brazil

[3]Department of Obstetrics and Gynecology, Federal University of Bahia (UFBA, Salvador, Brazil

[4]Department of Obstetrics and Gynecology, Campinas University (UNICAMP, Campinas, Brazil

Correspondence
Marcelo Borges Cavalcante, Av. Washington Soares, 1321 - Edson Queiroz, CEP 60811-905, Fortaleza, CE, Brazil.
Email: marcelocavalcante.med@gmail.com

Abstract

Problem: Lymphocyte immunotherapy (LIT) emerged in the early 1980s as a new therapeutic proposal for couples with a history of recurrent miscarriages (RM). However, in the early 2000s, the effectiveness of LIT was questioned. Recently, meta-analyses have observed the effectiveness and safety of LIT in treating couples with RM. Some studies evaluated the use of LIT in recurrent implantation failure (RIF) in *in vitro* fertilization cycles.

Methods: This systematic and narrative review evaluated the data available in the literature regarding the efficacy and safety of the use of LIT. Searches in PubMed/Medline, Embase, and Cochrane Library databases were conducted, using the following keywords: "recurrent miscarriage," "lymphocyte immunotherapy," and "recurrent implantation failure".

Results: This review describes the historical aspects of LIT and discusses its protocols, mechanisms of action, side effects, complications, and current evidence of the effectiveness in cases of reproductive failure. It also discusses the use of LIT during the COVID-19 pandemic and new immunological therapies.

Conclusion: In the vast majority of studies, the use of LIT for RM couples has shown an improvement in pregnancy outcomes. The most of the current studies that support the evidence are quasi-experimental, with few randomized, double-blind studies (Level of evidence III). However, the current evidence are not convincing for the use of LIT in RIF patients.

KEYWORDS
lymphocyte immunotherapy, recurrent implantation failure, recurrent miscarriage, reproductive failure

1 | INTRODUCTION

Attempts to explain the enigma of fetal survival throughout pregnancy have aroused the interest of countless researchers over the past few decades. This topic remains controversial in the literature and at scientific events, such as the one that occurred in the "Fertile Battles" section of the recent edition of Fertility and Sterility, the official journal of American Society for Reproductive Medicine (ASRM). The key point of the debate was to understand the immunological mechanisms involved in the embryonic implantation and thus propose therapeutic options based on the clinical evidence of improvement in pregnancy results.[1,2]

In 1953, "Father of Reproductive Immunology," Sir Peter Brian Medawar first recognized that the paradoxical immune response

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between the pregnant woman and the embryo/fetus (a semi-allograft) is the central enigma of pregnancy. Sir Medawar brought this paradox into a sharp scientific question in terms of the newly emerging concepts in transplantation biology. He stated that "*The immunological problem of pregnancy may be formulated thus: how does the pregnant mother contrive to nourish within itself, for many weeks or months, a foetus that is an antigenically foreign body?*". According to the hypothesis of Medawar, the maternal immune system does not react against the fetus because of (a) the anatomical separation of the fetus from the mother, (b) the antigenic immaturity of the fetus, and (c) the immunological indolence or inertness of the mother.[3]

In 1966, Clarke and Kirby suggested that the antigenic disparity between the embryo and the mother is beneficial for pregnancy.[4] In the 1970s, the initial studies by Billingham and Beer which elucidated the mechanisms of embryonic implantation demonstrated that the specific maternal immune responses during pregnancy that interfered with fetal survival could be measured.[5,6] The first research and treatment protocol for couples with reproductive failures, especially for couples with recurrent miscarriage (RM), emerged in the 1980s.[7]

Currently, embryonic implantation, from an immunological point of view, has not yet been fully unveiled, but much progress has been achieved since Medawar's first inquiries. In this scenario, clinicians and patients expect that immunotherapies, with strong scientific evidence, can help a considerable percentage of couples with RM and recurrent implantation failure (RIF). However, the diagnosis of RM and RIF remains unclear and inconsistent, when evaluated established protocols.[1]

2 | LYMPHOCYTE IMMUNOTHERAPY AND RECURRENT MISCARRIAGE

2.1 | Recurrent miscarriage definition and the history of lymphocyte immunotherapy

Recurrent miscarriages is historically defined as the occurrence of three or more consecutive spontaneous abortions.[8] In 2009, ASRM defined RM as the occurrence of two or more consecutive spontaneous abortions.[9] This problem can affect up to 5% of couples in reproductive age. Patients with a high number of previous abortions have an increased risk of additional pregnancy losses. In accordance with the European Society of Human Reproduction and Embryology (ESHRE) and ASRM guidelines, only about half of the couples with a history of RM have a known cause.[10,11] The only immunological factor included in the international management guidelines for couples with RM is antiphospholipid syndrome. Some autoimmune conditions are strongly associated with RM, but the immunotherapies proposed for these conditions lack robust evidence to be included in international guidelines. The same is true for alloimmune conditions, where an association exists between uterine and systemic alloimmune factors, but therapeutic options with scientific evidence are limited.[10,11]

The first immunological therapy proposed for couples with a history of RM was immunotherapy with lymphocytes. In the early 1970s, studies observed improved outcomes of kidney transplantation in patients undergoing blood transfusions. Based on the theory of anti-rejection immunosuppression by Opelz et al.,[12,13] Taylor and Faulk reported the gestational success of three patients with a history of RM treated with plasma-rich leukocytes using unrelated donors in the early 1980s.[7] This therapeutic proposal was also suggested by Beer (1984) and Mowbray (1985).[14,15]

