



Differential Effects of Low-Dose Prednisolone Therapy and Lymphocyte Immunotherapy on Peripheral MDSC Subsets in Women with Recurrent Miscarriage

Fatemeh Rezayat¹, Nafiseh Esmaili^{1*}, Roya Sherkat^{2*}, Abbas Rezaei^{1,3}

¹Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran;

²Immunodeficiency Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Background: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of myeloid cells that play a prominent role in maintaining immune tolerance. Despite advances in reproductive immunology, the optimal management of recurrent miscarriage (RM) remains uncertain, highlighting the need to better understand the immunological mechanisms underlying this condition and the effects of available therapeutic approaches. .

Objective: To evaluate the effects of low-dose prednisolone therapy and lymphocyte immunotherapy (LIT) on peripheral MDSC subsets in women with RM during the pre-pregnancy period.

Methods: 100 women with RM were assigned to two treatment groups: a short-term low-dose prednisolone therapy group (n=50), and an LIT group (n=50). Peripheral blood samples were collected before and after treatment and analyzed using flow cytometry. MDSCs were identified as HLA-DR-CD33+CD66+ cells. Within the gated MDSC population, CD15+CD66+, and CD14+CD66+ cells were identified as granulocytic MDSCs (GR-MDSCs) and monocytic MDSCs (MO-MDSCs), respectively.

Results: LIT significantly reduced the percentage of total MDSCs ($P = 0.0362$), as well as the percentages of GR-MDSCs and MO-MDSCs (both $P < 0.0001$). Prednisolone therapy did not significantly alter the percentage of total MDSCs ($P = 0.1073$), but significantly reduced both GR-MDSCs ($P = 0.0121$) and MO-MDSCs ($P < 0.0001$). Following prednisolone therapy, the percentage of GR-MDSCs was significantly higher than that of MO-MDSCs ($P = 0.046$), whereas following LIT, the percentage of GR-MDSCs was significantly lower than that of MO-MDSCs ($P = 0.0002$).

Conclusion: Both Prednisolone therapy and LIT reduced GR-MDSCs and MO-MDSCs, although the relative effects on the two subsets differed between treatment groups. The biological significance of these changes and their relationship to subsequent pregnancy outcomes require further investigation.

Keywords: Myeloid-derived suppressor cell; recurrent miscarriage; lymphocyte immunotherapy; prednisolone

*Corresponding author:

Nafiseh Esmaili,
Department of Immunology,
School of Medicine, Isfahan
University of Medical Sciences,
Isfahan, Iran.

Email: nafesm5@gmail.com

Roya Sherkat,
Immunodeficiency Diseases
Research Center, Isfahan
University of Medical Sciences,
Isfahan, Iran.

Email: Sherkat@med.mui.ac.ir

Cite this article as:

Rezayat F, Esmaili N, Sherkat R,
Rezaei A. Differential Effects of
Low-Dose Prednisolone Therapy
and Lymphocyte Immunotherapy
on Peripheral MDSC Subsets
in Women with Recurrent
Miscarriage. *Iran J Immunol.*
2026; 23(3): ,
doi: 10.22034/iji.2026.111387.3205.

Received: 2026-04-07

Revised: 2026-06-01

Accepted: 2026-08-22

INTRODUCTION

In recent decades, myeloid-derived suppressor cells (MDSC) have emerged as critical regulators of immune responses in various pathological contexts, including cancer, inflammatory disorders, transplantation, and autoimmune diseases (1-6). MDSCs are a heterogeneous population of myeloid cells that exert potent immunomodulatory functions, primarily through the suppression of T-cell responses and the induction of immune tolerance. Human MDSCs express the common myeloid marker CD33 but do not express human leukocyte antigen DR (HLA-DR). (7-9). MDSCs consist of two sub-populations, including granulocytic MDSCs (GR-MDSC) that express CD11b, CD15, and CD66b markers, and CD14⁺ monocytic MDSCs (MO-MDSC) (10-12). Notably, healthy pregnancy is associated with an expansion of GR-MDSCs, and elevated proportions of these cells have been linked to favorable outcomes following in vitro fertilization (IVF) (13). In addition, reduced levels of immunosuppressive MDSC have been observed in both the peripheral blood and endometrium of patients with miscarriage (14-18). Accordingly, the abundance of MDSC, particularly GR-MDSC, appears to be critical for the establishment and maintenance of a healthy pregnancy.

Early miscarriage is defined as clinical pregnancy loss before 20 weeks of gestation, whereas recurrent miscarriage (RM) is characterized by three or more consecutive early spontaneous pregnancy losses (19). RM affects approximately 1–3% of couples attempting to conceive (20). Although numerous etiological factors have been implicated in the pathogenesis of RM, no identifiable cause is found in approximately 50% of cases (21). Women with a history of multiple miscarriages are at increased risk of subsequent pregnancy loss and poor IVF outcomes (22). Accordingly, successful pregnancy may be achieved through the integration of various therapeutic strategies

aimed at enhancing the maintenance of feto-maternal immune tolerance.(23-25).

Lymphocyte immunotherapy (LIT) involves the transition of a lymphocyte suspension isolated from paternal peripheral blood to enhance maternal immunomodulation (26). LIT is typically administered prior to conception over a course of approximately 6-10 weeks (11, 26-28). Immunosuppression of the maternal immune system through this approach may reduce the risk of miscarriages in RM patients. Corticosteroid and glucocorticoid therapies, including cyclosporine A, prednisone, or prednisolone, are well-established immunosuppressive agents (29). Prednisolone, along with other short-term, low-dose glucocorticoids, is utilized to promote feto-maternal immune tolerance (30). Despite extensive research, current treatment strategies have not been established as definitive therapies for RM, owing to the complexity of immune responses and the heterogeneity of the patient population, underscoring the need for further investigation. All therapeutic approaches for RM influence maternal immune cells, and MDSCs are among the immune cell populations that may be modulated during pregnancy. Alterations in the overall MDSC population, as well as in its granulocytic and monocytic subsets, may influence pregnancy outcomes. In the present study, we investigated the effects of two typical treatment approaches for RM—LIT and prednisolone therapy—on the proportion of granulocytic and monocytic MDSC subsets prior to conception.

MATERIALS AND METHODS

Study population

This single-blind, randomized clinical trial was conducted in Iran from April 2019 to May 2024. The study comprised a prospective cohort of 100 women referred to the immunology clinic at Alzahra Hospital in Isfahan. Participants were randomly assigned

to two treatment groups after providing informed consent. Although patients were aware of their treatment allocation, the laboratory technician responsible for sample collection and numbering was blinded to patient group assignments, thereby ensuring single-blind conditions. Samples were analyzed and examined anonymously.

The protocol was approved by the Iranian Registry of Clinical Trials Committee (IRCT registration number: IRCT20210115050041N1), and all participants provided written informed consent. Furthermore, ethical approval was obtained from the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1399.877). Women with RM were randomly allocated to two study groups using the random permutation block method with a block size of 6. One group received short-term low-dose prednisolone therapy (n=50),

while the other group underwent lymphocyte immunotherapy (LIT; n=50). All RM patients were assessed according to the inclusion and exclusion criteria detailed in Table 1.

Treatment Procedures and Sample Collection

Treatment protocols were administered according to the schedule outlined in Table 2. Participants in the PT group received oral prednisolone (5 mg once daily) and low-dose aspirin (80 mg once daily) for two months. Participants in the LIT group received three subcutaneous injections of paternal peripheral blood lymphocytes on days 1, 15, and 30, along with low-dose aspirin (80 mg once daily).

All participants initiated the intervention simultaneously during the follicular phase of the menstrual cycle. Blood samples were collected from all participants before treatment and again after completion of the two-month intervention period.

