Original Article

Iranian Journal of Immunology

December 2026; 23(4):

# Mucosal-Associated Invariant T (MAIT) Cells in Cases of Spontaneous and Recurrent Miscarriage

Doha H. Mahrous[1], Asmaa M. Zahran[2], Omnia El-Badawy[3,4], Ahmed M. Hamed[1], Asmaa S. Shaltout[3]*

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Assiut, Egypt; [2]Department of Clinical Pathology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt; [3]Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt; [4]Department of Basic Medical Sciences, Badr University in Assiut (BUA), Assiut, Egypt.

UNCORRECTED PROOF

## ABSTRACT

**Background:** Recurrent spontaneous miscarriage (RSM) is a reproductive complication in which immune dysregulation is thought to contribute to pregnancy loss. Although natural killer (NK) cells have been extensively studied, the role of mucosal-associated invariant T (MAIT) cells remains unclear.

**Objective:** To evaluate the distribution of phenotypically defined MAIT cells ($CD3^+V\alpha7.2^+CD161^{bright}$), NK subsets, and natural killer T (NKT)-like cells in the peripheral blood and decidual tissue of women with spontaneous miscarriage and RSM.

**Methods:** This exploratory, hospital-based case-control study included 39 women with first-trimester miscarriage (17 with RSM and 22 with spontaneous miscarriage) and 27 healthy pregnant controls. Peripheral blood was collected from all participants, and decidual tissue was obtained from miscarriage cases. Phenotypically defined MAIT cells ($CD3^+V\alpha7.2^+CD161^{bright}$ cells), NK cell subsets, and NKT-like cells were assessed by flow cytometry.

**Results:** Compared with healthy controls, women with miscarriage had higher circulating percentages of phenotypically defined MAIT cells ($p = 0.037$) and lower percentages of $CD3^+V\alpha7.2^+CD161^{dim}$ cells ($p = 0.011$). In decidual tissue, phenotypically defined MAIT cells were reduced, whereas $CD3^+V\alpha7.2^+CD161^{dim}$ and $CD3^+V\alpha7.2^+CD161^-$ cell populations were increased. $CD56^{bright}$ NK cells were enriched in the decidua, but $CD56^{dim}$ cells remained the predominant subset. Peripheral $CD3^+V\alpha7.2^+CD161^{dim}$ cells positively correlated with the number of previous miscarriages ($r = 0.348$, $p = 0.030$), whereas circulating $CD56^{bright}$ NK cells were negatively correlated with decidual MAIT and $CD3^+V\alpha7.2^+CD161^{dim}$ cells.

**Conclusion:** Miscarriage is associated with distinct systemic and decidual immune alterations involving phenotypically defined MAIT cells and NK cell subsets. These findings characterize the immune profiles associated with pregnancy loss. Further studies are needed to determine their prognostic or therapeutic relevance.

**Keywords:** Mucosal-Associated Invariant T (MAIT); Spontaneous; Recurrent Miscarriage

*Corresponding author:
Asmaa S. Shaltout, MD;
Department of Medical Microbiology & Immunology
Faculty of Medicine, Assiut University.
**Email:** asmaashaltout@aun.edu.eg

*Cite this article as:*
Mahrous DH, Zahran AM, El-Badawy O, Hamed AM, Shaltout AS. Mucosal-Associated Invariant T (MAIT) Cells in Cases of Spontaneous and Recurrent Miscarriage. *Iran J Immunol.* 2026; 23(4): ,
doi: 10.22034/iji.2026.112211.3265.

Received: 2026-07-19
Revised: 2026-10-31
Accepted: 2026-11-01

## INTRODUCTION

Spontaneous miscarriage is a common medical issue affecting women of reproductive age, with an estimated 23 million cases occurring globally each year (1). The emotional impact is particularly severe for women and couples who experience recurrent spontaneous miscarriage (RSM) (2). Although the overall prevalence of RSM in the general population is relatively low, it remains a deeply significant event for the individual woman who endures it (1).

Various immune cell types are thought to contribute to both spontaneous miscarriages and recurrent spontaneous miscarriages (RSM). Research indicates that women who experience miscarriages often have higher numbers and increased activity of Natural Killer (NK) cells in their blood and uterine lining. Multiple studies have demonstrated that elevated levels of uterine NK cells can create an inflammatory environment during implantation or later in pregnancy, which is a significant risk factor for recurrent miscarriage (3, 4).

Mucosal-associated invariant T (MAIT) cells are a subset of innate-like T cells known for their pro-inflammatory and cytotoxic functions. They are a significant component of the T cell population at mucosal surfaces and are most abundant in the liver, while constituting 0.1-10% of T cells in the bloodstream (5). MAIT cells are characterized by a semi-invariant T cell receptor (TCR) that carries the Vα7.2 α chain segment and pairs with a limited number of β chains. They recognize antigens presented by the MR1 protein (6).

MAIT cells, considered innate-like T lymphocytes, undergo functional modulation during pregnancy. In the decidua, their behavior and regulation more closely resemble those of conventional T cells, indicating controlled local immune activity. Conversely, in peripheral blood, MAIT cells show heightened functional activity, with characteristics that align more closely with innate immune responses (7).

A 2024 study by Wu et al. found that MAIT cells in the placentas of women experiencing preterm birth (PTB) are hyperactivated, as indicated by elevated levels of the marker CD69, compared with those in normal pregnancies. This heightened activation is linked to a functional shift: while these placental MAIT cells produce less of the key pro-inflammatory cytokine IFN-γ, they demonstrate increased immunosuppressive potential (8). This study is the first known investigation of MAIT cells in both spontaneous and RSM types of miscarriage.

The objective of this study was to analyze immune cells, including phenotypically defined MAIT cells, $CD3^{+}V\alpha7.2^{+}CD161^{dim/-}$ cells, NK cells, and NKT-like cells, in both peripheral blood and decidual tissue from patients who experienced either a spontaneous miscarriage or RSM. Because samples were obtained after miscarriage, the study was designed to characterize immune profiles associated with pregnancy loss rather than to determine whether these profiles preceded or caused it.

