Disease and Diagnosis

→ **Original Article**

The Association of NKp46-Positive uNK Cells With a Higher Risk of Recurrent Miscarriage and IVF Failure

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Abstract

Background: Uterine natural killer (uNK) cells have a significant impact on pregnancy and related complications. Given the importance of receptors in the activity of uNK cells, the present study aimed to determine the number of uNK cells and NKp46 (one of the most important NK cell-activating receptors) expression in the endometrium of women with recurrent miscarriage (RM) or a history of in vitro fertilization (IVF) failure.

Materials and Methods: This case-control study was performed on 48 participants, including 16 healthy controls, 27 cases with RM, and 5 cases with repeated implantation failure (RIF) during the mid-luteal phase according to a standardized diagnostic protocol. All participants were assessed using transvaginal ultrasound to determine embryo survival rate and confirm gestational age. Endometrial specimens were collected and subjected to immunohistochemistry (IHC) staining using an anti-human NKp46 antibody expressed by uNK cells.

Results: A significantly higher number of cells positive for NKp46 was obtained among two groups of cases versus healthy subjects (patients: 1.46 ± 0.78, controls: 0.82 ± 62, $P = 0.006$), and the number of CD56 + cells was significantly higher in patients than in controls (patients: 18.14 ± 7.14, controls: 11.71 ± 6.17, $P = 0.003$). Additionally, there was not a significant difference in the frequency ratio of NKp46 + NK cell subset to CD56 + uNK cells between the patients ($P = 0.59$) and control healthy group.

Conclusion: The increase in the number of uterine NK cells and their cytotoxic activity during implantation and early pregnancy, possibly resulting from an excessive expression of inflammatory cytokines, confirms a significant association between uNK cell activity and a higher risk of RM and RIF. Therefore, immunomodulatory treatments may benefit these patients.

Keywords: Natural killer cells, NCR1, Abortion, Recurrent implantation failure, Immunohisto chemistry

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Introduction

Endometrial receptivity is one of the critical biological processes attributed to successful pregnancy and embryo transfer (1). Abnormality in endometrial function can lead to various reproductive issues such as infertility, a tendency towards recurrent miscarriage (RM), and repeated failure of implantation. The etiology of spontaneous abortion remains unknown in approximately 50% of all subjects with RM and recurrent in vitro fertilization (IVF) failure. However, changes in the uterine environment are considered to be associated with these idiopathic disorders (2, 3). A variety of risk drivers, including endocrine disorders, anatomical and chromosomal abnormalities, and hemostatic imbalance, were attributed to spontaneous abortion. More importantly, the immune system and

immunological mediators were demonstrated to be involved in this condition. However, although there is accumulating evidence demonstrating the important role of immunological mediators in more than 50% of RM subjects, physicians only use the antiphospholipid syndrome screening test (4). IVF is a commonly-used procedure of assisted-reproductive technology. However, despite breakthroughs in assisted-reproductive technology, there is still one successful pregnancy out of three IVF cycles (5). Repeated implantation failure (RIF) is a clinical phenomenon characterized by a lack of implantation after the transfer of several embryos into the uterus. On the other hand, labor resembles an inflammatory response that includes the secretion of numerous cytokines/chemokines from the resident and infiltrating immune cells into the maternal/fetal

interface. Importantly, uterine natural killer (uNK) cells were demonstrated to be the largest proportion of resident leukocytes in the endometrium which play a protective role during pregnancy (6).

uNK cells are supposed to be CD56bright CD16-, which identify the trophoblast-specific HLA-G molecule through the expression of killer-cell immunoglobulin-like receptors (KIRs). uNK cells either stem from peripheral natural killer or are differentiated from CD34-positive cells present in the uterus under humoral effect during the first trimester of pregnancy (7). Lymphoid cells represent more than 40% of all human decidual immune cell subpopulations in early pregnancy, 50% to 90% of which are uNK cells (8). However, the number of uNK cells decreases in the second and third trimesters. There are controversial opinions about uNK functional activities, including cytotoxicity, production of cytokines and/or cytokine receptors, and gene expression, in recurrent abortion and IVF failure (9-11). Research has shown that uNK cell activity in the endometrium is regulated by both inhibitory receptors (such as NKG2A) and activating receptors (such as NKp30 and NKp46). (9). Some studies have indicated increased infiltration of uNK cells in women with abortion (8, 10, 12). However, there are studies demonstrating no change in the number of uNK cells in women with abortion (13). There are limited studies evaluating the expression levels of NKp46 (CD335 or NCR1), exclusively expressed by activated uNK cells, in non-fertile women with a history of recurrent spontaneous miscarriage and IVF failure. This study aimed to evaluate the number of uNK cells and cells expressing NKp46 in the endometrium of women with a history of RM or IVF failure considering the significant role of NKp46 in uNK cell cytotoxicity.