Table 1. Inclusion and exclusion criteria for study participants

Inclusion criteria	Exclusion criteria
Age 25 to 40 years	Pregnancy
BMI 24-30 kg/m ²	Smoking or alcohol consumption
Normal fertility history	Previous treatment with corticosteroids or immunotherapy
APCA titer < 10%	Autoimmune diseases (e.g., SLE, antiphospholipid antibody syndrome)
Three or more spontaneous miscarriages before 20 weeks of gestation	Acute infection with TORCH agents (e.g., Toxoplasma, HCV, Rubella, CMV, and HSV)

APCA: anti-paternal cytotoxic antibody, BMI: body mass index, SLE: systemic lupus erythematosus, HCV: hepatitis C virus, CMV: cytomegalovirus, HSV: herpes simplex virus

Table 2. Timetable for treatment procedures

Timepoint	PT Group (n=50)	LIT Group (n=50)
Pre-treatment (day 0)	First blood sampling	First blood sampling
Intervention period (2 months)	• Prednisolone 5 mg orally (one tablet daily) • Low-dose aspirin 80 mg orally (one tablet daily)	• 1st paternal lymphocyte transfer (subcutaneous injection, day 1) • 2nd paternal lymphocyte transfer (subcutaneous injection, ~day 15) • 3rd paternal lymphocyte transfer (subcutaneous injection, ~day 30) • Low-dose aspirin 80 mg orally (one tablet daily)
Post-treatment (day 60)	Second blood sampling	Second blood sampling

PT: prednisolone therapy, LIT: lymphocyte immunotherapy, SC: subcutaneous

At each sampling time point, 2 mL of peripheral blood was collected into sterile tubes containing EDTA as an anticoagulant. Peripheral blood mononuclear cells (PBMCs) were subsequently isolated using Ficoll–Hypaque density gradient centrifugation for flow cytometric analysis.

Flow cytometry

Two milliliters of fresh peripheral venous blood were collected into EDTA-anticoagulated tubes and stained directly with the following monoclonal antibodies according to the manufacturer's instructions: PerCP/Cyanine5.5-conjugated anti-human HLA-DR (Clone: L243), FITC-conjugated anti-human CD33 (Clone: P67.6), PE-conjugated anti-human CD66 (Clone: W20030D), FITC-conjugated anti-human CD15 (Clone: HI98), and FITC-conjugated anti-human CD14 (Clone: HCD14) (all from BioLegend, USA). Isotype controls were used to assess nonspecific antibody binding.

The prepared samples were analyzed using FACS Caliber flow cytometer (BD Biosciences San Jose, CA, USA) with CellQuest research software (Becton Dickinson) for primary data acquisition and analysis. The resulting data were subsequently analyzed using FlowJo software 10 (FlowJo, LLC). The gating strategy was as follows: first, debris was excluded and the cell population was gated based on forward scatter (FSC-A) versus side scatter (SSC-A). Next, HLA-DR-negative cells were identified using an HLA-DR versus SSC plot. Within this population, CD33⁺CD66⁺ double-positive cells were identified. Granulocytic and monocytic MDSC subsets were defined as CD15⁺CD66⁺ and CD14⁺CD66⁺ cells, respectively (31–33).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). The distribution of the data was assessed using an appropriate test of normality. For comparisons between two independent groups, the unpaired Mann–Whitney U test was used.

For paired comparisons of measurements obtained before and after treatment, the Wilcoxon signed-rank test was applied. The framework of mixed-effects nested ANOVA was used for data adjustment and one-way analysis of variance (ANOVA) was used for comparisons among more than two groups, as appropriate. For categorical variables (e.g., β -hCG titer > 25 IU/L, first-trimester abortion, full-term delivery, and receipt of additional treatments), comparisons between the LIT and prednisolone therapy groups were performed using the chi-square test when all expected cell counts were ≥ 5 . Fisher's exact test was used when any expected cell count was < 5. Odds ratios (ORs) and risk ratios (RRs), with corresponding 95% confidence intervals (CIs), were calculated to quantify the associations between treatment group and categorical outcomes. Graphical representations of the data were generated using GraphPad Prism version 9.0. Continuous variables are presented as mean $\pm$ standard error (SE). A two-tailed P value < 0.05 was considered statistically significant. Comparisons between the LIT and prednisolone therapy groups were performed as appropriate, and paired comparisons of cell subpopulation percentages before and after treatment were conducted within each treatment group.

RESULTS

Most of the women included in the present study had also participated in our previous study (34). The demographic characteristics of the participants are summarized in Table 3. No statistically significant differences were observed among the study groups with respect to age, body mass index (BMI), and history of previous miscarriages.

In the present study, HLA-DR⁺ CD33⁺ CD66⁺ cells were identified as MDSCs. Within this population, CD14-positive and CD15-positive MDSCs were defined as monocytic and granulocytic MDSC subsets, respectively (Figures 1 A, 1C, 2A, and 2C).

Table 3. Clinical and demographic characteristics of the participants

	PT n=50	LIT n=50	P value
Age (year)	32.46 ± 0.58	31.92 ± 0.56	0.095 (ns)
BMI (kg/m ²)	26.10 ± 0.27	26.36 ± 0.27	0.865 (ns)
Previous IVF Failures	0.76 ± 0.14	0.80 ± 0.17	0.954 (ns)
Previous Miscarriages	3.76 ± 0.22	3.08 ± 0.17	0.059 (ns)

PT: Prednisolone therapy, LIT: Lymphocyte Immunotherapy, BMI: Body Mass Index, IVF: In vitro fertilization, ns: not significant, Data are presented as mean ± standard error (SE). $P < 0.05$ was considered statistically significant.

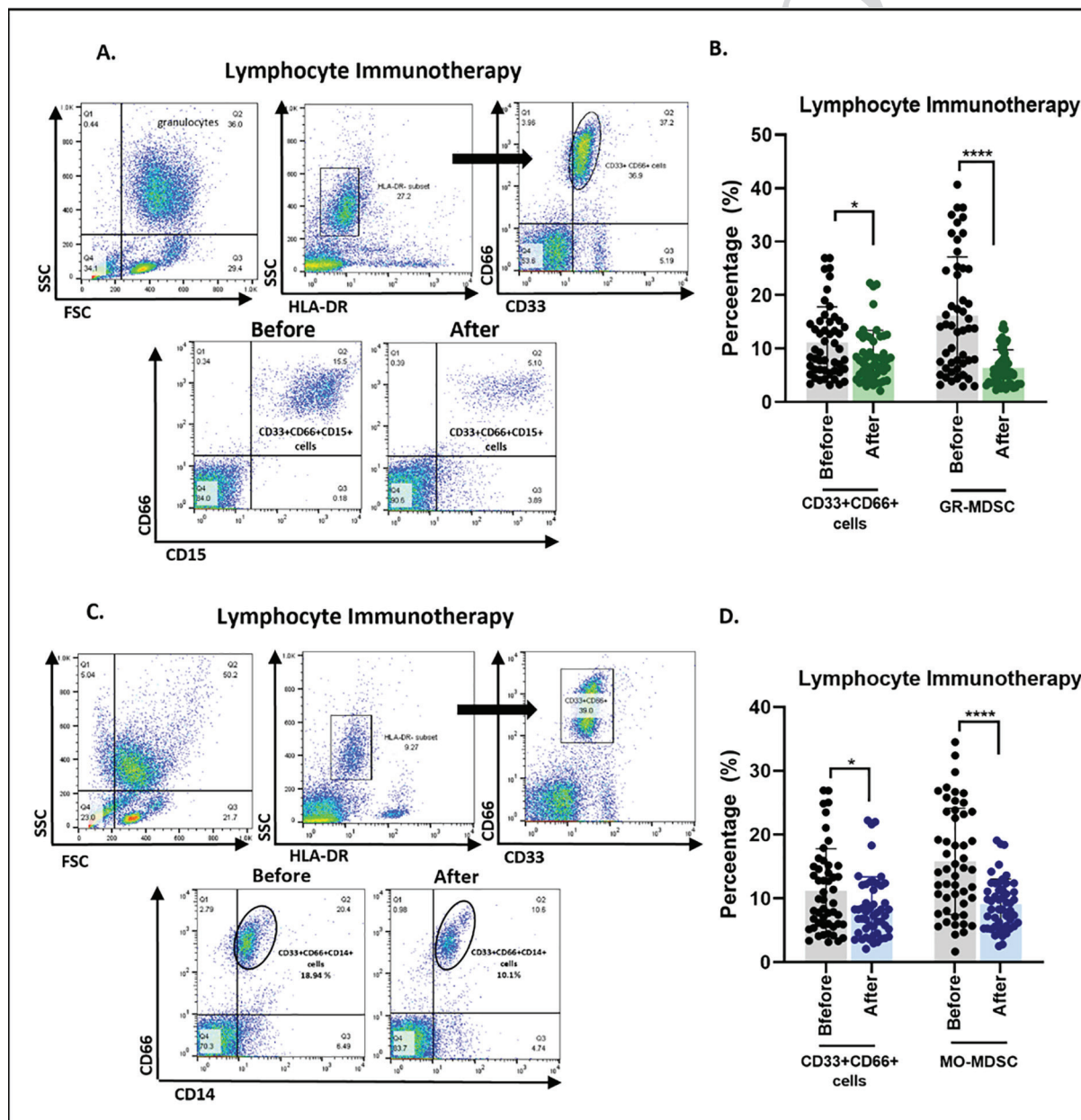


Figure 1. Changes in granulocytic and monocytic myeloid-derived suppressor cell (GR-MDSCs and MO-MDSCs) subsets following lymphocyte immunotherapy, as assessed by flow cytometry. According to the gating strategy, MDSCs were identified as HLA-DR⁺CD33⁺CD66⁺ cells (A, C). Within the MDSC population, CD15⁺CD66⁺ and CD14⁺CD66⁺ were identified as GR-MDSCs and MO-MDSCs, respectively. Lymphocyte immunotherapy significantly decreased the percentages of total MDSCs, GR-MDSC and MO-MDSC subsets (B, D). * P value < 0.05 , **** P value < 0.0001