## PATIENTS AND METHODS

**Study populations**. This exploratory hospital-based case-control immunophenotyping study was approved by the Ethical Review Board of Assiut Medical School, Assiut University, Egypt (IRB No. 17101313). The study adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment.

The study included 39 women in their first trimester who presented with vaginal bleeding at a tertiary university hospital. Of these, 17 had a history of three or more recurrent spontaneous abortions occurring before 28 weeks of gestation (9), while 22 had experienced fewer than three spontaneous abortions. The current miscarriage was included in this count. All previous losses were clinically confirmed as intrauterine pregnancies through ultrasound visualization of an intrauterine gestational sac,

UNCORRECTED PROOF

thereby excluding biochemical pregnancy losses. The study also included 27 women with normal, uncomplicated pregnancies of comparable gestational age who attended the hospital for antenatal care follow-up visits as a control group.

Pregnant women were eligible for inclusion if they reported a stable marital relationship with the same partner, had a history of regular menstrual cycles before pregnancy, and conceived naturally. Women were excluded if any identifiable cause of recurrent miscarriage was present, including anatomical defects, infections, autoimmune factors, endocrine disorders, or chromosomal abnormalities.

All participants underwent thorough history taking, clinical examination, and ultrasound examination. A full history was obtained from each participant, including age, body mass index, residence, and drug therapy.

**Sampling procedures.** Samples were obtained from all women admitted to the emergency unit for evacuation of uterine contents for missed or inevitable miscarriage. Two mL whole blood samples were collected before evacuation, and decidua specimens were obtained by uterine wall curettage during evacuation. For the control group, only whole blood samples were collected.

Decidual specimens were rinsed with phosphate-buffered saline (PBS) and then kept in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, and 5% fetal bovine serum (GIBCO BRL, Thermo Fisher Scientific, USA) until transport to the laboratory.

**Flow cytometry methodology.** Tissue samples were finely minced and enzymatically digested with 3 mL of collagenase type Ia (Thermo Fisher Scientific, USA) and 2 mL of PBS for 40 min at 37°C in a shaking water bath at 100 r.p.m. The resulting cell suspension was filtered, and after centrifugation, the cell pellet was treated with red blood cell lysis buffer and then washed with PBS.

Both the processed decidua specimens and 100 µl of the whole blood samples were incubated with 10 µl of each of allophycocyanin (APC)-H7 conjugated anti-CD3(Becton Dickinson (BD), USA), phycoerythrin (PE) conjugated anti-CD56, V450 conjugated anti-CD16 (BD, USA), fluorescein 6-isothiocyanate (FITC) conjugated anti-Vα7.2 (TRAV1-2) (Beckman Coulter, France), and APC conjugated anti-CD161 (EXBIO, Czech Republic), for 15 min at 4°C in dark. Lysis solution was then added to the mixture, and the sample was centrifuged at 1500 r.p.m. for 1 min; the supernatant was discarded. The pellet was washed by adding PBS, then centrifuged at 1500 r.p.m. for 1 min, and the supernatant was discarded.

Flow cytometric analysis was performed on a FACSCanto flow cytometer using FACSDIVA 8.0.1 (BD, USA). The FACSCanto underwent daily performance checks and calibration with BD Cytometer Setup and Tracking (CS&T) beads, per the manufacturer's recommendations.

Events were first selected using a forward-scatter/side-scatter gate to obtain a lymphocyte-enriched population, followed by a gating strategy based on the surface expression of CD3 and Vα7.2 as MAIT cell lineage phenotypic markers. The developmental marker CD161 enabled evaluation of phenotypically defined MAIT cells ($CD3^{+}V\alpha7.2^{+}CD161^{bright}$), $CD3^{+}V\alpha7.2^{+}CD161^{dim,}$ and $CD3^{+}V\alpha7.2^{+}CD161^{-}$ cells. Because MR1 tetramer staining was not performed, $CD3+V\alpha7.2+CD161^{high}$ cells are referred to as phenotypically defined MAIT cells, and CD161 expression was used descriptively rather than as a definitive lineage marker. In addition, NKT-like cells ($CD3^{+}CD56^{+}CD16^{+}$) were measured within the $CD3^{+}$ population. Gating on $CD3^{-}$ lymphocytes, NK cell subsets were evaluated ($CD56^{bright}CD16^{bright/dim}$ and $CD56^{dim}CD16^{bright/dim}$). For each flow-cytometry variable, the denominator was the parent gate specified in the gating strategy. $CD56^{dim}$ and $CD56^{bright}$ NK-

UNCORRECTED PROOF

cell subsets were expressed as percentages of the total CD56-positive NK-cell gate. $CD56^{dim}$ NK cells comprise $CD56^{dim}$CD16bright and $CD56^{dim}CD16^{dim}$ cells, while $CD56^{bright}$ NK cells comprise $CD56^{bright}CD16^{bright}$ and $CD56^{bright}CD16^{dim}$ cells. Phenotypically defined MAIT ($CD3^{+}V\alpha7.2^{+}CD161^{bright}$), $CD3^{+}V\alpha7.2^{+}CD161^{-}$ and NKT-like cell percentages were expressed relative to their specified parent gates. The gating strategy is illustrated in Fig. 1.

*Statistical analysis*

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM).

Continuous variables were assessed for normality using the Shapiro–Wilk test. Normally distributed variables were compared with an independent-samples *t*-test and presented as mean ± standard deviation. Non-normally distributed variables were compared with the Mann–Whitney *U* test and reported as median with interquartile range. Categorical variables were presented as frequencies and percentages and compared using Pearson's chi-square test, Fisher's exact test, or the Fisher–Freeman–Halton exact test, as appropriate. Blood and decidual measurements from the same participant were compared using the Wilcoxon signed-rank test. The Spearman correlation coefficient was used, as appropriate, to assess associations between blood and decidual cell populations and other continuous study variables. A two-sided $p$ value $< 0.05$ was considered statistically significant.

