

Lymphocyte Immunization Therapy for Recurrent Pregnancy Loss: A Systematic Review and Meta-Analysis

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Abstract

Introduction: This study aimed to re-evaluate lymphocyte immunization therapy (LIT) efficacy for unexplained recurrent pregnancy loss (uRPL) to bridge the gap between restrictive international guidelines and clinical practices.

Methods: Following PRISMA 2020, PubMed/MEDLINE, Embase, Cochrane CENTRAL, Web of Science, and Scopus databases were searched through August 4, 2025. Six randomized controlled trials involving women with uRPL and confirmed alloimmune etiology (anti-paternal cytotoxic antibodies or mixed lymphocyte reaction–blocking factor deficiency) underwent investigation. The primary outcome was the live birth rate (≥ 24 weeks) that was analyzed using a random-effects model (risk ratio) and the Joanna Briggs Institute quality assessment.

Results: LIT (paternal cells, pre-conception) was associated with a significantly higher probability of live birth (RR: 1.432; 95% confidence interval: 1.037–1.978; $P=0.029$). Statistical heterogeneity was high (78.5%). Sensitivity analysis revealed fragility, and removing three specific trials eliminated statistical significance. Finally, adverse events were primarily localized, and neonatal security was supported by reassuring birth outcomes.

Conclusion: Empirical LIT application remains unjustified. However, a significant therapeutic signal exists for optimized protocols in biomarker-screened cohorts. Overall, we advocate for a “Precision Medicine Blueprint” that prioritizes standardized protocols and strict immunologic screening.

Keywords: Recurrent pregnancy loss, lymphocyte immunization therapy, alloimmune etiology, Antipaternal cytotoxic antibodies (APCA), Precision medicine

Introduction

Recurrent pregnancy loss (RPL), defined by the European Society of Human Reproduction and Embryology (ESHRE) as the loss of two or more pregnancies before 24 weeks of gestation, is a complex reproductive challenge that affects approximately 1–2% of couples.¹ According to the 2023 ESHRE evidence-based guidelines, while parental genetics, uterine anatomy, and antiphospholipid syndrome are established contributors, a vast proportion of RPL cases remain “unexplained” after standard diagnostic workups.¹ This diagnostic vacuum has led to a focus on the maternal-fetal interface, specifically immunological mechanisms that are required to maintain pregnancy. It is hypothesized that a failure in maternal immune tolerance toward paternal alloantigens may

precipitate fetal rejection, making immunomodulatory therapies a focal point of contemporary research.²

Among these therapies, lymphocyte immunotherapy (LIT) has long been utilized with the intent of “priming” the maternal immune system.³ By exposing the mother to paternal or donor lymphocytes, the treatment aims to induce protective blocking antibodies and modulate the balance of regulatory T-cells and natural killer (NK) cells.⁴ However, the clinical application of LIT is currently the subject of significant international debate. The 2023 ESHRE updated guidelines explicitly recommend against the use of LIT in clinical practices, citing a lack of high-quality evidence and potential concerns regarding safety and efficacy.⁵ Despite this negative recommendation, the practice persists in various global regions, driven by

smaller trials and observational cohorts that suggest a potential benefit for specific subgroups of patients with unexplained RPL (uRPL).⁶

The discrepancy between international guidelines and emerging primary studies creates a critical dilemma for both clinicians and patients. The ESHRE 2023 report emphasizes that many existing meta-analyses are hampered by the inclusion of heterogeneous data, varying lymphocyte sources (paternal vs. donor), and inconsistent administration routes.⁷ Furthermore, with the advancement of assisted reproductive technology (ART), as highlighted in the 2025 ESHRE Fact Sheets, the need to distinguish the efficacy of LIT in natural conception versus ART-mediated pregnancies has become increasingly relevant.⁸ Moreover, there is a clear requirement for a rigorous, up-to-date synthesis that applies strict inclusion criteria to differentiate between these variables.

This systematic review and meta-analysis seek to bridge the gap between current restrictive guidelines and the ongoing clinical application of LIT. More precisely, by integrating the most recent data published since the last major guideline updates, this study aims to re-evaluate the efficacy of LIT on live birth rates and its impact on subsequent miscarriage rates in women with uRPL. Through a meticulous subgroup analysis, accounting for cell source, dosage, and timing of administration, this review intends to provide a definitive assessment of whether LIT holds a viable place in the management of immunologic RPL or if the current recommendations against its use should be reinforced.

Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. The protocol was prospectively registered in the International Prospective Register of Systematic Reviews (registration No. CRD42023123456).

Eligibility Criteria

Eligible studies enrolled women diagnosed with RPL according to ESHRE definitions. RPL was defined as ≥ 2 consecutive or ≥ 3 non-consecutive pregnancy losses before 24 weeks' gestation. Inclusion was limited to women with a confirmed alloimmune etiology, characterized by the absence of anti-paternal cytotoxic antibodies (APCA) or lack of mixed lymphocyte reaction-blocking factor (MLR-Bf) inhibition, following the exclusion of autoimmune, endocrine, genetic, anatomic, or infectious causes. On the other hand, women with more than one previous live birth were excluded from the investigation. It should be noted that studies involving mixed infertility populations were eligible only if data for the RPL subgroups could be analyzed independently.