Table 4. Percentages of MDSC subsets before and after the intervention in the lymphocyte immunotherapy group

	Before Intervention	After Intervention	<i>P</i> value
	Mean ± SEM		
Total MDSCs population	11.20 ± 0.92	8.51 ± 0.68	0.036 *
MO-MDSCs (% of MDSCs)	15.84 ± 1.16	9.11 ± 0.55	<0.0001 ****
GR-MDSCs (% of MDSCs)	16.18 ± 1.53	6.41 ± 0.46	<0.0001 ****

MO-MDSC: Monocytic myeloid-derived suppressor cell, GR-MDSC: Granulocytic myeloid-derived suppressor cell. Data are presented as mean $\pm$ standard error of the mean (SEM). $P < 0.05$ was considered statistically significant; * P value<0.05, **** P value<0.0001

Table 5. Percentages of MDSC subsets before and after the intervention in the prednisolone therapy group

	Before Intervention	After Intervention	<i>P</i> value
	Mean ± SEM		
Total MDSC population	12.05 ± 1.25	14.19 ± 1.22	0.1073 ns
MO-MDSCs (% of MDSCs)	18.28 ± 1.84	7.81 ± 0.71	<0.0001 ****
GR-MDSCs (% of MDSCs)	19.94 ± 1.64	11.24 ± 1.16	0.0121 *

MO-MDSC: Monocytic myeloid-derived suppressor cell, GR-MDSC: Granulocytic myeloid-derived suppressor cell. Data are presented as mean $\pm$ standard error of the mean (SEM). $P < 0.05$ was considered statistically significant; ns, not significant; * P value<0.05=, **** P value<0.0001

Significant Reduction in Granulocytic and Monocytic MDSCs Percentage Following LIT

LIT significantly reduced the percentage of total MDSCs (CD33⁺CD66⁺HLA-DR⁺ cells) ($P=0.036$; Figure 1 and Table 4). Furthermore, LIT resulted in a significant reduction in both GR-MDSCs ($P < 0.0001$; Figure 1. B) and MO-MDSCs ($P < 0.0001$; Figure 1. D).

Prednisolone Treatment Significantly Reduced MDSC Subsets While Preserving the Overall MDSC Population

No statistically significant change was observed in the percentage of total MDSCs following prednisolone therapy (Table 5). However, prednisolone treatment significantly reduced the percentages of GR-MDSCs ($P=0.012$; Figure 2B) and MO-MDSCs ($P < 0.0001$; Figure 2D).

LIT Was More Effective than Prednisolone Therapy in Reducing the Proportion of GR-MDSCs

Before the intervention, there were no

statistically significant differences between the LIT and prednisolone therapy groups in the proportions of GR-MDSCs or MO-MDSCs within the MDSC population ($P=0.71$ and $P=0.6$, respectively; Figure 3). Following the interventions, a significant difference was observed between the two treatments groups, indicating a greater reduction in GR-MDSCs following LIT compared with prednisolone therapy. In the prednisolone therapy group, the percentage of GR-MDSCs remained significantly higher than that of MO-MDSCs ($P=0.046$; Figure 3A). Although prednisolone therapy significantly reduced both MDSC subsets, the reduction in MO-MDSCs was greater than that observed for GR-MDSCs.

However, in the LIT group, the marked reduction in the percentage of GR-MDSCs following treatment resulted in a significantly lower proportion of these cells compared with MO-MDSCs ($P=0.0002$; Figure 3B). These findings suggest that LIT produced a more pronounced reduction in GR-MDSCs, whereas prednisolone therapy had a greater effect on MO-MDSCs.

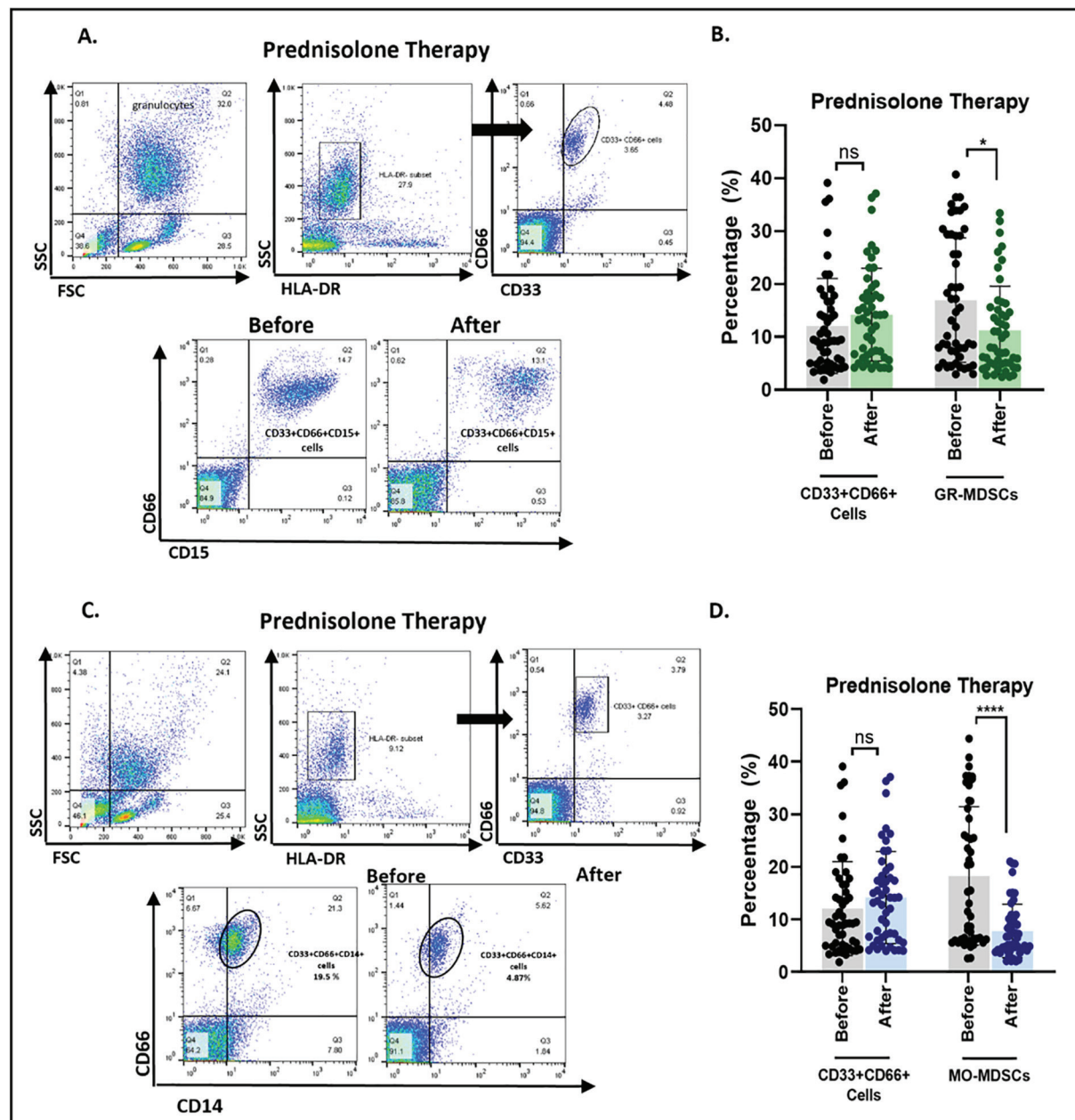


Figure 2. The changes in GR-MDSCs and MO-MDSCs subsets following prednisolone therapy, as assessed by flow cytometry. CD33⁺ HLA-DR⁺ cells were initially gated, followed by identification of CD33⁺ CD66⁺ cells as MDSCs. Within the MDSC population, CD15⁺ CD66⁺ and CD14⁺CD66⁺ cells were identified as GR-MDSC and MO-MDSC subsets, respectively (A, C). No statistically significant difference was observed in the percentage of total CD33⁺CD66⁺HLA-DR⁺ cell before and after prednisolone therapy. However, prednisolone therapy significantly decreased the percentages of GR-MDSCs (B) and MO-MDSCs (D) in the peripheral blood of treated women. GR-MDSC: Granulocytic myeloid derived suppressor cell, MO-MDSC: Monocytic myeloid derived suppressor cell, ns, not significant, **P* value<0.05, **** *P* value<0.0001

