

The consequences of this pathology are recurrent pregnancy loss (RPL), hypomenorrhea, amenorrhea, and miscarriage. Riemma *et al.* [1] conducted a randomized trial to evaluate the efficacy of *Echinacea angustifolia* (EA) and *Echinacea purpurea* (EP) supplementation combined with vaginal hyaluronic acid soft gel capsules in boosting the remission of cervical low-grade squamous intraepithelial lesions (L-SILs). The study found that adding an oral supplement of EP, EA, zinc, vitamin C, and polyphenols to vaginal hyaluronic acid soft gel capsules may improve the regression of CIN-1/L-SIL and colposcopic, vaginal, and cytologic parameters compared to using the supplement or capsules alone. At the same time, Vitale *et al.* [2] found that the diagnostic odds ratio for hysteroscopy compared to laparoscopic chromoperturbation was 43, with an area under the receiver operating characteristic curve of 0.93, indicating high diagnostic accuracy. Sensitivity and specificity were 88% and 85%, respectively, and the positive and negative likelihood ratios were 5.88 and 0.16, respectively. The authors conclude that interventional assessment enhances diagnostic accuracy compared to observational assessment and that the office setting is preferable to the operating room.

The physiological reasons behind the thinning of endometrial tissue (less than 7 mm) during repeat pregnancy are attributed to the diminished quantity of endometrial stem cells and the reduced levels of oestradiol [3, 4]. Vascular endothelial growth factor (VEGF) concentrations are lower, which causes a decrease in endometrial blood flow and a decrease in the rate of new blood vessel development [5]. As shown by Yang *et al.* [6], a low embryo implantation rate is associated with a thin endometrium.

De Franciscis *et al.* [7] assessed the impact of hysteroscopic metroplasty on reproductive outcomes in women with recurrent miscarriages. After 6 to 60 months of follow-up, the reported live birth rate was 50%, with a clinical pregnancy rate of 73% and a miscarriage rate of 23%. According to Dreisler and Kjer [8], adhesive lysis is the prevailing method for treating this illness. However, difficulties brought on by adhesions, such as insufficient blood flow and decreased metabolism, frequently limit the efficacy of this strategy [9].

Recurrent reproductive failure refers to two distinct conditions: recurrent miscarriage (RM) and recurrent implantation failure (RIF). Currently, over 50% of these incidents are attributed to un-

known causes [10]. Natural killer cells (NK cells) likely contribute to both RIF and RM. NK cells are the predominant immune cells responsible for the formation of the placental bed in the first stage of pregnancy's first trimester [11].

Lebovitz and Oriveto [12] have recorded instances of successful pregnancies with an endometrial thickness as low as 3.7 mm. However, a correlation between thin endometrium and low rates of trophoblast implantation has been shown in studies from the last century. Specifically, the success rates of *in vitro* fertilisation for female patients are 53% for those with an endometrial layer thickness below 9 mm and 77% for receivers with an endometrial thickness beyond 16 mm. Endometrial thickness fluctuates with age and is influenced by the frequency of menstrual cycles, assuming there are no underlying medical conditions. More precisely, in women under the age of 40, only 5% were discovered to have an endometrial layer thickness of less than 9 mm. However, among women aged 41-45, 25% of receivers had a thickness in the range of 5-8 mm [13].

This study aims to investigate the immunological mechanisms underlying recurrent miscarriages and repeated failed implantations in individuals diagnosed with thin endometrium syndrome.

Theoretical overview

The immunological response that takes place both locally and throughout the body affects the embryo implantation process. This immune response involves several substances, such as immunoglobulins, cytokines, hormones, and other components found in the lining of the uterus. The alignment of these elements is crucial for a successful foetal birth. Natural killer cells are crucial in this process. They are part of the lymphocytic series of the natural immune system and are classified as big granulocytes. These cells provide the immune response by exerting cytotoxic action by lysis of the target through activation of apoptosis and also synthesize tumour necrosis factor- α , IL-10, γ -IFN, and transforming growth factor- β [14].

