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Changes in the immunophenotype of endometrium during implantation window receptivity formation in healthy fertile women

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ABSTRACT

Introduction: Endometrial receptivity is largely determined by the immunophenotype of endometrium, especially uterine NK-cells (uNK). Immune component is directly involved in the formation of favourable microenvironment for the blastocyst implantation and placenta formation, but the way it changes during the maturation of endometrial tissue in healthy fertile women is still underexplored.

Methods: The endometrium was collected from 47 healthy oocyte donors after controlled ovarian stimulation: 23 women on the day of oocytes retrieval (OR) and 24 women on the term of implantation window (IW). The OR group was analysed, published previously and used as a comparison group to show the dynamic of changes. Isolated endometrial lymphocytes and peripheral blood samples were stained with monoclonal antibodies and analysed according to the three-color flow cytometry protocol.

Results: The proportion of NK-cells ($CD3^{-}CD56^{+}$) in endometrium grew significantly in the implantation window compared to the oocytes retrieval day. NK-cells acquired a more differentiated phenotype from the day of OR until IW: the expression of CD8 and CD158a significantly increased, while the expression of HLA-DR significantly decreased. Significant correlations between peripheral blood and endometrial NK-cells were found in CD8 expression during OR and IW, CD335(p46)neg and CD335(p46)++ subsets during IW term.

Discussion: Immunophenotype of receptive endometrium forms due to the accumulation of uNK-cells, which actively proliferate, become mature, differentiative, and ready to meet the embryo.
Endometrial immunophenotype is peculiar and specific but not autonomic and isolated.
Differentiation (CD8 on NK-cells), and activity (p46 on NK-cells) of peripheral blood lymphocytes is reflected in endometrial lymphocytes profile, and therefore the research of peripheral blood immunophenotype is relevant.

1. Introduction

The endometrium as the inner epithelial layer of the uterus plays a vital role in creating favourable conditions for all reproductive processes. The physiological functions of the uterine endometrium (uterine lining) are preparation for implantation, maintenance of pregnancy if implantation occurs, and menstruation in the absence of pregnancy [1]. Endometrial receptivity as the ability of endometrium to enable embryo implantation highly depends on immune cells, especially endometrial NK-cells (uterine NK-cells, uNK) [2].

The function of endometrium is regulated by hormones such as ovarian oestradiol and progesterone. Oestradiol promotes endometrial proliferation, while after ovulation, progesterone converts the oestradiol-primed endometrium into secretory tissue [3].

Endometrial tissue matures every menstrual cycle under the influence of hormones. At the same time, the implantation and maintenance of pregnancy are supported by both the hormones and the immune cells. Human endometrium is full of immune cells such as dendritic cells,

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macrophages, neutrophils, T- and B-cells, Natural Killers (NK) cells. NK-cells are especially important for the receptivity of endometrium [4]. Uterine natural killer cells (uNK) are the most abundant lymphocytes found in the decidua during implantation and in the first trimester of pregnancy. They are important for early placental development, especially trophoblast invasion and transformation of the spiral arteries [5].

The maturation process is characterized by morphological and transcriptional changes in the endometrium, expression of different cytokines/chemokines, growth factors and adhesion molecules and, admittedly, changes in the amount and phenotype of the immune cells [6–8].

Endometrium maturation disorders lead to defective endometrial receptivity. In some cases, even PGT-A (preimplantation genetically tested for aneuploidy) euploid embryos cannot successfully implant [9], which suggests that endometrial factor is responsible for their failure [10].

Physiological cyclical changes in the cellular component of the endometrium are widely studied in vitro or using mice models [11–13], or relative to different diseases [14,15]. However, the exact physiological processes occurring in the endometrial tissue of healthy women are still unclear.

The authors of this study performed this investigation to establish how the phenotype of endometrial immune cells changes and forms a receptive microenvironment for blastocyst implantation in healthy women. Terms of oocytes retrieval (OR – equivalent to ovulation in the natural cycle) and implantation window formation (IW) in stimulated cycle were selected as the two most critical points for successful pregnancy during the menstrual cycle.

In previous study, the authors showed some important immunophenotype characteristics of endometrium and compared those with blood [16], in the present study, the authors investigated new features during 2 periods and compared recent data with previous findings.

2. Materials and methods

2.1. Samples

Endometrial biopsy was performed on the group of healthy oocyte donors (n = 47), who had at least 1 successful pregnancy in anamnesis and had no incidents of infertility or pregnancy failure. Women were 24–30 years old; they did not have any autoimmune diseases, antibiotic therapy, or vaccination for at least 3 months prior to endometrial sampling.