2.2 | Lymphocyte immunotherapy: Composition and protocols

Lymphocyte immunotherapy (LIT) consists of a lymphocyte concentrate prepared from the peripheral blood of the partner or a third party to promote maternal immunomodulation that is favorable to embryonic implantation. The process of isolating lymphocytes from a whole blood sample was first described by Bøyum in 1968.[16] Most protocols for separating lymphocytes from whole peripheral blood use the density gradient protocol with Ficoll-Hypaque. In brief, defibrinated or anticoagulant-treated blood is diluted with an equal volume of a balanced salt solution. Diluted samples are layered carefully over Ficoll-Hypaque product and centrifuged for 30–40 min. Differential migration of cells during centrifugation results in the formation of layers containing different cell types: (a) The bottom layer includes erythrocytes; (b) the layer immediately above the bottom layer contains mostly granulocytes; and (c) at the interface between the plasma and the Ficoll-Hypaque layer, mononuclear cells coexist with other slowly sedimenting particles (eg, platelets).[17] The separation of normal whole human peripheral blood by Ficoll-Hypaque density gradient media typically yields a mononuclear cell preparation (60 ± 20% recovery of the mononuclear cells present in the original blood sample) with (a) 95 ± 5% mononuclear cells (T cells, B cells, natural killer (NK) cells, and monocytes), (b) >90% viability of the separated cells, (c) maximum 5% granulocytes, and (d) maximum 10% erythrocytes.[18]

The immunotherapy proposed by Taylor and Faulk consisted of the transfusion of leukocytes isolated from the partner's blood. Of the four patients, two received leukocyte transfusion in the preconception period. One patient received at 8 and 4 weeks before becoming pregnant; the other patient received at 25, 21, 17, and 13 weeks before becoming pregnant, and all patients received leukocyte transfusion during pregnancy (every 3 weeks up to 20–26 weeks).[7] Mowbray et al. used a lymphocyte concentrate prepared from the partner's blood and applied by three routes, intravenously, intradermal, and subcutaneous routes.[15] There are variations in the preparation of lymphocyte concentration, such as the origin of the blood donor (partner or third person), the storage of the blood sample (immediate use or stored overnight), the number of the applied lymphocytes, the administration route, and the frequency of immunizations.[17] Evidence shows that these variables can affect the efficacy of the therapy.[19-22]

2.3 | Efficacy of lymphocyte immunotherapy for recurrent miscarriage

Mowbray et al. (1985) published the first randomized, double-blind study that evaluated the effectiveness of LIT in couples with RM. The success rate was significantly higher in women injected with purified lymphocytes prepared from their husbands' blood (17/22, 77%) than in those injected with their lymphocytes (10/27, 37%) ($p = 0.01$). The selection criterion for treatment was the absence of anti-paternal lymphocyte antibodies. Specifically, 100–900 × 10^6 partner's lymphocytes were suspended in 5 ml of medium, with 3 ml given intravenously, 1 ml intradermally, and 1 ml subcutaneously. The patients were immunized only once (ie, before pregnancy), and pregnancies that occurred within 12 months were evaluated. The presence of partner anti-lymphocyte antibodies was observed in 37 of 49 treated patients (75.5%). In the discussion of the results, the authors suggested that the ideal regimen of immunization was two or three separate doses of cells from small amounts of blood.[15]

In 1991, Susan Cowchock published an editorial in the American Journal of Reproductive Immunology, analyzing three studies that used LIT in women with a history of RM. The meta-analysis of live-born delivery rates from published studies comparing control patients and treated women (immunized with at least 100 million paternal mononuclear cells) revealed a significant improvement in the treated group (90/135, 67%) compared with the control group (49/100, 49%) ($p = 0.001$).[23] In 1993, Fraser et al. published a meta-analysis including four studies, three using LIT (paternal or third-party leukocytes), and another using trophoblast membrane immunotherapy. The statistical analysis of all studies did not show significant improvement in the treated group when compared to the controls [odds ratio (OR) 1.3, 95% confidence interval (CI) 0.77–2.3], not even when the study that used the trophoblast membrane was excluded (OR: 1.8, 95% CI 0.96–3.4).[24] The meta-analysis by Fraser et al. was criticized by Cowchock and Smith, who recalculated the analysis of the three studies using immunotherapy with lymphocytes in the same software (True Epistat, Richardson, TX) but found a different result (OR 1.8, 95% CI 1.003–3.342).[25]

In 1994, The Recurrent Miscarriage Immunotherapy Trialist Group (RMITG) published the results of LIT from 15 centers, including nine randomized trials (seven of which were double-blind) that were independently assessed by two separate data analyses. In the first analysis, the inclusion criteria were three or more prior miscarriages, no more than one live birth (with any partner), no identified non-immunologic causes, and no evidence of simultaneous cointervention (ie, aspirin, heparin, corticosteroids, progesterone). In the second analysis, the inclusion criteria were women and her present partner who had lost three or more intrauterine pregnancies at less than 20 weeks' gestation and who had not had more than one liveborn with this same partner.[26] RMITG concluded that LIT improved the rate of live births in patients with a history of RM (three or more consecutive miscarriages), with no more than one live birth with any partner (OR: 1.16, 95% CI 1.04–1.34, $p = 0.031$), or no more than one live birth with the current partner (OR: 1.21, 95% CI 1.04–1.37, $p = 0.024$). According to the patient's obstetric history, the success rate was significantly higher in the primary RM group (OR: 1.206; 95% CI 1.028–1.414, $p = 0.025$) but not in the secondary RM group (at least one live birth) (OR: 1.005; 95% CI 0.759–1.331, p = NS). When primary RM and secondary RM were combined, the result was significant (OR: 1.164; 95% CI 1.014–1.335, $p = 0.019$).[26]