No Significant Difference in Pregnancy Outcomes between the LIT and Prednisolone Therapy Groups

As shown in Table 6, participants were followed for one year, and pregnancy outcomes were assessed during the follow-up period. However, some participants received

additional treatments during this period, which may have influenced pregnancy outcomes and potentially confounded the effects of LIT or prednisolone therapy. The proportion of participants achieving β -hCG > 25 IU/L was numerically higher in the prednisolone group (80.00% vs. 66.67%), but

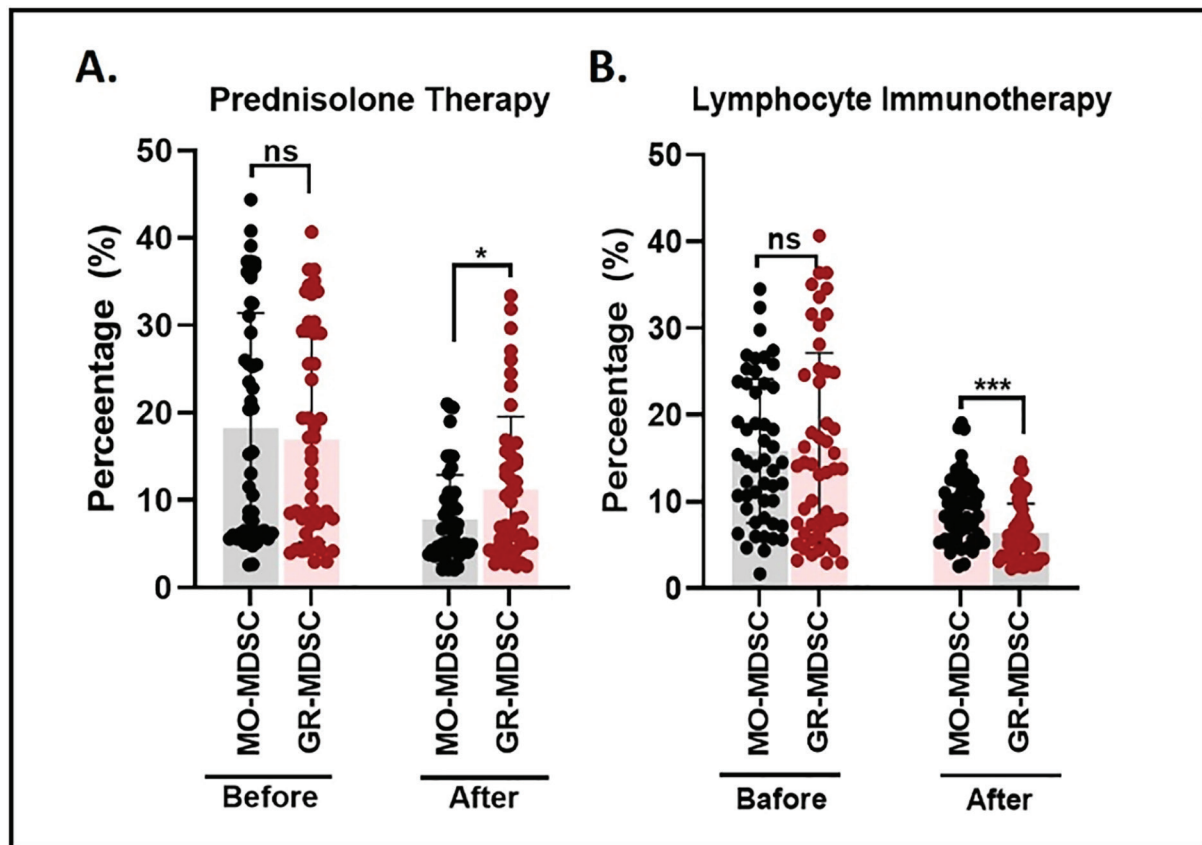


Figure 3. Comparison of GR-MDSC and MO-MDSC proportions following Lymphocyte immunotherapy and oral administration of prednisolone. The proportion of GR-MDSCs was significantly higher than that of MO-MDSCs following prednisolone therapy (A, $P = 0.046$), whereas the proportion of was significantly lower than that of MO-MDSCs following LIT (B, $P = 0.0002$). GR-MDSC: Granulocytic myeloid-derived suppressor cell, MO-MDSC: Monocytic myeloid-derived suppressor cell, P value > 0.05 = not significant (ns), $*P$ value < 0.05 , $***P$ value < 0.001

Table 6. Pregnancy outcomes during the one-year follow-up period in the LIT and prednisolone therapy groups

Outcome	LIT (n=48) n (%)	Prednisolone (n=50) n (%)	P value	OR (95% CI)	RR (95% CI)
β-hCG titer > 25 IU/L	32 (66.67)	40 (80.00)	0.138	0.50 (0.20–1.25)	0.83 (0.65–1.07)
First-trimester abortion	3 (6.25)	5 (10.00)	0.716	0.60 (0.14–2.65)	0.63 (0.16–2.47)
Full-term delivery	17 (35.42)	16 (32.00)	0.721	1.17 (0.51–2.65)	1.11 (0.63–1.94)
Received additional treatments	8 (16.67)	21 (42.00)	0.006	0.28 (0.11–0.71)	0.40 (0.20–0.80)

LIT: lymphocyte immunotherapy; β-hCG: beta-human chorionic gonadotropin; OR: odds ratio; RR: risk ratio; CI: confidence interval. Data are presented as n (%). Categorical variables were compared using the chi-square test when all expected cell frequencies were ≥ 5 ; Fisher's exact test was used when any expected frequency was < 5 . We considered $P < 0.05$ statistically significant. *Two participants in the LIT group were lost to follow-up due to unrelated infectious diseases. Limitation: The study may have been underpowered to detect statistically significant differences in some pregnancy outcomes due to the small sample size

the difference was not statistically significant ($P=0.138$; OR=0.50, 95% CI: 0.20–1.25; RR=0.83, 95% CI: 0.65–1.07). Similarly, no significant differences were observed for first-trimester abortion (6.25% vs. 10.00%; $P=0.716$;

OR=0.60, 95% CI: 0.14–2.65; RR=0.63, 95% CI: 0.16–2.47) or full-term delivery (35.42% vs. 32.00%; $P=0.721$; OR=1.17, 95% CI: 0.51–2.65; RR=1.11, 95% CI: 0.63–1.94). Notably, the proportion of participants