The endometrium was collected from 23 women on the day of oocytes retrieval (OR), which is equivalent to ovulation in the natural cycle, and 24 women on the term of implantation window (IW) in the stimulated cycle to analyse the markers of endometrial lymphocytes and compare them with the same markers on peripheral blood lymphocytes.

The group of oocyte donors on the oocytes retrieval term (OR, n = 23) was examined in 2019 and used as a comparison group to show the dynamic of immunophenotype changes. A long luteal protocol using GnRH-a (diphereline 3.75 mg) and recombinant gonadotropin (Gonal-F) was applied for controlled ovarian hyperstimulation. Endometrial samples were obtained by pipelle biopsy immediately after oocytes retrieval, 34–36 h after hCG trigger (Ovitrel 250 mg) [16].

In the new experimental group – oocyte donors on the implantation window term (IW, n = 24) – all donors were initially prepared for the egg donation program during 9–10 days by Follitropin alfa (Gonal F, Industria Farmaceutica Serono for Serono International Switzerland 900 IU/1.5 ml) administration in antagonist protocols.

The dosage of gonadotropins (150–300 IU) was chosen on individual parameters (AMH level, antral follicle count, weight).

Daily antagonist Ganirelix 0.25 mg (Orgalutran, Organon Farma-Fertigung, Netherland/Germany 0.25mg/0.5 ml) was added on Day 6 of stimulation.

Ovulation trigger Triptorelin (Diferelin Beaufor-Ipsen Pharma Biotec France 0.1 ml) was prescribed in a dosage of 0.3 ml when relevant criteria had been reached (at least 3 follicles measuring 17 mm).

The mean duration of ovarian stimulation was 9.5 days.

The egg was retrieved by follicle aspiration under ultrasound guidance.

After egg retrieval, all donors did not take any hormonal therapy for luteal phase support.

Endometrial pipelle biopsy was performed on Days 5–6 after the egg retrieval (“Day 0”). These periods are typically used as standard embryo transfer days, or implantation window days.

In both groups, the biopsy procedure was performed by Pippele catheter (Pippele Endometrial Biopsy Curette for Endometrial Sampling Sterile JS China), and the biopsy samples were submerged in 20 ml Dulbecco’s phosphate buffered saline (+4 to +9 °C).

The protocols used in both groups are classic for embryo transfer, the principal difference between groups is the timing of the biopsy – immediately after oocytes retrieval in OR group and on Days 5–6 after the egg retrieval in IW group.

1 woman was excluded from IW group due to the symptoms of severe hyperstimulation, final n = 23.

Peripheral blood samples were collected by venipuncture in the amount of 6 ml on the same day 20–30 min prior to the endometrial biopsy and stored in BD Vacutainer Heparin Tubes.

The procedure of endometrial biopsy was performed at the Reproductive Medicine Clinic “Nadiya”, Kyiv, Ukraine according to the bioethical agreement. The local ethical committee’s approval pursuant to the Declaration of Helsinki 1975 on human experimentation and patient’s informed consent were obtained (Minutes No. 3–18 of the Meeting of the ethical committee of Reproductive Medicine Clinic “Nadiya” dated 26.01.2018; Minutes No. 6 of the Meeting of the Medical Ethics Committee of IPAG NAMS of Ukraine dated 17.12.2017).

2.2. Method of investigating the peripheral blood lymphocytes

Lymphocyte subsets were investigated according to three-color flow cytometry in whole blood on the same day the collection of samples was performed. 80 μl of whole blood were stained with 5 μl of monoclonal antibodies conjugated with fluorochromes FITC, PE, PE-Cy-5 (BD Biosciences, USA) in the following combinations: CD158aFITC/CD56PE/CD3PE-Cy5, CD8FITC/CD56PE/CD3PE-Cy5, HLA-DRFITC/CD56PE/CD3PE-Cy5, HLA-DRFITC/CD8PE/CD3PE-Cy5, CD16FITC/CD56PE/CD3PE-Cy5, CD3FITC/CD335(NKp46)PE/CD56PE-Cy5. After staining within 20 min, samples were vortexed for 30 s with 0.5 ml Lysing solution (BD Biosciences, USA). After lysis, samples were washed with 10 ml of PBS (Phosphate-buffered saline, Sigma-Aldrich). The samples were analysed with a BD FACScan flow cytometer using CellQuest software (BD Biosciences, USA).

2.3. Method of investigating the endometrial lymphocytes

Endometrial lymphocytes were investigated in the same way as described in the previous study [16], on the same day when the biopsy was performed.