Recurrent Miscarriage Immunotherapy Trialist Group also showed a significant improvement in the rate of live births when the patients had antibodies against the lymphocytes of their partners before pregnancy (RR: 1.37, 95% CI 1.10–1.58, $p = 0.007$) or after LIT (RR: 1.17, 95% CI 1.06–1.27, $p = 0.003$). However, the live birth rate was decreased among patients with autoimmune disorders (positive ANA and/or antiphospholipid antibodies) (RR: 0.64, 95% CI 0.38–0.98, $p = 0.039$), the number of pregnancy loss [for each pregnancy loss greater than three, live birth was 15% less likely (RR: 0.85, 95% CI 0.78–0.93, $p = 0.0001$)], and the number of pregnant women who underwent intravenous immunotherapy (RR: 0.79, 95% CI 0.66–0.91, $p = 0.0007$).[26] The RMITG study showed a need for stratification of patients with RM by identifying characteristics associated with a poor prognosis for immunotherapy. The study concluded that "*We, in summary, conclude that paternal leukocyte immunotherapy appears to represent a useful therapy. The treatment is, however, effective in only a small proportion of women with unexplained recurrent spontaneous abortion.*"[26]

The Recurrent Miscarriage Study (REMIS) published in 1999 was a multicenter, randomized, double-blind study, with patients recruited from six centers, from 1992 to 1997. A total of 171 patients were randomized (86 in the treated group and 85 in the control group). In the analysis of all 171 patients, the success rate was 36% in the LIT group and 48% in the control group (OR: 0.60, 95% CI 0.33–1.12, $p = 0.108$). In the analysis including only pregnant women, the authors demonstrated a negative impact on the group undergoing immunization with lymphocytes; the success rate was 46% in the treatment group and 65% in the control group (OR: 0.45, 95% CI 0.22–0.91, $p = 0.026$).[27] The patients in the treated group were immunized once a week for the first 2 weeks of their menstrual cycle, with the concentrate containing about 200 × 10^6 lymphocytes from the partner, prepared from whole blood stored overnight at 1–6°C. Lymphocytes were diluted in 5 ml of saline and were administered intravenously (3 ml), subcutaneously (two applications, 0.5 ml each) and intradermally (two applications, 0.5 ml each). The immunization protocol was repeated for patients who did not become pregnant over an interval of 6 months. The search for maternal anti-paternal HLA antibodies was performed 2 weeks or more after immunization; however, it was not used as an exclusion criterion in the treated group.[27] The REMIS was criticized by several authors due to serious flaws in the inclusion/exclusion criteria (patients with autoimmune changes were not excluded) and in the laboratory protocol for preparing and applying immunizations (lymphocyte concentrate prepared and stored overnight, and

applied intravenously, subcutaneous, and intradermal).[28-30] Clark et al. showed a negative impact on the protocol used in the REMIS. The lymphocyte vaccine prepared from blood and stored has a low concentration of lymphocytes with CD200 on its surface, reducing the anti-abortion effect.[28] Other studies have shown that the storage of peripheral blood at room temperature increases the concentration of Th1 interleukins, which may make it difficult to balance the Th1/Th2 responses.[31,32]

In 2001, a Cochrane meta-analysis evaluated immunological therapies in cases of RM, including immunization with lymphocytes. The last update of this meta-analysis in 2014 included 12 studies that performed immunotherapy with paternal lymphocytes, totaling 641 participants, including 316 treated women and 325 in the placebo group. The treatment effect on the live birth rate was not significant, with an OR of 1.22 and a CI of 0.89–1.69. In this same Cochrane meta-analysis, no improvement in the rate of live births was observed when assessing the use of immunotherapy with unrelated donor lymphocytes, with an OR of 1.39 and a CI of 0.68–2.82. In the Cochrane meta-analysis, Ober et al. observed a negative impact from immunotherapy, whereas all the other studies found a positive impact or no significant difference between the treated and control groups.[33]

Pandey et al. performed a vast literature review of 53 studies (14 randomized and 39 non-randomized) on the efficacy of LIT in the treatment of couples with RM. The meta-analysis of all 53 studies revealed a significant difference in efficacy between the treated group (67% success rate, 2478/3701) and the control group (36%, 440/1198) ($p < 0.001$). The meta-analysis of randomized studies only revealed a significantly higher success rate in the treated group (68%) than in the placebo group (54%) ($p < 0.02$). Pandey et al. also discussed aspects of different protocols related to the effectiveness of LIT, such as the time of immunization (only before, only during or before, and during pregnancy), administration route, and concentration of lymphocytes in each immunization. They observed better results when immunizations were performed before and during pregnancy, administered intradermally or intravenously at a concentration greater than or equal to 100×10^6 lymphocytes per dose.[20] In 2016, Liu et al. published a new meta-analysis of 18 studies, including 1738 patients (739 patients in the LIT group and 999 patients in the control group). They observed a higher live birth rate in the LIT group (77.8%, 575/739) than in the control group (46.1%, 461/999) (OR: 3.74, 95% CI 3.07–4.57, $p < 0.00001$). The evaluation of the effectiveness of LIT among different populations revealed an efficacy of LIT in the population of Asian women (OR: 5.09, 95% CI 4.05–6.40, $p < 0.00001$), but no improvement in the European and American populations (OR: 1.45, 95% CI 0.97–2.17, $p < 0.00001$).[19] Our group carried out a meta-analysis including published studies until 2017 and confirmed the beneficial effect of LIT in the treatment of RM.[21]

The meta-analysis of studies included in the two most relevant meta-analyses (Cochrane and Liu et al.)[19,33] together with the data recently published by our group[34] revealed a positive impact of LIT on the number of live births in patients with a history of RM (OR: 3.22, 95% CI 2.74–3.78, $p < 0.00001$) (Table 1) (Figure 1).