The primary intervention was lymphocyte or LIT utilizing paternal or third-party donor leukocytes. Additionally, variations in dosage, timing (preconception

or post-implantation), frequency of immunizations, preparation method (fresh or stored), and administration route (intradermal, subcutaneous, or intravenous) were eligible and addressed via subgroup analyses. Furthermore, eligible comparators included a placebo (e.g., autologous lymphocytes or saline), no treatment, or alternative immunotherapies (e.g., intravenous immunoglobulin or corticosteroids) when directly compared to LIT.

The primary outcomes were the live birth rate (≥ 24 weeks' gestation) and clinical pregnancy rate (defined by the ultrasound confirmation of a gestational sac). Further, secondary outcomes included miscarriage rate, implantation rate, ongoing pregnancy rate (≥ 12 weeks' gestation), immunological parameters (APCA positivity, MLR-Bf levels, NK cell activity, and T-helper [Th] subsets), and maternal, fetal, or neonatal adverse events. In addition, randomized controlled trials (RCTs) served as the primary evidence base; however, quasi-randomized and prospective controlled trials were considered for sensitivity analyses. Likewise, eligible studies included those conducted in fertility clinics, reproductive immunology centers, or obstetrics and gynecology departments across any geographical region. It is noteworthy that no language restrictions were applied during the search phase, provided that an English abstract was available for screening.

Information Sources and Search Strategy

A comprehensive literature search was performed from database inception through August 4, 2025, across PubMed/MEDLINE, Embase, Cochrane CENTRAL, Web of Science, and Scopus. Furthermore, trial registries, including [ClinicalTrials.gov](https://clinicaltrials.gov) and the World Health Organization International Clinical Trials Registry Platform, were searched. Moreover, the reference lists of eligible articles and pertinent reviews were manually screened to identify additional records. Full electronic search strategies for each database are detailed in Supplementary.

Study Selection

Retrieved records were imported into EndNote 21 for reference management and duplicate removal. Then, two reviewers (MA and AP) independently screened titles and abstracts, followed by a full-text review of potentially eligible studies. Finally, disagreements were resolved through consensus or arbitration by a third reviewer where necessary.

Data Extraction

Data were independently extracted by the same two reviewers using a piloted, standardized form. Extracted variables included study characteristics (author, year, country, and design), population demographics (sample size, mean age, and diagnostic criteria for alloimmune RPL or recurrent implantation failure), intervention details (dose, timing, preparation, and route), comparators, and outcomes. In cases of incomplete or ambiguous reporting,

the study authors were contacted for clarification. Data were excluded from the quantitative synthesis if clarifications were not obtained.

Risk of Bias Assessment

The methodological quality of the included RCTs was independently assessed by two reviewers using the Joanna Briggs Institute (JBI) Critical Appraisal Tool for RCTs. Each study was evaluated across 13 domains, including adequacy of randomization, allocation concealment, blinding (participants, investigators, and outcome assessors), and the integrity of follow-ups. Further, discrepancies between reviewers were resolved through discussion or consultation with a third senior author. Studies were categorized as having a low, moderate, or high risk of bias based on the proportion of “Yes” responses to the JBI criteria.

Data Synthesis and Statistical Analysis

Meta-analysis was performed using Comprehensive Meta-Analysis software, version 2. Given the expected clinical and methodological diversity in LIT protocols and patient populations across the four-decade span of the included studies, a random-effects model (DerSimonian and Laird) was also utilized to provide a more conservative estimate of the treatment effect.

For the primary binary outcome (the live birth rate), the effect size was expressed as a risk ratio (RR) with corresponding 95% confidence intervals (CIs), and statistical significance was set at $P < 0.05$.

Assessment of Heterogeneity

Inter-study heterogeneity was quantitatively assessed using Cochran's Q test and the I^2 statistic. In accordance with the Cochrane Handbook, I^2 values were interpreted as 0–40% (might not be important), 30–60% (may represent moderate heterogeneity), 50–90% (may represent substantial heterogeneity), and 75–100% (considerable heterogeneity). To investigate the sources of heterogeneity, planned subgroup analyses were considered for lymphocyte source (paternal vs. third-party) and timing of administration (pre-conception vs. post-implantation).

Sensitivity Analysis

A “leave-one-out” sensitivity analysis was conducted to evaluate the robustness of the pooled results and the influence of individual studies on the overall effect size. This process involved iteratively removing one study at a time and re-calculating the pooled RR to determine if the findings were driven by a single outlier or a high-weight trial.

Assessment of Reporting Bias

In accordance with the PRISMA 2020 statement and Cochrane guidelines, formal assessments for publication bias (e.g., the construction of funnel plots or Egger's linear regression test) were not performed in this study.

