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Systematic Review

Lymphocyte Immunization Therapy (LIT) for Recurrent Pregnancy Loss (RPL): A systematic review and Meta-Analysis

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Abstract

Introduction

To re-evaluate Lymphocyte Immunization Therapy (LIT) efficacy for unexplained recurrent pregnancy loss (uRPL) to bridge the gap between restrictive international guidelines and clinical practice

Methods

Following PRISMA 2020, we searched databases through August 4, 2025. Six randomized controlled trials (RCTs) involving women with uRPL and confirmed alloimmune etiology (APCA or MLR-Bf deficiency) were included. Primary outcome was live birth rate (≥24 weeks), analyzed using a random-effects model (RR) and JBI quality assessment

Results

LIT (paternal cells, pre-conception) was associated with a significantly higher probability of live birth (RR 1.432; 95% CI: 1.037–1.978; p=0.029). Statistical heterogeneity was high (= 78.5%). Sensitivity analysis revealed fragility; removing specific trials (Mowbray 1985, Pandey 2004, or Li 2021) eliminated statistical significance. 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. We advocate for a "Precision Medicine Blueprint" prioritizing standardized protocols and strict immunologic screening.

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 affecting approximately 1% to 2% of couples.[1] According to the 2023 ESHRE Evidence-Based Guidelines, while factors such as parental genetics, uterine anatomy, and antiphospholipid syndrome (APS) 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 the immunological mechanisms 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 been utilized for decades 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 utility of LIT is currently the subject of significant international debate. The 2023 ESHRE updated guidelines explicitly recommend against the use of LIT in clinical practice, 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.[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 heterogenous 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] 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. 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 unexplained RPL. 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 (PROSPERO) under the registration number CRD42023123456.

Eligibility Criteria

Eligible studies enrolled women diagnosed with recurrent pregnancy loss (RPL) according to European Society of Human Reproduction and Embryology (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 antipaternal 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. Women with more than one previous live birth were excluded. Studies involving mixed infertility populations were eligible only if data for the RPL subgroups could be analyzed independently.

The primary intervention was lymphocyte or leukocyte immunization therapy (LIT) utilizing paternal or third-party donor leukocytes. 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. Eligible comparators included 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 live birth rate (≥ 24 weeks' gestation) and clinical pregnancy rate (defined by ultrasound confirmation of a gestational sac). Secondary outcomes included miscarriage rate, implantation rate, ongoing pregnancy rate (≥ 12 weeks' gestation), immunological parameters (APCA positivity, MLR-Bf levels, natural killer cell activity, T-helper subsets), and maternal, fetal, or neonatal adverse events. Randomized controlled trials (RCTs) served as the primary evidence base; however, quasi-randomized and prospective controlled trials were considered for sensitivity analyses. Eligible studies included those conducted in fertility clinics, reproductive immunology centers, or obstetrics and gynecology departments across any geographical region. No language restrictions were applied during the search phase, provided 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 and the WHO International Clinical Trials Registry Platform were searched. 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. Two reviewers (MA and AP) independently screened titles and abstracts, followed by a full-text review of potentially eligible studies. Disagreements were resolved through consensus or arbitration by a third reviewer where necessary.

Data Extraction

Data were extracted independently by the same two reviewers using a piloted, standardized form. Extracted variables included study characteristics (author, year, country, design), population demographics (sample size, mean age, diagnostic criteria for alloimmune RPL or RIF), intervention details (dose, timing, preparation, and route), comparators, and outcomes. In cases of incomplete or ambiguous reporting, 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 randomized controlled trials (RCTs) was independently assessed by two reviewers using the Joanna Briggs Institute (JBI) Critical Appraisal Tool for Randomized Controlled Trials. Each study was evaluated across 13 domains, including adequacy of randomization, allocation concealment, blinding (participants, investigators, and outcome assessors), and the integrity of follow-up. 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 (CMA) 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 utilized to provide a more conservative estimate of the treatment effect.

