

Review Article

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NK Cells and Fertility Immunological Aspects Review

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ABSTRACT

The basic functions of NK cells circulating in peripheral blood and present in immune organs are production of proinflammatory cytokines and cytotoxicity against transformed cells after viral infection, during neoplastic process. Tissue resident NK cells (liver, lungs, lymph nodes, uterus) are phenotypically and functionally distinct in relation to the tissue. Uterus NK cells (uNK) are involved in endometrial transformation during the embryo implantation and progressing pregnancy. There are divided into subpopulations – endometrial NK (eNK) and decidual NK (dNK1, dNK2, dNK3) and memory NK cells after pregnancies (PMdNK). The study of NK cells showed different combination of phenotype markers and relation to function – shifted for cytokines production or for the cytotoxicity. The interesting type of receptors important for uNK function are killer-immunoglobulin-like receptors (KIR) with inhibitory or activating properties. The inhibitory profile KIR receptors (KIR AA) are believed to be unfavourable marker for the embryo implantation and pregnancy as activation of uNK is needed for proper decidual vascularisation (remodelling of spiral arteries), the embryo implantation, induction and regulation of local maternal-foetal tolerance. The disorders of NK number and function are associated with pregnancy complication like recurrent implantation failure (RIF) or recurrent pregnancy loss (RPL). The diagnostic and therapeutic approaches in women with RIF or RPL are including the NK cells characteristics and activity.

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Abbreviations

ART: Assisted Reproductive Technology
CIDP: Chronic Inflammatory Demyelinating Polyneuropathy
CLP: Common Lymphoid Progenitors
CRP: Clinical Pregnancy Rate
DC: Dendritic Cells
DNAM1: DNAX Accessory Molecule 1
DSCs: Decidual Stromal Cells
DN: Double Negative CD4–CD8– T Lymphocytes
ELP: Early Lymphoid Progenitors
ETPs: Early Thymic Precursors
ESCs: Endometrial Stromal Cells
EVT: Extravillous Trophoblast
GM-CSF: Granulocyte–Monocyte Colony-Stimulating Factor
HSCs: Hematopoietic Stem Cells
ILC1: Innate Lymphoid Cells Type 1
IFN γ : Interferon- γ
IFZ: Interfollicular Zone
ITP: Autoimmune Thrombocytopenia
IVIG: Intravenous Immunoglobulins (Prepared for Therapy)
IVF: In Vitro Fertilisation
KIR: Killer Immunoglobulin-Like Receptors
LBR: Live Birth Rate
LILRB1: Leukocyte Immunoglobulin-Like Receptor

eMSCs: Endometrial Mesenchymal Stem Cells
MMN: Multifocal Motor Neuropathy
MHC-I: MHC Class I Determinants
NK: Natural Killer
NKT: Natural Killer T Cells
cNK: Conventional NK Cells
ihNK: Intrahepatic Resident NK Cells
luNK: Lung Resident NK Cells
lnNK: Lymph Node Resident NK Cells
tNK: Thymic Resident NK Cells
uNK: Uterine Resident NK Cells
iNKT: Invariant NKT Cells
eNK: Endometrial NK Cells
dNK: Decidual NK cells
PMdNK: Pregnancy Memory dNK
Prf1: Perforin
RIF: Recurrent Implantation Failure
RPL (RM): Recurrent Pregnancy Loss (Recurrent Miscarriages)
SCF: Stem Cell Factor
SpA: Spiral Arterioles
SASP: Senescence-Associated Secretory Phenotype
TNF: Tumour Necrosis Factor
TRAIL: TNF-Related Apoptosis-Inducing Ligand
TRM: Tissue-Resident Memory T Cells
TGF- β : Transforming Growth Factor
VEGF: Vascular Endothelial Growth Factor

NK Cells-Basic Role in Immune System

The NK cells consist a small population (up to 10-15%) of circulating mononuclear cells with crucial role in the antiviral defence. Their main functions are production of cytokines and direct cytotoxicity of transformed cells with the viral infection or the neoplastic process. NK cells develop in bone marrow and in secondary lymphoid structures such as tonsils, lymph nodes, and spleen. They belong to the group of the innate lymphoid cells, preferentially type 1 (ILC1). The following stages of NK cell development and maturation in bone marrow includes the expression of specific line determinants (e.g. CD244 in early precursors and along further stages, CD122 in stage 2b of development, up to expression of CD16 and CD56 in mature NK cells and KIR in adaptive NK cells) with loss of determinants typical for early precursors (e.g. CD34). The phenotype of mature NK cells shows the expression of: CD122, CD107a, CD27, CD244, CD49b, CD11b, CD62L, CD226, NKG2D, NK1.1, NKG2A/C, NCR1, Ly49, with loss of CD27 in end stage of maturation [1].

NK cells are often divided into circulating conventional NK cells (cNK) with ontogeny mainly in bone marrow and non-conventional NK cells localised in tissues (tissue resident NK cells – trNK). The main mechanism of elimination of the infected or transformed cells by stimulated NK cells is based on recognition and direct lysis of target cells with cytotoxic mechanism [2-7].

The function of NK cells is regulated with germline coding activating and inhibitory receptors and soluble mediators including cytokines and growth factors produced by other cells within regulatory network. The initiation of immune response is based on close co-operation of NK cells and dendritic cells (DC); however, this regulation is bi-directional as cytokines produced by DC are necessary for development and function of NK cells [2]. The activating receptors (NKG2D and NCRs) are present on all subtypes of NK cells and recognize self-ligands or antigens from pathogens. Induced self-ligands in human NK cells include non-classical MHC class I complex (MIC-A, MIC-B). The main function of NCRs present on NK cells is recognition of viral proteins. The signals from tissues are important for regulation of the ligands for NKG2D and NCRs expression, however, these signals are coming from stressed of infected epithelial, endothelial cells and monocytes. Based on flow cytometry technique of NK study with assay of CD56 and CD16 expression on the surface, NK cells are divided into: CD56brightCD16- or CD56dimCD16+ with different distribution among the cNK and trNK cells. NK cell showing phenotype CD56dimCD16+ present strong cytotoxic activity whereas NK cells CD56brightCD16- display low cytotoxic activity and high immunomodulatory properties and cytokines production [1,3,6].

The interaction between DC and NK cells is critical for regulation of the adaptive immune system responses. cNK cells producing Type-1 IFN α/β activates DCs, what results in the production and trans-presentation of IL-15/IL15R α complex on the surface of plasmacytoid DC. The back signal is based on presentation of IL-15/IL-15R α complex to complex of IL-15R α /IL-2R β /IL-2R γ on NK cells. This signal is one of necessary transition steps from the innate to the adaptive immune response. The interaction between DC and cNK cells leading to production of Type-1 interferons directs DC to maturation and supports priming Th1 cells in lymph nodes [3,1].