Endometrial tissue samples were carefully pipetted in PBS (Phosphate-buffered saline, Sigma-Aldrich). Every pipetting step ended with centrifugation at 400*g* for 10 min to remove the debris.

1 ml pipette tip was cut at the edge so that the diameter of the tip orifice was 4 mm and endometrial tissue was washed 10–15 times by pipetting in PBS (+4 °C) to obtain free endometrial cells in PBS solution. The next pipette tip had a hole diameter of 2–3 mm and tissue pieces were pipetted again. At the last step of pipetting, the tip orifice was 1–2 mm and pieces were pipetted without resistance, i.e., every next step was done with a smaller diameter of the tip orifice. Endometrial tissue was disaggregated and flocculated.

7 ml of PBS (+4 °C) were added, mixed with the obtained suspension, and left for 3–5 min for sedimentation of big aggregates. Cellular

supernatant was carefully layered onto 3 ml of Histopaque-1077 in 15 ml conical centrifuge tube and centrifuged at 400 g for 30 min at room temperature. After centrifugation, the upper layer was carefully aspirated and opaque interface containing endometrial lymphocytes was carefully transferred into a clean tube. The cells were washed by 10 ml of PBS. Cells pellet was resuspended in 0.5 ml of patients' own serum.

Final cell suspension in the amount of 80 μl was analysed by flow cytometry with the same monoclonal antibodies and protocol as shown in the Method of investigating the peripheral blood lymphocytes (see above).

Gating strategies are presented in Supplementary Figs. 1–6. The lymphocyte gate was at least 10,000 events.

2.4. Statistical analysis

Statistical analysis was performed using GraphPad Prism 9. The significance of differences was calculated according to the Mann-Whitney test (unpaired, non-parametric, two-tailed), with 95% confidence interval (CI). Correlations were calculated using Pearson and Spearman correlation. Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Immunophenotype of endometrial NK-cells

3.1.1. Quantity of NK-cells in endometrium (uNK) during oocytes retrieval and implantation window

The proportion of NK-cells ($CD3^{-}CD56^{+}$) in isolated endometrial lymphocytes grew significantly in the implantation window compared to the oocytes retrieval day (two-tailed p-value <0.0001) (Fig. 1.).

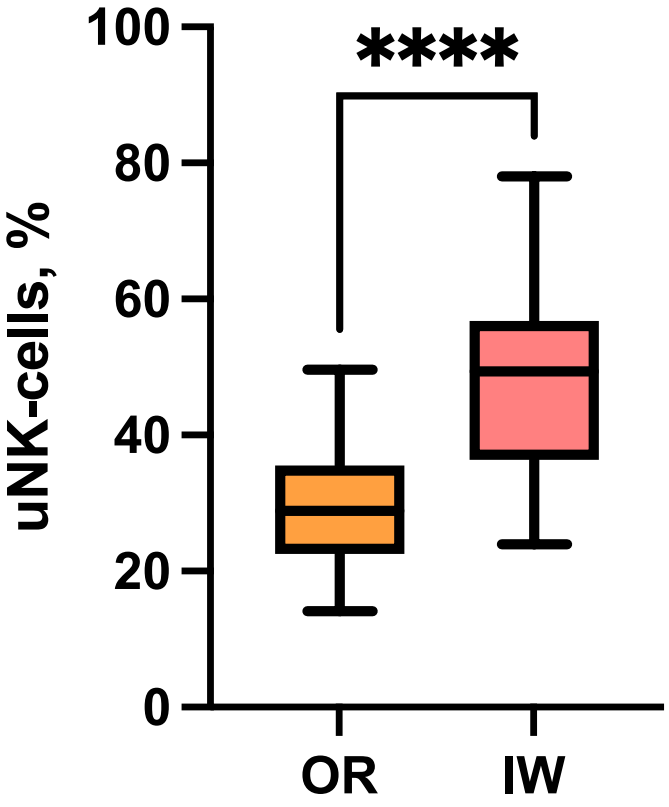


Fig. 1. Changes in the quantity of $CD3^{-}CD56^{+}$ uNK-cells in isolated endometrial lymphocytes during implantation window formation.
Comparison of the number of $CD3^{-}CD56^{+}$ NK-cells in isolated endometrial lymphocytes during oocytes retrieval and implantation window terms; **** – significantly different, two-tailed p-value <0.0001.

3.1.2. HLA-DR expression on NK-cells in endometrium during oocytes retrieval and implantation window

The study found an extremely decreased expression of HLA-DR on NK-cells ($CD3-CD56^{+}$) during implantation window formation compared to the oocytes retrieval period (two-tailed p-value = 0.0002) (Fig. 2A.).