Uterine NK cells in the endometrium of non-pregnant women remain inactive during the menstrual cycle but have the ability to transform and become active in preparation for pregnancy [15]. After the embryo is implanted, uterine NK cells play a crucial role in placental development by assisting in the invasion of trophoblasts and the remodelling

of the spiral arteries. This enables the embryo to receive oxygen and nutrients from the maternal blood supply [16]. The key is to achieve a delicate equilibrium between excessive and insufficient infiltration of uterine tissue in order to prevent the onset of arterial hypertension and limit foetal growth. Trophoblast resonance cells exhibit initial expression of histocompatibility complex antigens, including atypical HLA-E (human leukocyte antigen) and HLA-G molecules, as well as classical HLA-C molecules, which possess genetic variations from both the father and mother. The existence of these antigens can provoke an immune response from the maternal organism [17]. The interaction between NK cells and receptors can be classified into three categories: CD94/NKG2, leukocyte, and killer immunoglobulin-like receptor (KIR). Stimulation of uterine NK cells triggers the production of cytokines. As a consequence of initiating the activation cascade through the HLA-C2 antigen, NK cells release a colony-stimulating factor that guarantees the movement of trophoblast cells. During pregnancy, the role of NK cells undergoes alterations. Initially, they generate growth factors. Around 8-10 weeks, they release cytokines such as gamma interferon (γ -IFN). By 12-14 weeks, they start producing interleukins such as IL-1b, IL-6, and IL-8 [18].

Multiple ideas exist about the genesis of maternal NK cells, including the development of maternal hematopoietic stem cells, the conversion of mature peripheral NK cells through chemokine activities, and the differentiation from immature peripheral NK cells that are transferred from the circulation [19]. NK cells express CD56, CD16, and CD8 receptors (the latter similar to T-cells) on their surface. Maternal NK cells express tissue-specific markers CD49a and CD56 and, unlike peripheral NK cells, are rarely CD16 carriers [20]. Peripheral NK cells demonstrate cytotoxic activity and release cytokines that neutralise tumours and virus particles [21].

Maternal NK cells, unlike peripheral NK cells, carry immunoregulatory potential. They exhibit low cytotoxicity against tumours and are not at all cytotoxic to trophoblast cells because they cannot form active synapses to release perforin. Perforin is a cytotoxic protein that triggers caspase and non-caspase mechanisms of apoptosis in target cells. If trophoblast cells become infected with a virus, uterine NK cells form active synapses and trigger the perforin cascade in trophoblasts [22]. When the quantity of uterine NK cells surpasses the standard

value, there is a rise in the levels of angiogenic substances, leading to excessive blood flow and the initiation of oxidative stress on trophoblast cells [23]. Maternal NK cells have a tendency to release inflammatory cytokines that are associated with T-helper (Th1) cytokines. At the same time, they have a depressed synthesis of anti-inflammatory Th2 cytokines that support the normal course of pregnancy. RM and RIF phenomena may also be caused by maternal HLA-C incompatibility with maternal KIR receptors, which leads to an alternative, incompatible with pregnancy, activation of uterine NK [24].

According to Di Donato *et al.* [25], endometrial cancer management has seen significant advances in recent years, including the use of sentinel node mapping, molecular and genomic profiling, and immunotherapy. Sentinel node mapping has replaced lymphadenectomy in surgical staging, accurately identifying nodal involvement in low- and high-risk patients. Molecular characterization guides treatment in advanced or metastatic diseases. MMRd/MSI-H is an important biomarker, with approximately 30% of patients harbouring this alteration. Ultra-mutated (POLE) and hyper-mutated (MSI-H) profiles predict responses to immune checkpoint inhibitors. At the same time, Bogani *et al.* [26] identify that molecular/genomic profiling is the most accurate method to assess the prognosis of endometrial cancer patients. Radiomic profiling allows for the extraction of mineable, high-dimensional data from clinical radiological images, thus providing noteworthy information regarding tumour tissues. In recent years, the adoption of molecular and genomic profiling has provided a practice change in the management of endometrial cancers. However, the costs and turnaround time associated with the execution of NGS represent the main barriers to the adoption of molecular testing. Inositols are insulin-sensitizing compounds that have regulatory functions in human reproduction. Etrusco *et al.* [27] examine that myo-inositol (myo-ins) mediates the granulosa response to follicle-stimulating hormone stimuli and plays a crucial role in determining oocyte maturation, while D-chiro-inositol is involved in the regulation of ovarian steroidogenesis and, at high concentrations, may be detrimental for oocyte quality. Supplementation with myo-ins during assisted reproductive technologies reduces the total amount of gonadotropins used in polycystic ovary syndrome (PCOS) and non-PCOS women and leads to im-