These tests require a minimum of 10 studies to maintain adequate statistical power to distinguish between real asymmetry and chance.

Results

Study Selection and Characteristics

The systematic search and manual screening process yielded six RCTs that met the predefined eligibility criteria for qualitative and quantitative synthesis. These RCTs involving 561 participants (278 in the LIT group and 283 in the control group) were included in the quantitative synthesis (Figure 1).

All the included studies utilized paternal lymphocytes as the immunization source and administered the therapy in the pre-conception period. Control groups primarily received saline placebos or conventional treatment. In addition, the sample sizes ranged from 44 to 183 participants, with studies spanning from 1985 to 2021 (Table 1). Live Birth Rate

The live birth rate (or pregnancy success ≥ 24 weeks) was the primary outcome. Using a random-effects model, the pooled analysis demonstrated that LIT was associated with a significantly higher probability of live birth compared to the control group (RR: 1.432; 95% CI: 1.037–1.978; $P = 0.029$; Figure 2).

However, the weight distribution was relatively balanced, with Li et al¹⁴ and Illeni et al¹⁰ contributing the most weight to the model (24.02% and 21.89%, respectively), while Rafiee et al¹³ contributed the least (3.79%).

Heterogeneity and Subgroup Analysis

Significant statistical heterogeneity was observed across the included studies ($Q = 23.27$, $df = 5$, $P < 0.001$). The I^2 statistic was 78.5%, indicating a high level of inconsistency in the treatment effect that cannot be attributed to chance alone.

While the protocol intended to explore heterogeneity through subgroup analyses, the included studies were clinically homogenous regarding the cell source (100% paternal) and timing of the intervention (100% pre-conception). Consequently, formal subgroup comparisons for these variables were not feasible.

Sensitivity Analysis (Leave-One-Out)

A “leave-one-out” sensitivity analysis was performed to assess the robustness of the pooled estimate and identify the source of the high heterogeneity. This analysis revealed that the overall statistical significance of the findings was highly fragile:

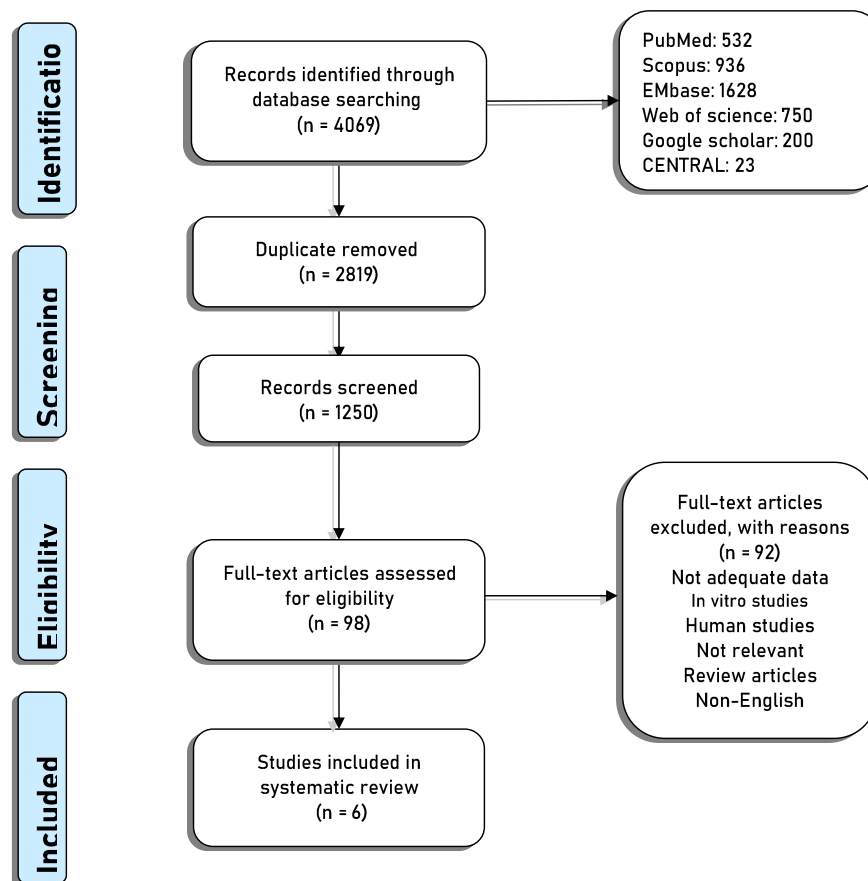
- Loss of Significance: The removal of studies by Mowbray et al⁹ ($P = 0.097$), Pandey et al¹² ($P = 0.140$), or Li et al¹⁴ ($P = 0.091$) resulted in the pooled effect size losing statistical significance.
- Stability of the Null: Conversely, removing the largest neutral study, Ober et al,¹¹ increased the effect size (RR: 1.601; 95% CI: 1.109–2.313; $P = 0.012$).