2.4 | Predictors of lymphocyte immunotherapy success

Historically, LIT has been indicated in patients with a history of RM. However, patients with RM are quite heterogeneous, which may interfere with the effectiveness of LIT. Additionally, other factors, such as the lymphocyte preparation method, its concentration, route of administration, and the frequency of immunization, have been reported to be associated with the efficacy of LIT.[19,20] There is strong evidence for the apparent effectiveness of LIT that is determined by the selection criteria for LIT candidates, and the preparation and administration protocol of LIT.[19-21,26] Most studies used clinical (epidemiological profile of the couple) and laboratory criteria for the selection of patients. Initial studies in reproductive immunology admitted a possible association between the compatibility of HLA antigens between the couple and a high risk of pregnancy loss. Thus, the early studies included couples with HLA histocompatibility and no maternal antibodies against paternal lymphocytes.[15,20,26]

Recently, Hajipour et al reviewed the potential patient selection criteria for LIT already described in the literature; they are as follows: higher levels of TNF-α (>400 pg/mL), negative for mixed lymphocyte reaction blocking antibodies (MLR-BAbs), lack of chromosome abnormities history, absence of pretreatment paternal antibodies, presence of anti-paternal cytotoxic antibodies (APCA) after immunization, absence of antinuclear autoantibodies, absence of antithyroglobulin (TgAb) autoantibodies, number of previous miscarriages, maternal and paternal age, CD4 + CD25 + Treg cells levels, Th17 level, and lack of anatomical and hormonal defects.[17]

The number of previous miscarriages and autoimmune disease/abnormalities is associated with the outcomes of LIT.[26,35] In RMITG, the first multicenter study of the effectiveness of LIT in the treatment of RM, a lower live birth rate was noticed in patients who had five or more pregnancy losses and positive autoantibodies (ANA and anticardiolipin antibodies).[26] Recently, we have evaluated the predictive factors for pregnancy success in 752 women with a history of RM who underwent LIT. A total of 421 women successfully carried the pregnancy to term (60%, 421/752). The multivariate analysis demonstrated that age, the number of previous miscarriages, the presence of autoantibodies, and thrombophilia were negatively associated with the success of LIT. Secondary RM alone was not a predictive factor of LIT success or failure; however, secondary RM among women with a history of five or more previous miscarriages is a predictor of LIT success (OR: 10.24; 95% CI: 1.9–55.8; $p = 0.007$).[36]

The frequency of LIT in relation to pregnancy is another predictor of therapeutic success.[19,20] Assessing the gestational outcome of 1096 patients with a history of RM in a quasi-randomized study, we observed that the beginning of the LIT, only after the diagnosis of pregnancy, was associated with a worse outcome (success rate of 34.1%). Gestational success was observed in 74.2% of cases when

TABLE 1 Summary of studies included in the recent meta-analyses (Cochrane and Liu et al.)[19,33] and data by Sarno et al.,[34] on the effect of LIT in the treatment of couples with RM

Author	Year	LIT group *n* (%)	Control group *n* (%)	Comments
Mowbray et al[15]	1985	17/22 (77.3)	10/27 (37)	Treatment: LIT from partner's blood, before pregnancy Control: LIT from the patient's blood Route: IV +ID + SC
Cauchi et al[75]	1991	13/21 (62)	19/25 (76)	Treatment: LIT from partner's blood, before pregnancy Control: Saline Route: IV +ID + SC
Clark et al[76]	1991	7/11 (64)	2/7 (29)	Treatment: LIT from partner's blood, before pregnancy Control: Saline Route: ID
Ho et al[77]	1991	33/42 (78.5)	32/49 (65.3)	Treatment: LIT from partner's blood, before pregnancy Control: LIT from the patient's blood Route: ID
Gatenby et al[78]	1993	13/19 (68.4)	10/22 (45.5)	Treatment: LIT from partner's blood, before pregnancy Control: LIT from the patient's blood Route: IV +ID + SC
Christiansen et al[79]	1994	29/43 (67.4)	10/23 (43.5)	Treatment: LIT from two third-party blood, before pregnancy Control: LIT from the patient's blood Route: IV
Illeni et al[80]	1994	10/16 (62.5)	11/14 (78.5)	Treatment: LIT from partner's blood, before pregnancy Control: LIT from the patient's blood Route: IV +ID + SC
Kilpatrick et al[81]	1994	8/12 (66.7)	6/10 (60)	Treatment: LIT from partner's blood, before and during pregnancy Control: LIT from the patient's blood Route: IV +ID + SC
Reznikoff et al[82]	1994	17/26 (65.4)	14/26 (53.8)	Treatment: LIT from partner's blood, before and during pregnancy Control: LIT from the patient's blood Route: IV +ID + SC
Scott et al[83]	1994	6/10 (60)	5/12 (41.7)	Treatment: LIT from partner's blood, before pregnancy Control: Saline Route: IV
Stray-Pederson et al[84]	1994	24/33 (72.7)	22/31 (70.9)	Treatment: LIT from partner's blood, before and during pregnancy Control: LIT from the patient's blood Route: IV +ID + SC
Carp et al[85]	1997	5/11 (45.5)	11/31 (35.5)	Treatment: LIT from partner's blood, before pregnancy Control: No treatment Route: IV +ID + SC
Ober et al[27]	1999	31/68 (45.6)	41/63 (65.1)	Treatment: LIT from partner's blood, before pregnancy Control: Saline Route: IV +ID + SC
Pandey et al[86]	2002	12/14 (85.7)	1/5 (20)	Treatment: LIT from partner's blood, before pregnancy Control: LIT from patients' blood or saline Route: ID
Hong et al[87]	2003	18/21 (85.7)	2/8 (25)	Treatment: LIT from partner's blood, before and during pregnancy Control: No treatment Route: ID
Pandey et al[20]	2004	21/25 (84)	6/20 (30)	Treatment: LIT from partner's blood, before pregnancy Control: LIT from the patient's blood Route: IV + ID + SC + IM
Yanping et al[88]	2011	41/49 (83.7)	24/45 (53.3)	Treatment: LIT from partner's blood, before and during pregnancy Control: No treatment Route: ID