The main activation of cNK cells is based on cytotoxicity regulated, among other, by signal from killer immunoglobulin-like receptors (KIR) reacting with ligand of MHC class I determinants. KIR receptors are present on NK cells and, in part, of NKT subpopulation with genes located on chromosome 19 in the leukocyte receptor complex (LRC). Up to now, KIR gene family consists 15 loci (KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL2, KIR3DL3, KIR2DS1, KIR3DL1/S1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5) and 2 pseudogenes (KIR2DP1, KIR3DP1). KIR genes exist in sever allelic form – now (December 2020) the known number of alleles was 1110. The functions of these receptors are associated with their structure – activating receptors have a short cytoplasmic fragment (marked as S), inhibitory receptors show long cytoplasmic fragment (marked as L). This general rule has one exception – KIR2DL4 receptor may act as activator or inhibitor depending of the conducted signal. KIR genotypes are divided into two haplotypes – A and B depending of genes composition. Haplotype A containing mainly inhibitory receptors with one activating KIR2DS4 in contrary to B haplotype containing different combination of activating genes mainly (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5 and KIR3DS1).

There are more than 40 haplotypes described as B type. The ligands for KIR receptors are determinants of HLA class I – HLA-C, HLA-B. Ligand-receptor coupling as complex is sending the signal and according this signal NK cells are activated or not activated to react against the target cells. KIR as a marker indicating function of NK cells is important for haematology, transplantology (transplantation of hematopoietic stem cells and solid organs) and oncology (cancer therapy). Moreover, the results of studies showed the haplotypes of KIR as important for normal reproduction, primary infertility, implantation failure and recurrent miscarriages [8,9].

The description of trNK is associated with the tissue where they are localized e.g. lung NK, liver NK, lymph node NK, thymus and uterus NK cells. The differences between cNK and trNK are shown in Table 1. The special subpopulations of NK cells are thymic NK cells (tNK) precisely described according their phenotype. The function of tNK cells is not very well known; however, they migrate to other tissues – bone marrow, liver and spleen. What is interesting, there are present in relatively high percentage in the mesenteric lymph nodes. The known activity of tNK cells is based on production of cytokines including IFN γ and regulation of the T lymphocytes ontogeny through different mechanisms. The elimination of negatively selected thymocyte with perforin/granzyme, Fas/FasL/TNF related apoptosis inducing ligand (TRAIL) or producing cytokine and chemokines maybe involved tNK cells in this regulatory process. Liver resident NK cells (ihNK) represent up to 50% of hepatic lymphocytes, what suggests important role of these cells. In description these cells show low expression of perforin and granzyme B indicating prevalence of non-cytotoxic function. The effector function of ihNK cells is associated with production of high number of inflammatory cytokines. Lung NK cells (luNK) are present in about 20% of lymphocyte population localised in the lungs.

Table 1: Characteristics NK Cells Subpopulations Acc

Parameter of NK cells	Conventional (cNK)	Lymph node (lnNK)	Thymic (tNK)	Liver NK (ihNK)	Lung NK (luNK)	Uterine (uNK)
Phenotype determinants	NCR1-3, NKG2D, DNAM1, CD56, CD16, CD62L, IL-15R $\alpha/\beta/\gamma$	NCR1, CXCR6, CD56, CD16, CD158b, IL-2R $\alpha/\beta/\gamma$	NCR1, NKG2D, CD56, IL-7R $\alpha/\beta/\gamma$	NCR1, DNAM1, NKG2A, CXCR3, CXCR6, CCR4, TRAIL, FasL, CD56, CD69, CD94,	NCR1, CD16, CD49a, CD56, CD57, CD69, CD103	NCR1 CD56, CD49a, CD103, CD94
Transcription factor: surface nucleus	IL-15R $\alpha/\beta/\gamma$ Nfil3, Id2, PU.1, Ers-1, Eomes, Tbx21	IL-2R $\alpha/\beta/\gamma$ Id2, Eomes, Tbx1/2 Tbx21	IL-7R $\alpha/\beta/\gamma$ Nfil3, Id2, Gata3	IL-15R $\alpha/\beta/\gamma$ Tbx21, Eomes, Hobit, Blimp1, AhR	IL-15R $\alpha/\beta/\gamma$ Tbx21, Eomes, Hobit,	IL-15R $\alpha/\beta/\gamma$ Eomes
Function	Cytotoxicity Inflammatory cytokine production Chemokine production	Prevention of tumour metastasis Th1 polarization, Interaction with myeloid cells	Help T cell development Clear failed thymocytes Interaction with stromal cells	Interaction with Kupfer cells Antiviral hepatitis response Promotion of tolerogenic T cells	Antiviral response Epithelial regeneration Cytotoxicity Inflammatory cytokine production	Placental-vascular remodelling Supporting fetal growth Production of IL-22

The luNK cells are involved in two activities – one is associated with lungs protection the second is presented as anti-pathogens activity. Mycobacterial infection, cancer (e.g. non-small-cell-lung cancer) and other respiratory tract infections are changing of luNK cells receptors and determinants expression for better and more effective activity. NK cells resident in lymph nodes (lnNK) is probably in precursor stage and further mature within lymph node. The lnNK consisted about 5% of lymphocytes, however, the number of lymph nodes in human body means, that lnNK cells localised within lymph node provide marked part of total NK cells populations. Circulating NK cells (CD56bright) enter into lymph node using endothelial venules and afferent lymph vessels, recruited by CCL19 and CCL21 chemokines highly expressed in lymph node. The precise role of lnNK cells is not known. Uterine NK cells (uNK) are well characterised and different from circulating cNK cells in multiple aspects including KIR receptors. The role of uNK cells during implantation of embryo and pregnancy include the formation of foetal-maternal interface and placental vascular remodelling are described below [3].

NK Cells in Physiology of Reproductive System

The origin of uNK cells is not known up to now, moreover, observations showed presence of uNK cells in different tissues suggesting multiple origin. One of NK cells subpopulations present in uterus non-pregnant or pregnant are NK cells expressing CD3 – typical determinant of T lymphocytes. This population is called the natural killer T cells (NKT) with probably involvement in process of immune tolerance mechanisms development in uterus. NKT cells, as subpopulation of NK cells, is divided into subgroup – invariant NKT (iNKT) and non-invariant – non-iNKT. The most of NKT population is present the decidua, however, there are noted in liver and peripheral blood. In the characteristics of iNKT, the production of TNF, IFN γ and IL-4 are induced in response to the stimulation with α -Galactosylceramide.

Another interesting of NKT characteristic is CD4 (determinant typical for T helper lymphocytes) expression on part of this subpopulation. NKT cells expressing CD4 (CD4+) demonstrate the immunoregulatory function, when the remaining NKT cells not expressing CD4 (CD4-) are acting as effector cells. The iNKT cells are recognised as important in maintaining pregnancy and regulation of immune system during this pregnancy. The number of NKT in decidua is about ten times higher than in peripheral

blood with function of balancing between activity of Th1/Th2 T lymphocytes. These NKT cells in decidua expressed CD3/CD161. Maternal iNKT cells interacted with CD1 present on surface of trophoblast cells and it might be one of many mechanisms involved in placenta formation and embryo implantation [10]. This subpopulation of NK cells – decidua NK cells (dNK) are present from the formation of decidua up to end of pregnancy in large number, decreasing after delivery.