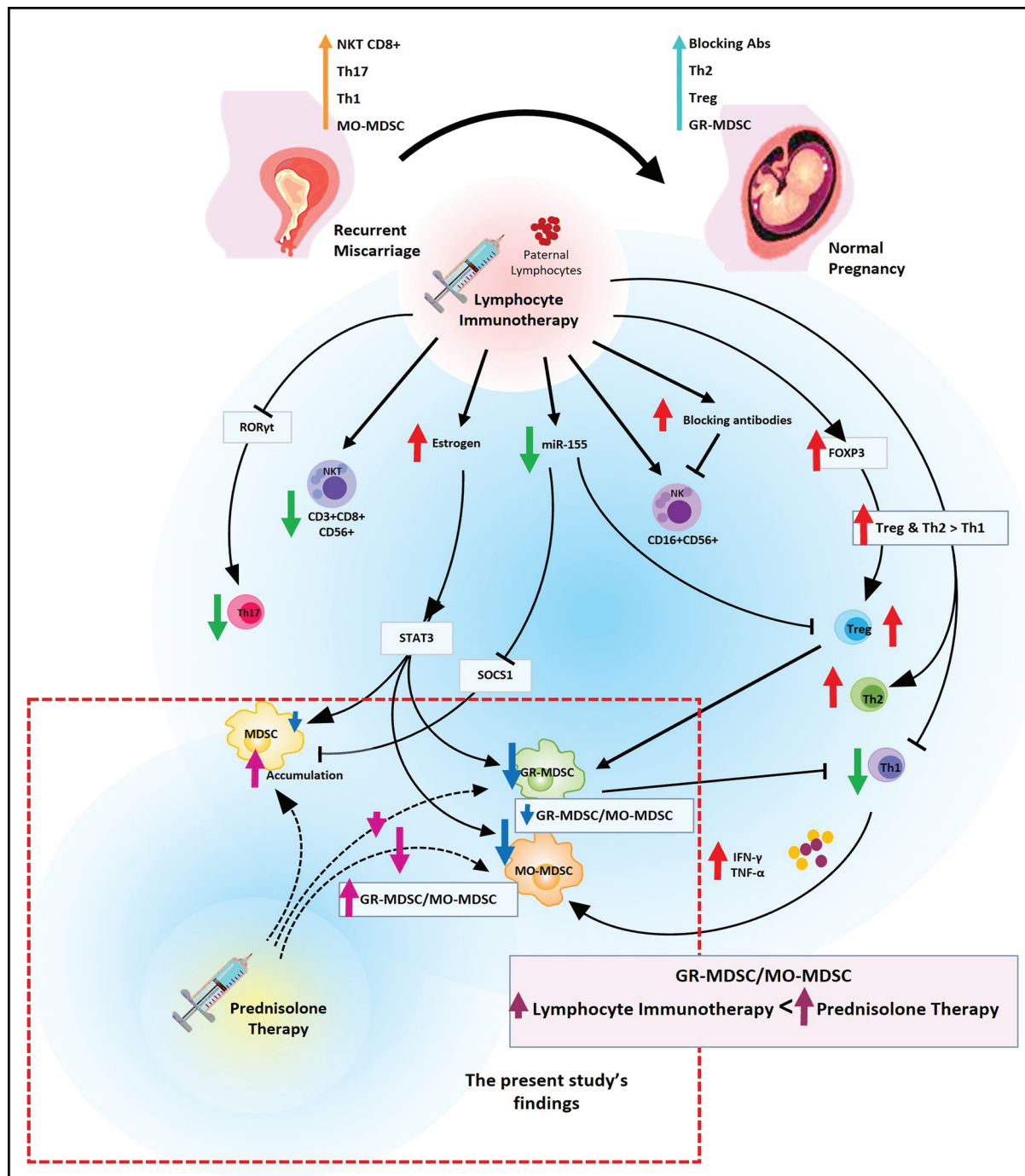


Figure 4. Schematic representation of the mechanisms investigated in the present study and the proposed mechanisms underlying the therapeutic effects of prednisolone and lymphocyte immunotherapy (LIT) in women with recurrent miscarriage (RM). LIT increases the levels of Th2 cells, regulatory T cells (Tregs), estrogen, FOXP3, and blocking antibodies, while decreasing GR-MDSCs, the GR-MDSC/MO-MDSC ratio, Th17 cells, monocytic MDSCs (MO-MDSCs), Th1 cells, NKT cells, NK cells, CD3+CD8+CD56+ cells, miR-155, and RORyt. Prednisolone therapy increases MDSC accumulation and the GR-MDSC/MO-MDSC ratio, primarily through a greater reduction in MO-MDSCs. The present study's findings (red dashed box) highlight the opposing effects of LIT and prednisolone on the GR-MDSC/MO-MDSC ratio: LIT decreases this ratio (↓), whereas prednisolone increases it (↑). Through these pathways, both treatments may establish a safe microenvironment that supports normal pregnancy. Dashed arrows and molecular mediators (e.g., STAT3, SOCS1, miR-155) represent proposed or hypothetical mechanisms that require further experimental validation. LIT: lymphocyte immunization therapy, RM: recurrent miscarriage, Tregs: regulatory T cells, GR-MDSCs: granulocytic myeloid-derived suppressor cells, MO-MDSCs: monocytic myeloid-derived suppressor cells, NKT: natural killer T cell.

who received additional treatments was significantly higher in the prednisolone therapy group (42.00% vs. 16.67%; $P=0.006$; $OR=0.28$, 95% CI: 0.11–0.71; $RR=0.40$, 95% CI: 0.20–0.80). These findings should be interpreted with caution, as the study may have been underpowered to detect statistically significant differences in some pregnancy outcomes due to the small sample size.

DISCUSSION

In the last several decades, studies have demonstrated the involvement of MDSCs at various stages of pregnancy and during the neonatal period, as well as in gynecological disorders such as first-trimester miscarriage, recurrent implantation failure (RIF), and preeclampsia (13, 35-37).

MDSCs have been implicated in immune suppression, regulation of inflammatory response, trophoblast implantation, and angiogenesis (13, 36, 38-41). Elevated levels of estradiol and progesterone have been reported to increase the proportion of MDSCs in the decidua of pregnant women compared with non-pregnant women and may promote their recruitment during pregnancy (14, 42). GR-MDSCs accumulate at the feto-maternal immune interface during healthy pregnancies and may contribute to the establishment and maintenance of feto-maternal immune tolerance (43). MDSCs can modulate the activity of other immune cells, particularly T and NK cells, primarily through suppression of their functions (41, 44). Kostlin et al. demonstrated that GR-MDSCs accumulate in the maternal peripheral blood throughout pregnancy in both mice and humans (13). Conversely, a reduction in the number or suppressive function of these cells during early pregnancy has been associated with RM (36, 40, 45). The distribution and characteristics of MDSCs across different stages of pregnancy have also been investigated in several studies (14, 36, 40, 46). Available evidence indicates that GR-MDSCs play an important role in

maintaining feto-maternal tolerance (47-49). Given their potential involvement in the implantation process, modulation of the GR-MDSC population during the pre-pregnancy period may also influence implantation and the success of embryo transfer.

The early stages of a successful pregnancy depend on the embryo's ability to attach to and invade the endometrium. The complex interplay among maternal decidual stromal cells, decidual glandular epithelial cells, and various immune cell populations within the feto-maternal immune microenvironment is crucial for the trophoblast migration and invasion and for the development of decidual vasculature (54, 47, 48). Maintaining a delicate balance between pro- and anti-inflammatory immune cell populations, including T cells, NK cells, and MDSCs, is essential for successful implantation and placentation (40, 49–53). Furthermore, the high levels of estrogen and progesterone produced by the maternal corpus luteum and, later, the fetal placenta influence immune cell function during pregnancy (55). In the present study, women with a history of recurrent miscarriage were evaluated during the pre-pregnancy period. Before treatment, no statistically significant differences were observed in the proportions of MO-MDSCs and GR-MDSCs between the treatment groups.

Based on our findings of the present study, oral administration of low-dose prednisolone for two months before pregnancy in women with RM significantly reduced the proportions of both GR-MDSCs and MO-MDSCs within the MDSC population. Two distinct observations emerged from these data. First, with respect to the relative proportions of the two subsets after treatment, the proportion of GR-MDSCs remained significantly higher than that of MO-MDSCs ($P=0.046$). Second, with respect to the magnitude of change from baseline, MO-MDSCs showed a greater reduction than GR-MDSCs. Thus, although both MDSC subsets decreased following prednisolone therapy, the relative dominance of GR-MDSCs within the MDSC

population was preserved, while the absolute reduction was more pronounced for MO-MDSCs. Prednisolone, a glucocorticoid, has anti-inflammatory and immunosuppressive properties (50) and has been investigated as an immunomodulatory treatment in women with recurrent miscarriage. A meta-analysis of randomized controlled trials reported that prednisolone was associated with improved pregnancy outcomes and a reduced miscarriage rate in women with unexplained recurrent miscarriage (29). Experimental and clinical evidence has demonstrated the potential effects of glucocorticoid supplementation on reproductive outcomes in selected infertility and recurrent pregnancy loss (RPL) populations undergoing assisted reproductive treatment, although varied observations in clinical trials arise from heterogeneity in therapeutic approaches (51, 52). In the present study, prednisolone therapy significantly reduced the proportions of both GR-MDSCs and MO-MDSCs within the MDSC population. However, the proportion of GR-MDSCs remained significantly higher than that of MO-MDSCs following treatment. Thus, although both MDSC subsets decreased following prednisolone therapy, MO-MDSCs showed a greater reduction than GR-MDSCs. LIT has different immunomodulatory effects from prednisolone therapy and has been investigated as a treatment for recurrent miscarriage (34). Proposed mechanisms of LIT include modulation of maternal T and NK cell activity (53-56), induction of anti-T-cell receptor (TCR) idiotype antibodies (55, 56), and inhibition of mixed lymphocyte reaction (57-60). These immunomodulatory effects have been proposed to contribute to the establishment of maternal immune tolerance during pregnancy (22, 61-63). Researchers have utilized allogeneic lymphocytes, particularly paternal lymphocytes, in lymphocyte immunotherapy (LIT) to induce maternal immune responses that may promote tolerance to paternal and fetal antigens. This maternal immunomodulation involves a shift from Th1 to Th2 cells, increased

concentrations of anti-inflammatory cytokines, and inhibition of NK cells. Another notable mechanism of LIT is the promotion of anti-paternal cytotoxic antibodies (APCA), anti-idiotypic antibodies (Ab2), and mixed lymphocyte reaction blocking antibodies (MLR-Bf) (64-66). However, the clinical significance of blocking antibodies and their role in preventing maternal immune rejection of the embryo remain uncertain.