provements in oocyte quality and maturation, embryo development, and an increase in the rate of successful pregnancies. Myo-ins also act directly on spermatogenesis, improving sperm performance *in vitro* and *in vivo*.

The results of Urman *et al.* [28] indicate that both myo-inositol (MYO) and D-chiro-inositol (DCI) have potential benefits for improving fertility in both men and women. For women, myo-inositol has been found to help improve insulin resistance and manage the symptoms of polycystic ovary syndrome (PCOS), which can contribute to infertility. A study has shown that myo-inositol supplementation can reduce the amount of gonadotropins needed and shorten the length of ovarian stimulation in women undergoing IVF. For men, myo-inositol has been shown to improve sperm count, motility, capacitation, acrosome reaction, and mitochondrial membrane potential. The antioxidant and insulin-sensitizing properties of myo-inositol and D-chiro-inositol may also be beneficial for treating male infertility caused by oxidative stress or metabolic disorders.

The immunological response during embryo implantation involves a complex interplay of immunoglobulins, cytokines, hormones, and natural killer cells in the uterine lining. These cells are crucial for facilitating placental development by aiding trophoblast invasion and arterial remodeling, ensuring adequate maternal-foetal exchange. Imbalances in NK cell activity can impact pregnancy outcomes, potentially leading to conditions like arterial hypertension or restricted foetal growth. Understanding the regulatory role of these cells is essential, as their dysregulation may contribute to reproductive disorders such as recurrent miscarriage or implantation failure.

MATERIALS AND METHODS

75 female patients took part in the study to identify the pathogenetic features of implantation disorders. The main group was divided into two subgroups: one with 30 infertile patients and the other with 25 subfertile patients. The inclusion criteria for this study were carefully defined to ensure homogeneity within each subgroup. The infertile group (RIF) comprised patients who had undergone multiple unsuccessful *in vitro* fertilization (IVF) cycles, failing to achieve pregnancy following embryo transfer despite repeated attempts.

Diagnosis confirmation was based on negative results of beta-chorionic gonadotropin testing on the 14th day post-embryo transfer. Exclusion criteria included the absence of genetic abnormalities in both partners, no anatomical barriers to conception, and the absence of other contraindications to pregnancy planning. The sub-fertile group (RPL) included patients with documented instances of recurrent pregnancy loss (2-3 miscarriages), confirmed by positive pregnancy tests and uterine ultrasound findings on day 3 post-menstrual delay. The selection criteria for this subgroup specifically targeted individuals with a history of miscarriage, aiming to study factors potentially contributing to recurrent pregnancy loss. The exclusion criteria for the sub-fertile group (RPL) involved excluding patients with known causes of infertility such as untreated endocrine disorders, anatomical abnormalities of the uterus, and genetic factors contributing to recurrent pregnancy loss. These criteria aimed to ensure that the study focused specifically on investigating factors potentially associated with recurrent pregnancy loss in patients without these known causes of infertility.