The typical NK cells (CD3-) resident in uterine (uNK) are usually divided in relation to endometrium without pregnancy (endometrial NK – eNK) or localised in the decidua – dNK cells in different periods of pregnancy (early pregnancy or term pregnancy). The number of eNK cells is changing during menstrual cycle reaching 70% of endometrial leukocytes during secretory phase of menstrual cycle with the important role of depletion of senescent cells accumulated during the menstrual cycle (Figure 1) [6,11-13].

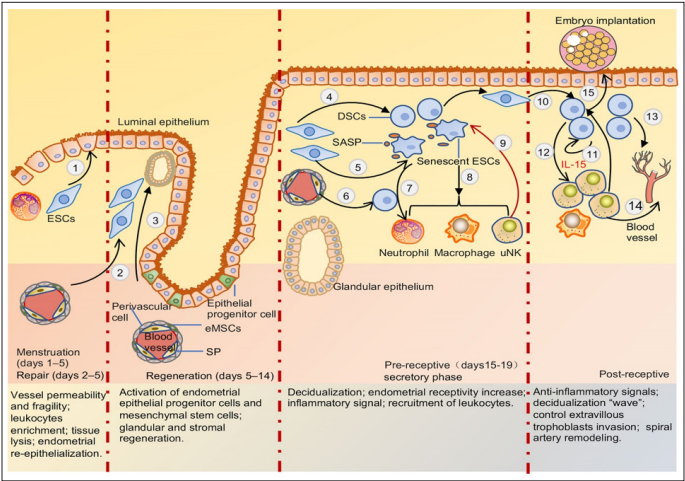


Figure 1: Human Endometrium in Menstrual Cycle and Embryo Implantation. Acc

The physiological roles of eNK are focused on the elimination of decidual senescent cells followed by conversion of endometrial signals from pro-inflammatory into anti-inflammatory maintaining the homeostasis of endometrium. The clearance of the senescent

decidual cells by eNK is enhanced by increased level of the hyaluronian in the implanted embryo. The embryo without proper implantation is producing less amount of the hyaluronian what resulted in inhibition of eNK activity. Both observations suggested possible eNK role in determining the embryo fate during the implantation process. Moreover, eNK cells participate in development blood vessels in the endometrium with secretion of CCL2 chemokine under regulation by the estrogen. Summarising these roles of eNK cells, the disorders or lack of these functions may be one of high-risk factors leading to the complications during pregnancy [11,14,15].

There are cumulating observations suggested that eNK are differentiating into dNK cells after the implantation of the embryo. However, similarity in markers indices possibility of dNK1 localised in endometrial tissue being inter-differentiated cells between eNK and dNK. Based on characteristic with the method of single-cell sequencing three different subtypes of dNK were selected and described (Table 2, Figure 2) [6,16,11]. The molecules produced by dNK cells (dNK1 – SPINK2, CD39, CD73, dNK2 and dNK3 - ANX1) demonstrate anti-inflammatory properties inhibiting the immune activation at the maternal-foetal interface. Observation of activity of uNK cells in following pregnancies suggested memory of these cells (PMdNK) similarly to the memory of whole population of the innate lymphoid cells (ILC). In following pregnancies uNK cells localised in the endometrium expand producing high amount of IFN γ and VEGF α mediators of the placenta development [6].

Table 2: Endometrial NK cell (eNK) and Subpopulations of Decidual NK Cells Acc

e- d- PTd-NK subtypes and localization	Surface markers	Ligands for KIR and NKG	Other markers	Secreted mediators and produced molecules
eNK endometrium (early and middle stage of menstrual cycle) immature	CD49a, CD9, CD69, NKp30, Nkp46, KIR2DL1, KIR2DL3, KIR3DL2/S2, KIR3DL1, , NKG2A/C/E	HLA-C2 HLA-C1 HLA-Bw4 HLA-E	CD39, CD103 CXCR3, CXCR4 LILRB1,	CCL2, IFN γ , granzyme B, perforin (upon stimulation of IL-2 and IL-15)
dNK1 endometrium	CD56+, CD9+, CD49a+, CD69+, CD94+, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS1, KIR2DS4, NKp30, NKp44, NKp46, NKG2A/C/D LILRB1	HLA-E (CD94) HLA-C2 HLA-C1 HLA-C1 HLA-C2 HLA-C2 (some alleles) HLA_E HLA-G	CD39, CYP26A1, B4GALNT1, CSF1 CD103	IL-1b, IL-4, IL-6, IL-8, IL-10, IL-13, GM-CSF, IFN γ SPINK2, CD39, CD73
dNK2 Early decidua	CD56, CD49a NKp30, NKp44, NKp46 KIR2D2/3, KIR2DL4, KIR2DS1 NKG2A/C/D,	HLA-C1 HLA-G HLA-C2	ANXA1, ITGB2, XCL1 CD9, CCL5, CCL4, KLRB1	RANTES, TNF, Ang1, Ang2
dNK3 term decidua	KIR2DS4, NKG2A/C/D/E NKp44, NKp46 NKp30, KLRB1, TIGIT	HLA-C2 HLA-E	CD160, ANX1, KLRB1, CD103, XCL1, CCL5, CXCR4 ITGB2	VEGFc, PIGF, TGFb1, TNF, MIP1a, MIP1b
PTdNK (pregnancy-trained memory NK)	CD56, CD49a NKp30, NKp44, NKp46 NKG2A/C/D LILRB1 KIR2DL1 KIR2DL2/L3 KIR2DL2/L3/S2	HLA-E HLA-G HLA-C2 HLA-C1		IFN γ , VEGF α