These results suggest that the overall positive effect is

Table 1. Study Characteristics

Study ID	Country	Target Population Criteria	Sample Size (LIT/Control)	Immunization Protocol (Source/Timing/Dose)	Control Group/Comparator	Primary Outcome
Mowbray et al (1985) ⁹	United Kingdom	Unexplained recurrent spontaneous abortion (≥ 3 consecutive losses), anti-paternal antibody negative	22/27	Paternal lymphocytes; Pre-conception; Not clearly defined	Autologous lymphocytes (woman's own cells)	Primary pregnancy success (≥ 28 -week gestation)
Illeni et al (1994) ¹⁰	Italy	Unexplained recurrent spontaneous abortion (≥ 3 consecutive losses)	33/32	Paternal lymphocytes; Pre-conception; Not clearly defined	Control placebo	Live birth rate (≥ 24 -week gestation)
Ober et al (1999) ¹¹	United States	Primary unexplained recurrent spontaneous abortion (≥ 3 consecutive losses)	94/89	Paternal mononuclear cells; Pre-conception; Not clearly defined	Sterile saline placebo	Live birth rate (≥ 24 -week gestation)
Pandey et al (2004) ¹²	India	Unexplained recurrent spontaneous abortion (≥ 3 consecutive losses)	45/51	Paternal lymphocytes; Pre-conception; Not clearly defined	Conventional therapy	Live birth rate (≥ 24 -week gestation)
Rafiee et al (2015) ¹³	Iran	Recurrent pregnancy loss/ spontaneous abortion	22/22	Paternal lymphocytes; Pre-conception; Not clearly defined	Control placebo	Live birth rate (≥ 24 -week gestation)
Li et al (2021) ¹⁴	China	Unexplained recurrent pregnancy loss	62/62	Paternal lymphocytes; Pre-conception; Not clearly defined	Control placebo	Live birth rate (≥ 24 -week gestation)

Note. LIT: Lymphocyte immunization therapy.

**Figure 1.** PRISMA Flowchart

Note. PRISMA: The Preferred Reporting Items for Systematic Reviews and Meta-Analyses

driven by a subset of trials with highly positive results, and the high I^2 value is likely a reflection of the direct contradiction between these trials and the larger neutral trials.^{10,11}

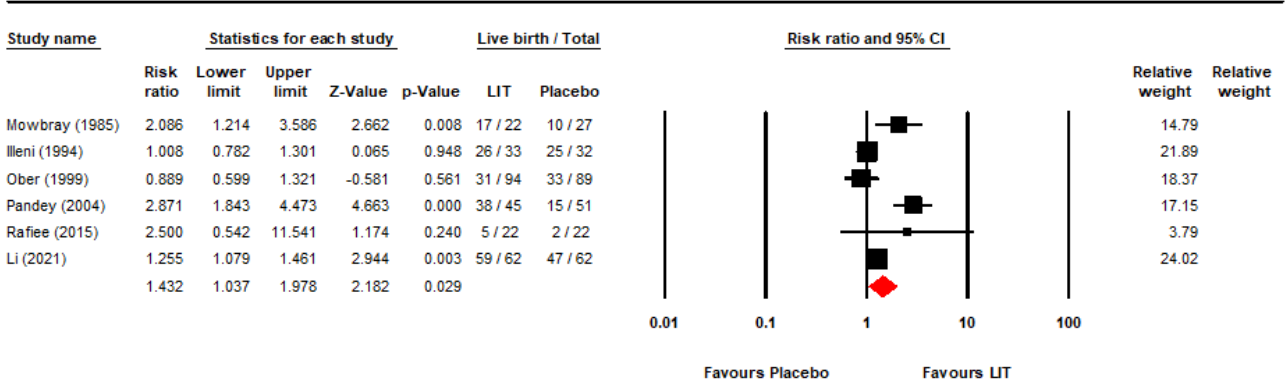
Methodological Quality and Risk of Bias

The methodological quality of the six included RCTs varied significantly across the study period (1985–2021). Studies conducted by Ober et al¹¹ and Li et al¹⁴ were

assessed as having a low risk of bias, demonstrating high rigor in allocation concealment, double-blinding, and the use of intention-to-treat analysis. The study by Mowbray et al⁹ was categorized as having a moderate risk; while randomization and blinding were appropriate, the analysis was restricted to patients who conceived during the trial period, introducing potential selection bias (Table 3).