(Continues)

TABLE 1 (Continued)

Author	Year	LIT group *n* (%)	Control group *n* (%)	Comments
Lin et al[89]	2012	33/42 (78.6)	17/42 (40.5)	Treatment: LIT from partner's blood, before and during pregnancy Control: No treatment Route: SC
Aiwu et al[90]	2013	250/297 (84.2)	254/591 (42.9)	Treatment: LIT from the third party or partner's blood, before and during pregnancy Control: Traditional Chinese medicine Route: SC
Bin et al[91]	2013	32/39 (82.1)	18/39 (46.2)	Treatment: LIT from partner's blood, before and during pregnancy Control: No treatment Route: ID
Sarno et al[34]	2019	452/752 (60.1)	114/344 (33.1)	Treatment: LIT from partner's blood, before and/or during pregnancy Control: No treatment Route: ID

RM, recurrent miscarriage; LIT, lymphocyte immunotherapy; Routes: IV, intravenous; ID, intradermal; SC, subcutaneous; IM, intramuscular.

the LIT was given only before pregnancy and 78.3% when the LIT was given before and during pregnancy. Liu et al. also highlighted greater effectiveness of LIT when administered before and during pregnancy (OR: 4.67, 95% CI 3.70–5.90, $p < 0.00001$) compared with immunization only before pregnancy (OR: 2.00, 95% CI 1.39–2.88, $p = 0.0002$).[34] Other meta-analyses that also evaluated gestational success in relation to the timing of treatment agreed that the best results were obtained with the pre-gestational initiation of LIT and its maintenance in the first months of pregnancy.[19,20]

Pandey et al. assessed the relationship between the concentration of lymphocytes and the effect of LIT. They observed a dose-dependent effect of LIT, with lymphocyte concentrations greater

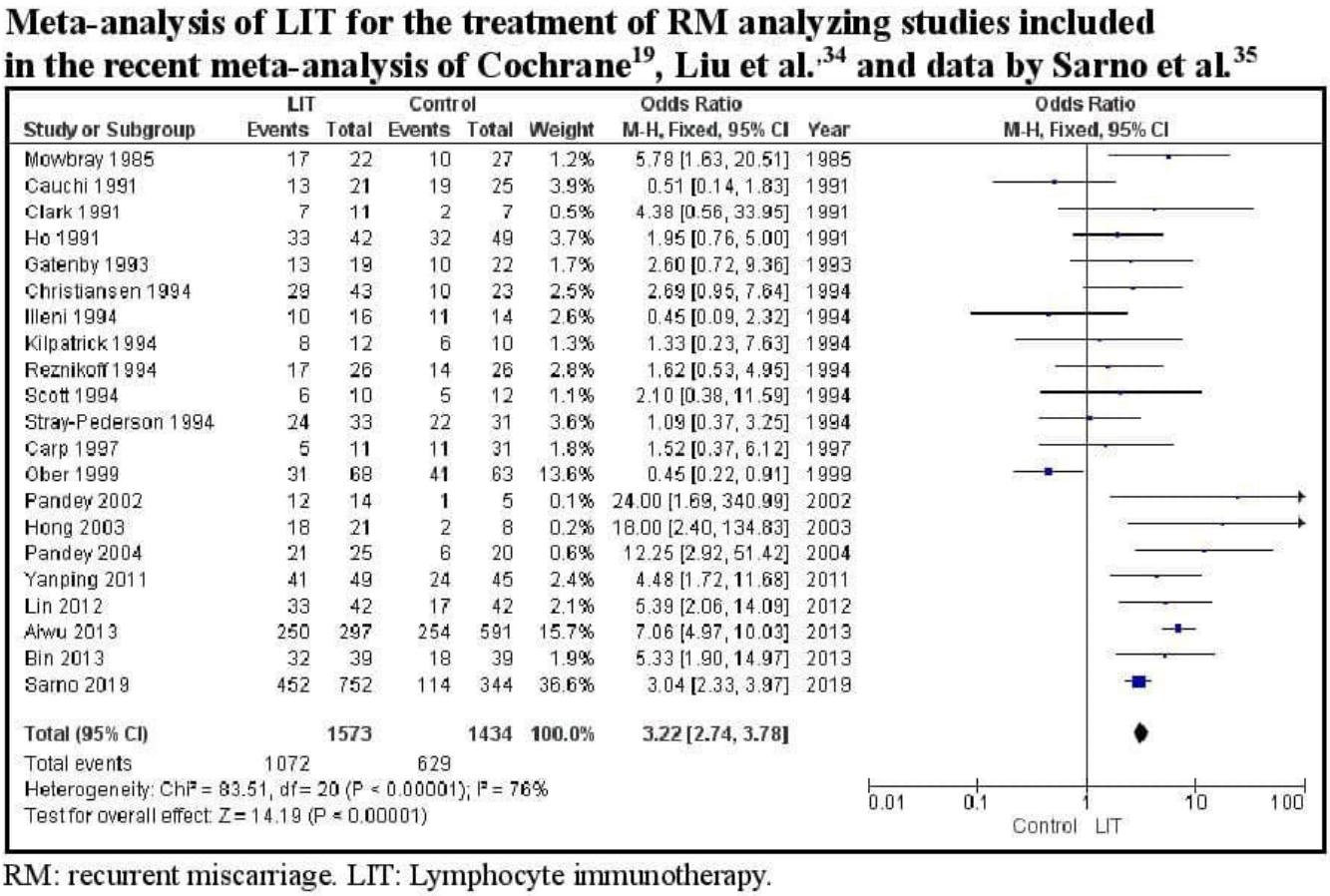

FIGURE 1 Meta-analysis of LIT for the treatment of RM analyzing studies included in the recent meta-analysis of Cochrane,[19] Liu et al.,[33] and data by Sarno et al[34]

than 100 × 10^6 lymphocytes/dose exerting the optimal effects.[20] However, Liu et al. observed that the effectiveness of LIT decreases when the immunization concentration exceeds 100 × 10^6 lymphocytes/dose (OR: 1.52, 95% CI 1.04–2.22, p = 0.03) compared with less than 100 × 10^6 lymphocytes/dose (OR: 5.25, 95% CI 4.16–6.64, p < 0.00001).[19] Pandey et al. also observed that applying the lymphocyte concentrate intradermally and intravenously is effective. Low levels of response were observed when immunization was performed subcutaneously, intracutaneously, and intramuscularly.[20]