The control group comprised 20 patients who exhibited no reproductive problems and had a minimum endometrial thickness of 8 mm on the 20th to 24th days of their menstrual cycle. The endometrium was classified as thin if its thickness was less than 7 mm between days 20 and 24 of the menstrual cycle. The mean age of the patients in the study group was 34.1 ± 4.3 years, whereas the mean age of the control group was 32.8 ± 3.6 years. 30% of the main group exhibited menstrual cycle abnormalities (Table 1).

Table 1. Baseline characteristics.

Characteristic	Experimental Group	Control Group
Number of participants	55	20
Mean age (years)	34.1 ± 4.3	32.8 ± 3.6
Sub-groups included	Infertile (n = 30); subfertile/RPL (n = 25)	None
% with menstrual cycle abnormalities	30%	Unknown
Relevant reproductive history	Failed IVF (n = 30); RM (n = 25)	No reproductive problems
Endometrial thickness	Unknown	≥ 8 mm

A comprehensive medical record was created, consisting of examination results and patient complaints; medical history related to physical and reproductive health; general and gynaeco-

logical health information; findings from a pelvic ultrasound; endometrial hysteroscopy (including biopsy and histology) using equipment from Karl Storz, a German company; karyotyping; thrombophilia testing; enzyme immunoassay for detecting genital infections; and determination of lupus anticoagulant presence. The typical ultrasonography criteria included a uniform endometrial structure, alignment with the specific day of the menstrual cycle, and the lack of hypo- and hyper-echogenic inclusions.

The immunological component of the inquiry entailed analysing uterine endometrial lymphocytes by a pipelle biopsy, employing a Goldstein catheter. Immunocompetent cells were isolated from endometrial tissue using a non-enzymatic technique. Endometrial pieces were inserted in a Medicon container (Westop Dickenson, USA), and phosphate buffer was introduced. The mixture was then homogenised for many minutes using a Medimachine (Westop Dickenson, USA) until it was destroyed. The obtained homogeneous cell suspension underwent gradient centrifugation using Ficol-verografin (density = 1.078) for a total of 30 minutes. The authors put the monoclonal antibodies CD8PE, CD16PE, and CD56PE into the tubes that had the mononuclear cells. These antibodies were linked to PE-phycoerythrin so that they could stain and bind to surface receptors. Membrane permeabilization was conducted using Cytotfix/Cytoperm solution, followed by the application of antibodies targeting γ -IFN, IL-1, and IL-10, which were labelled with FITC, also known as fluorescein isothiocyanate, for the purpose of labelling and binding intracellular receptors.

An enzyme-linked immunosorbent assay (ELISA) was used to analyse cytokines. In addition, the intracellular synthesis of cytokines (IL-1, IL-10, and γ -IFN) was analysed by specific lymphocyte populations, including intracellular cytokine staining and flow cytometry. Flow cytometry is an adaptable analytical method that is extensively employed in the fields of biology and medicine to assess the properties of individual cells within populations containing heterogeneity. As cells pass through a flow cytometer, flow cytometry permits the simultaneous measurement of numerous physical and chemical properties by staining cells with fluorescent dyes or antibodies that target specific markers. The utilisation of laser illumination and detectors in this procedure enables the swift eval-

uation of various parameters, including cell count, viability, cell cycle distribution, immunophenotyping, apoptosis detection, and functional assays. Flow cytometry is an indispensable tool in numerous scientific fields, including cell biology, immunology, oncology, and more, due to its exceptional throughput and accuracy. Its insights into cellular functions and dynamics are invaluable.

The main method that helped reach the aim of the study was a BD FACS CALIBUR flow cytometer from the USA and the CELLQuest software. It was used to measure the number of cytotoxic lymphocytes in the endometrium and the amount of γ -IFN, IL-1, and IL-10 that they produced inside cells. Statistical processing of the results was performed by determining the student coefficient and its corresponding level of reliability depending on the number of degrees of freedom. The samples were deemed to have a statistically significant difference when the significance value was less than 0.05.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. A study was approved by Ethics Commission of the Al-Farabi Kazakh National University.