In contrast, no significant changes were found in the expression of HLA-DR on T-lymphocytes ($CD3^{+}$) during IW compared to OR (two-tailed p-value = 0.5547) (Fig. 2B.), expression was quite high and stayed elevated.

Initially, the group of implantation window (IW) term included 24 women but 1 woman was excluded due to the symptoms of severe hyperstimulation. In this woman's case, the expression of HLA-DR on endometrial NK-cells was extremely high, which testifies to the strength of the hormonal influence on endometrial immunophenotype.

3.1.3. CD8 and CD158a expression on NK-cells in endometrium during oocytes retrieval and implantation window

On uNK-cells ($CD3^{-}CD56^{+}$), the CD158a expression significantly increased during implantation window formation compared to the oocytes retrieval term (two-tailed p-value <0.0001) (Fig. 2C.), as well as CD8 expression (two-tailed p-value = 0.0021) (Fig. 2D.)

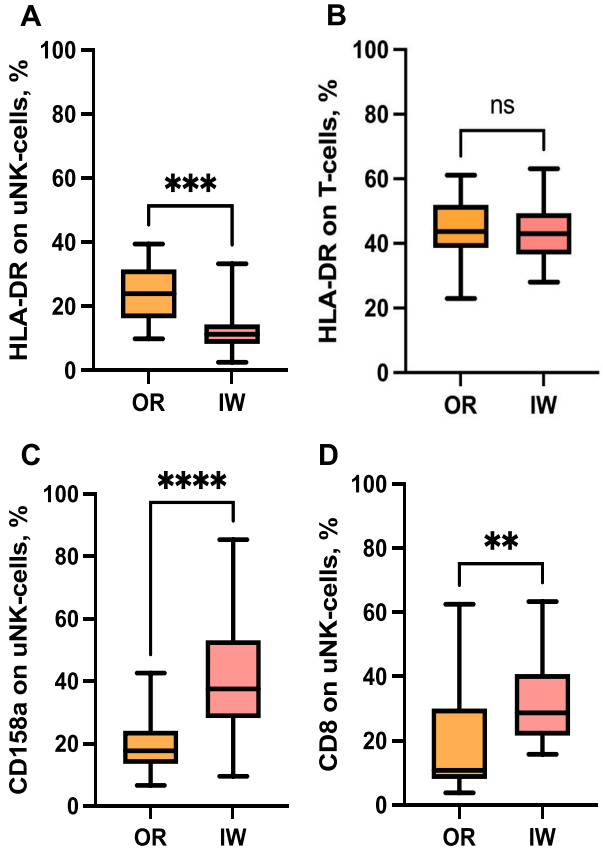


Fig. 2. Main changes in immunophenotype of endometrial lymphocytes during implantation window term (IW) compared with oocytes retrieval term (OR).
A – decrease of HLA-DR expression on endometrial uNK-cells ($CD3^{-}CD56^{+}$)); *** – significantly different, two-tailed p-value <0.001. B – stable HLA-DR expression on endometrial T-lymphocytes ($CD3^{+}$); ns – not significant, two-tailed p-value >0.05. C – increased CD158a expression on endometrial uNK-cells ($CD3^{-}CD56^{+}$); **** – significantly different, two-tailed p-value <0.0001. D – increased CD8 expression on endometrial uNK-cells ($CD3^{-}CD56^{+}$); ** – significantly different, two-tailed p-value <0.01.

3.2. Immunophenotype of peripheral blood NK-cells

3.2.1. Quantity of NK-cells in peripheral blood (pbNK-cells) during oocytes retrieval and implantation window

When comparing oocytes retrieval term and implantation window, no significant changes were found in the proportion of the NK-cells ($CD3^{-}CD56^{+}$) in peripheral blood (two-tailed p-value = 0.2689) (Supp. Fig. 7.).

3.2.2. HLA-DR, CD8 and CD158a expression on NK-cells in peripheral blood during oocytes retrieval and implantation window

The expression of HLA-DR, CD8 and CD158 on peripheral blood NK-cells did not change significantly between oocytes retrieval and implantation window term.

3.3. Immunophenotype of T-cells ($CD3^{+}$) during oocytes retrieval and implantation window

The proportion of T-cells ($CD3^{+}$) decreased significantly in isolated endometrial lymphocytes (two-tailed p-value <0.0001) for obvious reasons – uNK-cells became a dominant population close to the implantation window term.