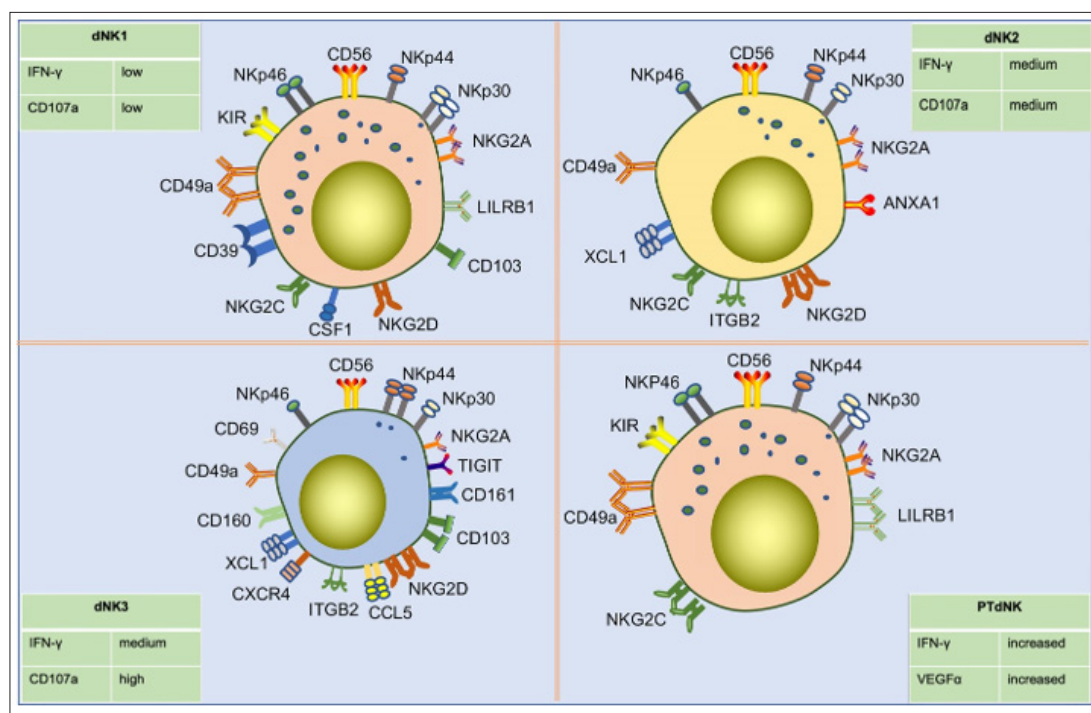


Figure 2: Subpopulations of Human dNK Cells in Early Pregnancy (acc. Xi3 2022)

Subpopulations of human dNK cells in early pregnancy and their differences in surface receptor expression profiles. Single-cell sequencing showed that dNK cells in early pregnancy were divided into three subpopulations, including dNK1, dNK2, and dNK3. dNK1 cells are CD56^{bright} CD49a^{high} KIR^{high} NKG2A^{high} LILRB1^{high} CD39^{high} phenotype, about 55% of total dNK cells. dNK2 cells are CD56^{bright} CD49a⁺ KIR^{low} NKG2A^{high} LILRB1-CD103- phenotype, about 15% of total dNK cells. dNK3 cells are CD49a⁺ CD69^{high} CD103^{high} CD161^{high} KIR^{low} NKG2A^{low} NKP44⁺ NKG2D^{high} phenotype, about 15% of total dNK cells. Additionally, three dNK types have different degranulation capacity and IFN-γ secretion capacities in response to nonspecific stimulation. Pregnancy-trained memory NK cells (PTdNK) are found in the decidua of multiple pregnancies, like dNK1, with CD56^{bright} KIR^{high} NKG2A^{high} LILRB1^{high} NKG2C^{high} phenotype. In the presence of IL-15, the interaction of NKG2C with HLA-E and LILRB1 with HLA-G induced increased amounts of IFN-γ and VEGFa secreted by PTdNK. And *IFNG* and *VEGFA* were found to have increased accessibility in PTdNK cells by ATAC-seq analysis.

The dNK cells from early pregnancy showed marked changes in comparison to eNK from the secretory phase. In the studies the altered transcripts level, changes in RNA splicing resulting in expression of rather inhibitory isoform of individual cytotoxicity receptors were noted. Ligation of NKP30 or NKP44 therefore induces the inhibitory response in dNK. This may contribute to the low cytotoxicity including the trophoblast [16]. dNK cells along the pregnancy, especially in late phase (last trimester, term pregnancy), decreased in the number changing the expression of determinants. The expression of CD16 and CD57 is lack, the expression of KIR, LILRB1 and 9kDa is lower, the expression of CD9 remains unchanged [16,11].

The function of dNK in first trimester of pregnancy is associated with formation of the placenta to support nutrients and oxygen for the developing embryo. In this stage, the most important is formation the new net of blood vessels – the normal small spiral

arteries are transformed into large capacity, the low resistance thin-walled blood vessels. This process requires co-operation of variety of cells and cytokines as signals. The dNK cells presented in a high number, localised close to the uterine spiral arteries are playing the important role due to regulation of invasive ability and motility of EVT cells and secretion of the cytokines list. The one of possible mechanism is based on secretion by dNK the angiogenic factors like metalloproteinases (MMP), angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), the placental growth factor (PLGF), IFNg, VEGFa and VEGF-C leading to destruction of the vascular smooth muscles and the extracellular matrix. Ang1, Ang2 and TGF-b are inducing the differentiation of spiral artery vascular smooth muscles and increase motility to migrate into the decidual stroma. Moreover, dNK cells are able to promote the recruitment and migration of EVT by secretion of other factors – IL-8, IP-10 (CXCL10), CCL5. It indicated the basic role of dNK and their products in the process of the arterial remodelling during the placenta formation. The co-operation of dNK and EVT is bi-directional, so the receptors on dNK cells and ligand expressed by EVT are regulated each other. In this regulation, the receptors of KIR family are involved in activation of dNK cells and stimulation of the cytokine's secretion e.g. KIR2DL4 present on dNK cells binds to HLA-G determinant expressed on EVT cells and activates the nuclear factor kappa-B (NF-kb) pathway.

In the results, dNK cells convert into the secretory phenotype and secrete large amount of pro-inflammatory cytokines (TNF, IL-1b, IFNγ) and pro-angiogenic factors (IL-6, IL8) to promote the angiogenesis. The interaction of some KIRs receptors with HLA-C induce the secretion of GM-CSF important for promotion of the trophoblast cells invasion. The vascular remodelling in placenta is, at least, partially associated with interaction of dNK cells (maternal) and the trophoblast cells (foetal) [11,10,16,14,17,18]. The repertoire of factors secreted by dNK included the growth promoting factors (GPF), osteoglycin, osteopontin in the response to stimulation, what is one of many mechanisms supporting and promoting the implantation and the foetal development. However,

active dNK cells are cytotoxic, what is not required due to the establishing of immune tolerance during pregnancy. The problem is important because high number of dNK cells with cytotoxic activity in maternal-foetal interface may inhibit the proliferation of trophoblasts cells. The lower cytotoxic activity of dNK cells is an effect of balance between the inhibitory and the activating signals.

The regulatory function of dNK cells is associated with NCRs (NKp46, NKp30, NKp44) specific function e.g. NKp44 display inhibitory activity, NKp30 promote the cytokines secretion, only one – NKp46 activates dNK cells. Moreover, the trophoblast cells may interact with dNK cells reducing the expression of NKG2D and NKp80 what resulted in the functional inhibition of dNK cells [11,16,10]. dNK cells are interacting and cooperating with other immune cells - B lymphocytes, dendritic cells, monocytes and T lymphocytes. The key receptor for this co-operation is CD39 present on dNK cells. CD39 combined with CD73 degrades extracellular ATP to extracellular adenosine with the significant immunosuppressive effects. This mechanism is possible as CD39 is present on dNK cells and CD73 is expressed on the endothelial and EVT cells [14].