Materials and Methods
Study Design
This is a case-control study that was carried out between January and June 2019 to determine the number of uNK cells and NKp46 expression in women with RM or a history of IVF failure referred to Imam Khomeini Hospital in Ahvaz, southwest Iran.

Participants
A total of 48 participants, aged 20 to 51 years, participated in this study. The participants included 16 healthy controls (normal pregnancy with ≥ one previous successful pregnancy and no history of abortion or infertility), 27 cases (with ≥ two previous unexplained recurrent abortions confirmed by vaginal ultrasound and hysteroscopy), and 5 cases (with ≥ three IVF failures). Unexplained pregnancy loss is defined as two or more spontaneous abortions before the 20th week of pregnancy.

Inclusion and Exclusion Criteria

Potential participants, in both case and control groups, were eligible for the study if they met the following inclusion criteria: being non-smoker and non-alcohol user with an anatomically normal uterus, not having uterine infection, cancer, thyroid dysfunctions, systemic lupus erythematosus, serum anti-ß2 glycoprotein, and anti-cardiolipin antibodies, being in the luteal phase of the cycle during sampling, receiving no hormonal medication in the preceding 6 months, and having a history of at least two miscarriages or three unsuccessful cycles of IVFs. Participants in the control group should have had no history of miscarriage and a minimum of one successful pregnancy. Patients with septic miscarriage, recorded endocrinopathies such as thyroid or prolactin abnormalities, diabetes, uterine anomalies, cancer, and a history of hormonal contraception use during the last 6 months before the last pregnancy were excluded from the study.

Procedure
After obtaining written informed consent, eligible patients were included in the study. Endometrial biopsies were performed using a pipelle suction curette during the mid-luteal phase (on days 21 to 24 of the menstrual cycle).

Immunohistochemistry
Immunohistochemistry (IHC) was used to assess the expression profile of CD56 and NKp46. In brief, endometrial biopsy specimens were immersed in 10% neutral-buffered formalin to fix for nearly 24 hours, embedded in paraffin wax, and cut into 3-4 μm thick sections with a microtome (Leica RM2235; Leica, Wetzlar, Germany). All paraffin-embedded sections were mounted onto 3-Triethoxysilane-propylamin slides (Sigma Chemical Co.; Poole, UK), dewaxed in xylene, and rehydrated in descending concentrations of alcohol. Antigen retrieval was performed using a microwave (800W) for 25 minutes (10). After washing, tissue sections were quenched in 3% hydrogen peroxide diluted in methanol for 7 minutes to inhibit endogenous peroxidase activity. Slides were washed in PBS and incubated with protein blocker (Biopharmadx, Germany) (10 minutes for CD56 and 1 minute for NKp46). Afterwards, the primary antibodies were added to CD56 monoclonal mouse anti-human CD56 antibody (diagnostic bio-systems, Pleasanton, CA, USA) for 90 minutes at 37°C and to NKp46/NCR1 polyclonal goat anti-human NKp46 antibody (R&D Systems, Minneapolis, MN, USA) at 3 μg/mL, and incubated overnight at + 4 °C in a humidified chamber, respectively. Samples were incubated for 1 hour with the HRP-labeled secondary antibody (rabbit anti-IgG mouse for CD56 and donkey anti-IgG goat antibody) at 37 °C. The peroxidase reaction was achieved with chromogen DAB (3.3′ diaminobenzidine