## RESULTS

*Basic obstetric and clinical characteristics of women with recurrent and spontaneous miscarriage and the controls*

The table shows no significant differences in age, BMI, gestational age, residence, or obstetric history, indicating well-matched groups. Notably, lymphocyte counts are significantly lower in the study group ($p$=0.02), suggesting a potential difference in baseline immune status. Other variables, including residence, parity, previous evacuations, and basic labs, are comparable. Overall, most baseline characteristics are balanced, except for lymphocyte levels, which may influence immune-related outcomes (Table 1).

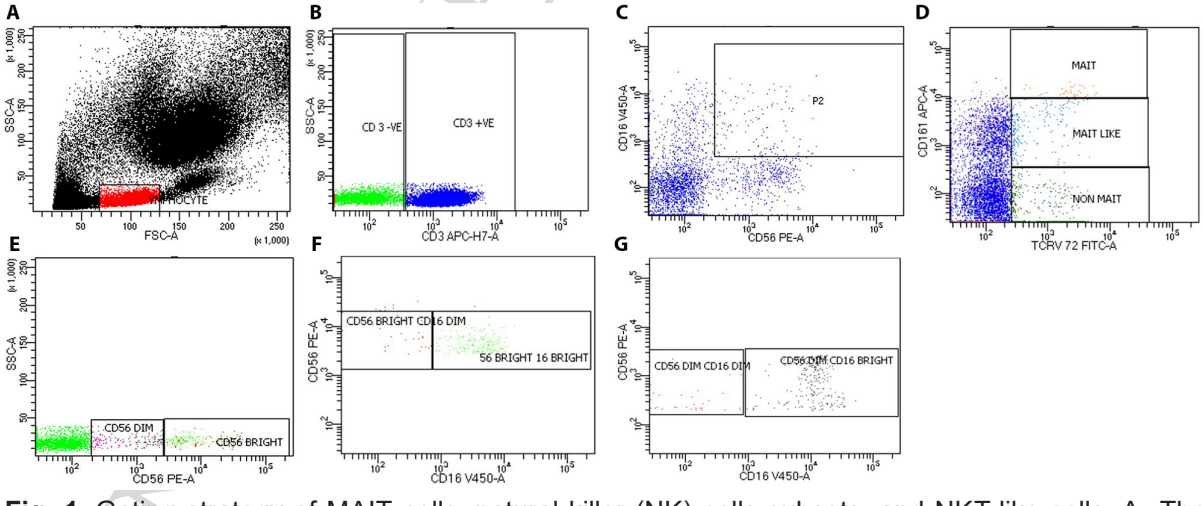



**Fig. 1.** Gating strategy of MAIT cells, natural killer (NK) cells subsets, and NKT-like cells: A- The lymphocyte population was selected by drawing a region on the forward scatter (FSC)/side scatter (SSC) dot plot; B-Then, regions were drawn to select $CD3^{+}$ and $CD3^{-}$ cells; C-The percentage of NKT-like cells (CD3+CD56+CD16+) was assessed within the $CD3^{+}$ population; D-The percentages of different T cell subsets within the $CD3^{+}$ population were assessed based on the relative expression of CD161 and Vα7.2 [$CD3^{+}V\alpha7.2^{+}CD161^{high}$ MAIT cells, $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells, and $CD3^{+}V\alpha7.2^{+}CD161^{-}$ cells]; E-G-The frequency of NK cell subsets was evaluated among $CD3^{-}$ lymphocytes ($CD56^{dim}CD16^{bright/dim}$) and ($CD56^{bright}CD16^{bright/dim}$) NK cells.

UNCORRECTED PROOF

**Table 1. Baseline characteristics of the study and control groups.**

| Variable | Study group (n=39) | Control group (n=27) | *p*-value |
|---|---|---|---|
| Age (years) | 28.00 (21.00–32.50) | 27.00 (23.00–30.00) | 0.969 |
| BMI (kg/m²) | 27.68 (25.63–29.72) | 30.10 (26.46–33.56) | 0.073 |
| GA (weeks) | 11.00 (9.00–12.00) | 10.00 (8.50–11.00) | 0.198 |
| Hemoglobin (g/dL) | 11.00 (9.85–12.00) | 10.75 (10.00–12.15) | 0.982 |
| Platelets (×10³/µL) | 222.56 ± 80.59 | 261.86 ± 70.35 | 0.053 |
| TLC (×10³/µL) | 9.90 (7.10–12.00) | 10.00 (6.47–13.75) | 0.609 |
| Lymphocytes % | 43.30 (24.60–58.00) | 76.30 (63.35–78.15) | 0.020* |
| Residence | | | 0.756 |
| Rural | 26 (66.7%) | 17 (63.0%) | |
| Urban | 13 (33.3%) | 10 (37.0%) | |
| Drug therapy | | | 1.000 |
| Yes | 1 (2.6%) | 0 (0.0%) | |
| No | 38 (97.4%) | 22 (81.5%) | |
| Missing | 0 (0.0%) | 5 (18.5%) | |
| Parity | | | 0.866 |
| P1 | 10 (25.6%) | 8 (29.6%) | |
| P2 | 9 (23.1%) | 6 (22.2%) | |
| P3 | 9 (23.1%) | 9 (33.3%) | |
| P4 | 6 (15.4%) | 3 (11.1%) | |
| P5 | 1 (2.6%) | 0 (0.0%) | |
| P0 | 4 (10.3%) | 1 (3.7%) | |
| Previous evacuation | | | 0.800 |
| 0 | 21 (53.8%) | 12 (44.4%) | |
| 1 | 10 (25.6%) | 10 (37.0%) | |
| 2 | 4 (10.3%) | 2 (7.4%) | |
| 3 | 4 (10.3%) | 3 (11.1%) | |

Continuous variables are presented as mean ± standard deviation (SD) when both groups met the normality assumption and as median (interquartile range [IQR]) otherwise. Categorical variables are presented as frequency (percentage). Missing data are reported separately and were excluded from the corresponding statistical analysis independent-samples t test was used for normally distributed continuous variables, and the Mann–Whitney U test was used for non-normally distributed variables. The chi-square test was used when expected cell counts were adequate; Fisher's exact test was used for sparse categorical tables. GA, gestational age; BMI, body mass index; TLC, total leukocyte count. * *P-value* < 0.05 was considered statistically significant.