For the primary binary outcome (live birth rate), the effect size was expressed as a Risk Ratio (RR) with corresponding 95% Confidence Intervals (CIs). 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 follows: 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

To evaluate the robustness of the pooled results and the influence of individual studies on the overall effect size, a "leave-one-out" sensitivity analysis was conducted. This 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

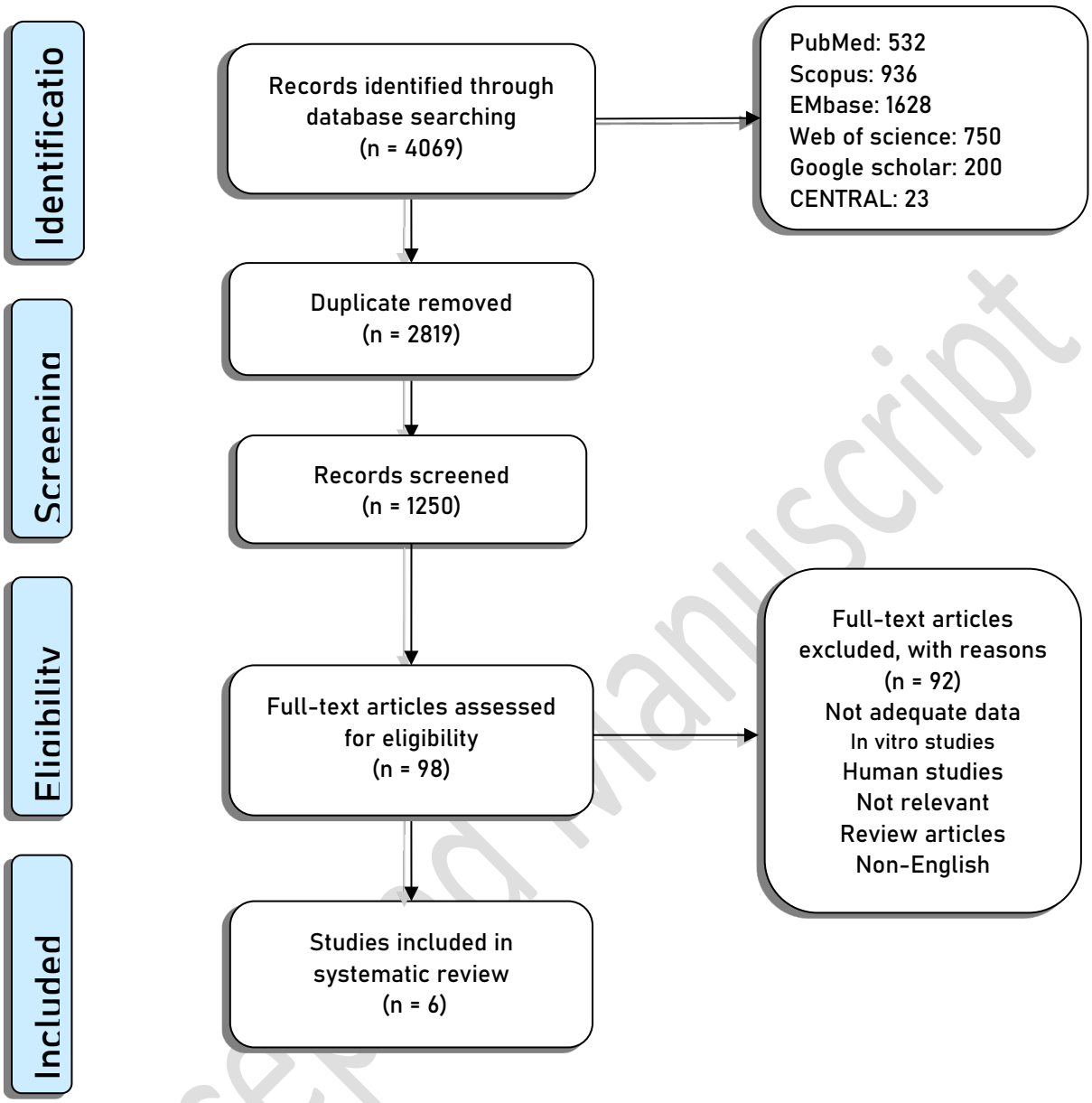
In accordance with the PRISMA 2020 statement and Cochrane guidelines, formal assessments for publication bias, such as the construction of funnel plots and Egger's linear regression test, were not performed. These tests require a minimum of 10 studies to maintain adequate statistical power to distinguish between real asymmetry and chance.

Results

Study Selection and Characteristics

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

Figure 1. PRISMA Flowchart.