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tetrahydrochloride; Biopharmadx, Germany) and discontinued by adding water after 5 minutes. Finally, the slides were counterstained with hematoxylin (DAKO Corporation, Carpinteria, CA), dehydrated in ascending concentrations of alcohol, cleared in xylene, mounted with a non-aqueous mounting medium (Entellan, Merck, German), and verified by optical microscopy. Positive specimens (lymphoma tissue) were used for every set to assess the validity of the immunostaining method. In addition, the phosphate-buffered saline (PBS) buffer, instead of primary antibodies, was used as the negative control. Two experienced pathologists independently analyzed all samples using microscopes (Optika, Italy). Endometrial glands and stroma were evaluated for confirmation of the day of the menstrual cycle according to Noyes et al (14). All stained cells of 10 views of each specimen were counted under a microscope at 400x magnification. The ratio between positive, CD56 or NKp46 cells (brown stain) and total endometrial stromal cells (blue stain) were calculated.

Statistical Analysis

The data collected in this study were analyzed using Prism version 7. Data were represented as mean ± standard deviation of the mean (SD) in the case of normally distributed raw data or median (IQR) for non-normal distribution. Mann-Whitney test was used to compare data obtained from women with RM or RIF with those obtained from the control group. P values less than 0.05 were considered to be statistically significant.

Results
Demographic Characteristics

A total of 48 participants, including patient and control groups, with a history of RM or RIF were enrolled in this study. Table 1 indicates the demographic characteristics of the women with reproductive failure (the patient group) and fertile women (the control group). No significant differences were found in the age or BMI of women between the two groups ($P > 0.05$).

The Percentage of CD56-Positive Cells in the Patient

Table 1. Demographic Data of Patient and Control Groups

	Case (n = 32)	Control (n = 16)
Age (y)	30.9 ± 6.0	35.6 ± 4.4
Number of gravidities	2.5 (2-3.75)	3 (3-4.75)
Number of deliveries	0 (0-1)	3 (3-4.75)
Number of miscarriages	2 (2-3)	0
Gestational age of miscarriages (wk)	8 (6-10)	
Time after last miscarriage (mon)	11 (5-15)	
BMI (kg/m²)	27.5 ± 4.1	29.0 ± 2.7

BMI, body mass index.
Note: All data are shown as mean ± standard deviation or median (IQR).

and Control Groups

Figure 1 shows IHC staining for CD56 marker in women with RIF and IVF failure as compared with the fertile women. A significantly higher number of CD56-positive uNK cells were found in the endometrial samples obtained from females with infertility and recurrent abortion as compared with those obtained from controls as shown in Figure 2 (patients: 18.14 ± 7.14 and controls: 11.71 ± 6.17; $P = 0.003$).

The Percentage of Nkp46-Positive Cells in the Patient and Control Groups

Figure 3 shows IHC staining for the NKp46 marker in women with RIF and IVF failure as compared with fertile women.

A significantly higher number of NKp46-positive cells was found in the patient group as compared with the control group (patients: 1.46 ± 0.78, controls: 0.82 ± 0.62, Figure 4), showing a statistically significant difference between the two means ($P = 0.006$).

The Percentage Ratio of Nkp46- and CD56-Positive Cells

As illustrated in Figure 5, the percentage ratio of NKp46-positive cells to CD56-positive cells in the endometrial stroma was found to be 0.08 ± 0.5 and 0.07 ± 0.6 in the patient and control groups, respectively. However, this difference was not statistically significant ($P = 0.59$).