*Analysis of the proportions of phenotypically defined MAIT, NK cell subsets, and NKT-like cells in the blood of patients and controls*

The patient group had a significantly higher percentage of phenotypically defined MAIT cells ($p$=0.037) than the control group, whereas $CD3^+V\alpha7.2^+CD161^-$ cells were significantly lower in patients ($p$=0.011). Other cell types showed no significant differences between the two groups (Table 2).

*Analysis of the proportions of phenotypically defined MAIT, NK, and NKT-like cells in the decidua compared with blood*

For comparisons between peripheral blood and decidual immune-cell populations, only participants with complete paired measurements from both compartments were included. Thus, the analysis presented in Table 3 was restricted to 33 complete blood–decidua pairs. The percentages of phenotypically defined MAIT cells were significantly lower in the decidua. However, $CD3^+V\alpha7.2^+CD161^{dim}$ and $CD3^+V\alpha7.2^+CD161^-$ cells were significantly higher in the decidua. $CD56^{dim}$ NK cells were significantly lower in the decidua than in peripheral blood, whereas $CD56^{bright}$ NK cells were significantly higher in the decidua. $CD56^{dim}$ NK cells had a higher percentage than $CD56^{bright}$ in both blood and

UNCORRECTED PROOF

**Table 2. Blood phenotypically defined MAIT, NK subsets and NKT like cells in the recurrent and spontaneous miscarriage patients and control group**

| Study group (n= 39) | Control group *P*-value (n= 27) |
|---|---|
| Phenotypically defined MAIT cells (%) 0.400 (.100-1.500) | 0.100 (0.100-0.300) 0.037* |
| $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells (%) 1.600(1.100-3.500) | 1.800 (1.00-3.200) 0.932 |
| $CD3^{+}V\alpha7.2^{+}CD161^{-}$ cells (%) 0.900(0.700-1.500) | 1.700(1.00-3.800) 0.011* |
| $CD56^{dim}$ NK cells (%) 88.90(83.60-91.90) | 86.80(79.20-93.60) 0.493 |
| $CD56^{dim}CD16^{bright}$NKcells (%) 19.40(10.00-50.80) | 28.40(15.40-48.60) 0.411 |
| $CD56^{dim}CD16^{dim}$ NK cells(%) 69.30(35.40-75.00) | 57.70(21.70-76.50) 0.368 |
| $CD56^{bright}$ NK cells (%) 7.30(3.80-10.90) | 8.60(4.90-17.80) 0.102 |
| $CD56^{bright}CD16^{bright}$ NK cells (%) 2.60(1.100-5.100) | 4.00(0.800-10.300) 0.159 |
| $CD56^{bright}CD16^{dim}$ NK cells (%) 3.50(1.60-4.70) | 4.20(2.00-7.30) 0.157 |
| NKT-like cell (%) 1.00 (0.400- 2.400) | 0.900(0.600-2.900) 0.457 |

**P*-value was significant if < 0.05, CD: cluster of differentiation; MAIT: Mucosal Associated Invariant T, NK: Natural killer cells.

**Table 3. Decidua vs. blood Phenotyically defined MAIT, NK, and NKT like cells in in the recurrent and spontaneous miscarriage patients**

| No. of pairs 33 | Blood median (iqr) | Decidua median (iqr) | *p* value |
|---|---|---|---|
| Phenotyically defined MAIT cells (%) | 0.400(.100-1.50) | 0.000(0.000-0.300) | <0.001* |
| $CD3^{+}V\alpha7.2^{+}CD161^{dim}$cells (%) | 1.60(1.10-3.50) | 10.20(1.800-37.550) | <0.001* |
| $CD3^{+}V\alpha7.2^{+}CD161^{-}$cells | 0.900(0.700-1.500) | 5.500(3.400-8.800) | <0.001* |
| $CD56^{dim}$ NK cells (%) | 88.90(83.60-91.90) | 51.40(35.050-81.050) | <0.001* |
| $CD56^{dim}CD16^{bright}$ NK cells (%) | 19.40(10.00-50.80) | 11.90(3.900-19.100) | 0.001* |
| $CD56^{dim}CD16^{dim}$ NK cells (%) | 69.30(35.40-75.00) | 33.700(22.20-67.650) | 0.026* |
| $CD56^{bright}$ NK cells (%) | 7.30(3.80-10.90) | 10.70(3.70-42.650) | 0.031* |
| $CD56^{bright}CD16^{bright}$ NK cells (%) | 3.05(1.02-6.00) | 0.100(0.000-0.800) | < 0.001* |
| $CD56^{bright}CD16^{dim}$ NK cells (%) | 3.65(1.80-5.60) | 10.60(3.650-40.600) | < 0.001* |
| NKT like cells (%) | 1.00(400-2.40) | 13.00(3.7500-37.150) | <0.001* |

**P*-value was significant if < 0.05. iqr: interquartile range, CD: cluster of differentiation; MAIT: Mucosal-Associated Invariant T, NK: Natural killer cells.

decidua; NKT-like cells were significantly higher in the decidua (Table 3).