Previous studies have suggested that LIT may influence estrogen levels and modulate cellular immune responses in women with unexplained recurrent miscarriage (uRM), with potential effects on implantation outcomes (67). Estrogen signaling has also been implicated in the regulation of MDSCs. Activation of the STAT3 pathway in bone marrow myeloid precursors through the estrogen receptor can promote the expansion of MDSCs with potent immunosuppressive activity toward T cells. In addition, estrogen plays a key role in the accumulation and functional regulation of MDSCs in systemic lupus erythematosus (SLE), although the effects may differ among MDSC subsets. MO-MDSCs have been shown to promote inflammation in the pathogenesis of SLE by modulating the Th17/Treg cell balance (68). Liu et al. evaluated women with uRM and reported changes in the Th1/Th2 ratio and Treg cell levels following LIT, using the Th1/Th2/Treg paradigm as an indicator of therapeutic effectiveness (61). In contrast, another study found no significant differences in the absolute numbers of peripheral T, B, or NK cells between women receiving LIT and controls (69).

Aslanian et al reported that LIT in women with recurrent pregnancy loss (RPL) increased FoxP3 mRNA expression, decreased ROR γ t mRNA expression and NK-cell cytotoxicity, and reduced miR-155 expression (70). MicroRNAs play important roles in immune cell maturation, differentiation, and function, and have also been implicated in pregnancy and reproductive disorders, including RPL, endometriosis, preeclampsia, and

ovarian cancer (70, 71). miR-155 has been shown to promote the accumulation and immunosuppressive activity of MDSCs in the tumor microenvironment, partly through repression of SOCS1 and modulation of CD4⁺ Foxp3⁺ Treg generation (72). An association between MDSCs and Th17 cells has also been reported in Ehrlich ascites carcinoma, suggesting these cell populations may interact in certain pathological settings (73). Although pregnancy and the tumor microenvironment share some features of immune regulation, whether similar interactions occur during pregnancy or in women with RPL remains unclear. In the study by Aslanian et al., LIT was associated with reduced ROR γ t, together with increased FoxP3 expression and Treg cell frequency (70, 74). These findings raise the possibility that changes in the Th17/Treg balance following LIT may be related to alterations in MDSC subsets.

The LIT protocol may influence the outcomes observed across studies (10). The protocol used in the present study was similar to that described by Liu et al. (69) with respect to the administration of a low-dose of fresh lymphocyte injection (10⁷ cells per administration), intradermal injection, and lymphocyte isolation procedures. In the present study, although LIT significantly reduced both MO-MDSCs and GR-MDSCs, the proportion of MO-MDSCs remained higher than that of GR-MDSCs after treatment. The mechanisms underlying this differential response remain unclear. One possible explanation is that LIT may influence hormonal or immune pathways that differentially regulate MDSC subsets; however, estrogen levels were not assessed in the present study, and this possibility therefore requires further investigation. Liu et al. reported changes in the Th1/Th2/Treg cell profile and cytokine levels following LIT (69). In contrast, in our previous study, we did not observe significant changes in TGF- β or IFN- γ concentrations following LIT (34). This discrepancy may be related to differences in the immunological characteristics of the

study populations, including the abnormal Th1/Th2/Treg cells profile reported among participants in the study by Liu et al.

Prednisolone therapy significantly reduced the proportions of both MO-MDSCs and GR-MDSCs, with a greater reduction in MO-MDSCs, whereas LIT resulted in a greater reduction in GR-MDSCs. The mechanisms underlying these differential effects on MDSC subsets remain unclear and warrant further investigation. Overall, the findings indicate that both LIT and low-dose prednisolone therapy can alter MDSC subset proportions during the pre-pregnancy period in women with RM.

In the present study, we investigated changes in the peripheral blood ratio of MO-MDSCs to GR-MDSCs in women prior to pregnancy. No significant difference in pregnancy outcomes was observed between the LIT and low-dose prednisolone groups during the follow-up period. Further prospective studies are needed to clarify the relationship between pre-pregnancy modulation of MDSC subsets and subsequent pregnancy outcomes.

In this study, no statistically significant differences were observed between the LIT and prednisolone therapy groups in β -hCG titer > 25 IU/L, first-trimester abortion, or full-term delivery during the one-year follow-up period. These findings are consistent with previous studies reporting that neither LIT nor low-dose prednisolone is clearly superior to the other in improving pregnancy outcomes in women with RM (75). The lack of a significant difference between the two treatment groups may reflect patient population heterogeneity, differences in treatment protocols across studies, or the complex and multifactorial nature of RM (76, 77). Notably, the proportion of participants who received additional treatments during the follow-up period was significantly higher in the prednisolone therapy group than in the LIT group. This finding may reflect differences in clinical response to the two treatment strategies and suggests that

women in the prednisolone therapy group may have required further interventions to achieve or maintain pregnancy. However, this observation should be interpreted cautiously, as the study was not primarily powered to detect differences in the need for additional treatments, and the use of additional therapies may have confounded the observed pregnancy outcomes. Although LIT and prednisolone had opposing effects on the GR-MDSC/MO-MDSC ratio, this did not translate into significant differences in pregnancy outcomes. Further prospective studies with larger sample sizes and standardized protocols for additional treatments are needed to clarify the relationship between pre-pregnancy modulation of MDSC subsets and subsequent pregnancy outcomes.

CONCLUSION

In women with recurrent miscarriage, short-term low-dose prednisolone therapy and LIT were associated with significant changes in the proportions of MDSC subpopulations in women with RM during the pre-pregnancy period. Both treatments reduced MO-MDSCs and GR-MDSCs, although the magnitude of reduction differed between the two subsets and treatment groups. No significant difference in pregnancy outcomes was observed between the two treatment groups during follow-up. These findings suggest that both treatments modulate MDSC subsets, which may have implications for pre-conception immunomodulation in RM. Further prospective studies with larger sample sizes and standardized protocols are needed to clarify the relationship between pre-pregnancy MDSC modulation and subsequent pregnancy outcomes.

REFERENCES

1. Arocena AR, Onofrio LI, Pellegrini AV, Carrera Silva AE, Paroli A, Cano RC, et al. Myeloid-derived

- suppressor cells are key players in the resolution of inflammation during a model of acute infection. *Eur J Immunol.* 2014;44(1):184-94.
2. Beury DW, Parker KH, Nyandjo M, Sinha P, Carter KA, Ostrand-Rosenberg S. Cross-talk among myeloid-derived suppressor cells, macrophages, and tumor cells impacts the inflammatory milieu of solid tumors. *J Leukoc Biol.* 2014;96(6):1109-18.
3. Buessow SC, Paul RD, Lopez DM. Influence of mammary tumor progression on phenotype and function of spleen and in situ lymphocytes in mice. *J Natl Cancer Inst.* 1984;73(1):249-55.
4. Dai J, El Gazzar M, Li GY, Moorman JP, Yao ZQ. Myeloid-derived suppressor cells: paradoxical roles in infection and immunity. *J Innate Immun.* 2015;7(2):116-26.
5. Drujont L, Carretero-Iglesia L, Bouchet-Delbos L, Beriou G, Merieau E, Hill M, et al. Evaluation of the therapeutic potential of bone marrow-derived myeloid suppressor cell (MDSC) adoptive transfer in mouse models of autoimmunity and allograft rejection. *PLoS One.* 2014;9(6):e100013.
6. Nakamura I, Shibata M, Gonda K, Yazawa T, Shimura T, Anazawa T, et al. Serum levels of vascular endothelial growth factor are increased and correlate with malnutrition, immunosuppression involving MDSCs and systemic inflammation in patients with cancer of the digestive system. *Oncol Lett.* 2013;5(5):1682-86.
7. Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, et al. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J Immunol.* 2001;166(1):678-89.
8. Ochoa AC, Zea AH, Hernandez C, Rodriguez PC. Arginase, prostaglandins, and myeloid-derived suppressor cells in renal cell carcinoma. *Clin Cancer Res.* 2007;13(2 Pt 2):721s-26s.
9. Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res.* 2001;61(12):4756-60.
10. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun.* 2016;7:12150.
11. Condamine T, Dominguez GA, Youn JI, Kossenkova AV, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol.* 2016;1(2).
12. Dumitru CA, Moses K, Trellakis S, Lang S,