NK Cells in Male Reproduction

Male infertility is associated with the inflammation, the infection in majority of cases and, in less extent, cancers of the reproduction male system. The NK and NKT cells are present in the testes, the epididymis, the prostate and the semen in different percentage, however, there are shortage of the study in human. The NK and NKT role in the human reproductive system is associated with typical, obvious role of these cells against the viral infection and cancer transformed cells, what is shown as a suggestion based on the animal study in Table 3. NK and NKT cells present in the interstitial tissue of testis mediate the anti-tumour response and enable the surveillance in various cancers. In testes are the mechanisms “balancing” higher activity of NK cells – the Sertoli cells express and secrete the immunosuppressive factors including TGF β , activitin A, galectin-1 to inhibit the proliferation of NK cells. The epididymis is most common site of the intrascrotal inflammation believed to be the main cause of the male fertility disorders. NK and NKT cells are reported in the epididymis exclusively in the association with the adipose tissue. The animal studies showed activation of NK cells by high-fat diet. There are no data analysing the NK cells number, activation and specific role in the epididymis. The prostate is an immunocompetent organ with lymphocytes, NK cells, macrophages, mast cells found in the interstitium and the stroma of the gland. It seems, that these present immune cells are involved in the maintenance of the sterility of male reproductive tract and the prevention of the autoimmune reactions.

The prostatitis and the prostatic cancer are common urologic disorders associated with basic the role of NK cells located in the prostate gland. In the primary prostate cancers, the study of NK cells phenotype showed the expression of ligands for

the natural cytotoxicity receptors (NKp30, NKp46), not seen in the benign prostate hyperplasia. An increased number of NK cells in the prostate tumour was associated with the lower risk of progression, what support the opinion about protective role of NK cells against cancer. The interaction of tumour and immune cells e.g. NK cells are bi-directional. The new study of activity of NK cells demonstrated the low cytotoxic potential in patients with the prostatic cancer. It might be an effect of the tumour-driven immunosuppressive reaction leading to diminished and impaired NK cells the anti-cancer cytotoxic efficiency.

There are no data suggesting the specific role of NK in the prostate physiological function. The ejaculate is a complex secretion originating from the testis, epididymis and accessory sex glands including the seminal vesicle, the prostate, the bulbourethral glands and the urethral glands. The leukocytes are present in the fertile semen in about $1-5 \times 10^4/\text{ml}$. These leukocytes and their products such as the cytokines, the reactive oxygen species play regulatory role in the sperm function – motility, vitality, acrosome reaction and the sperm-oocyte fusion. There are few data regarding the presence of NK and NKT cells within the ejaculate and their role in the spermatozoa function. The significant percentage of NKT cells were detected in the ejaculate in chronic inflammation of the genital tract. In some studies, the significant correlation between iNKT cells and seminal IL-6, IFN γ was noted. Summarising, the suggestion that despite of heterogeneity of NKT cells in the genital tract, NKT cells are involved in the modulation of the inflammatory process [19].

NK Cells in Primary Infertility Including Implantation Failure and Recurrent Miscarriages

The term infertility is defined as the inability to achieve the pregnancy for period of 12 months despite the regular sexual intercourse without the contraceptive methods. Another important point is losing the pregnancy (miscarriages) within first trimester, what results in the lack of successful pregnancy and delivery of the healthy newborn. The both aspects of infertility are related to different mechanisms – in permanent lack of pregnancy the endometrium and mechanisms leading to the implantation following the development of placenta and foetus are the most important. The problem of losing pregnancy (RPL) is dependent of the endometrium as wells, however, other mechanisms associated with supporting implantation and the progress of the pregnancy are playing their roles. The studies were focused on the different aspects of the implantation failure (RIF) and the pregnancy loss (RPL). In both situation uNK cells are involved in the process of the embryo implantation and supporting the normal pregnancy (Figure 3). The study of peripheral blood NK cells in women with RIF and RPL showed (meta-analysis) the higher proportion of NK cells in women with RPL than in the control group – this difference is statistically significant [20,11]. Similar observations were noted in group women with RIF; however, available data were too small to performed the statistical analysis.

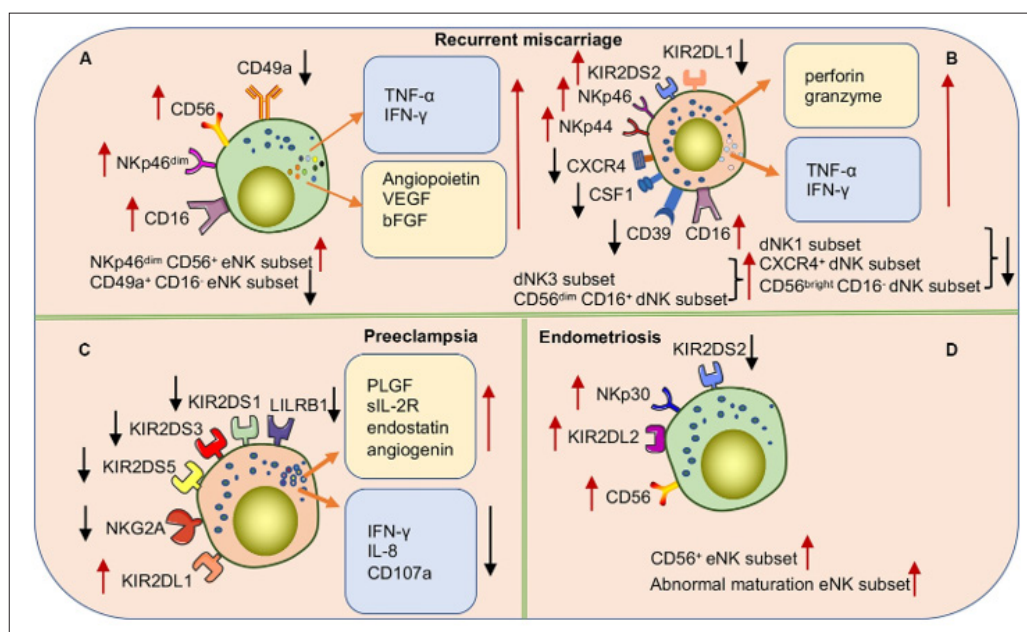


Figure 3: NK Cells in Pregnancy Complications acc

Subpopulations or functional alterations of human uNK cells in pregnancy complications. (A) NKp46^{dim} CD56⁺ eNK cell populations in RM patients are significantly increased while the CD49a⁺ CD16⁺ eNK cell populations significantly decrease [11]. In addition, there are a significant accumulation of various cytokines in eNK cells. (B) In women with RM, the expression of various NCRs and activating receptor KIR2DS2 is up-regulated, and the inhibitory receptor KIR2DL1 is down-regulated to activate dNK cells. The frequencies of dNK1 cell, CXCR4⁺ dNK cell, and CD56^{bright} CD16⁻ dNK cell subsets are significantly decreased while dNK3 cells and CD56^{dim} CD16⁺ dNK cell subsets significantly increased. (C) The cytokine secretion profile of dNK cells is markedly altered in preeclampsia patients. Downregulation of activating receptor expression on the surface of NK cells or upregulation of inhibitory receptors inhibits NK cell activation causing a high risk of developing PE. Especially when maternal NK cells carrying the KIR AA genotype are combined with embryos carrying the HLA-C2 gene. (D) In women with endometriosis, higher frequencies of CD56⁺ eNK cells and abnormal maturation eNK cells are found in eutopic endometrium. Upregulation of inhibitory receptors or downregulation of activating receptors leads to over suppression of eNK cell function associated with the occurrence and severity of endometriosis.