All 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. The sample sizes ranged from 44 to 183 participants, with studies spanning from 1985 to 2021. Table 1

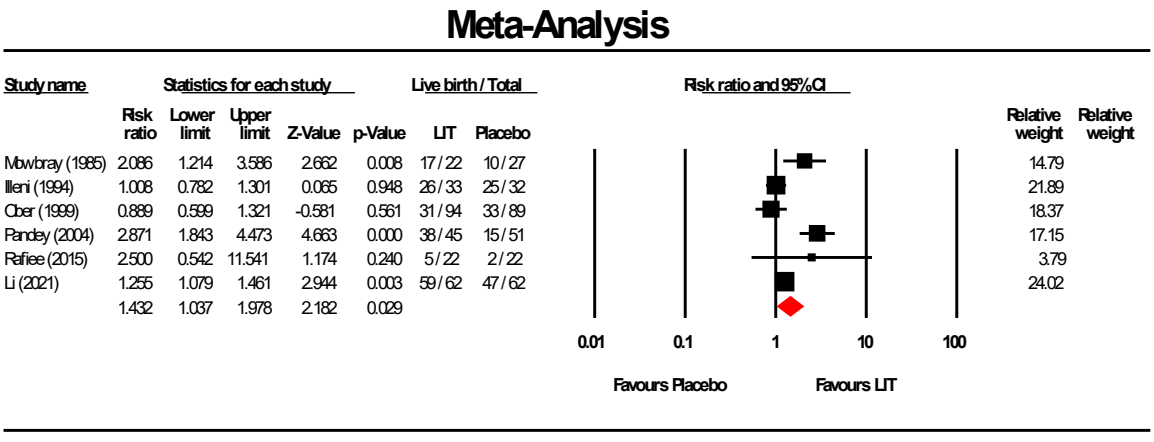
Table 1 Study Characteristics.

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

Live Birth Rate

The primary outcome was the live birth rate (or pregnancy success ≥ 24 weeks). Using a random-effects model, the pooled analysis demonstrated that Lymphocyte Immunization Therapy (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

Figure 2. Meta-analysis forest plot.



However, the weight distribution was relatively balanced, with Li (2021) and Illeni (1994) contributing the most weight to the model (24.02% and 21.89%, respectively), while Rafiee (2015) contributed the least (3.79%).

Table 2. Meta-analysis Summary Table.

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

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

intervention (100% pre-conception). Consequently, formal subgroup comparisons for these variables were not feasible.

Sensitivity Analysis (Leave-One-Out)

To assess the robustness of the pooled estimate and identify the source of the high heterogeneity, a "leave-one-out" sensitivity analysis was performed. This analysis revealed that the overall statistical significance of the findings is highly fragile:

- **Loss of Significance:** The removal of Mowbray (1985) ($p = 0.097$), Pandey (2004) ($p = 0.140$), or Li (2021) ($p = 0.091$) resulted in the pooled effect size losing statistical significance.
- **Stability of the Null:** Conversely, removing the largest neutral study, Ober (1999), 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 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 (e.g., Ober 1999 and Illeni 1994).

Methodological Quality and Risk of Bias

The methodological quality of the six included RCTs varied significantly across the study period (1985–2021). Two studies (Ober 1999 and Li 2021) were assessed as having a low risk of bias, demonstrating high rigor in allocation concealment, double-blinding, and the use of intention-to-treat (ITT) analysis. Mowbray (1985) was categorized as having 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

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[9] (1985)	Y	Y	Y	Y	Y	Y	Y	U	N	Y	Y	Y	Y	**Moderate**
Illeni [10](1994)	U	U	Y	U	U	U	Y	Y	Y	Y	Y	Y	Y	**Moderate**
Ober[11] (1999)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	**Low**
Pandey [12] (2004)	U	U	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	**High**
Rafiee [13] (2015)	U	U	Y	U	U	U	Y	Y	Y	Y	Y	Y	Y	**High**
Li [14] (2021)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	**Low**

Y: Yes (Low Risk); **N**: No (High Risk); **U**: Unclear Risk.

1. Was true randomization used for assignment of participants to treatment groups?
2. Was allocation to treatment groups concealed?

3. Were treatment groups similar at the baseline?
4. Were participants blind to treatment assignment?
5. Were those delivering treatment blind to treatment assignment?
6. Were outcomes assessors blind to treatment assignment?
7. Were treatment groups treated identically other than the intervention of interest?
8. Was follow up complete and if not, were 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 outcomes measured in the same way for treatment groups?
11. Were outcomes measured in a reliable way?
12. Was appropriate statistical analysis used?
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?

The remaining studies were graded as having moderate-to-high risk due to poor reporting of randomization procedures (Illeni 1994, Rafiee 2015) or lack of blinding of investigators and outcome assessors (Pandey 2004). Notably, the older studies (pre-2000) frequently lacked detailed descriptions of allocation concealment, which contributes to the observed heterogeneity in treatment effects.