- Brandau S. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. *Cancer Immunol Immunother.* 2012;61(8):1155-67.
13. Kostlin N, Kugel H, Spring B, Leiber A, Marme A, Henes M, et al. Granulocytic myeloid derived suppressor cells expand in human pregnancy and modulate T-cell responses. *Eur J Immunol.* 2014;44(9):2582-91.
 14. Kang X, Zhang X, Liu Z, Xu H, Wang T, He L, et al. Granulocytic myeloid-derived suppressor cells maintain feto-maternal tolerance by inducing Foxp3 expression in CD4+CD25-T cells by activation of the TGF-beta/beta-catenin pathway. *Mol Hum Reprod.* 2016;22(7):499-511.
 15. Netherby CS, Abrams SI. Mechanisms overseeing myeloid-derived suppressor cell production in neoplastic disease. *Cancer Immunol Immunother.* 2017;66(8):989-96.
 16. Sinha P, Clements VK, Bunt SK, Albelda SM, Ostrand-Rosenberg S. Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *J Immunol.* 2007;179(2):977-83.
 17. Zhao AM, Xu HJ, Kang XM, Zhao AM, Lu LM. New insights into myeloid-derived suppressor cells and their roles in feto-maternal immune cross-talk. *J Reprod Immunol.* 2016;113:35-41.
 18. Kostlin-Gille N, Gille C. Myeloid-Derived Suppressor Cells in Pregnancy and the Neonatal Period. *Front Immunol.* 2020;11:584712.
 19. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. *Acta Obstet Gynecol Scand.* 1977;56(3):247-53.
 20. Odendaal J, Quenby S, Sammaritano L, Macklon N, Branch DW, Rosenwaks Z. Immunologic and rheumatologic causes and treatment of recurrent pregnancy loss: what is the evidence? *Fertil Steril.* 2019;112(6):1002-12.
 21. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017. *Hum Reprod.* 2017;32(9):1786-801.
 22. Cavalcante MB, Sarno M, Barini R. Lymphocyte immunotherapy in recurrent miscarriage and recurrent implantation failure. *Am J Reprod Immunol.* 2021;85(4):e13408.
 23. Carp H. Immunotherapy for recurrent pregnancy loss. *Best Pract Res Clin Obstet Gynaecol.* 2019;60:77-86.
 24. Chen J, Liu B, Zhang Y, Ao L, Li Z, Qu B, et al. Effect of immunotherapy on patients with unexplained recurrent spontaneous abortion. *Ann Palliat Med.* 2020;9(5):2545-50.
 25. Woon EV, Day A, Bracewell-Milnes T, Male V, Johnson M. Immunotherapy to improve pregnancy outcome in women with abnormal natural killer cell levels/activity and recurrent miscarriage or implantation failure: A systematic review and meta-analysis. *J Reprod Immunol.* 2020;142:103189.
 26. Hajipour H, Nejabati HR, Latifi Z, Hamdi K, Bahrami-Asl Z, Fattahi A, et al. Lymphocytes immunotherapy for preserving pregnancy: Mechanisms and Challenges. *Am J Reprod Immunol.* 2018;80(3):e12853.
 27. Cavalcante MB, Sarno M, Araujo Junior E, Da Silva Costa F, Barini R. Lymphocyte immunotherapy in the treatment of recurrent miscarriage: systematic review and meta-analysis. *Arch Gynecol Obstet.* 2017;295(2):511-18.
 28. Opelz G, Terasaki PI. Second kidney transplants and presensitization. *Transplant Proc.* 1972;4(4):743-8.
 29. Dan S, Wei W, Yichao S, Hongbo C, Shenmin Y, Jiaxiong W, et al. Effect of Prednisolone Administration on Patients with Unexplained Recurrent Miscarriage and in Routine Intracytoplasmic Sperm Injection: A Meta-Analysis. *Am J Reprod Immunol.* 2015;74(1):89-97.
 30. Woynarowski M, Nemeth A, Baruch Y, Koletzko S, Melter M, Rodeck B, et al. Budesonide versus prednisone with azathioprine for the treatment of autoimmune hepatitis in children and adolescents. *J Pediatr.* 2013;163(5):1347-53 e1.
 31. Duffy A, Zhao F, Haile L, Gamrekashvili J, Fioravanti S, Ma C, et al. Comparative analysis of monocytic and granulocytic myeloid-derived suppressor cell subsets in patients with gastrointestinal malignancies. *Cancer Immunol Immunother.* 2013;62(2):299-307.
 32. Lv M, Wang K, Huang XJ. Myeloid-derived suppressor cells in hematological malignancies: friends or foes. *J Hematol Oncol.* 2019;12(1):105.
 33. Ren W, Zhang X, Li W, Feng Q, Feng H, Tong Y, et al. Circulating and tumor-infiltrating arginase 1-expressing cells in gastric adenocarcinoma patients were mainly immature and monocytic Myeloid-derived suppressor cells. *Sci Rep.* 2020;10(1):8056.
 34. Rezayat F, Esmaeil N, Rezaei A, Sherkat R. Contradictory Effect of Lymphocyte Therapy and Prednisolone Therapy on CD3(+)CD8(+)CD56(+) Natural Killer T Population in Women with Recurrent Spontaneous Abortion. *J Hum Reprod Sci.* 2023;16(3):246-56.
 35. Kostlin N, Schoetensack C, Schwarz J, Spring B, Marme A, Goelz R, et al. Granulocytic Myeloid-Derived Suppressor Cells (GR-MDSC) in Breast Milk (BM); GR-MDSC Accumulate in Human