*Analysis of the proportions of phenotypically defined MAIT, NK subsets, and NKT-like cells in blood and decidua by miscarriage frequency.*

In the patient group, 17 (43.59%) women had recurrent miscarriages, while 22 (56.41%) had spontaneous miscarriages. As shown in Table 4, most phenotypically defined MAIT, $CD3^{+}V\alpha7.2^{+}CD161^{dim}$, $CD3^{+}V\alpha7.2^{+}CD161^{-}$, and NK-cell subsets did not differ significantly between the two subgroups in peripheral blood. However, women with recurrent miscarriage had significantly higher percentages of $CD56^{dim}CD16^{bright}$ NK cells

UNCORRECTED PROOF

**Table 4. Blood Phenotyically defined MAIT, NK, and NKT like cells in patients based on the frequency of miscarriage**

| | Frequency of miscarriage | | *P*-value |
|---|---|---|---|
| | Recurrent (n= 17) Median(iqr) | Spontaneous (n= 22) Median(iqr) | |
| Phenotyically defined MAIT cells (%) | 0.300(0.050-1.700) | 0.450 (0.100-1.425) | 0.690 |
| $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells (%) | 2.100(1.200-3.600) | 1.400 (0.900-2.150) | 0.126 |
| $CD3^{+}V\alpha7.2^{+}CD161^{-}$ cells (%) | 1.200(0.650-2.550) | 0.850 (0.700-1.500) | 0.504 |
| $CD56^{dim}$ NK cells (%) | 89.300(83.25-91.400) | 88.500 (83.150-92.55) | 0.989 |
| $CD56^{dim}CD16^{bright}$ NK cells (%) | 26.00(17.150-61.950) | 17.400 (6.700-35.875) | 0.041* |
| $CD56^{dim}CD16^{dim}$ NK cells (%) | 60.800(23.600-71.700) | 73.150(53.650-75.100) | 0.079 |
| $CD56^{bright}$ NK cells (%) | 7.800(3.900-12.650) | 4.950 (1.600-10.275) | 0.228 |
| $CD56^{bright}CD16^{bright}$ NK cells (%) | 3.900(2.300-6.200 | 1.400 (0.225-4.300) | 0.025* |
| $CD56^{bright}CD16^{dim}$ NK cells (%) | 3.800(1.800-4.500) | 3.150(1.575-4.975) | 0.600 |
| NKT like cells (%) | 1.400(0.600-3.250) | 0.700 (0.200-1.375) | 0.027* |

**P*-value was significant if < 0.05. iqr : interquartile range, CD: cluster of differentiation; MAIT: Mucosal-Associated Invariant T, NK: Natural killer cells.

**Table 5. Decidua Phenotyically defined MAIT, NK, and NKT like cells in patients based on the frequency of miscarriage**

| | Frequency of miscarriage | | *P* value |
|---|---|---|---|
| | Recurrent (n= 15) Median (iqr) | Spontaneous (n= 18) Median (iqr) | |
| Phenotyically defined cells (%) | 0.000 (0.000-0.200) | 0.050 (0.000-0.450) | 0.212 |
| $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells (%) | 9.300 (1.200-27.600) | 13.900 (2.425-38.800) | 0.437 |
| $CD3^{+}V\alpha7.2^{+}CD161^{-}$ cells (%) | 6.100 (2.600- 8.800) | 5.450(3.850-9.575) | 0.928 |
| $CD56^{dim}$ NK cells (%) | 66.400 (27.700-93.100) | 49.950 (36.925-70.925) | 0.386 |
| $CD56^{dim}CD16^{bright}$ NK cells (%) | 11.900(3.300-19.400) | 10.550 (3.875-17.450) | 0.437 |
| $CD56^{dim}CD16^{dim}$ NK cells (%) | 45.600(16.400-73.700) | 33.650 (26.925-47.925) | 0.814 |
| $CD56^{bright}$ NK cells (%) | 21.800(0.300- 43.900) | 9.700 (4.350-38.525) | 0.828 |
| $CD56^{bright}CD16^{bright}$ NK cells (%) | 0.100 (0.000-0.800) | 0.450 (0.075-0.900) | 0.269 |
| $CD56^{bright}CD16^{dim}$ NK cells (%) | 21.800 (0.300-43.400) | 9.300 (3.975-37.500) | 0.842 |
| NKT like cell (%) | 7.100 (3.900-34.100) | 19.450 (3.575-38.100) | 0.613 |

**P*-value was significant if < 0.05. iqr: interquartile range, CD: cluster of differentiation; MAIT: Mucosal-Associated Invariant T, NK: Natural killer cells.

($p$ = 0.041), $CD56^{bright}CD16^{bright}$ NK cells ($p$ = 0.025), and NKT-like cells ($p$ = 0.027) compared with those with spontaneous miscarriage. Similarly, women with either spontaneous or recurrent miscarriages showed insignificant differences in the percentages of phenotypically defined decidual MAIT, NK cell subsets, and NKT-like cells (Table 5).

*Correlations of NK Cell subsets, phenotypically defined MAIT cells, and $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells in blood and decidua*

A significant positive correlation was observed between $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells in the blood and the number of abortions (r=0. 348, $p$=0.030). Conversely, a negative correlation was observed between $CD56^{bright}$ NK cells in the blood and both phenotypically defined MAIT cells (r=-0.507, $p$=0.003) and $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells (r=-0.379, $p$=0.030) in the decidua, as shown in Fig. 2.

## DISCUSSION

Many reports demonstrate a profound impact of immune cells, such as NK and T cells, on implantation fate (10, 11). However, the

UNCORRECTED PROOF

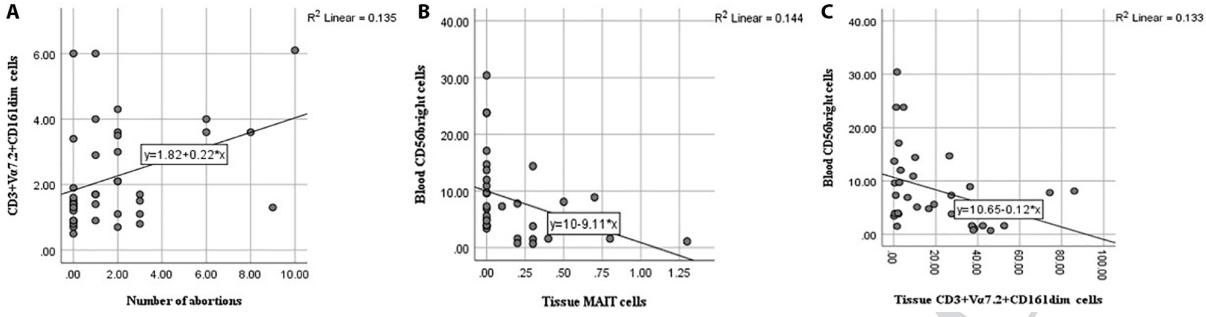



**Fig. 2.** Correlations among phenotypically defined MAIT, $CD3^+V\alpha7.2^+CD161^{dim,}$ and NK cell subsets in blood and decidua. (A): correlation between $CD3^+V\alpha7.2^+CD161^{dim}$ cells in the blood and the number of abortions; (B): correlation between $CD56^{bright}$ NK cells in the blood and phenotypically defined MAIT cells in decidua; (C): correlation between $CD56^{bright}$ NK cells in the blood and $CD3^+V\alpha7.2^+CD161^{dim}$ cells in decidua