Discussion

Uterine natural killer cells produce angiogenic factors during the first trimester of gestation, presumably playing a significant role in the successful implantation. In addition, uNK cells were found to be associated with human reproductive disorders, especially repeated miscarriage, recurrent implantation failure, preeclampsia, and fetal growth restriction (15). This study aimed to examine the number of uNK cells and the expression profile of NKp46 in the endometrium of women with a history of RM or IVF failure. The CD56 marker is classified as an isomorph of the neural cell adhesion molecule, which is expressed on neural-originated cells, conventional cytotoxic T lymphocytes, and NK cells. Both peripheral blood and endometrium are sources of natural killer cells that express the surface marker CD56 (16,17). Studies revealed that about 80% of the uNK cells express the CD56 bright CD16- phenotype. CD56-positive cells were demonstrated to have a regulatory action, although the NCR1 or NKp46-positive cells display a cytotoxic activity (18, 19). Studies demonstrated that increased cytotoxicity level of peripheral blood NK cells in human beings is associated with an increased risk of spontaneous abortion (20). Patients aged less than 35 years with unexplained recurrent abortion demonstrated a lower risk of spontaneous abortion as compared with those aged over 35 years (21). The BMI of patients participated
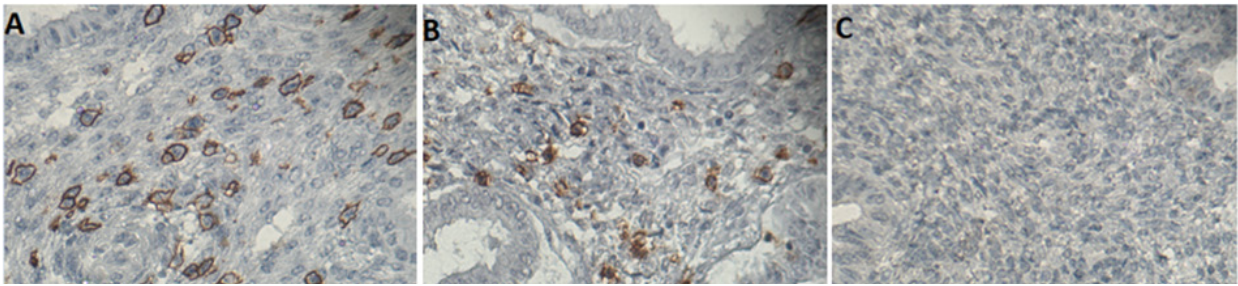
Figure 1. Immunohistochemistry Staining to Detect the CD56 Marker in Women With Recurrent Miscarriage and IVF Failure and Fertile Women. An illustration of cell surface localization of CD56 in endometrial stroma derived from (A) patients, (B) fertile women, and (C) negative control (treated with PBS buffer instead of specific primary antibody) (original magnification × 400)

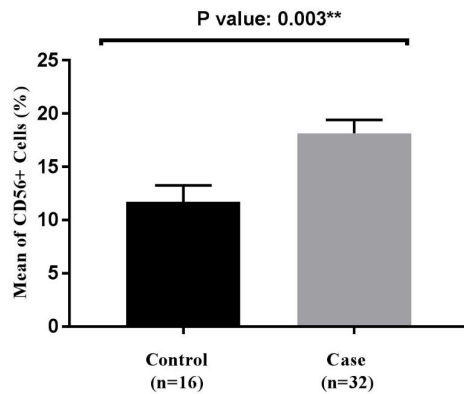**Figure 2.** The Differences Between CD56-Positive Cells in the Endometrial Stroma Derived From Patients With Recurrent Miscarriage and IVF Failure as Compared With Fertile Women. **CD56 expression was significantly higher in the patient group as compared with fertile women

in this study was calculated to be 27.58 ± 4.19 kg/m^2. This could be related to the standards used in this study, where we excluded a number of risk factors that render the patients susceptible to RM or IVF failure, especially diabetes mellitus, obesity, and thyroid abnormalities (22).

Furthermore, as compared with healthy fertile women, evidence from our study on representative RM and RIF disorders indicated immunological changes, especially inducible expression of activating NKp46 receptor on NK cells along with an increased number of corresponding CD56 bright NK cells in these high-risk subjects. In fact, it can be concluded-that NK cells are necessary for embryo implantation and successful pregnancy despite controversial previous evidence. Accordingly, there were contradicting empirical results from different studies. For example, Giuliani et al (10) detected a high expression level of CD16 and NKp46 positive uNK subsets in individuals with a history of abortion but they did not find any difference in CD56, which is not consistent with the findings of this study and the study conducted by Gao and Wang (23) in which a high expression level of CD56 was detected in unexplained recurrent spontaneous abortion. Liu et al also showed no correlation between the number of uNK cells and the outcomes of pregnancy in women with a history of RM and IVF failure (24). However, in another study (25), decreased expression