2.5 | Lymphocyte Immunotherapy immune mechanism

Recurrent miscarriages is a complex reproductive condition that involves genetic, anatomical, genetic, hormonal, metabolic, and immunological factors (auto- and alloimmune). Studies have demonstrated the participation of several immune pathways in this process.[20] Based on the initial hypothesis that HLA histocompatibility increases the risk of pregnancy losses, maternal-fetal histocompatibility was proposed to be an underlying cause for the low production of maternal anti-paternal cytotoxic antibodies (APCA), anti-idiotypic antibodies (Ab2), and mixed lymphocyte reaction blocking antibodies (MLR-Bf). The lack of maternal immune recognition to paternal antigens would leave trophoblast antigens exposed to maternal NK and T-cell attacks. Some authors observed that LIT stimulated the production of APCA, Ab2, and MLR-Bf, thereby downregulating maternal interleukin-2 receptors and inhibiting T lymphocytes (Table 2).[20,37-58]

Gestational success depends on a balanced Th1/Th2 immune response. Numerous studies described that women with a history of RM have an imbalance in Th1/Th2 immune response with predominant inflammatory Th1 cytokines. The reestablishment of the balance by reducing Th1 response and increasing Th2 cytokines is another mechanism of action described for LIT.[20,44] Gharesi-Fard et al. showed the effectiveness of leukocyte therapy in primary but not in secondary RSA patients. They described in the co-culture study of PBMC from women with her partners' PBMC that after LIT, the levels of IFN-γ and TNF-α were significantly reduced in the culture supernatant as compared with those of baseline. These authors also suggested that an elevated level of TNF-α (>400 pg/ml) is a good laboratory criterion for selecting women with RM who would benefit from LIT.[59,60]

Natural killer cells are directly involved in embryonic implantation. Studies suggested that the hyperactivity of NK cells in peripheral blood and the endometrial layer is related to reproductive failure, including RM. Other authors disagree with this association between NK cells and pregnancy loss. Lymphocyte immunotherapy, intravenous immunoglobulin, and anti-TNF drugs have been utilized for the immunomodulation of NK cells to improve pregnancy outcomes.[61,62] Liang et al. described the effect of LIT in reducing the activity of NK cells and improving pregnancy outcomes.[44] Progesterone-induced blocking factor (PIBF) is a molecule with inhibitory effects on cell-mediated immune reactions. Studies demonstrated that low concentrations of PIBF are associated with the risk of RM. Check et al. found that the levels of PIBF increased in patients undergoing LIT.[58] The balance between Th17 cells and $CD4^+CD25^+$ regulatory T cells (Treg) is another mechanism involved in embryonic implantation. The action of Treg is mediated by anti-inflammatory cytokines, such as IL-10 and TGF-β. Women with a history of RM show a reduction in peripheral blood Treg cells. Studies have shown that LIT can reduce the levels of Th17 cells and raise the level of Treg cells.[55]

2.6 | Safety of lymphocyte immunotherapy for recurrent miscarriage

Lymphocyte immunotherapy uses a lymphocyte concentrate isolated by density gradient (Ficoll-Hypaque) and centrifugation, where the

TABLE 2 Mechanisms of action involved in lymphocyte immunotherapy

Mechanisms of Lymphocyte Immunotherapy	References
Production of anti-paternal cytotoxic antibodies (APCA)	33,34
Production of anti-idiotypic antibodies (Ab2)	35
Production of mixed lymphocyte reaction blocking antibodies (MLR-Bf)	36-39
Inhibition in the level of Th1 cytokines	40-42
Inhibition of natural killer cell activity	40,41,43-45
Production of anti-TCR idiotypic antibodies	43,46
T-cell suppression	41,46,48
Decrease in the level of maternal IL-2 receptor	47,49
Shift Th1 to Th2 type immunity	40-42
Reduction in the level of IL-6	50
Reduction in the concentration of peripheral Th17 cells	41,51
Increase in the concentration of regulatory T cells (CD4+CD25+)	41,51,52
Increase in progesterone-induced blocking factor (PIBF)	53,54