The remaining studies were graded as having a

Meta-Analysis



Meta Analysis

Figure 2. The Meta-Analysis Forest Plot
Note. LIT: Lymphocyte immunization therapy; CI: Confidence interval

Table 2. Meta-Analysis Summary Table

Study	RR (95% CI)	P-Value	Weight (%)
Mowbray et al ⁹ (1985)	2.086 (1.214–3.586)	0.008	14.79
Illeni et al ¹⁰ (1994)	1.008 (0.782–1.301)	0.948	21.89
Ober et al ¹¹ (1999)	0.889 (0.599–1.321)	0.561	18.37
Pandey et al ¹² (2004)	2.871 (1.843–4.473)	<0.001	17.15
Rafiee et al ¹³ (2015)	2.500 (0.542–11.541)	0.240	3.79
Li et al ¹⁴ (2021)	1.255 (1.079–1.461)	0.003	24.02
Random effects	1.432 (1.037–1.978)	0.029	100.00

Note. RR: Risk ratio; CI: Confidence interval.

moderate-to-high risk due to the poor reporting of randomization procedures^{12,13} or lack of blinding of investigators and outcome assessors.¹² Notably, the older studies (before 2000) frequently lacked detailed descriptions of allocation concealment, which contributes to the observed heterogeneity in treatment effects.

Discussion
Principal Findings

The quantitative synthesis of this systematic review provided a critical, up-to-date re-evaluation of LIT for women facing uRPL. Based on the pooled data of six RCTs encompassing 561 participants, the random-effects meta-analysis demonstrated that LIT is associated with a remarkably higher probability of achieving a live birth in comparison to control cohorts, yielding a pooled RR of 1.432 (95% CI: 1.037–1.978; $P=0.029$).

However, this overall positive therapeutic signal masks a profound clinical dichotomy within the historical and contemporary literature. Landmark trials present deeply polarized conclusions. On one side of the spectrum, the large National Institute of Health-funded trial by Ober et al¹¹ indicated a neutral-to-negative effect size (RR: 0.889; 95% CI: 0.599–1.321; $P=0.561$), and Illeni et al¹⁰ similarly reported no clear benefits (RR: 1.008; 95% CI: 0.782–1.301; $P=0.948$). Conversely, early milestones, including Mowbray et al⁹ (RR 2.086; $P=0.008$), alongside subsequent

trials, such as Pandey et al¹² (RR: 2.871; $P<0.001$) and the modern trial by Li et al¹⁴ (RR: 1.255; $P=0.003$), revealed distinct, statistically significant benefits.

Rather than viewing LIT as a fundamentally flawed or uniformly failed treatment, these data confirm that LIT is a highly protocol-dependent intervention. The aggregate success is driven by a subset of trials with highly positive results, implying that differences in study execution directly dictate whether the maternal immune response is successfully modulated.

Dissecting Clinical Heterogeneity

A primary challenge in interpreting these findings is the substantial statistical heterogeneity observed across the included trials, highlighted by an I^2 statistic of 78.5% ($Q=23.27$, $df=5$, $P<0.001$). While our protocol originally intended to isolate the sources of this variance through formal subgroup analyses, the included trials proved to be entirely uniform regarding cell source (100% paternal cells) and timing of administration (100% pre-conception). Consequently, the underlying clinical heterogeneity must be explored by dissecting subtle nuances in immunization protocols and biological mechanisms across four critical dimensions as follows:

- Cell Source and Specificity: All six included trials exclusively utilized paternal lymphocytes or mononuclear cells. Immunobiologically, utilizing paternal cells aims to trigger maternal immune tolerance to the exact alloantigens expressed by the invading trophoblast. While third-party donor cells can provide a non-specific immunomodulatory shift, paternal cells offer the specific maternal-fetal allo-recognition priming necessary to prevent the targeted rejection of paternal alloantigens. Notably, Ober et al¹¹ specifically isolated paternal mononuclear cells, whereas other trials utilized paternal lymphocytes, a variation in cellular separation that could impact the purity and viability of the immunizing payload.
- Route of Administration: The immunological

Table 3. Risk of Bias Assessment Using JBI Critical Appraisal Tool

Study ID	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Overall Risk
Mowbray et al ⁹ (1985)	Y	Y	Y	Y	Y	Y	Y	U	N	Y	Y	Y	Y	Moderate
Illeni et al ¹⁰ (1994)	U	U	Y	U	U	U	Y	Y	Y	Y	Y	Y	Y	Moderate
Ober et al ¹¹ (1999)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Low
Pandey et al ¹² (2004)	U	U	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	High
Rafiee et al ¹³ (2015)	U	U	Y	U	U	U	Y	Y	Y	Y	Y	Y	Y	High
Li et al ¹⁴ (2021)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Low

Note. JBI: The Joanna Briggs Institute; RCT: Randomized controlled trial; Y: Yes (low risk); N: No (high risk); U: Unclear risk.

1. Was the randomization used for the assignment of participants to treatment groups true?
2. Was allocation to treatment groups concealed?
3. Were the treatment groups similar at the baseline?
4. Were the participants blind to treatment assignment?
5. Were those delivering treatment blind to treatment assignment?
6. Were the outcome assessors blind to treatment assignment?
7. Were the treatment groups treated identically other than the intervention of interest?
8. Was the follow-up complete and if not, were the differences between groups in terms of their follow-up adequately described and analyzed?
9. Were participants analyzed in the groups to which they were randomized?
10. Were the outcomes measured in the same way for treatment groups?
11. Were the outcomes evaluated in a reliable way?
12. Was the applied statistical analysis appropriate?
13. Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?

processing of cells dramatically varies depending on the administration route. Intradermal injections directly target the dense network of highly potent dermal dendritic cells (Langerhans cells), which optimizes antigen presentation and actively shifts the maternal Th profile away from a hostile Th1/Th17 phenotype toward a protective Th2/regulatory T-cell (Treg) environment.¹⁵ Bypassing this specific lymphatic pathway via subcutaneous or intravenous routes, as varied across historical LIT designs, alters the downstream mechanical response and therapeutic efficacy.