of NKp46 in the blood and endometrium of women with an experience of abortion was reported. The main reason behind these different findings is not clear however increased expression of NKp46 is more reliable because activation of NK results in more expression of the activator NKp46 receptors in targeted cases. This has been approved through mAbmediated blocking NCR1/NKp46 receptor on NK cells results in defective killing tumor cells. Only a few studies reported non-significant changes in the number of peripheral natural killer cells and NKp46 which is a cell-surface receptor expressed on NK cells in women with a history of RIF versus healthy women. All these studies reported that the peripheral blood level of NK cells is not a reliable marker to define the events in the uterus beyond uterine/decidual type NK cells. Other factors for the absence of similar infallible conclusions among various studies are related to different measurement methods. Apart from being invasive, this procedure is time-consuming. Additionally, it can be difficult to use stratified sampling. We suggest that the evaluation of NK endometrial stromal cells by immunohistochemical method during the luteal phase is a good estimation of the risk of abortion in women with RM and RIF and interventional therapeutic approaches should be used based on the results. Diagnosis of immunological problems in patients with pregnancy problems leads to treatment with immunomodulatory drugs. Glucocorticoid drugs (prednisolone) (26), intravenous immunoglobulin (IVIg) (27), and paternal lymphocyte therapy (through the stimulation of the immune system by paternal antigens) have been used to treat both RM and RIF complications that lead to a decrease in cytotoxicity activity and the number of uNK cells besides positive regulation of cytokine production. In the endometrium of women with RM, uNK cells and blood vessel maturation are increased and prednisolone treatment reduces uNK cells and endometrial spiral artery development in the endometrium of these patients (28). Paternal lymphocyte therapy reduces abortion in women with recurrent abortion. One of the effects of lymphocyte therapy is the reduction of NK cells (29, 30) and their cytotoxicity (31). The effect of IVIg has also been investigated on mice in addition to
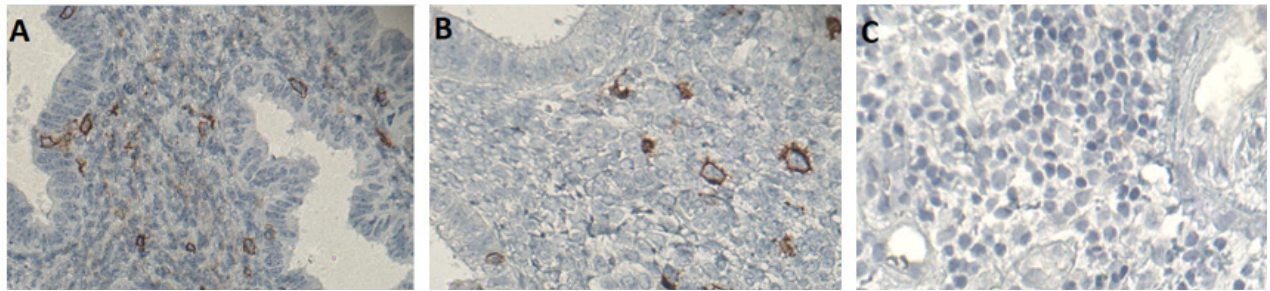
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Figure 3. Immunohistochemistry Staining to Detect the NKp46 Marker in Women With Recurrent Miscarriage and IVF Failure. An illustration of cell surface localization of NKp46 in the endometrial stroma derived from (A) patients, (B) fertile women, and (C) negative control (treated with PBS buffer instead of specific primary antibody) (original magnification × 400)
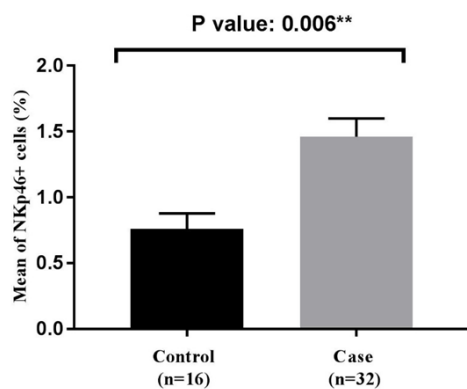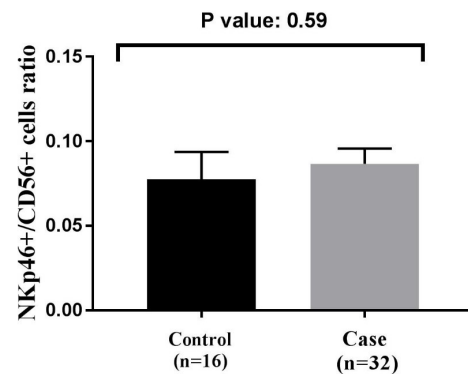