potential role of MAIT and NKT-like cells in this process remains poorly defined. Data on changes in MAIT cell levels in spontaneous miscarriage and RSM in peripheral blood and decidua biopsies are scarce. The present study enrolled 39 women with miscarriages (study group). In addition, 27 women with normal, uncomplicated pregnancies of comparable gestational age were recruited as a control group. Compared with the control group, patients had significantly higher circulating phenotypically defined MAIT cells and lower $CD3^+V\alpha7.2^+CD161^-$ cells. Moreover, patients' decidua showed lower phenotypically defined MAIT cells and markedly higher $CD3^+V\alpha7.2^+CD161^{dim}$ and $CD3^+V\alpha7.2^+CD161^-$ cell frequencies than in blood, irrespective of miscarriage type (spontaneous or RSM).

To date, evidence on the role of MAIT cells in pregnancy-related complications remains limited. Nevertheless, one study suggested a potential association between MAIT cells and recurrent spontaneous miscarriage (RSM). The authors analyzed several decidual and peripheral leukocyte subsets, including NK cells, MAIT cells, and macrophages. Consistent with our findings, they reported a higher frequency of circulating MAIT cells in patients with RSM than in those with normal pregnancy. These cells exhibit a highly immune-activated phenotype indicative of an enhanced pro-inflammatory status. However, in the decidua, their analysis focused primarily on NK cells and macrophages and did not address potential changes in MAIT cells (12).

During the first trimester of normal pregnancy, MAIT cells are significantly less abundant in the decidua than in blood. However, decidual MAIT cells exhibit an immunomodulatory phenotype, with higher expression of the immune checkpoint markers PD-1 and CTLA-4 than in blood (7), suggesting that MAIT cells are regulated to balance immunotolerance and restrict unwanted T cell activation at the fetal-maternal interface.

In contrast, decreased PD-1 and increased perforin expression indicated an activated phenotype in peripheral blood MAIT cells from early-onset pre-eclamptic patients compared with those from normal pregnancy (13), reflecting a loss of immunomodulation. Another possible explanation for the decreased frequency of phenotypically defined MAIT cells in the decidua could be altered CD161 expression, shifting the distribution of $V\alpha7.2^+$ T cell phenotypes. Persistent exposure to bacterial antigens has been associated with functional exhaustion and alterations in MAIT cell frequency, as previously described in patients infected with HIV-1,14. Still, these mechanisms cannot be evaluated using the data obtained in our study. Supporting this hypothesis, our study demonstrated a significant increase in $CD3^+V\alpha7.2^+CD161^{dim}$ and $CD3^+V\alpha7.2^+CD161^-$ cells within the decidua.

UNCORRECTED PROOF

Previous studies have reported that these phenotypically defined populations may show increased expression of activation markers and enhanced cytotoxic potential compared with MAIT cells (13,14), suggesting that their increased representation in the decidua may contribute to a locally activated immune environment. However, given the cross-sectional nature of our study, these findings do not establish a direct phenotypic transition or conversion of phenotypically defined MAIT cells into $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ or $CD3^{+}V\alpha7.2^{+}CD161^{-}$ cells.

Although no meaningful differences were detected in peripheral $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cell levels between patients and controls, or between patients with spontaneous vs. RSM in blood or decidua, a positive correlation was observed between the percentage of $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells and the number of miscarriages. Additionally, decidual $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells inversely correlated with blood $CD56^{bright}$ NK cells, a regulatory subset critical for vascular remodeling (15). These associations may suggest a relationship between $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells and immune-regulatory alterations associated with pregnancy loss. However, the present cross-sectional data cannot establish a causal or disease-amplifying role.

During early pregnancy, decidual natural killer (NK) cells are the predominant leukocyte population at the maternal–fetal interface. Unlike peripheral NK cells, these decidual cells have reduced cytotoxic activity but actively produce immunoregulatory mediators and cytokines essential for spiral artery remodeling, trophoblast invasion, and proper fetal development (15).

$CD56^{dim}$ NK cells are highly granular and cytotoxic. Conversely, $CD56^{bright}$ NK cells are characterized by robust cytokine production and contain fewer lytic granules, indicating a primarily immunoregulatory function, whereas $CD56^{dim}$ NK cells serve predominantly cytotoxic roles (16).

$CD56^{bright}CD16^{dim}$ NK cells predominate in the endometrium during the luteal phase, providing regulatory activity critical for maintaining pregnancy (17). Our findings align with this profile: $CD56^{bright}$ NK cells were significantly enriched in the decidua compared with blood, consistent with active recruitment during decidualization (18).

In decidual samples from women with miscarriage, $CD56^{dim}$ NK cells remained the predominant NK cell subset, regardless of miscarriage type. This finding indicates a difference in the distribution of NK cell subsets. Notably, the $CD56^{dim}CD16^{dim}$ frequency exceeded that of the $CD56^{dim}CD16^{bright}$ subset. However, because decidual tissue from healthy pregnancies was not available, we cannot determine whether this distribution differs from that of normal pregnancy or reflects changes occurring after pregnancy loss. One hypothesis, which requires functional confirmation, is that activated CD56dimCD16bright NK cells lose CD16 expression (19,20). Similar dysregulation of phenotypically defined MAIT cells may further contribute to an inflammatory decidual environment and impair the immune mechanisms that normally support pregnancy maintenance.