Recurrent Implantation Failure (RIF)

The majority of available data about NK cells in RIF is based on NK study in the peripheral blood, what is only suggestive for the special role of uNK and dNK cells. The number and the activity of peripheral NK cells including the expression of granzyme B, perforin, receptors associated with function (NKG2D, NKp30, NKp46, CD158a, b) in women with RIF did not show the significant differences as compare to the fertile women [21]. Another approach was based on the histopathology of endometrial biopsies assayed with CD56, CD138 and BCL-6 staining. The results indicated the increase of CD56⁺ cells number (uNK) in women with RIF, what is in agreement with other data [9].

The assays of the expression of KIR receptors on NK cells showed two haplotypes: A and B: A – have fixed gene content, B – are much more diverse in total number of genes, their combinations and number of the activating genes. In haplotype A there are genes

encoding only the inhibitory receptors – KIR2DL1, KIR2DL3, KIR3DL1, KIR2DP1. In the haplotypes B the genes are encoding, in majority, the activating KIR receptors – at least following receptors: KIR2DS1, KIR2DS2, KIR2DL2, KIR2DL5A, KIR2DL5B, KIR2DS3, KIR2DSA5, KIR3DS1. The study of NK cells in women with RIF or RPL showed high frequency of KIR AA haplotype, what now is used as marker of the risk of RIF or RPL [11,18]. The statistical significance of KIR AA relation to the infertility in comparison to KIR AB or KIR BB haplotypes was noted in a group of 397 women with the unexpected infertility, 113 women with RIF and 175 women with RPL. Similar association of KIR AA haplotype with the presence of HLA-C2 of foetus and high risk of the miscarriages are presented in other studies [17,15,22].

Recurrent Miscarriages (RPL)

The eNK are involved in the process of decidualisation and revascularisation, so the question about the role eNK in RPL was undertaken in many studies. The increased number of eNK suggested the increased process of the spiral arteries vascularisation and probably the defective vascular transformation as an effect. In the consequences, the early maternal-foetal circulation is opened, what caused the excessive oxidative stress, leading to the pregnancy failure. The expressions of angiopoietin, VEGFα and basic fibroblast growth factor (bFGF) were significantly higher in eNK cells of women with RPL than in normal reproductive females supporting these observations and such conclusions [11].

However, the associations between eNK population expressing NKp46 CD56 and RPL are non-conclusive – there are data about higher proportion of these cells and, in opposite, the lower expression of this receptor in women at risk of RPL. The expression of NKp46 is related to NK cell function – high expression is suggesting the cytokine production as main function of these NK cells, low expression is associated with the cytotoxicity. The balance between these two different functionally NK subpopulations are crucial for the proper regulation of endometrium. Moreover, the cytokines production in women with RPL was altered as compare to normal fertility women, as ratios of TNF/IL-4, IFN-γ/IL4, TNF/IL-10, and IFN-γ/IL10 were significantly elevated in eNK of women with RPL [11,10,22,23,17,24,20,21].

The other studies of NK cells are showing the increased IFN γ level in the serum women with RIF and RPL, although this increase was statistically significant only for the group with RPL [21]. The data about dNK in the endometrium women with RPL showed decreased frequency of CD56 $^{+}$ CD16 $^{-}$ dNK cells in the decidua, whereas the frequency of CD56 $^{+}$ CD16 $^{+}$ dNK cells was significantly increased. The proportion of CD56 $^{+}$ CD16 $^{-}$ dNK and CD56 $^{+}$ CD16 $^{+}$ dNK cells highly expressing NKp44 and NKp46 receptors was significantly higher, shifting of dNK cells to marked cytotoxic activity against the target cells [11]. Moreover, dNK cells isolated from patients with RPL in vitro were found to secrete higher levels of TNF, hindering the endometrial decidualization [22].

The Endometriosis

The endometriosis is one of known factors with the increased risk of RPL, what was shown in a cohort of 29563 women with the diagnosis of endometriosis against 295630 women without. In women with the endometriosis ectopic NK cells are less cytotoxic that in healthy women, in the contrary to NK cells from ectopic lesion, when these cells are highly cytotoxic especially in the deep infiltrating endometriosis form. In women with the endometriosis and RIF or RPL the increased expression of NKp46 and CD16 on NK cells is noted, what means higher cytotoxic activity with decrease of angiogenesis-promoting activity. These active cytotoxic NK cells (CD16, NKp46) are present in high number in women with the endometriosis complicated by RIF or RPL [25].

Therapy Including Modifications of NK Cells Phenotype and Function

The implantations failure (RIF), the recurrent miscarriages (RPL) are a big problem for couples and for their doctors. Including possible problematic points along the normal stages of fertilization, embryo growing, implantation and progress of pregnancy, the different therapies modulating endometrium were used. Some of these approaches were taking in account the number and functional role of uNK cells, so these therapies were selected and discussed below.

Corticosteroids

The NK cells, including uterine uNK cells, are involved in the implantation process and early pregnancy, however, the role of these cells and their activation in the early pregnancy complications, the implantation problems and the miscarriages remain speculative in many aspects. The therapeutic interventions in RIF and RPL including uNK cells are based on possible modification or inhibition of uNK activity, believed as overactivity in these cases. The first suggested intervention for modulation of the immune response in RPL was associated with use of steroids as the method of the inflammatory process suppression. Moreover, the presence of glucocorticoid receptors on uNK cells showed possibility of the reduction of uNK number, the normalization or decrease of the cytokine's expression and secretion by these cells leading to the suppression of the proinflammatory endometrial state. In the group of women with RPL treated with steroids (double-blind placebo randomised controlled clinical trial) the significant increase of live birth rate (70.3% against 9.2% in placebo group) was obtained.

The study of NK cells (peripheral blood) in group of women with RPL showed the decrease of NK number and lower CD16 expression on these cells suggesting the suppression of cytotoxic activity what might be associated with the improvement of pregnancy rate [26]. The indication for prednisolone was RPL

(clinical trial) and suspension of the hyperactive immune system associated with the presence of autoantibodies circulating in serum, without clinical symptoms of the autoimmune disease. The low dose of steroids was introduced for 21 days in menstrual cycle before fertilization and during the first trimester of pregnancy without side effects, what is important due to use of steroids in otherwise healthy women [27,10,28]. The effect of steroids on NK cells in general and uNK cells as the target of this therapy, is important, although, it is not only one mechanism initiated with steroids, what have to be concerned.

Growth-Factors – Granulocyte Colony Stimulating Factor (G-CSF)

The first indication for the intrauterine infusion of G-CSF (accophil, filgramostin) was too thin endometrium. The obtained results in the improvement of endometrium thickness were promising. The further studies showed the positive results in the increase of live birth rate (LBR), but the difference between the study group and the placebo did not reach the statistical significance. The study of DC, Treg cells and NK cells showed the increase of number of DC and Treg cells and, what seemed more important, the modulation of selected KIRs expression on NK cells especially, when “unfavourable” KIR receptors are in binding with HLA-C on the foetal cells [10,28,24]. However, this result was not proved in clinical trial so, this therapy is not recommended.