Figure 4. The Differences Between NKp46-Positive Cells in the Endometrial Stroma Derived From Patients With Recurrent Miscarriage and IVF Failure, as Compared With Fertile Women. **NKp46 expression was significantly higher in the patient group compared to fertile women

Figure 5. The Percentage Ratio of the NKp46- to CD56-Positive Cells in the Endometrial Stroma. No significant differences were found in the percentage rate of Nkp46- to CD56-positive cells in the endometrial stroma derived from patients with RM and RIF, as compared with the healthy controls

humans. In a study on mice, IVIg was found to reduce miscarriage by affecting NK (32). On the other hand, measurement of Nk cells before treatment is essential because research has shown that the success rate of IVF with IVIG therapy was high in people with elevated Th1: Th2 and/or CD56 (+) cells, and in people with normal Nk or Th1: Th2, IVIG treatment had no effect (33). New strategies for modulation of immune responses against the fetus include the use of monoclonal antibodies (anti-TNF), cytokines (granulocyte colony-stimulating factor), granulocyte macrophage colony-stimulating factor), and immunosuppressive factors (tacrolimus and cyclosporine) (34). It is necessary to investigate the effect of these treatments on Nk levels in the endometrium of patients. It is also recommended that the number of uNK cells and the expression of NKp46 should be evaluated after the use of these medications. Further, considering ever-changing face regarding the amount of uNK and pursued immunological cytotoxic effects are proposed for normal pregnancy.

Conclusion
The increased number of uNK cells and their elevated cytotoxic activity during implantation and early pregnancy in the patient group showed a significant association between the activity of uNK cells and a higher risk of RM and RIF. Given the role of these immune cells in pregnancy abnormalities, prescribing specific immunomodulatory drugs can decrease the risk of these diseases. Larger case-control studies are required to assess endometrial function using endometrial tissue specimens derived from healthy control subjects without RM and RIF to investigate the association between uNK cells and human reproductive disorders and develop new therapeutic approaches.

Authors' Contribution
Conceptualization: Maryam Matouri, Mehri Ghafourian, Farideh Moramezi.
Data curation: Maryam Matouri, Farideh Moramezi.
Formal analysis: Maryam Matouri.
Investigation: Maryam Matouri.
Project administration: Mehri Ghafourian, Ata Ghadiri.
Supervision: Mehri Ghafourian, Farideh Moramezi.
Writing–original draft: Maryam Matouri.
Writing–review & editing: Maryam Matouri, Mehri Ghafourian.

Competing Interests

Authors declare that they have no conflict of interests.

Ethical Approval

This study was performed in accordance with the recommendations of ethical guidelines (IR.AJUMS.REC.1397.712). All study procedures were carried out in accordance with the ethical standards of the Declaration of Helsinki. The research protocol was approved by the Research Ethic Committee of Ahvaz Jundishapur University of Medical Sciences. All participants signed the informed consent form after receiving explanations about the study.

Funding

Informed Consent

All participants signed the informed consent form after receiving explanations about the study.

References

1. Teh WT, McBain J, Rogers P. What is the contribution of embryo-endometrial asynchrony to implantation failure? J Assist Reprod Genet. 2016;33(11):1419-30. doi: 10.1007/s10815-016-0773-6.

2. Boujenah J, Salakos E, Pinto M, Shore J, Sifer C, Poncelet C, et al. Endometriosis and uterine malformations: infertility may increase severity of endometriosis. Acta Obstet Gynecol Scand. 2017;96(6):702-6. doi: 10.1111/aogs.13040.

3. Farghali MM, El-Kholy AL, Swidan KH, Abdelazim IA, Rashed AR, El-Sobky E, et al. Relationship between uterine natural killer cells and unexplained repeated miscarriage. J Turk Ger Gynecol Assoc. 2015;16(4):214-8. doi: 10.5152/jtgga.2015.0082.

4. Sen A, Kushnir VA, Barad DH, Gleicher N. Endocrine autoimmune diseases and female infertility. Nat Rev Endocrinol. 2014;10(1):37-50. doi: 10.1038/nrendo.2013.212.