RESULTS

The critical consequence of Ascherman syndrome is a high blood flow rate in the radial arteries and inhibition of endometrial vasculature development. Under such conditions, various metabolic links, including uterine lymphocyte activity and haematopoiesis, are impaired. Ultrasound diagnostics detected thin endometrial syndrome in all individuals in the main group. The RPL participants had an average tissue thickness of 5.9 mm, while the RIF sample showed an average tissue thickness of 6.8 mm. The value of 11.2 mm (**Figure 1**) determined the value in the control sample. The statistics clearly indicate a significant difference between the main and control groups, emphasising the influence of endometrial thickness on both unsuccessful implantation and the ability to carry a foetus.

Moreover, heterogeneous structures were detected in 53% of the patients, and in 37%, the endome-

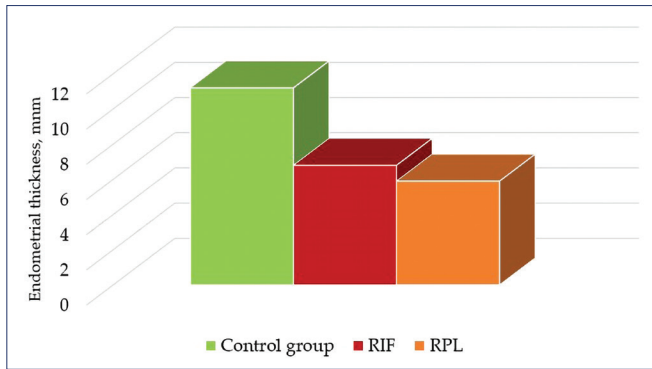


Figure 1. Endometrial thickness in patients with pregnancy pathology and controls, determined by ultrasonography.

trial structure did not correspond to the norm on the current day of the menstrual cycle. According to hysteroscopy data, changes in the endometrial structure were observed exclusively in all the recipients of the main group. Namely, endometritis (oedemas, small polyps less than 1 mm in size) was detected in 82% of cases. In 32% of patients, there were uterine adhesions that were visible as fibrosis with lymphoid inclusions without vascular mesh and as spiral asymmetry of the uterine cavity. Cervical polyps and cystic masses were found in 16%. Myoma of the submucosal layer was diagnosed in 4%. Weak vascular filling, pallor, and thinness of the endometrial tissue were traced in the RIF group.

There is a disagreement among experts about the specific kind of white blood cells present in the reproductive tract. These variances arise from variations in the determination methods, the stages of the menstrual cycle during the collection process, and the gathering of components from different areas of the reproductive system. In general, the leukocytes number per gram of endometrial tissue is always higher than in the fallopian tubes, endocervix, and exocervix. The dominant immunocompetent cells of the endometrium are T lymphocytes (including regulatory T-Treg), natural killer NK, macrophages, dendritic cells, neutrophils, and mast cells. Changes in their ratio are strictly associated with infertility, failure to conceive, and other complications. These cells are carriers of different marker receptors and produce cytokines with different, sometimes opposite, functional loads. NK cells envelop the arteries and glands within the endometrium. They experience apoptosis during the specific part of the menstrual cycle characterised by a decline in progesterone levels. Given that there are no receptors for progesterone on the surface of these cells, the hormone regulates their

numbers through a spectrum of cytokines and stromal cells [29].

NK lymphocytes carry two types of CD56 markers: dim and bright. 95% of dim marker cells also have CD16 receptors and are highly cytotoxic. Bright-marker cells synthesize many cytokines with low cytolytic activity. CD16 markers, in addition to natural killer cells, are expressed by endometrial neutrophils. However, the number of these cells is only 6-15% [38]. T-lymphocytes with the CD8 marker account for 66% of the total endometrial T-cell population. Their cytotoxic ability to kill cells remains strong during the phase of cell growth and decreases during the phase of hormone secretion [29].

At the next stage, the spectrum of cytotoxic lymphocytes (endometrial lymphocytes) with surface markers CD8, CD16, and CD56 (usually natural killers, T-killers) and the intracellular synthesis of cytokines (IL-1, IL-10, and γ -IFN) were studied. All indicators of the main group were on average an order of magnitude lower than those of the main group, but no statistically significant difference was proven between the data obtained for RIF and RPL.