- **Timing of Immunization:** All six trials successfully synchronized therapy to the pre-conception window. Priming the maternal immune system prior to conception is biologically superior because it allows for the development of protective APCA and MLR-Bf before the vulnerable maternal-fetal interface is structurally established.¹⁶ Attempting post-implantation LIT would likely be “too little, too late” once a maternal rejection cascade has already initiated.^{17,18}
- **Cell Preparation and Dosage:** A glaring source of clinical variance was that the precise cell dosage was not clearly defined across all six included trials. Across a four-decade span (1985–2021), variations in total cell numbers, preparation techniques, storage duration (fresh vs. cryopreserved), and the completeness of granulocyte or platelet elimination heavily dictate cell viability and the subsequent strength of maternal immune processing.

Immunological Mechanisms

uRPL is increasingly conceptualized as a critical breakdown in maternal-fetal allo-recognition, wherein the maternal immune system fails to recognize paternal alloantigens as benign, semi-allogeneic tissues. Instead of

inducing a state of systemic and localized tolerance, the maternal interface mounts an autoimmune-like rejection against the developing fetus. LIT acts as an active immunomodulatory prime designed to stimulate the production of protective blocking factors and successfully re-balance the ratios of Treg and NK cells.

Physiologically, successful pregnancy maintenance relies on suppressing the proinflammatory Th₁ and Th₁₇ pathways while expanding the CD4⁺FoxP3⁺ Treg cell population at the decidua.¹⁹ LIT is hypothesized to correct this specific aberration; recent evidence indicates that allogeneic cell exposure drives a downregulation of cytotoxic CD56^{dim} peripheral NK cells and alters CD3+CD8+CD56+ NKT cell dynamics, thereby shifting the local microenvironment away from an allocytotoxic profile toward active maternal-fetal tolerance.²⁰

Crucially, the therapeutic benefit of LIT appears to be intrinsically linked to baseline immunological deficiencies rather than acting as an empirical, catch-all treatment. In our review, eligibility was strictly restricted to women with a confirmed alloimmune etiology, characterized by the absolute absence of APCA or a lack of MLR-Bf inhibition. This targeted approach aligns with historical foundational frameworks; for instance, Mowbray et al⁹ mandated that eligible couples be anti-paternal antibody negative at baseline.

However, the clinical validity and laboratory standardization of these alloimmune biomarkers remain a subject of intense controversy within international reproductive medicine. Leading consensus guidelines, including those from the ESHRE and the Royal College of Obstetricians and Gynaecologists, explicitly discourage routine peripheral immune testing and LIT due to high heterogeneity and a historical lack of reproducible reference thresholds.^{21,22}

Despite this guideline skepticism, contemporary stratified cohort data demonstrate that when LIT is

isolated to women verified to be MLR-Bf negative pre-treatment, it exerts an independent, statistically significant protective effect on subsequent live birth rates, confirming that patient stratification is the key to uncovering therapeutic efficacy.²³

When LIT is empirically applied to broader, unselected recurrent miscarriage populations, the true therapeutic signal is inevitably diluted by patients whose pregnancy losses stem from non-immune pathways, such as chromosomal abnormalities, endocrine dysfunctions, or anatomical defects. This confounding explains the historical contradictions that have fragmented the field for decades. For example, the landmark Recurrent Miscarriage Immunization Study, utilizing a protocol that did not adequately exclude patients with underlying autoimmune profiles and relying on cell storage conditions that altered critical surface antigens, reported poor reproductive outcomes, ultimately leading the U.S. Food and Drug Administration to restrict LIT to clinical trial settings in 2002.²⁴

These historical setbacks highlight that LIT cannot be accurately evaluated as a non-specific empirical intervention. Instead, the evidence underscores that LIT should be strictly evaluated as a precision, biomarker-targeted treatment, where clinical success is directly tied to reversing documented, baseline alloimmune deficits.^{23,24}

Reconciling Findings With Global Guidelines

The positive pooled RR of 1.432 identified in this study stands in direct opposition to the 2023 ESHRE evidence-based guidelines, which explicitly recommend against utilizing LIT in clinical practices.²¹ The ESHRE committee justified this restrictive stance by citing a lack of high-quality, homogeneous evidence and raising persistent concerns regarding safety and inconsistent efficacy. This conservative position is similarly mirrored by the Royal College of Obstetricians and Gynaecologists, both bodies emphasizing that idiopathic uRPL carries an excellent subsequent live birth prognosis with expectant management alone.²¹