Platelet-Rich Plasma (Prp)

The first indications for use of PRP (experimental at the beginning) were problems with prolonged healing of wounds in the regenerative medicine. The effects of PRP showed shorter time of healing wounds and regeneration what was encouraging to use PRP in the therapy of infertility including weak ovary function. The platelets are short-living small structures released from the megakariocytes' cytoplasm in the bone marrow. Following activation, the platelets show cytoplasmic – cytoskeletal activity leading to the morphological transformation and facilitating their aggregation. It is associated with release of the different molecules from the cytoplasmic granules of platelets. The list of these molecules included: the cytokines, growth factors (e.g. IL-8, chemokine-ligand 5), platelets-derived growth factor (PDGF), TGF β , VEGF, insulin-like growth factor, epidermal growth factor (EGF). This list is interesting because part of these molecules is associated with regulating effect on the NK phenotype and function [24-28].

The study of the interaction between platelets and NK in the cancer surveillance showed bi-directional signalling with mainly the inhibitory effect on NK cells. The platelets are able to downregulate of NKG2D expression, what is one of the NK inhibitory mechanisms, might be due to releasing of NKG2DL (NKG2D ligand) in the soluble form. Moreover, the platelets are releasing large amount of active TGF β supporting downregulation of NKG2D resulting in the inhibition of NK cytotoxicity [22]. In women with the infertility and disorders of ovary function, PRP are indicated with perspective of the improvement of ovary function, higher clinical pregnancy rate [29-31]. The interaction between NK cells (including uNK cells) and PRP is imported for the positive results of PRP infusion, however, the precise mechanisms still need the study and elucidation.

Immunoglobulins (IVIG)

The preparations of the human immunoglobulin contain more than 90% of IgG isolated from plasma or sera of the healthy blood donors. It means, that the main role of the immunoglobulin preparate is supplementation of the ready specific antibodies to common pathogen, as basic therapy in patients with the

immunodeficiencies of humoral immunity. However, the effect of immunoglobulins given to patients is much wider including the immunomodulation and regulation of immune system. The receptors F(ab)2 are reacting with target - antigen, Fc receptor type I (FcγRI) and type III (FcγRIII) are involved in regulatory mechanisms – the neutralisation of autoantibodies, triggering of expansion of Treg, the inhibition of maturation of DC and synthesis of the own immunoglobulins, the regulation (mainly inhibition) of complement cascade activation, the reduction of NK number and activity including cytotoxicity. The immunoglobulins are used in 2 main indications – the substitution in low dose (0.4-0.6g/kg b.w.) in patients with the immunodeficiency and in the immunosuppressive therapy in high dose (1.0-2.0g/kg b.w.) in autoimmune diseases (e.g. Kawasaki disease, ITP, Evans syndrome, haemolytic anaemia, chronic autoimmune neurological diseases – CIDP, MMN).

At the beginning of this supportive therapy, the immunoglobulin was used in the infertile women with the diagnosis autoimmune diseases or the autoimmune process before clinical onset of disease (e.g. high level of ATPO, ATG, antiphospholipid antibodies). The immunoglobulins (IVIG) in therapy for RPL and RIF were used in different doses, schedules, what effected in non-conclusive results. However, the meta-analysis showed the higher rate of pregnancy, although, the wide range of immunoglobulin dose (0.25g -1.0g/kg b.w.), the different protocols and schedules for the infusions are difficult for the analysis and interpretations. The IVIG are commonly used as adjuvant to ART, up to now, without enough data for the statistical analysis of results. The effect of IVIG on NK cells (peripheral blood) showed decreased of percentage within lymphocyte population from >12% (above normal value) to <12% (normalisation). Moreover, the cytotoxic activity of NK cell was significantly inhibited [32,26]. It seems that, IVIG are still one of the offers for women with RPL, RIF and disorders of the immune regulation, although, the reaching of best results are possible after very careful individual analysis of case as individualised “patient-tailored” therapy [33,32,10,24,28].

Other Approaches – Activated Peripheral Blood Mononuclear Cells (PBMC)

The indication for intrauterine administration of activated PBMC (48 h in vitro culture with presence of 10IU/ml hCG) was RIF (tree or more implantation failures). The idea of this method was the immunomodulation of the endometrium for better receptivity. PBMC produce the long list of the cytokines and other regulatory molecules. The regulation of Th1/Th2 balance for Th1 side is expected and believe to be associated with the increase of supporting cytokines level in time of the embryo implantation. The results of this therapy were shown as the increase of implantation and pregnancy rates in women with previous RIF. In another group of women treated with intrauterine PBMC infusion the percentage of miscarriages significantly decreased [24]. There are no studies of NK and uNK phenotypes and activity in patients after infusion of with PBMC, however, it is obvious, that increased production of Th1 profile cytokines effected the uNK cells number and function. The other way of PBMC administration was subcutaneous injections. The adverse reactions of this method were the immunisation against PBMC antigens and the allergic reactions including anaphylaxis [24-28].

Vitamin E and sildenafil were used with the expectation of improvement of the uterine artery blood flow and improvement of thin endometrium. There results were confusing - from one side with the increase of the endometrium thickness in majority of patients to the other observations with no positive results at all [24]. The one of hypothesis of miscarriages (RPL) and RIF is the

hyperactive immune system what, in consequence, suggested use of the immunosuppressive therapy. The use of tacrolimus (typical immunosuppressive therapy after HSCT and organ transplantation) showed positive results correcting immunological imbalance in Th1/Th2 ratio, however, the inhibitory activity of immune system and the side effects of such therapy have to be considered, especially in women trying to be pregnant [24].

The use of intralipid (fat emulsion) were based on the suppression of cytokines production (decreased level in the serum during this therapy) and the NK cells activity. This last one was not studied, so this effect on NK cell is only suggested. Similar to other methods supporting the positive effect of infertility therapy the intralipid infusions are no standardized procedure [28].

Conclusions

The role of NK and uNK in fertility is complex and multidirectional through the cytokines production and direct activity. The study of these roles and trials of modulations, “corrections” of these cells are far from the success. However, the use of more than one therapeutic approach is a chance and possibility to increase life birth rate in women with diagnosis of infertility [3,11,34,2].