In peripheral blood, no significant changes in the number of T-cells ($CD3^{+}$) were observed.

When comparing oocytes retrieval term and implantation window, the expression of HLA-DR and CD158 on T cells ($CD3^{+}$) changed non-significantly both in the endometrium and in peripheral blood (Supp. Fig. 8).

However, the study found that the quantity of cytotoxic T-cells ($CD3^{+}CD8^{+}$) decreased significantly during the implantation window formation both in the endometrium (two-tailed p-value <0.0001) and in peripheral blood (two-tailed p-value = 0,0023) (Fig. 3.).

3.4. Correlations between peripheral blood and endometrial lymphocytes

The study found that the CD8 expression on NK-cells in peripheral blood highly correlated with the same on endometrial NK-cells during OR (Pearson correlation r = 0.6538, two-tailed p-value <0.001, 95% CI: 0.3206 to 0.8430; Spearman correlation r = 0.5027, two-tailed p-value = 0.02, 95% CI: 0.08971 to 0.7682). This correlation stays significant during IW (Pearson correlation r = 0.7095, two-tailed p-value <0.001, 95% CI: 0.4202 to 0.8679; Spearman correlation r = 0.7233, two-tailed p-value <0.001, 95% CI: 0.4328 to 0.8777) (Fig. 4.).

The population of cytotoxic T-cells ($CD3^{+}CD8^{+}$) correlated significantly between endometrium and peripheral blood during OR term (Pearson correlation r = 0.4267, two-tailed p-value = 0.0476, 95% CI: 0.006220 to 0.7190). However, during the implantation window term, this correlation became non-significant (Supp. F. 9.).

The study did not find any significant correlation between the expression of HLA-DR on peripheral blood cytotoxic T-cells ($CD3^{+}CD8^{+}$) and endometrial cytotoxic T-cells ($CD3^{+}CD8^{+}$) during implantation window term (Supp. F. 10B.)

A certain number of samples (n = 15) from the group of implantation window term was analysed to find any features in CD335 (p46) expression on peripheral blood NK-cells and uNK.

A significant correlation was found between p46neg subsets among NK-cells in peripheral blood and endometrium (Pearson correlation r = 0.5786, two-tailed p-value = 0.04, 95% CI: 0.04060 to 0.8565) (Fig. 5A.), as well as p46++ subsets (Pearson correlation r = 0.5785,

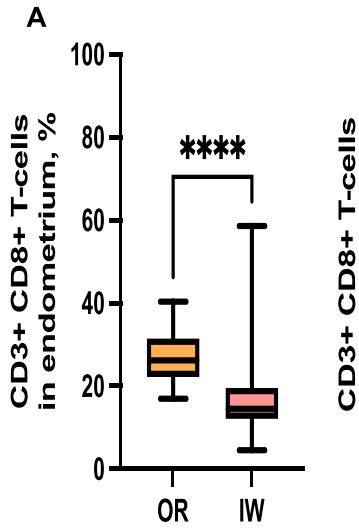


Fig. 3. Changes in the quantity of $CD3^{+}CD8^{+}$ T-lymphocytes during implantation window term (IW) compared with oocytes retrieval term (OR).
A – decrease of $CD3^{+}CD8^{+}$ population in isolated endometrial lymphocytes; **** – significantly different, two-tailed p-value <0.0001. B – decrease of $CD3^{+}CD8^{+}$ population in peripheral blood; ** – significantly different, two-tailed p-value<0.01.