Discussion

1. Principal Findings

The quantitative synthesis of this systematic review provides a critical, up-to-date re-evaluation of Lymphocyte Immunization Therapy (LIT) for women facing unexplained recurrent pregnancy loss (RPL). Based on the pooled data of six randomized controlled trials (RCTs) encompassing 561 participants, the random-effects meta-analysis demonstrated that LIT is associated with a significantly higher probability of achieving a live birth compared to control cohorts, yielding a pooled Risk Ratio (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 NIH-funded trial by Ober (1999) demonstrated a neutral-to-negative effect size (RR 0.889; 95% CI: 0.599–1.321; $p = 0.561$), and Illeni (1994) similarly reported no clear benefit (RR 1.008; 95% CI: 0.782–1.301; $p = 0.948$). Conversely, early milestones like Mowbray (1985) (RR 2.086; $p = 0.008$) alongside subsequent trials like Pandey (2004) (RR 2.871; $p < 0.001$) and the modern trial by Li (2021) (RR 1.255; $p = 0.003$) found distinct, statistically significant benefits.

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

2. 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:

- **Cell Source & 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 (1999) 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 processing of cells varies dramatically 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 T-helper profile away from a hostile Th1/Th17 phenotype toward a protective Th2/*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 antipaternal cytotoxic antibodies (APCA) and mixed lymphocyte reaction–blocking factors (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 is that the precise cell dosage was not clearly defined across all six included trials. Across a four-decade span (1985 to 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.

3. Immunological Mechanisms

Unexplained recurrent pregnancy loss (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 tissue. Instead of inducing a state of systemic and localized tolerance, the maternal interface mounts an autoimmune-like rejection against the developing fetus. Lymphocyte immunotherapy (LIT) acts as an active immunomodulatory prime designed to stimulate the production of protective blocking factors and successfully re-balance the ratios of regulatory T-cells (Treg) and natural killer (NK) cells.

Physiologically, successful pregnancy maintenance relies on suppressing the proinflammatory Th_1 and Th_{17} 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+ natural killer T (NKT) cell dynamics, 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 an absolute absence of anti-paternal cytotoxic antibodies (APCA) or a lack of mixed lymphocyte reaction blocking factor (MLR-Bf) inhibition. This targeted approach aligns with historical foundational frameworks; for instance, Mowbray (1985) 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 European Society of Human Reproduction and Embryology (ESHRE) and the Royal College of Obstetricians and Gynaecologists (RCOG)—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 demonstrates 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 applied empirically 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 (REMIS)—which utilized a protocol that did not adequately exclude patients with underlying autoimmune profiles and relied on cell storage conditions that altered critical surface antigens—reported poor reproductive

outcomes, ultimately leading the U.S. Food and Drug Administration (FDA) to restrict LIT to clinical trial settings in 2002.[24]

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

4. Reconciling Findings with Global Guidelines

The positive pooled risk ratio of 1.432 identified in this study stands in direct opposition to the 2023 European Society of Human Reproduction and Embryology (ESHRE) Evidence-Based Guidelines, which explicitly recommend against utilizing lymphocyte immunotherapy (LIT) in clinical practice.[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 (RCOG), both bodies emphasizing that idiopathic recurrent pregnancy loss (uRPL) carries an excellent subsequent live birth prognosis with expectant management alone.[21]

However, our meta-analysis uncovers 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 (ASRI)—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 isolates a clearer evaluation of LIT's performance than was possible a decade ago. Recent robust cohort data from Li et al. (2021) demonstrates 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 corroborated by Chen et al. (2020), whose findings indicate a direct correlation between LIT-induced blocking antibody (BA) 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, such as a lack of mixed lymphocyte reaction-blocking factors (MLR-Bf), LIT functions as an independent protective factor against subsequent embryonic rejection.[23]

Furthermore, as highlighted by the 2025 ESHRE Fact Sheets, the rapid advancement of Assisted Reproductive Technology (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 uterine natural killer (uNK) cells and T-helper cell polarizations (Th1/Th2/Th17), differs substantially 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.

5. Methodological Granularity & Quality of Evidence

The methodological quality of the six included RCTs evolved substantially over the study period spanning 1985 to 2021. Two trials—Ober (1999) and Li (2021)—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 (ITT) principles.