Th1: T-Helper 1. Th1: T-Helper 1

TABLE 3 Side effects and complications of lymphocyte immunotherapy

	Recurrent Miscarriage Immunotherapy Trialist Group[26] (Year: 1994)			Kling et al[63] (Year: 2006)
Local maternal complications	**LIT Group (*n* = 1149)**	**Control group (*n* = 410)**	***p***	**LIT group (*n* = 2587)**
Redness, *n* (%)	–	–	–	2371 (92)
Itching, *n* (%)	–	–	–	2362 (91)
Swelling, *n* (%)	–	–	–	1697 (66)
Burning sensation, *n* (%)	–	–	–	779 (30)
Blisters at injection sites, *n* (%)	–	–	–	360 (14)
Axillary lymphadenopathy, *n* (%)	–	–	–	200 (8)
Discomfort or pain in immunized arm, *n* (%)	–	–	–	41 (1.6)
Hematoma at injection site, *n* (%)	–	–	–	2 (<0.1)
Systemic maternal complications				
Fatigue, *n* (%)	–	–	–	48 (1.85)
"Influenza"/Flu-like symptoms, *n* (%)	9 (0.78)	00 (00)	–	46 (1.78)
Headache, *n* (%)	–	–	–	31 (1.20)
Elevated temperature or fever, *n* (%)	–	–	–	27 (1.04)
Dizziness, bad circulation, *n* (%)	–	–	–	25 (0.96)
Autoimmune	3 (0.26)	1 (0.24)	NS	8 (0.40)[a]
Alloimmune	4 (0.35)	1 (0.24)	NS	–
Nausea/vomiting/diarrhea, *n* (%)	–	–	–	15 (0.58)
Skin rashes, *n* (%)	–	–	–	14 (0.54)
Transfusion reaction, *n* (%)	6 (0.52)	0 (00)	–	–
Viral transmission, *n* (%)	2 (0.17)	1 (0.24)	NS	–
Labial herpes simplex, *n* (%)	–	–	–	5 (0.19)
Hot flushes with GnRH (endometriosis), *n* (%)	–	–	–	4 (0.15)
Lymphadenopathy neck, *n* (%)	–	–	–	4 (0.15)
Pain in one leg/groin/lower back, *n* (%)	–	–	–	3 (0.11)
Loss of scalp hair, *n* (%)	–	–	–	1 (0.04)
Sleeplessness, *n* (%)	–	–	–	1 (0.04)
Allergic conjunctivitis, *n* (%)	–	–	–	1 (0.04)
Allergic asthma, breathlessness, *n* (%)	–	–	–	1 (0.04)
Depression, *n* (%)	–	–	–	1 (0.04)
Urinary tract infection, *n* (%)	–	–	–	1 (0.04)
"Pretibial dots", *n* (%)	–	–	–	1 (0.04)
Newborn/Infant complications				
Congenital anomalies, *n* (%)	17 (1.48)	11 (2.68)	0.011	–
Preterm birth, *n* (%)	8 (0.69)	2 (0.48)	NS	–
IUGR, *n* (%)	6 (0.52)	5 (1.22)	NS	–
Neonatal death, *n* (%)	2 (0.17)	0	–	–
Failure to thrive, *n* (%)	1 (0.08)	0	–	–
Neonatal thrombocytopenia, *n* (%)	2 (0.17)	0	–	–

[a]A total of 1914 patients were evaluated after 2 years of immunotherapy: 8/1.914 (0.4%).

final product consists of lymphocytes, with a trace amount of other elements of the peripheral blood (erythrocytes, platelets, granulocytes, and monocytes). The peripheral blood donor for the preparation of LIT is usually the patient's partner or a third-party donor. Thus, the donor selection process and the potential side effects/complications observed in LIT are similar to those of blood transfusion and the use of blood products. In addition to the risks related to blood transfusion, other systemic and local adverse reactions have been described.

In the multicenter study—RMITG, the prevalence of systemic maternal complications in the treated group was 2.1% versus 0.5% in the control group. Flu-like symptoms, transfusion reaction (including fever), and viral transmission (hepatitis and cytomegalovirus) were the most frequent systemic maternal complications (Table 3); however, local complications were not found. The authors reported that in regard to side effects and complications of LIT, the benefit of LIT was five to six times greater than the risk.[26] In the RMITG study, the frequency of major neonatal complications was similar between the treated and control groups, namely 3% (36/1149) in the treated group and 4% (18/410) in the control group (p = 0.232). Interestingly when neonatal complication was analyzed separately, the prevalence of congenital anomalies was statistically more frequent in the control group (2.68%, 11/410) than in the treated group (1.48%, 17/1149) (p = 0.01). Prematurity and intrauterine uterine growth restriction were commonly observed neonatal complications in both groups (Table 2).[26] Kling et al. described the local and systemic side effects of intradermal allogeneic LIT in 2587 patients, followed up to 4 weeks after vaccination. Frequent reactions observed at the injection sites were redness (92%), itching (91%), and swelling (66%). Local reactions appeared within the first 3 weeks after the intradermal injection and lasted an average of 5–15 days (Table 3). Fatigue (1.9%), "influenza-" like symptoms (1.8%), headache (1.2%), elevated temperature (1.04%), and dizziness (0.96) were the most frequent systemic maternal reactions (Table 3).[63]

The long-term effects of LIT were also assessed by the RMITG study and Kling et al. The prevalence of autoimmune diseases was similar between the treated and control groups. The follow-up study revealed an increased incidence of 0.1% per year, similar to that observed among women with reproductive problems. Although the LIT presents a theoretical risk of graft-versus-host disease and post-transfusion purpura, these complications were not observed in the treated group.

Additionally, the incidence of malignant diseases was not different in the treated group as compared to the control group.[26,63,64] Despite the precaution of the transmittable diseases, in 2018, five Chinese patients were reported to be infected with HIV after LIT. The investigation found flaws in the treatment protocol, including the delay in the diagnosis of HIV in blood donor, a failure in the control of infectious disease screening before the preparation of the vaccine, and the reuse of blood tubes during vaccine processing.[65]

Recently, with the emergence of COVID-19, the safety of LIT has been brought into question again. The risk of transmission of SARS-CoV-2 by blood products is still poorly understood. Chang et al. did not observe any case of transmission of COVID-19 in a group of 187 women, residents of the city of Wuhan, China, who underwent LIT from their partner's blood in the period February 2020 to May 2020.[66] However, given the possibility of transmission of COVID-19 and the lack of knowledge about the impact of LIT on the immune response to SARS-CoV-2, a group of specialists in reproductive immunology suggested the suspension of this therapy during the pandemic period.[67]