- BM and Modulate T-Cell and Monocyte Function. *Front Immunol.* 2018;9:1098.
36. Nair RR, Sinha P, Khanna A, Singh K. Reduced Myeloid-derived Suppressor Cells in the Blood and Endometrium is Associated with Early Miscarriage. *Am J Reprod Immunol.* 2015;73(6):479-86.
 37. Ostrand-Rosenberg S, Sinha P, Figley C, Long R, Park D, Carter D, et al. Frontline Science: Myeloid-derived suppressor cells (MDSCs) facilitate maternal-fetal tolerance in mice. *J Leukoc Biol.* 2017;101(5):1091-101.
 38. Mandruzzato G, Antsaklis A, Botet F, Chervenak FA, Figueras F, Grunebaum A, et al. Intrauterine restriction (IUGR). *J Perinat Med.* 2008;36(4):277-81.
 39. Pan T, Zhong L, Wu S, Cao Y, Yang Q, Cai Z, et al. 17beta-Oestradiol enhances the expansion and activation of myeloid-derived suppressor cells via signal transducer and activator of transcription (STAT)-3 signalling in human pregnancy. *Clin Exp Immunol.* 2016;185(1):86-97.
 40. Verma P, Verma R, Nair RR, Budhwar S, Khanna A, Agrawal NR, et al. Altered crosstalk of estradiol and progesterone with Myeloid-derived suppressor cells and Th1/Th2 cytokines in early miscarriage is associated with early breakdown of maternal-fetal tolerance. *Am J Reprod Immunol.* 2019;81(2):e13081.
 41. Zhao H, Kalish F, Schulz S, Yang Y, Wong RJ, Stevenson DK. Unique roles of infiltrating myeloid cells in the murine uterus during early to midpregnancy. *J Immunol.* 2015;194(8):3713-22.
 42. Li C, Chen C, Kang X, Zhang X, Sun S, Guo F, et al. Decidua-derived granulocyte macrophage colony-stimulating factor induces polymorphonuclear myeloid-derived suppressor cells from circulating CD15⁺ neutrophils. *Hum Reprod.* 2020;35(12):2677-91.
 43. Zhang Y, Wang X, Zhang R, Wang X, Fu H, Yang W. MDSCs interactions with other immune cells and their role in maternal-fetal tolerance. *Int Rev Immunol.* 2022;41(5):534-51.
 44. Fu B, Tian Z, Wei H. TH17 cells in human recurrent pregnancy loss and pre-eclampsia. *Cell Mol Immunol.* 2014;11(6):564-70.
 45. Ren J, Zeng W, Tian F, Wu F, Zhang S, Liu X, et al. Differential gene expression profile in monocytic myeloid-derived suppressor cells at maternal-fetal interface in a mouse model of spontaneous abortion. *J Cell Physiol.* 2019;234(7):10789-99.
 46. Pan T, Liu Y, Zhong LM, Shi MH, Duan XB, Wu K, et al. Myeloid-derived suppressor cells are essential for maintaining feto-maternal immunotolerance via STAT3 signaling in mice. *J Leukoc Biol.* 2016;100(3):499-511.
 47. Gantt S, Gervasi A, Jaspán H, Horton H. The role of myeloid-derived suppressor cells in immune ontogeny. *Front Immunol.* 2014;5:387.
 48. Hu C, Zhen Y, Pang B, Lin X, Yi H. Myeloid-Derived Suppressor Cells Are Regulated by Estradiol and Are a Predictive Marker for IVF Outcome. *Front Endocrinol (Lausanne).* 2019;10:521.
 49. Jiang H, Zhu M, Guo P, Bi K, Lu Z, Li C, et al. Impaired myeloid-derived suppressor cells are associated with recurrent implantation failure: A case-control study. *J Reprod Immunol.* 2021;145:103316.
 50. Schuckman H, DeJulius DP, Blanda M, Gerson LW, DeJulius AJ, Rajaratnam M. Comparison of intramuscular triamcinolone and oral prednisone in the outpatient treatment of acute asthma: a randomized controlled trial. *Ann Emerg Med.* 1998;31(3):333-8.
 51. Li T, Yuan Y, Liu H, Lu Q, Mu R. Glucocorticoids improve the pregnancy rate and outcome in women with unexplained positive autoantibodies: a systematic review and meta-analysis. *Frontiers in Medicine.* 2022;9:819406.
 52. Sun Y, Cui L, Lu Y, Tan J, Dong X, Ni T, et al. Prednisone vs placebo and live birth in patients with recurrent implantation failure undergoing in vitro fertilization: a randomized clinical trial. *Jama.* 2023;329(17):1460-68.
 53. Khonina NA, Broitman EV, Shevela EY, Pasman NM, Chernykh ER. Mixed lymphocyte reaction blocking factors (MLR-Bf) as potential biomarker for indication and efficacy of paternal lymphocyte immunization in recurrent spontaneous abortion. *Arch Gynecol Obstet.* 2013;288(4):933-7.
 54. Liang P, Mo M, Li GG, Yin B, Cai J, Wu T, et al. Comprehensive analysis of peripheral blood lymphocytes in 76 women with recurrent miscarriage before and after lymphocyte immunotherapy. *Am J Reprod Immunol.* 2012;68(2):164-74.
 55. Takakuwa K, Goto S, Hasegawa I, Ueda H, Kanazawa K, Takeuchi S, et al. Result of immunotherapy on patients with unexplained recurrent abortion: a beneficial treatment for patients with negative blocking antibodies. *Am J Reprod Immunol.* 1990;23(2):37-41.
 56. Wilczynski JR, Radwan P, Tchorzewski H, Banasik M. Immunotherapy of patients with recurrent spontaneous miscarriage and idiopathic infertility: does the immunization-dependent Th2 cytokine overbalance really matter? *Arch Immunol Ther Exp (Warsz).* 2012;60(2):151-60.
 57. Agrawal S, Kishore R, Halder A, Sharma A, Sharma RK, Das V, et al. Outcome of pregnancy in women with recurrent spontaneous abortion following immunotherapy with allogeneic lymphocytes. *Hum Reprod.* 1995;10(9):2280-4.

58. Behar E, Carp H, Livneh A, Gazit E. Anti-idiotypic IgM antibodies to anti-HLA class I antibodies in habitual abortion. *Am J Reprod Immunol.* 1991;26(4):143-6.
59. Cavalcante MB, Sarno M, Niag M, Pimentel K, Luz I, Figueiredo B, et al. Lymphocyte immunotherapy for recurrent miscarriages: Predictors of therapeutic success. *Am J Reprod Immunol.* 2018;79(6):e12833.
60. Regan L, Braude PR, Hill DP. A prospective study of the incidence, time of appearance and significance of anti-paternal lymphocytotoxic antibodies in human pregnancy. *Hum Reprod.* 1991;6(2):294-8.
61. Liu Z, Xu H, Kang X, Wang T, He L, Zhao A. Allogenic Lymphocyte Immunotherapy for Unexplained Recurrent Spontaneous Abortion: A Meta-Analysis. *Am J Reprod Immunol.* 2016;76(6):443-53.
62. Mowbray JF, Gibbings C, Liddell H, Reginald PW, Underwood JL, Beard RW. Controlled trial of treatment of recurrent spontaneous abortion by immunisation with paternal cells. *Lancet.* 1985;1(8435):941-3.
63. Wong LF, Porter TF, Scott JR. Immunotherapy for recurrent miscarriage. *Cochrane Database Syst Rev.* 2014;2014(10):CD000112.
64. Ghaebi M, Nouri M, Ghasemzadeh A, Farzadi L, Jadidi-Niaragh F, Ahmadi M, et al. Immune regulatory network in successful pregnancy and reproductive failures. *Biomedicine & Pharmacotherapy.* 2017;88:61-73.
65. Piccinni M-P. T cells in normal pregnancy and recurrent pregnancy loss. *Reproductive biomedicine online.* 2006;13(6):840-44.
66. Karami N, Boroujerdnia MG, Nikbakht R, Khodadadi A. Enhancement of peripheral blood CD56dim cell and NK cell cytotoxicity in women with recurrent spontaneous abortion or in vitro fertilization failure. *Journal of reproductive immunology.* 2012;95(1-2):87-92.
67. Li J, Gu Y, Zhang S, Ju B, Wang J. Effect of Prepregnancy Lymphocyte Active Immunotherapy on Unexplained Recurrent Miscarriage, Pregnancy Success Rate, and Maternal-Infant Outcome. *Biomed Res Int.* 2021;2021:7878752.
68. Ren J, Hou Y, Wang T. Roles of estrogens on myeloid-derived suppressor cells in cancer and autoimmune diseases. *Cell Mol Immunol.* 2018;15(7):724-26.
69. Liu S, Gu X, Weng R. Clinical effect of lymphocyte immunotherapy on patients with unexplained recurrent spontaneous abortion. *Immun Inflamm Dis.* 2021;9(4):1272-78.
70. Aslanian-Kalkhoran L, Kamrani A, Alipourfard I, Chakari-Khiavi F, Chakari-Khiavi A, Aghebati-Maleki L, et al. The effect of lymphocyte immunotherapy (LIT) in modulating immune responses in patients with recurrent pregnancy loss (RPL). *Int Immunopharmacol.* 2023;121:110326.
71. Zolfaghari MA, Motavalli R, Soltani-Zangbar MS, Parhizkar F, Danaii S, Aghebati-Maleki L, et al. A new approach to the preeclampsia puzzle; MicroRNA-326 in CD4(+) lymphocytes might be as a potential suspect. *J Reprod Immunol.* 2021;145:103317.
72. Chen S, Wang L, Fan J, Ye C, Dominguez D, Zhang Y, et al. Host miR155 promotes tumor growth through a myeloid-derived suppressor cell-dependent mechanism. *Cancer Res.* 2015;75(3):519-31.
73. Chatterjee S, Das S, Chakraborty P, Manna A, Chatterjee M, Choudhuri SK. Myeloid derived suppressor cells (MDSCs) can induce the generation of Th17 response from naive CD4+ T cells. *Immunobiology.* 2013;218(5):718-24.
74. Sarkesh A, Sorkhabi AD, Parhizkar F, Soltani-Zangbar MS, Yousefi M, Aghebati-Maleki L. The immunomodulatory effect of intradermal allogeneic PBMC therapy in patients with recurrent spontaneous abortion. *J Reprod Immunol.* 2023;156:103818.
75. Lima J, Guerreiro J, Ângelo-Dias M, Serra SS, Costa T, Marto N, et al. Efficacy of therapeutic interventions for idiopathic recurrent pregnancy loss: a systematic review and network meta-analysis. *Frontiers in Medicine.* 2025;12:1569819.
76. Liu Z, Xu H, Kang X, Wang T, He L, Zhao A. Allogenic lymphocyte immunotherapy for unexplained recurrent spontaneous abortion: a meta-analysis. *American Journal of Reproductive Immunology.* 2016;76(6):443-53.
77. Gupta SN, Palanivel V, Chari S, PS J. # 216: High Live Birth Rates After Low Dose Lymphocyte Immunization Therapy (LD-LIT) with Partner Lymphocytes in Recurrent Miscarriage Patients. *Fertility & Reproduction.* 2023;5(04):613-13.