References

1. Abel AM, Yang Ch, Thakar MS, Malarkannan S (2018) Natural killer cells: development, maturation and clinical utilization. Review. *Frontiers in Immunology* 13: 9.
2. Erick TK, Brossay L (2016) Phenotype and functions of conventional and non-conventional NK cells. *Current opinion in immunology* 38: 67-74.
3. Hashemi E, Malarkannan S (2020) Tissue-resident NK cells: development, maturation, and clinical relevance. *Cancers* 12: 1553.
4. Ali A, Gyurova IE, Waggoner SN (2019) Mutually assured destruction: the cold war between viruses and natural killer cells. *Curr Opin Virol* 34: 130-139.
5. Ratajczak W, Niedźwiedzka-Rystwej P, Tokarz-Deptuła B, Deptuła W (2018) Immunological memory cells. *Central European Journal of Immunology* 43: 194-203.
6. Favaro RR, Phillips K, Delaunay-Danguy R, Ujčić K, Markert UR (2022) Emerging concepts in innate lymphoid cells, memory, and reproduction. *Frontiers in immunology* 13: 824263.
7. Waggoner SN, Reighard SD, Gyurova IE, Cranert SA., Mahl SE, et al. (2016) Roles of natural NK cells in antiviral immunity. *Curr Opin Virol* 16: 15-23.
8. Dębska-Zielkowska J, Moszkowska G, Zieliński M, Zielińska H, Dukat-Mazurek A, et al. (2021) KIR receptors as key regulators of NK activity in health and disease. *Cells* 10: 1777.
9. Ekemen S, Comunoglu C, Kayan CK, Bilir E, Cavusoglu I, et al. (2023) Endometrial staining of CD56 (uterine natural killer), BCL-6 and CD138 (plasma cells) improve diagnosis and clinical pregnancy outcomes in unexplained infertility and recurrent IVF failures. *Diagnostics* 13: 1557.
10. Shojaei Z, Jafarpour R, Mehdizadeh S, Bayatipoor H, Pashangzadeh S, et al. (2022) Functional prominence of natural killer cells and natural killer T cells in pregnancy and infertility: A comprehensive review and update. *Pathology-Research and Practice* 238: 154062.
11. Xie M, Li Y, Meng YZ, Xu P, Yang YG, et al. (2022) Uterine natural killer cells: a rising star in human pregnancy regulation. *Frontiers in Immunology* 13: 918550.
12. Feyaerts D, Kuret T, van Cranenbroek B, van der Zeeuw-Hingrez S, van der Heijden OW, et al. (2018) Endometrial natural killer (NK) cells reveal a tissue-specific receptor

- repertoire. *Human reproduction* 33: 441-451.
13. Manaster I, Mizrahi S, Goldman-Wohl D, Sela HY, Stern-Ginossar N, et al. (2008) Endometrial NK cells are special immature cells that await pregnancy. *J Immunol* 181: 1869-1876.
14. Mani S, Garifallou J, Kim SJ, Simoni MK, Huh DD, et al. (2024) Uterine macrophages and NK cells exhibit population and gene-level changes after implantation but maintain pro-invasive properties. *Frontiers in immunology* 15: 1364036.
15. Robertson SA, Moldenhauer LM, Green ES, Care AS, Hull ML (2022) Immune determinants of endometrial receptivity: a biological perspective. *Fertility and sterility* 117: 1107-1120.
16. Huhn O, Zhao X, Esposito L, Moffett A, Colucci F, et al. (2021) How do uterine natural killer and innate lymphoid cells contribute to successful pregnancy?. *Frontiers in immunology* 12: 607669.
17. Gil Laborda R, de Frías ER, Subhi-Issa N, de Albornoz EC, Meliá E, et al. (2024) Centromeric AA motif in KIR as an optimal surrogate marker for precision definition of alloimmune reproductive failure. *Scientific reports* 14: 3354.
18. Wasilewska A, Grabowska M, Moskalik-Kierat D, Brzoza M, Laudański P, et al. (2023) Immunological aspects of infertility—the role of KIR receptors and HLA-C antigen. *Cells* 13: 59.
19. Duan YG, Gong J, Yeung WS, Haidl G, Allam JP (2020) Natural killer and NKT cells in the male reproductive tract. *Journal of reproductive immunology* 142: 103178.
20. Kolanska K, Suner L, Cohen J, Kraiem YB, Placais L, et al. (2019) Proportion of cytotoxic peripheral blood natural killer cells and T-cells large granular lymphocytes in recurrent miscarriages and repeated implantation failure. *Arch Immunol Therapy Exper* 67: 225-236.
21. Zargar M, Ghafourian M, Behrahi F, Nikbakht R, Salehi AM (2024) Association of recurrent implantation failure and recurrent pregnancy loss with peripheral natural killer cells and interferon-gamma level. *Obstetrics Gynecology Science* 67: 112-119.
22. Andreescu M, Frincu F, Plotogea M, Mehedințu C (2023) Recurrent abortion and the involvement of killer-cell immunoglobulin-like receptor (KIR) genes, activated T cells, NK abnormalities, and cytokine profiles. *Journal of Clinical Medicine* 12: 1355.
23. Kofod L, Lindhard A, Hviid TVF (2018) Implications of uterine NK cells and regulatory T cells in the endometrium of infertile women. *Human Immunology* 79: 693-701.
24. Benkhalifa M, Joao F, Duval C, Montjean D, Bouricha M, et al. (2022) Endometrium immunomodulation to prevent recurrent implantation failure in assisted reproductive technology. *International Journal of Molecular Sciences* 23: 12787.
25. S, Wang H, Li D, Li M (2024) An estrogen-NK cells regulatory axis in endometriosis related to infertility and miscarriage. *Int J Mol Scie* 25: 3362.
26. Gomaa MF, Elkholy AG, El-Said MM, Abdel-Salam NE (2014) Combined oral prednisolone and heparin versus heparin: the effect on peripheral NK cells and clinical outcome in patients with unexplained recurrent miscarriages. A double-blind placebo randomised controlled trial. *Arch Gynecol Obstet* 290: 757-762.
27. Bequet YLB, Lashley EEO’N, Goddijn M, van der Hoorn M-LP (2023) The role of uterine natural killer cells in recurrent pregnancy loss and possible treatment options. *Fertility and Sterility* 120: 945-947.
28. Melo P, Thornton T, Coomarasamy A, Granne I (2022) Evidence for the effectiveness of immunologic therapies in women with subfertility and/or undergoing assisted reproduction. *Fertility and Sterility* 117: 1144-1159.
29. Maurer S, Ferrari de Andrade L (2020) NK cell interaction with platelets and myeloid cells in the tumor milieu. *Frontiers in Immunology* 11: 608849.
30. Serdarogullari M, Raad G, Makieva S, Liperis G, Fraire-Zamora JJ, et al. (2024) Revitalizing female fertility: platelet-rich plasma—hype or hope?. *Reproductive BioMedicine Online* 49: 103813.
31. Mehrafza M, Pourseify G, Yousefi TZ, Raoufi A, Jalali SS, et al. (2024) The efficiency of introducing intrauterine infusion of autologous platelet-rich plasma versus granulocyte colony-stimulating factor in repeated implantation failure patients: an unblinded randomised clinical trial. *Int J Fertil Steril* 18: 30-34.
32. Moraru M, Carbone J, Alecsandru D, Castillo-Rama M, Garcia-Segovia A, et al. (2012) Intravenous immunoglobulin treatment increased live birth rate in a Spanish cohort of women with recurrent reproductive failure and expanded CD56+ cells. *Amer J Reproductive Immunol* 68: 75-84.
33. Ho YK, Chen HH, Huang CC, Lee CI, Lin PY, et al. (2020) Peripheral CD56+ CD16+ NK cell populations in the early follicular phase are associated with successful clinical outcomes of intravenous immunoglobulin treatment in women with repeated implantation failure. *Frontiers in Endocrinology* 10: 937.
34. De Carolis C, Perricone C (2010) NK cells, autoantibodies and immunologic infertility: A complex interplay. *Clinic Rev, Allerg Immunol* 39: 166-175.

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