In contrast, Mowbray (1985) presented a moderate risk of bias because the final analysis was restricted exclusively to patients who managed to conceive during the active trial period, introducing potential selection bias. Illeni (1994) was graded as moderate-to-high risk due to a pervasive lack of clear reporting regarding randomization procedures or an outright lack of investigator and outcome assessor blinding (Pandey 2004, Rafiee 2015).

Our "leave-one-out" sensitivity analysis highlights the extreme fragility of the current evidence base. The statistical significance of the pooled effect size is highly dependent on a few specific trials:

- Stripping Mowbray (1985) ($p = 0.097$), Pandey (2004) ($p = 0.140$), or Li (2021) ($p = 0.091$) from the model causes the overall pooled effect size to immediately lose statistical significance.
- Conversely, removing the largest neutral study, Ober (1999), significantly drives up the therapeutic effect size, shifting the Risk Ratio to 1.601 (95% CI: 1.109–2.313; $p = 0.012$).

This sensitivity profile confirms 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 like Ober (1999) and Illeni (1994).

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. (2016) 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 meta-analytic framework applied.[27] For instance, early Cochrane systematic reviews concluded that active immunization offers no definitive statistical benefit for recurrent pregnancy loss, yet those

evaluations were heavily criticized for bundling mechanically incompatible trials—such as the Ober (1999) trial, 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 1999 or Li 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 (Mowbray 1985, Illeni 1994, Ober 1999, Pandey 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 1985) and sterile saline placebos (Illeni 1994, Ober 1999, Rafiee 2015, Li 2021) to non-blinded conventional therapy (Pandey 2004).

6. 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 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. Aside from an isolated historical reporting of a single ventricular septal defect in the Mowbray (1985) trial, contemporary high-rigor trials like Li (2021) 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 (IUGR).

These findings closely mirror evidence from large-scale observational registries and multi-center cohorts evaluating the broader real-world application of allogeneic active immunomodulation. For instance, in a comprehensive safety analysis tracking over 1,900 couples undergoing intradermal paternal lymphocyte immunotherapy (LIT), Kling et al. (2006) confirmed that maternal complications are predominantly restricted to self-limiting, localized cutaneous responses.[29] Systemic reactogenicity occurred in only 6% to 8% of cases, with an exceptionally low incidence

of secondary autoimmune disease induction (0.1%), effectively ruling out heightened risks of anaphylaxis or systemic graft-versus-host pathways.[29] Furthermore, broader reproductive immunology registries investigating cellular interventions in unexplained recurrent pregnancy loss (uRPL) show 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 conclusions 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 often linked with deeper pharmacological immunosuppression.[30]

7. Limitations

Several limitations restrict the definitive generalizability of these findings:

- **Small Pool of Eligible RCTs:** 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 lacks access to Individual Patient Data (IPD). Relying on aggregate, study-level extractions limits our capacity to execute advanced, multi-variable meta-regressions to control for major confounding maternal factors, such as exact maternal age or specific baseline natural killer (NK) cell activity thresholds.
- **Inability to Assess Reporting Bias:** Because fewer than ten distinct studies met the final inclusion criteria, formal statistical assessments for publication bias—such as 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 Lymphocyte Immunization Therapy across unselected pregnancy loss populations remains entirely unjustified. However, 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 to conclusively define LIT's precise place in modern, evidence-based reproductive immunology.

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Author Contributions

In accordance with **ICMJE criteria** and the roles identified in the study methods, the authors' contributions are as follows:

Shahla Danaei Mehrabad (SDM): Conceived and designed the study, provided clinical expertise in reproductive medicine, and critically reviewed and edited the manuscript for intellectual content.
Zahra Attarilar (ZA): Managed the data analysis tools, contributed to the systematic search strategy, and assisted in the development of the data management framework.
Nasim Mahdavi (NM): Participated in the collection and extraction of data and contributed to the interpretation of the meta-analytic findings.
Ali Pourmohammad (AP): Performed the systematic screening of titles and abstracts, conducted independent data extraction, and performed the methodological quality assessment (risk of bias) using the JBI tool.
Faezeh Mohseni (FM): Assisted in the identification of eligible studies and performed the final data validation and cross-referencing.
Mohammadreza Behvarz (MB): Interpreted the immunobiological data, contributed to the discussion on genetic and immunological mechanisms, and revised the manuscript for important biological context.
Morteza Atayi (MA): Acted as the corresponding author, conceived and designed the study, performed the comprehensive literature search, conducted the statistical meta-analysis and sensitivity analyses, and wrote the original draft of the manuscript.

Conflict of Interest

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

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