5. Fortin CS, Leader A, Mahutte N, Hamilton S, Léveillé MC, Villeneuve M, et al. Gene expression analysis of follicular cells revealed inflammation as a potential IVF failure cause. J Assist Reprod Genet. 2019;36(6):1195-210. doi: 10.1007/s10815-019-01447-4.

6. Yockey LJ, Iwasaki A. Interferons and proinflammatory cytokines in pregnancy and fetal development. Immunity. 2018;49(3):397-412. doi: 10.1016/j.immuni.2018.07.017.

7. Jabrane-Ferrat N. Features of human decidual NK cells in healthy pregnancy and during viral infection. Front Immunol. 2019;10:1397. doi: 10.3389/fimmu.2019.01397.

8. Tang AW, Alfirevic Z, Quenby S. Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. Hum Reprod. 2011;26(8):1971-80. doi: 10.1093/humrep/der164.

9. Lash GE, Bulmer JN. Do uterine natural killer (uNK) cells contribute to female reproductive disorders? J Reprod Immunol. 2011;88(2):156-64. doi: 10.1016/j.jri.2011.01.003.

10. 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. Am J Reprod Immunol. 2014;72(3):262-9. doi: 10.1111/aji.12259.

11. Quenby S, Farquharson R. Uterine natural killer cells, implantation failure and recurrent miscarriage. Reprod Biomed Online. 2006;13(1):24-8. doi: 10.1016/s1472-6483(10)62012-3.

12. Vacca P, Moretta L, Moretta A, Mingari MC. Origin, phenotype and function of human natural killer cells in pregnancy. Trends Immunol. 2011;32(11):517-23. doi: 10.1016/j.it.2011.06.013.

13. Freitag N, Pour SJ, Fehm TN, Toth B, Markert UR, Weber M, et al. Are uterine natural killer and plasma cells in infertility patients associated with endometriosis, repeated implantation failure, or recurrent pregnancy loss? Arch Gynecol Obstet. 2020;302(6):1487-94. doi: 10.1007/s00404-020-05679-z.

14. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Am J Obstet Gynecol. 1975;122(2):262-3. doi: 10.1016/s0002-9378(16)33500-1.

15. Fukui A, Kamoi M, Funamizu A, Fuchinoue K, Chiba H, Yokota M, et al. NK cell abnormality and its treatment in women with reproductive failures such as recurrent pregnancy loss, implantation failures, preeclampsia, and pelvic endometriosis. Reprod Med Biol. 2015;14(4):151-7. doi: 10.1007/s12522-015-0207-7.

16. Van Acker HH, Capsomidis A, Smits EL, Van Tendeloo VF. CD56 in the immune system: more than a marker for cytotoxicity? Front Immunol. 2017;8:892. doi: 10.3389/fimmu.2017.00892.

17. Gharibi T, Hosseini A, Marofi F, Oraei M, Jahandideh S, Abdollahpour-Alitappeh M, et al. IL-21 and IL-21-producing T cells are involved in multiple sclerosis severity and progression. Immunol Lett. 2019;216:12-20. doi: 10.1016/j.imlet.2019.09.003.

18. Stabile H, Fionda C, Gismondi A, Santoni A. Role of distinct natural killer cell subsets in anticancer response. Front Immunol. 2017;8:293. doi: 10.3389/fimmu.2017.00293.

19. Toth B, Vomstein K, Togawa R, Böttcher B, Hudalla H, Strowitzki T, et al. The impact of previous live births on peripheral and uterine natural killer cells in patients with recurrent miscarriage. Reprod Biol Endocrinol. 2019;17(1):72. doi: 10.1186/s12958-019-0514-7.

20. Karami N, Boroujerdnia MG, Nikbakht R, Khodadadi A. Enhancement of peripheral blood CD56(dim) cell and NK cell cytotoxicity in women with recurrent spontaneous abortion or in vitro fertilization failure. J Reprod Immunol. 2012;95(1-2):87-92. doi: 10.1016/j.jri.2012.06.005.