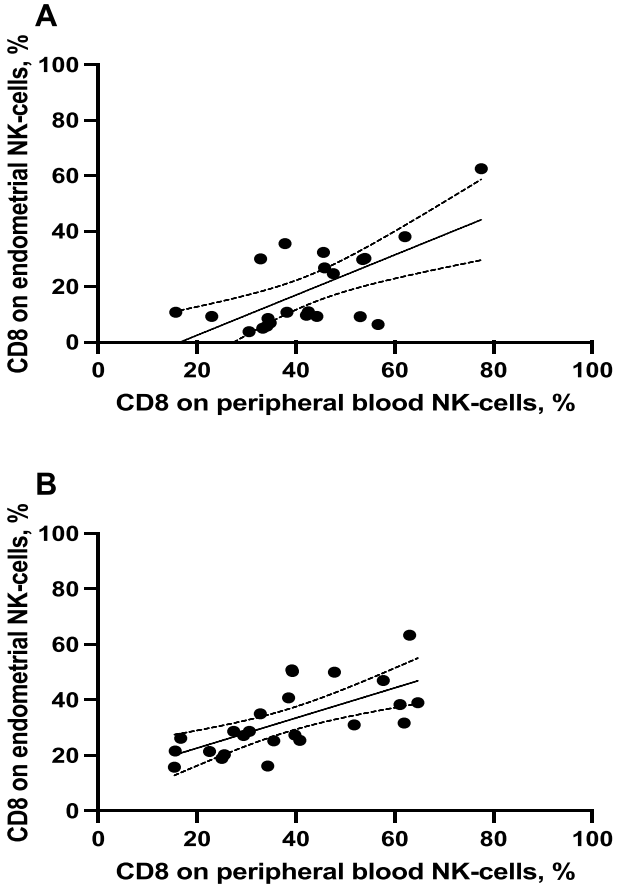


Fig. 4. Comparison of correlation levels between CD8 expression on NK-cells in peripheral blood and endometrium during OR and IW term
A – CD8 expression on NK-cells ($CD3^{-}CD56^{+}$) in peripheral blood and endometrium correlated significantly (Pearson correlation r = 0.6538, two-tailed p-value <0.001, 95% CI: 0.3206 to 0.8430; Spearman correlation r = 0.5027, two-tailed p-value = 0.02, 95% CI: 0.08971 to 0.7682) during the oocytes retrieval term (OR). B – CD8 expression on NK-cells ($CD3^{-}CD56^{+}$) in peripheral blood and endometrium correlated significantly (Pearson correlation r = 0.7095, two-tailed p-value <0.001, 95% CI: 0.4202 to 0.8679; Spearman correlation r = 0.7233, two-tailed p-value <0.001, 95% CI: 0.4328 to 0.8777) during implantation window term (IW). Expression of CD8 on NK-cells increases during maturation of endometrium, but correlation with peripheral blood NK-cells only become stronger.

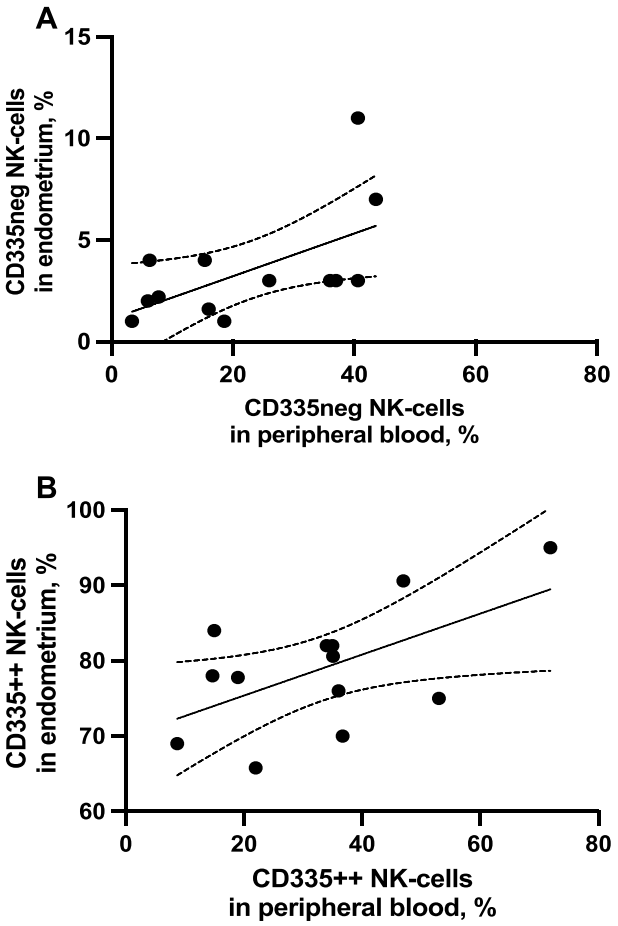


Fig. 5. Correlation between CD335(p46) subsets of NK-cells in peripheral blood and endometrium during implantation window term
CD335 (p46) expression on NK-cells ($CD3^{-}CD56^{+}$) forms 3 main subpopulations – bright, dim, and negative NK-cells. A – CD335neg NK-cells subpopulation correlated significantly between peripheral blood and endometrium (Pearson correlation r = 0.5786, two-tailed p-value = 0.04, 95% CI: 0.04060 to 0.8565). B – CD335bright NK-cells subpopulation correlated significantly between peripheral blood and endometrium (Pearson correlation r = 0.5785, two-tailed p-value = 0.04, 95% CI: 0.04046 to 0.8565).

two-tailed p-value = 0.04, 95% CI: 0.04046 to 0.8565) (Fig. 5B.).