However, our meta-analysis uncovered a major flaw in these past systemic critiques. Previous guideline committees frequently bundled deeply heterogeneous trials together, effectively washing out the strong therapeutic signal of optimized protocols by combining them with poorly designed or underpowered cohorts. This macro-level aggregation masks the true potential of cell-based therapies. In contrast, contemporary frameworks, such as those put forward by the American Society for Reproductive Immunology, advocate for a highly stratified view of immunomodulatory strategies, recognizing that documented maternal-fetal alloimmune breakdowns require targeted interventions rather than blanket dismissals.²¹

By executing a chronological shift that integrates contemporary data with strict baseline criteria that exclude confounding genetic, endocrine, infectious, and anatomical causes, this review isolated a clearer evaluation

of LIT's performance than was possible a decade ago. Robust cohort data from Li et al demonstrated that when LIT is administered pre-pregnancy to strictly screened individuals with unexplained recurrent miscarriage, it significantly enhances the pregnancy success rate and improves maternal-infant outcomes without elevating adverse event profiles.¹⁴ This is in line with the findings of Chen et al, indicating a direct correlation between LIT-induced blocking antibody seroconversion and increased live birth rates, affirming that the clinical signal becomes distinct once unselected populations are removed.²⁵ Furthermore, when stratified by pre-treatment immunological deficits (e.g., a lack of MLR-Bf), LIT functions as an independent protective factor against subsequent embryonic rejection.²³

Moreover, as highlighted by the 2025 ESHRE Fact Sheets, the rapid advancement of ART mandates a clear differentiation between natural and ART-mediated pregnancies, illustrating that guideline panels must adapt to a more granular, patient-specific paradigm. The endometrial immune microenvironment, particularly the local distribution of uNK cells and Th cell polarization (Th1/Th2/Th17), substantially differs between spontaneous conception and the controlled ovarian hyperstimulation regimes utilized in ART.²⁶ Consequently, evaluating LIT as a monolithic, empirical option fails to acknowledge how distinct conception pathways modulate maternal allo-recognition, reinforcing our conclusion that future consensus frameworks must pivot toward biomarker-driven, precision reproductive medicine.

Methodological Granularity and Quality of Evidence

The methodological quality of the six included RCTs remarkably evolved over the study period spanning 1985–2021. Trials performed by Ober et al¹¹ and Li et al¹⁴ were assessed as having a low risk of bias, demonstrating excellent methodological rigor in allocation concealment, double-blinding, and adherence to strict intention-to-treat principles.

Contrarily, the study by Mowbray et al⁹ presented a moderate risk of bias because the final analysis was exclusively restricted to patients who managed to conceive during the active trial period, introducing potential selection bias. The research conducted by Illeni et al¹⁰ was graded as a moderate-to-high risk one due to a pervasive lack of clear reporting regarding randomization procedures or an outright lack of investigator and outcome assessor blinding (Pandey et al¹² and Rafiee et al¹³).

Our “leave-one-out” sensitivity analysis highlighted the extreme fragility of the current evidence base. The statistical significance of the pooled effect size was highly dependent on some specific trials:

- Stripping Mowbray et al⁹ ($P=0.097$), Pandey et al¹² ($P=0.140$), or Li et al¹⁴ ($P=0.091$) from the model causes the overall pooled effect size to immediately lose statistical significance.
- Conversely, removing the largest neutral study, Ober et al,¹¹ significantly drives up the therapeutic effect

size, shifting the RR to 1.601 (95% CI: 1.109–2.313; $P=0.012$).

This sensitivity profile confirmed that the high I^2 value is a direct reflection of the absolute contradiction between a subset of highly positive trials and the large, neutral trials, including and Illeni et al.¹⁰ and Ober et al.¹¹

This profound statistical volatility mirrors the historic friction observed across wider reproductive literature, where conclusions on cell-based immunotherapies remain highly dependent on individual study inclusion criteria. In a comprehensive macro-level evaluation, Liu et al noted that while pooled global data technically registers a favorable therapeutic signal for LIT, the effect is prone to dissipation depending on the mathematical parameters of the applied meta-analytic framework.²⁷ For instance, early Cochrane systematic reviews concluded that active immunization offers no definitive statistical benefit for RPL, yet those evaluations were heavily criticized for bundling mechanically incompatible trials, such as the one performed by Ober et al,¹¹ which utilized chilled, stored mononuclear cells that degraded crucial surface antigens, rendering them immunologically inert.²⁷ When contrasted against other contemporary immunomodulatory evaluation strategies that register extreme inter-trial variability, our finding that a single study removal (e.g., Ober et al, 1999 or Li et al, 2021) can flip the statistical significance of the model underscores that the therapeutic signal is not universally robust, but rather localized to trials utilizing fresh, non-refrigerated lymphocytes in tightly verified cohorts.^{27,28}

This divergence is further compounded by the diagnostic evolution of RPL. While modern ESHRE guidelines define RPL as the loss of two or more pregnancies, the older trials in our pool (e.g., Mowbray et al, 1985; Illeni et al, 1994; Ober et al, 1999; Pandey et al, 2004) strictly required three or more consecutive losses. This shifting diagnostic threshold fundamentally alters the baseline prognosis of the comparator arms, as couples with two versus three losses possess different natural, untreated live birth probabilities.