21. Zhao X, Jiang Y, Wang L, Li Z, Li Q, Feng X. Advances in understanding the immune imbalance between T-lymphocyte subsets and NK cells in recurrent spontaneous abortion. Geburtshilfe Frauenheilkd. 2018;78(7):677-83. doi: 10.1055/a-0634-1813.

22. Dendana M, Bahia W, Finan RR, Al-Mutawa M, Almawi WY. Association of adiponectin gene variants with idiopathic recurrent miscarriage according to obesity status: a case-control study. J Transl Med. 2018;16(1):76. doi: 10.1186/s12967-018-1453-3.

23. Gao Y, Wang PL. Increased CD56(+) NK cells and enhanced Th1 responses in human unexplained recurrent spontaneous abortion. Genet Mol Res. 2015;14(4):18103-9. doi: 10.4238/2015.December.22.36.

24. Liu B, Mariee N, Laird S, Smith J, Li J, Li TC. The prognostic value of uNK cell count and histological dating in the mid-luteal phase of women with reproductive failure. Eur J Obstet Gynecol Reprod Biol. 2014;181:171-5. doi: 10.1016/j.ejogrb.2014.07.010.

25. Fukui A, Funamizu A, Fukuhara R, Shibahara H. Expression of natural cytotoxicity receptors and cytokine production on endometrial natural killer cells in women with recurrent pregnancy loss or implantation failure, and the expression of natural cytotoxicity receptors on peripheral blood natural killer cells in pregnant women with a history of recurrent pregnancy loss. J Obstet Gynaecol Res. 2017;43(11):1678-86. doi: 10.1111/jog.13448.

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26. Quenby S, Kalumbi C, Bates M, Farquharson R, Vince G. Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. Fertil Steril. 2005;84(4):980-4. doi: 10.1016/j.fertnstert.2005.05.012.

27. Laird S. The Role of Natural Killer Cells in Human Fertility. Royal College of Obstetricians and Gynaecologists (RCOG); 2016.

28. Lash GE, Bulmer JN, Innes BA, Drury JA, Robson SC, Quenby S. Prednisolone treatment reduces endometrial spiral artery development in women with recurrent miscarriage. Angiogenesis. 2011;14(4):523-32. doi: 10.1007/s10456-011-9237-x.

29. Szpakowski A, Malinowski A, Cieślak J, Nowak M, Wilczyński JR, Banasik M, et al. [Influence of paternal lymphocyte immunization on the selected subpopulations of peripheral blood lymphocytes in women with recurrent spontaneous abortions of unknown etiology]. Ginekol Pol. 2003;74(4):288-96. [Polish].

30. Szpakowski A, Malinowski A, Zeman K, Wilczyński J, Kolasa D, Nowak M, et al. [The influence of paternal lymphocytes immunization on percentage of peripheral blood CD16+/ CD56+cells in women with primary recurrent spontaneous abortion]. Ginekol Pol. 2001;72(12):1063-8. [Polish].

31. Kwak JY, Gilman-Sachs A, Moretti M, Beaman KD, Beer AE. Natural killer cell cytotoxicity and paternal lymphocyte immunization in women with recurrent spontaneous abortions. Am J Reprod Immunol. 1998;40(5):352-8. doi: 10.1111/j.1600-0897.1998.tb00065.x.

32. Tanaka J, Kitashoji A, Fukunaga Y, Kashihara J, Nakano A, Kamizono A. Intravenous immunoglobulin suppresses abortion relates to an increase in the CD44bright NK subset in recurrent pregnancy loss model mice. Biol Reprod. 2016;95(2):37. doi: 10.1095/biolreprod.116.138438.

33. Winger EE, Reed JL, Ashoush S, El-Toukhy T, Ahuja S, Taranissi M. Elevated preconception CD56+16+and/or Th1:Th2 levels predict benefit from IVIG therapy in subfertile women undergoing IVF. Am J Reprod Immunol. 2011;66(5):394-403. doi: 10.1111/j.1600-0897.2011.01018.x.

34. Abdolmohammadi-Vahid S, Danaii S, Hamdi K, Jadidi-Niaragh F, Ahmadi M, Yousefi M. Novel immunotherapeutic approaches for treatment of infertility. Biomed Pharmacother. 2016;84:1449-59. doi: 10.1016/j.biopha.2016.10.062.