Additionally, the inverse correlation between circulating $CD56^{bright}$ NK cells and decidual $CD3^{+}V\alpha7.2^{+}CD161^{dim}$ cells indicates that dysregulation of these immune populations may be coordinated and may contribute to recurrent spontaneous miscarriage.

In the present study, most circulating phenotypically defined MAIT, $CD3^{+}V\alpha7.2^{+}CD161^{dim}$, $CD3^{+}V\alpha7.2^{+}CD161^{-}$, and NK-cell populations did not differ significantly between women with RSM and those with spontaneous miscarriage. However, significantly higher levels were observed in $CD56^{dim}CD16^{bright}$ NK cells, $CD56^{bright}CD16^{bright}$ NK cells, and NKT-like cells in RSM. The predominance of nonsignificant findings may indicate that several immune alterations accompanying miscarriage are shared features of pregnancy loss rather than changes specific to recurrence.

UNCORRECTED PROOF

Nevertheless, RSM is a heterogeneous clinical and biological condition, and immune abnormalities may be present only in a subset of affected women. Moreover, the relatively small sample size, single-center design, cross-sectional nature of the study, and assessment of immune cells after pregnancy loss may have limited the ability to detect modest differences between the two groups. Accordingly, the absence of significant differences in most populations should not be interpreted as evidence of complete biological equivalence between RSM and spontaneous miscarriage.

NKT-like cells play an important role in maintaining pregnancy. Although they are absent from the nonpregnant endometrium (21), physiological studies have shown their accumulation in the decidua during early pregnancy (22). Dysregulation of these cells has also been associated with disruption of the Th1/Th2 cytokine balance and the development of a pro-inflammatory environment in RSM (23). In our study, NKT-like cells were significantly more abundant in the peripheral blood of women with RSM than in those with spontaneous miscarriage. They were further enriched in decidual tissue compared with peripheral blood.

Previous studies examining NKT-like or $CD3^+CD56^+CD16^+$ populations in miscarriage and recurrent pregnancy loss have reported inconsistent findings. Hosseini *et al.* found a lower frequency of $TCR\alpha\beta^+$ NKT-like cells but a higher frequency of $CD16^+$ NKT-like cells in women with unexplained recurrent spontaneous abortion compared with fertile controls (24). Van den Heuvel *et al.* reported that elevated circulating $CD3^+CD56^+$ NKT-cell numbers were associated with poorer reproductive outcomes. These numbers declined following successful treatment (25). Zhou et al. found that higher circulating $CD3^+CD56^+CD16^+$ NKT-like cell frequencies predicted better IVF outcomes (26). This heterogeneity across studies likely reflects differences in gating strategy, patient population, and sampling timing.

*Strengths and Limitations*

This study has several important strengths. To the best of our knowledge, it is the first to provide a comprehensive comparative analysis of phenotypically defined MAIT cells, NK cell subsets, and NKT-like cells in paired peripheral blood and decidual samples from women with spontaneous and recurrent miscarriage, offering valuable insights into both systemic and local immune alterations associated with pregnancy loss. However, several limitations should be acknowledged. MAIT cell identity was not confirmed by MR1 tetramer staining. The relatively small sample size and single-center design may limit the generalizability of the findings. In addition, the absence of decidual tissue from healthy pregnant controls, owing to ethical constraints, precludes definitive conclusions regarding the observed decidual immune cell alterations. Furthermore, the study did not include long-term follow-up to evaluate the relationship between these immune cell populations and subsequent pregnancy outcomes. No therapeutic interventions were investigated; therefore, the clinical applicability of these findings for guiding treatment remains to be established. The flow cytometry panel did not include a fluorescent viability dye, CD45 staining, fluorescence-minus-one controls, or doublet discrimination. Doublets may falsely increase double-positive events. However, viability was >90% by Trypan Blue, populations were identified within lineage-defined gates, and identical processing and gating were applied to all groups. Finally, the functional characteristics of the dysregulated immune cell populations, such as cytokine production and cytotoxic activity, were not assessed, limiting mechanistic interpretation of their role in miscarriage. Future multicenter studies with larger cohorts, longitudinal follow-up, and functional immune profiling are warranted to validate and extend these findings.

UNCORRECTED PROOF

## CONCLUSIONS

In this study, women with miscarriage showed differences in phenotypically defined MAIT, $CD3^{+}V\alpha7.2^{+}CD161^{dim}$, and NK-cell frequencies in blood and decidual tissue. These findings suggest that immune changes may accompany pregnancy loss; however, they do not establish whether these changes contributed to miscarriage or occurred afterward. Similar findings in women with recurrent and non-recurrent miscarriage may indicate shared immune features, although the small sample size and patient variability should be considered. Larger longitudinal studies using functional assays are needed to clarify the significance of these immune populations and their possible prognostic or therapeutic relevance.

## ACKNOWLEDGMENT

The authors acknowledge receipt of financial support for the research from Assiut Faculty of Medicine Research Grant (Code ????) Office.

## AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed in this study are available from the corresponding author upon reasonable request.