Figure 2 reflects the degree of endometrial receptivity in the main and control samples due to different expression levels of cytotoxic and suppressor lymphocyte markers. In particular, in the reimplantation subgroup, the expression of the CD8 receptor was 18 times lower, in contrast to the fertile sample, and in the RPL subgroup, this index was 12 times lower ($p < 0.01$). The presence of NK cells, as shown by the CD16 marker, exhibited a tendency to decline. Specifically, the number of NK cells in RIF patients was 1.5 times lower compared to the control group. However, no statistically significant difference was seen. While the control group had more CD8-lympho-

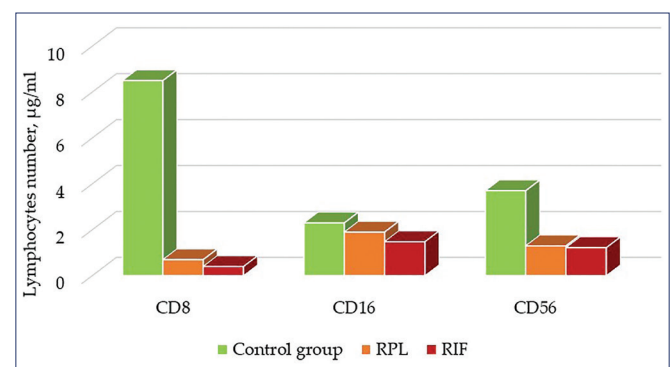


Figure 2. Lymphocytes number.

cytes compared to other cells, the main sample had more cells with CD16 and CD56 markers. In particular, the highest CD16 count (1.9) among patients with recurrent failure to conceive. Subsequently, the levels of cytokines were measured, namely the “inflammatory cytokine” IL-1 and the “anti-inflammatory cytokine” IL-10 (Figure 3). Interleukin-1 has a wide spectrum of activity and is formed in activated monocytes, macrophages, and lymphocytes. It triggers and controls the immune response, acts as a trigger of the inflammatory cascade and pyrogenic reaction, stimulates the expression of other interleukins, activates NK cells, and regulates haematopoiesis. Only the short-term activity of this cytokine is effective. Prolonged action triggers a “cytokine storm” that can lead to organ damage and death. Interleukin-10 is an antagonist of interleukin-1 and interferon gamma. It is mostly synthesized by activated CD8 T-lymphocytes. One of its mechanisms of action is to stimulate humoral immunity through the activation of B-lymphocytes and the modulation of antibody synthesis. At the same time, this protein inhibits the synthesis of interleukin-1, Th1 cytokines, antigen presentation, and expression of second histocompatibility complex molecules, which shows its protective effect against excessive activation of the immune cascade [29].

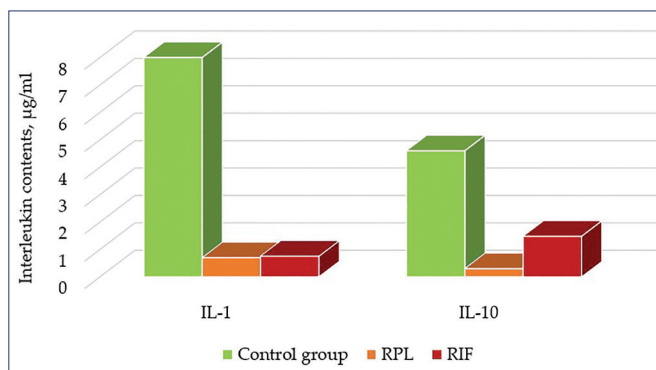


Figure 3. Interleukin contents.