3 | LYMPHOCYTE IMMUNOTHERAPY AND RIF

Recurrent implantation failure is a condition only applicable to patients undergoing assisted reproductive technology. Nowadays, the most commonly accepted definition is a failure to achieve a clinical pregnancy after the transfer of at least four good quality embryos, with three or more fresh or frozen embryo transfer cycles, in women under the age of 40.[68] The number of couples with RIF is unclear, and different causes are attributed to this reproductive issue.[68] Auto- and alloimmune factors have been listed as possible causes of failure of embryonic implantation in IVF cycles. Women with active autoimmune diseases and a breakdown in self-tolerance mechanisms, such as disorders in NK cells (peripherical and/or endometrial), regulatory T cells, and interleukins, have a high risk of failure in embryonic implantation.[68] Lédée et al. described changes in the endometrial immune profile in 81% of patients with RIF, including 56% presenting over immune response and 25% low immune response; thus, immunological therapies can be an alternative in improving pregnancy outcomes in women with a history of RIF.[69]

The use of LIT in women with a history of RIF was first described in 1992 by Hasegawa et al. The authors described three couples with RIF (three to four previous IVF-ET failures), where the women were negative for MLR-blocking antibody. Three patients received two intradermal injections of 100×10^6 paternal lymphocytes prior to the index IVF-ET cycle. One of the three couples successfully delivered a live-born infant at 37th week of gestation weighing 3306 gm, while two of 14 couples with similar characteristics (RIF and MLR-blocking antibody negative) who did not undergo immunotherapy (results not published by the authors) delivered live-born infants.[70] It was concluded that LIT could be beneficial in embryonic implantation. Carp et al. disagreed with Hasegawa's proposal, claiming that no similarities exist in the immunological mechanisms between cases of implantation failure and RM. However, the authors responded to the criticism by explaining that immune etiology was an underlying etiology of RIF, evidenced by hyporesponsive to LIT among these women.[71] During the 8th Congress of the ALPS ADRIA Society for Immunology of Reproduction, Kling et al. presented their 3-year experience (1996–1998) of treating couples with RIF with LIT. The authors showed a cumulative birth rate of 38% after at least two additional ETs, the result, 25% higher than that in couples who did not receive LIT.[72] Despite encouraging initial findings, the current literature lacks the efficacy of LIT in cases of RIF.

In 2006, Yoshioka et al. proposed the use of autologous peripheral blood mononuclear cells (PBMCs) by intrauterine infusion (72 h before embryo transfer) in cases of RIF to promote local immunomodulation. Considering that lymphocytes represent more than 95% of PBMCs, therapy with PBMCs is often considered LIT.[73] They observed that the treated-PMBC group had higher clinical pregnancy rate (CPR) (41.2% [7/17] vs. 11.1% [2/18]; p = 0.042), implantation rate (IR) (23.4% [11/47] vs. 4.1% [2/49]; p = 0.011), and live birth rate (LBR) (35.3% [6/17] vs. 5.5% [1/18]; p = 0.028) compared with

the control group. Subgroup analysis, according to age, revealed no difference in the results between the treated and control groups in women ≥40 years old. However, in patients <40 years, improvements in CPR (66.7% [6/9] vs. 15.4% [2/13]; p = 0.014), IR (37.5% [9/27] vs. 5.4% [2/37]; p = 0.003), and LBR (55.6% [5/9] vs. 7.6% [1/13]; p = 0.013) with PMBC treatment were more evident.[73]

Recently, Pourmoghadam et al. published a meta-analysis evaluating the effectiveness of immunotherapy with PBMCs in cases of RIF. Five studies were included two randomized controlled trial studies, and three quasi-experimental (non-randomized) studies, totaling 514 patients in the treated group and 659 patients in the control group. The authors observed an improvement in all outcome variables assessed in the group undergoing therapy with PBMCs. Improved implantation rates were observed in the subgroup analysis of randomized studies (OR: 2.47, 95% CI 1.31–4.67, p = 0.005) and non-randomized studies (OR: 1.59, 95% CI: 1.04–2.42, p = 0.03). A significant reduction in abortion rate was also reported in the randomized studies (OR: 0.42, 95% CI 0.23–0.77, p = 0.005).[74]

4 | CONCLUSION AND NEW PERSPECTIVES

The efficacy of LIT for the treatment of couples with RM has been studied for decades, with the vast majority of studies showing improved gestational outcomes. However, the results available in the literature are not convincing for the use of LIT in patients with RIF. The most of the current studies that support the evidence are quasi-experimental, with few randomized, double-blind studies (Level of evidence III). The theoretical basis and mechanistic action of LIT have been described in many reports, explaining its clinical impact on RM and RIF.

However, the controversies for the evidence of its effectiveness are present due to several factors: the methodological quality of studies, lack of consensus on laboratory controls, heterogeneity of the study population, and the different treatment protocols. Current evidence suggests that women with a history of primary RM (two or more pregnancy loss) of idiopathic cause (without autoantibodies) are candidates for LIT. The ideal protocol should perform immunizations with lymphocytes from the partner, with about 100 million lymphocytes per dose. The best outcomes are observed when the LIT is performed before and during pregnancy.

Based on recent guidelines for RM from international societies, half of the women with a history of RM remain unknown; hence, no treatment is recommended, which makes these couples very frustrated. Therefore, studies with robust methodological designs with specific treatment protocols and strict inclusion and exclusion criteria based on prognostic factors are needed. These studies should also attest to the safety of LIT in the short- and long-term complications, if any.

Currently, new immunological therapies for patients with reproductive failures propose an intrauterine immunomodulation. Several endometrial biomarkers are being described as promising patient selection criteria for the group of patients who can benefit from that therapies. Among the current immune therapies under investigation, the intrauterine infusion of autologous PBMCs shows encouraging results.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ORCID

Marcelo Borges Cavalcante https://orcid.org/0000-0001-9943-9731

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