4. Discussion

NK-cells and T-cells are crucial in decidual angiogenesis, trophoblast migration, and immune tolerance during pregnancy. Dysregulation of endometrial/decidual immune cells is strongly associated with infertility, miscarriage, and other obstetric complications [17].

NK-cells in endometrium – uterine Natural Killer cells (uNK) – show strikingly different phenotypic characteristics from NK cells in peripheral blood (pbNK). In receptive endometrium of healthy women, uNK-cells are predominantly $CD56^{bright}$, express markers of tissue residency (CD49a and CD9) and many canonical NK cell markers (NKG2A, NKG2D and NKp46) but mostly lack CD16 and CD57 [18].

uNK-cells and pbNK-cells are different, but their origin is not autonomic – this is confirmed by correlations of certain markers that are discussed below.

It is well-known that uNK-cells are the main lymphocytes subset in the decidual tissue. Up to 70% of decidual leukocytes are $CD56^{+}$ [19]. The results of the present study showed that uNK cells became a dominant lymphocyte population even earlier, in the endometrium during implantation window formation. A few studies investigated cyclic changes in the number of uNK cells in healthy fertile women, and our results undoubtedly confirm previous findings of increasing quantity of uNK-cells during the secretory phase [20,21].

The increase in NK-cells population is interconnected with certain changes in their phenotype.

HLA-DR is an MHC class II molecule. Its expression on NK-cells is associated with their activation, antigen presentation, and high functional activity [22–25].

Previously, it was shown that the high expression of HLA-DR on peripheral blood NK-cells is associated with IVF-failure (in vitro fertilisation failure) [26].

The newly found results of this study showed that in the case of physiological maturation of endometrium, HLA-DR expression on uNK-cells substantially decreased; during implantation window development, uNK changed their immature phenotype and became less reactive, which forms favourable conditions for embryo implantation.

In addition, HLA-DR on NK-cells was documented as the marker of inflammation [27] but in endometrium it looks like a marker of NK-cells proliferation because it decreased on NK-cells during implantation window formation, while there were no significant changes in HLA-DR expression on T-cells ($CD3^{+}CD56^{-}$), and therefore it cannot be described as “inflammation”.

At the same time, NK-cells acquired a more differentiated phenotype from the day of oocytes retrieval (which is equivalent to ovulation in the natural cycle) until the implantation window. This is shown by the increased expression of CD8 and CD158a on uNK cells in IW group compared to OR.

The NK lymphocytes express only CD8aa variant, and it is possible to clear-cut CD8-positive population from CD8-negative subset [28]. A considerable part of NK population is invariably CD8-positive. The role of CD8 receptors on NK cells is understudied.

However, previously, it was found that both hypo- and hyper-NK lymphocyte CD8 expression “accentuations” in peripheral blood are associated with poor IVF outcome [29].

In this study, the authors showed that changes in CD8 expression on uNK are significant and constitute a necessary part of physiological maturation of endometrial tissue.

CD158a (KIR2DL1) is one of the inhibitory KIRs (Killer Ig-Like Receptors). Ligation of inhibitory NK cell receptors with their HLA class I ligands is indispensable for educating NK-cells [30]. Decreased frequency of KIR2DL1+ NK-cells (combined with HLA-C2) expression was found in patients with recurrent miscarriage compared to controls [31].

It was observed that decidual NK-cells are educated through the interaction of maternal HLA-C with KIR2DL1 [32]. This study found that in the physiological menstrual cycle, the expression of CD158a increased upon approaching the term of implantation window formation, which probably signifies the beginning of uNK-cells “education” to acquire some kind of functional competence before pregnancy occurs. However, it was shown that the expression of KIRs by all uNK subsets reached the top in the first trimester of pregnancy compared to non-pregnant endometrium and third-trimester decidua [33]. Trophoblast cells express only HLA-C, so NK-cells must have something to recognize it.

When comparing oocytes retrieval term and implantation window we found a significantly decreased quantity of cytotoxic T-cells ($CD3^{+}CD8^{+}$) both in the endometrium and in peripheral blood, this paradox requires further study.

Previous studies showed that cytolytic activity of endometrial $CD8^{+}$ T cells is maintained during the proliferative phase of the menstrual cycle, but this activity weakens in the secretory phase without any drop in the $CD8^{+}$ cell number [34]. Another research showed that peripheral blood $CD3^{+}CD8^{+}$ T cells were marginally or not significantly changed during the natural menstrual cycle in general [35]. Apparently, the phenomenal change in the percentage of $CD3^{+}CD8^{+}$ T-lymphocytes was noticed due to the certain hormonal influence during the ovarian stimulation in the present study.