A statistically significant inhibition of the synthesis of the above cytokines ($p < 0.05$) was noticeable in the main group. It should be noted that RIF patients were found to have a higher content of interleukins than RPL women. Namely, the levels of IL-1 production were 11 and 10 times lower in the subfertile and infertile groups, respectively, in contrast to the control sample. The amount of IL-10 (compared to controls) is 15 and 3 times lower in RPL

and RIF patients, respectively. The production of this anti-inflammatory cytokine is insufficient for a positive course of implantation. The interaction between anti-inflammatory cytokines and interferons in the context of viral infections plays a pivotal role in disease pathogenesis. Anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β), regulate immune responses by dampening inflammation and promoting tissue repair. Conversely, interferons, including type I (IFN- α , IFN- β) and type II (IFN- γ), are crucial in initiating antiviral defences and modulating immune cell activity. The dynamic balance between these cytokines influences the severity and progression of viral infections, highlighting their intricate roles in immune regulation and disease outcomes.

The IL-1/IL-10 index exhibited a trend to decrease; however, no statistically significant distinction was observed across the subgroups. The decrease in the index is likely due to a decrease in the synthesis of chemicals that both reduce inflammation and promote inflammation. The IL-1 index prevails over IL-10 in both the control group and patients with RPL. However, in cases of unsuccessful re-implantation, the level of IL-10 is twice as high as IL-1. This difference may be related to the protective reaction of anti-inflammatory cytokine synthesis to inhibit the inflammatory cytokine. The decrease in IL-10 concentration is associated with a decrease in CD8 marker lymphocytes that produce it.

Interferon-gamma is found in intra-epithelial endometrial neutrophils carrying the CD16 marker [30]. This protein has antiviral activity and stimulates the synthesis of oligoadenylate synthase, which, in turn, activates the production of endoribonuclease. The latter cleaves the viral ribonucleic acid (RNA). In addition, interferon “turns on” a kinase that phosphorylates the translation initiation factor of the viral protein. This leads to the inhibition of viral protein production. In addition, γ -IFN has a cytotoxic effect in the G1 phase of the cell cycle; it protects normal, uninfected fibroblasts from the action of NK and stimulates certain immunocompetent links of the immune response [31]. Intracellular production of γ -IFN by different lymphocyte cell lines was also significantly decreased in the main group. CD56, CD8, and CD16 cytotoxic lymphocytes in the RIF subgroup synthesized 16, 35, and 7 times less interferon than in the control group (Figure 4). In the RPL sample, the level of synthesis was suppressed to a lesser ex-

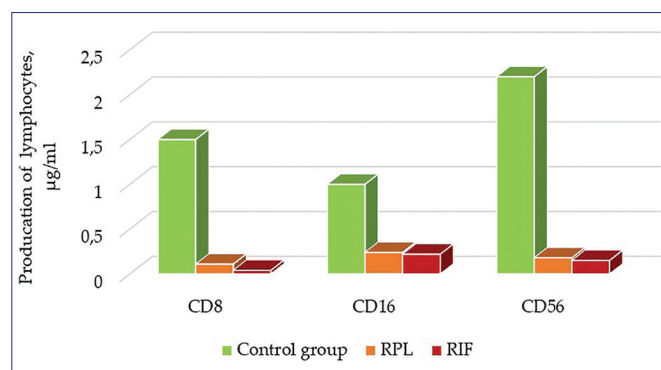


Figure 4. Production of gamma interferon by different lymphocytes.

tent: 12 times for CD56 lymphocytes, 13 times for CD8 lymphocytes, and 4 times for CD16 NK cells. Lymphocytes with the CD56 marker synthesized the most γ -IFN in the control group, whereas cells with the CD16 receptor synthesized the most in the main sample.