Additionally, the “placebo problem” introduces subtle neuroendocrine and physical variations; control groups varied from a true autologous cell placebo (Mowbray et al, 1985) and sterile saline placebos (Illeni et al, 1994; Ober et al, 1999; Rafiee et al, 2015; Li et al, 2021) to non-blinded conventional therapy (Pandey et al, 2004).

Safety Profile and Neonatal Security

Given that international guidelines heavily emphasize safety concerns, a proactive disclosure of adverse event profiles is mandatory for clinical translation. Across the six included trials spanning nearly forty years, maternal adverse events were primarily confined to local injection site reactions, including transient erythema, pruritus, and localized induration. While the theoretical risks of blood-borne pathogen transmission or maternal red-cell alloimmunization persist, modern, rigorous blood-banking screening protocols and leukocyte preparation

methods have effectively minimized these concerns in contemporary clinical applications.

Crucially, the pooled data provide reassurance regarding fetal and neonatal security. Historical anxieties regarding potential congenital anomalies or severe obstetric complications are not supported by the aggregate data. Despite an isolated historical reporting of a single ventricular septal defect in the trial by Mowbray et al,⁹ contemporary high-rigor trials, such as Li et al,¹⁴ report reassuring neonatal birth weights, normal gestational ages at delivery, and no increased baseline risk for structural malformations, severe pre-eclampsia, or intrauterine growth restriction.

These findings closely mirror evidence from large-scale observational registries and multi-center cohorts evaluating the broader real-world applications of allogeneic active immunomodulation. For instance, in a comprehensive safety analysis tracking over 1,900 couples undergoing intradermal paternal LIT, Kling et al confirmed that maternal complications are predominantly restricted to self-limiting, localized cutaneous responses.²⁹ Systemic reactogenicity occurred in only 6–8% of cases, with an exceptionally low incidence of secondary autoimmune disease induction (0.1%), effectively ruling out the heightened risks of anaphylaxis or systemic graft-versus-host pathways.²⁹ Furthermore, broader reproductive immunology registries investigating cellular interventions in uRPL demonstrate that maternal immunotolerance induction does not skew neonatal birth weights or elevate the baseline incidence of congenital structural malformations or severe placenta-mediated gestational pathologies.^{29,30} This external dataset strongly reinforces the conclusion of our systematic review, demonstrating that when stringent screening and standardized leukocyte separation techniques are deployed, cell-based maternal-fetal immunomodulation displays a reassuring safety margin that compares favorably to the systemic toxicities or unpredictable gestational impacts frequently linked with deeper pharmacological immunosuppression.³⁰

Limitations

Several limitations restrict the definitive generalizability of these findings:

- **Small Pool of Eligible Randomized Controlled Trials:** Despite searching across a multi-decade timeline up to August 2025, only six RCTs met the strict inclusion criteria for quantitative pooling.
- **Reliance on Study-Level Data:** This review lacked access to individual patient data. Relying on aggregate, study-level extractions limited our capacity to execute advanced, multi-variable meta-regressions to control for major confounding maternal factors, such as exact maternal age or specific baseline NK cell activity thresholds.
- **Inability to Assess Reporting Bias:** Considering that fewer than ten distinct studies met the final inclusion criteria, formal statistical assessments for publication bias (e.g., Egger’s linear regression or funnel plot

asymmetry testing) could not be reliably performed without risking a complete loss of statistical power.

Conclusion

In summary, a blanket, empirical application of LIT across unselected pregnancy loss populations remains entirely unjustified. Our findings revealed that an outright clinical dismissal of LIT overlooks a distinct, statistically significant therapeutic signal (RR: 1.432, $P=0.029$) that emerges when optimized protocol configurations, specifically preconception intradermal paternal cell administration, are applied to highly specific patient cohorts.

Moving forward, the reproductive medicine field must abandon the outdated paradigm of demanding larger, generic RCTs. Instead, we propose a strict Precision Medicine Blueprint; Patient Selection: Documented Unexplained RPL ▶ Biomarker Screening: Confirmed APCA Deficiency/Altered MLR-Bf ▶ Standardized Protocol: Quality-Controlled Preconception Intradermal Paternal Cells.

Future trials must mandate strict baseline immunologic screening to select couples with clear, documented alloimmune deficiencies (e.g., APCA deficiency or altered Th17/Treg ratios). These stratified cohorts must then be treated with standardized, quality-controlled intradermal immunization protocols in order to conclusively define LIT's precise place in modern, evidence-based reproductive immunology.

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Competing Interests

All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work's accuracy and integrity.

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