On the other hand, when comparing the OR and IW groups, no

significant changes were found in the CD158a expression on peripheral blood NK-cells (uNK-cells require more "teaching", so they express more KIRs compared to pbNK). The study found that levels of NK- and T-lymphocytes as well as CD3CD4 subsets in endometrium are not associated with peripheral blood lymphocytes levels.

These findings raise the question whether the endometrium is absolutely autonomic in its lymphoid component and whether the phenotype of peripheral blood lymphocytes reflects the endometrial one. In turn, the negative answer to the latter question contradicts the necessity of investigating the peripheral blood as such.

To eliminate this conundrum, this study performed an additional comparison of expression of markers on peripheral blood and uNK cells.

The study found a significant correlation between the CD8aa expression on NK-cells in peripheral blood and endometrium during both OR and IW terms.

In earlier attempts to find immunophenotypic commonalities in blood and endometrium, a significant positive correlation was found in levels of $CD8^+$ in NK cells ($CD8^+$ [%] in $CD56^+CD3^-$) between endometrium and peripheral blood [16].

It explains previous results regarding the unfavourable role of accentuated CD8 expression on NK in peripheral blood for implantation – for reasons yet to be investigated, both high and low levels of CD8 on NK-cells are disadvantageous for reproduction [29]. Thus, the findings of this study point to the reason – the same CD8 phenotype on pbNK and uNK.

We showed previously significant correlation between HLA-DR expression on peripheral blood cytotoxic T-cells ($CD3^+CD8^+$) and endometrial cytotoxic T-cells ($CD3^+CD8^+$) on the day of oocytes retrieval [16]. This accentuation on peripheral blood T-cells is also associated with IVF failures [26] because it probably represents unfavourable conditions for implantation in endometrium. Furthermore, in men, it was reported as a marker of adverse health conditions during adaptation to stressful environment in Antarctic Expedition [36]. However, in the present study, we observed that during the implantation window term, this correlation became non-significant. Therefore, obtained correlations still require a deeper study to clarify all possible links between the immunophenotype of lymphocytes in peripheral blood and endometrium.

Finally, the study found a significant correlation between p46neg subsets among NK-cells in peripheral blood and endometrium as well as p46++ subsets. NKp46 (CD335) is a major NK cell-activating receptor involved in the elimination of target cells [37,38] and NK cell maturation [39]. This is strange because pbNK and uNK are dramatically different in their expression of p46 (CD335) – almost all uNK cells are CD56++CD335++, meanwhile pbNK are mostly CD56dimCD335dim.

Some studies suggested that accentuations of NKp46 expression are associated with reproductive failure, such as recurrent pregnancy loss and implantation failures [40,41]. In our recent multicentre blind study, we proved that an accentuated CD335 expression on peripheral blood NK-cells, either increased or decreased, was associated with embryo implantation failure; balanced CD335 levels formed a condition favourable for implantation [42]. Therefore, for further research, the authors of this study propose investigating the function of p46 expression on endometrial NK-cells, as well as correlations between NKp46neg and NKp46++ subsets in endometrium and peripheral blood.

4.1. Limitations of the study

Both groups of healthy oocyte donors were prepared with certain ovarian stimulation. In contrast to the natural cycle, the exact time of ovulation and implantation window was known, which helped us to perform the biopsy and investigate the immune cells just in time.

All donors did not take any additional hormonal therapy for luteal phase support; thus, their conditions were not natural, but similar to the naturally occurring. It remains possible, that certain hormonal influence in the process of controlled ovarian stimulation could have an impact on the phenotype of immune cells. However, the protocols used in both groups are classic for embryo transfer; as a result of it, receptive endometrium is formed. Receptivity and successful implantation of embryo indicate that this endometrium is functionally identical to physiological.

5. Conclusions

This study showed that the immunophenotype of receptive endometrium forms due to the accumulation of NK-cells, decrease in the HLA-DR expression and increase in the CD8, CD158a expression on these cells. In the term of implantation window, the NK-cells in endometrium finish proliferating, become mature, differentiative, and ready to meet the embryo.

The endometrial immunophenotype is peculiar and specific but not autonomic and isolated.

Differentiation (CD8 on NK-cells) and activity (p46 on NK-cells) of peripheral blood lymphocytes is reflected in the endometrial lymphocytes profile. Thus, the research of peripheral blood immunophenotype is prudent and reasonable, although receptivity is determined by the state of the endometrium.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2023.10.002.

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