## REFERENCES

1. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, et al. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. The Lancet. 2021 May 1;397(10285):1658-67.
2. Coomarasamy A, Dhillon-Smith RK, Papadopoulou A, Al-Memar M, Brewin J, Abrahams VM, et.al. Recurrent miscarriage: evidence to accelerate action. The Lancet. 2021 May 1;397(10285):1675-82.
3. Giuliani E, Parkin KL, Lessey BA, Young SL, Fazleabas AT. Characterization of uterine NK cells in women with infertility or recurrent pregnancy loss and associated endometriosis. American journal of reproductive immunology. 2014 Sep;72(3):262-9.
4. Wang F, Jia W, Fan M, Shao X, Li Z, Liu Y,et. al. Single-cell immune landscape of human recurrent miscarriage. Genomics, proteomics & bioinformatics. 2021 Apr;19(2):208-22.
5. Nel I, Bertrand L, Toubal A, Lehuen A. MAIT cells, guardians of skin and mucosa? Mucosal immunology. 2021 Jul 1;14(4):803-14.
6. Huang S, Martin E, Kim S, Yu L, Soudais C, Fremont DH, Lantz O, et.al. MR1 antigen presentation to mucosal-associated invariant T cells was highly conserved in evolution. Proceedings of the National Academy of Sciences. 2009 May 19;106(20):8290-5.
7. Raffetseder J, Lindau R, Van der Veen S, Berg G, Larsson M, Ernerudh J. MAIT cells balance the requirements for immune tolerance and anti-microbial defense during pregnancy. Frontiers in immunology. 2021 Aug 9;12:718168.
8. Wu M, Li F, Zhou T, Zhao J, Jiang L, Zhang H, Wang W, Cheng X, Wu X, Xiong M, Weng X. Dysregulation of mucosal-associated invariant T cells correlates with altered placental microenvironment in preterm birth. Molecular Human Reproduction. 2024 Mar 1;30(3):gaae006.
9. La X, Wang W, Zhang M, Liang L. Definition and multiple factors of recurrent spontaneous abortion. In Environment and Female Reproductive Health 2021 Feb 2 (pp. 231-257). Singapore: Springer Singapore
10. Kong CS, Ordoñez AA, Turner S, Tremaine T, Muter J, Lucas ES,et.al. Embryo biosensing by uterine natural killer cells determines endometrial fate decisions at implantation. The FASEB Journal. 2021 Apr;35(4):e21336.
11. Hu X, Wang Y, Mor G, Liao A. Forkhead box P3 is selectively expressed in human trophoblasts and decreased in recurrent pregnancy loss. Placenta. 2019 Jun 1;81:1-8.
12. Wang F, Jia W, Fan M, Shao X, Li Z, Liu Y, et.al. Single-cell immune landscape of human recurrent miscarriage. Genomics, proteomics & bioinformatics. 2021 Apr;19(2):208-22.
13. Meggyes M, Szanto J, Lajko A, Farkas B, Varnagy A, Tamas P,et.al. The possible role of CD 8+/Vα7. 2+/CD 161++ T (MAIT) and CD 8+/Vα7. 2+/CD 161lo T (MAIT-like) cells in the pathogenesis of early-onset pre-eclampsia. American Journal of Reproductive Immunology. 2018 Feb;79(2):e12805.
14. Leeansyah E, Ganesh A, Quigley MF,

UNCORRECTED PROOF

Sönnerborg A, Andersson J, Hunt PW,et.al. Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. Blood, The Journal of the American Society of Hematology. 2013 Feb 14;121(7):1124-35.
15. Ander SE, Diamond MS, Coyne CB. Immune responses at the maternal-fetal interface. Science immunology. 2019 Jan 11;4(31):eaat6114.
16. Poli A, Michel T, Thérésine M, Andrès E, Hentges F, Zimmer J. CD56bright natural killer (NK) cells: an important NK cell subset. Immunology. 2009 Apr;126(4):458-65.
17. Bulmer JN, Lash GE. Uterine natural killer cells: time for a re-appraisal?. F1000Research. 2019 Jul 2;8:F1000-aculty.
18. van den Heuvel M, Peralta C, Bashar S, Taylor S, Horrocks J, Croy BA. Trafficking of peripheral blood CD56bright cells to the decidualizing uterus—new tricks for old dogmas?. Journal of reproductive immunology. 2005 Oct 1;67(1-2):21-34.
19. El-Badawy O, Helmy AS, Abbas AM, Zahran AM, Afifi NA, Abdel-Rahim MH. Concordance between peripheral and decidual NK cell subsets and killer immunoglobulin-like receptors in women with recurrent spontaneous miscarriages. Journal of reproductive immunology. 2020 Aug 1;140:103130.
20. Amand M, Iserentant G, Poli A, Sleiman M, Fievez V, Sanchez IP, Sauvageot N, Michel T, Aouali N, Janji B, Trujillo-Vargas CM. Human CD56dimCD16dim cells as an individualized natural killer cell subset. Frontiers in immunology. 2017 Jun 19;8:699.
21. Ito K, Karasawa M, Kawano T, Akasaka T, Koseki H, Akutsu Y, et.al. Involvement of decidual Vα14 NKT cells in abortion. Proceedings of the National Academy of Sciences. 2000 Jan 18;97(2):740-4.
22. Boyson JE, Rybalov B, Koopman LA, Exley M, Balk SP, Racke FK,et. al. CD1d and invariant NKT cells at the human maternal–fetal interface. Proceedings of the National Academy of Sciences. 2002 Oct 15;99(21):13741-6.
23. Khalaf WS, Mahmoud MR, Elkhatib WF, Hashem HR, Soliman WE. Phenotypic characterization of NKT-like cells and evaluation of specifically related cytokines for the prediction of unexplained recurrent miscarriage. Heliyon. 2021 Nov 1;7(11).
24. Hosseini S, Shokri F, Ansari Pour S, Khoshnoodi J, Jeddi-Tehrani M, Zarnani AH. Diminished frequency of menstrual and peripheral blood NKT-like cells in patients with unexplained recurrent spontaneous abortion and infertile women. Reproductive Sciences. 2019 Jan;26(1):97-108.
25. van den Heuvel MJ, Peralta CG, Hatta K, Han VK, Clark DA. Decline in number of elevated blood $CD3^{+}CD56^{+}$ NKT cells in response to intravenous immunoglobulin treatment correlates with successful pregnancy. American Journal of Reproductive Immunology. 2007 Nov;58(5):447-459.
26. Zhou J, Zhao X, Wang Z, et al. High circulating $CD3^{+}CD56^{+}CD16^{+}$ natural killer-like T cell levels predict a better IVF treatment outcome. Journal of Reproductive Immunology. 2013;97(2):197-203.

UNCORRECTED PROOF