The difference in the ratio of total gamma interferon synthesized by endometrial lymphocytes between the main sample subgroups was not statistically reliable. The index in the control group is, on average, 9 times greater (Figure 5). The control group exhibited a γ -IFN synthesis level that was proportional to IL-10 and half as high as IL-1. A similar trend can be observed in the RPL sample. Within the group of patients with RIF, the production of IL-10 is 3.5 times more than the production of γ -IFN. Such a decrease in the studied indices in the main group is indicative of functional insufficiency of the endometrium if its thickness is less than 7 mm. Consequently, this results in reduced responsiveness of the tissue, disturbance in its production of proteins, scarcity of signalling molecules, and compromised processes of both trophoblast implantation and the regulatory function of sex hormones. The high level of “inflammatory molecules” is what causes these things to happen. These molecules cause changes in the endometrium that are different in the infertile group from the non-fertile group. Consequently, cellular metabolism is impaired in the RIF group, leading to a significant deterioration of local immunity. IL-10 is known to be an inhibitor of inflammatory processes, while IL-1 is its stimulator [31]. It is likely that the higher content of IL-10 in RIF, compared with IL-10 in RPL and IL-1 in RIF, is evidence of an activated defence mechanism to counteract foetal failure. The occurrence of many miscarriages and subsequent reimplantation is associated with a reduction in cytokine levels and a decrease in the population of cytotoxic cells. Thus, the number of

CD8, CD16, and CD56-bearing lymphocytes and their production of interleukins 1, 10, and γ -IFN may be prognostic markers of a negative pregnancy course in Asherman’s disease.

In thin endometrium syndrome, the main cause of inhibition of the implantation process is the insufficient concentration of signalling molecules and disruption of the regulatory cascade of sex hormones. Subsequently, cytotoxic reactions are involved in the alteration of peri-implantation mechanisms. Therefore, rehabilitation measures in such patients should include two-stage preparation of the endometrium – the restoration of receptivity and subsequent induction of regeneration. Treatment at the primary stage should include the prevention of infections by immunomodulation and moderate antibacterial and antifungal therapy (to avoid the development of candidiasis). It is suggested that ultrasound therapy be used on the uterine cavity with solutions that contain gamma-interferon, immunophan, and saline with chlorhexidine to make the endometrium more responsive and sensitive. This is done to avoid infection complications. The next phase of treatment should involve the administration of biphasic hormone therapy, consisting of low-dose transdermal oestrogen and progesterone. It is advisable to prioritise the use of micronized progesterone, which has superior absorption and effectiveness when delivered vaginally. In this instance, the progesterone concentration in the endometrium is tenfold higher compared to intramuscular injection. This enhances progesterone’s ability to connect with endometrial receptors and boosts the likelihood of tissue implantation.

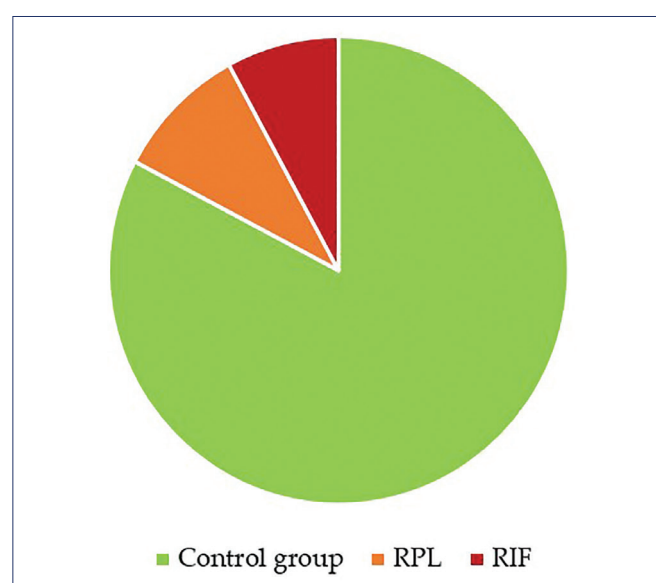


Figure 5. Total gamma interferon content, μ g/ml.

The findings suggest that monitoring immune cell profiles and cytokine levels, particularly IL-1 and IL-10, may aid in assessing endometrial receptivity in patients with thin endometrium syndrome experiencing recurrent implantation failure and miscarriages. Clinical practice should consider incorporating these biomarkers to personalize treatment strategies aimed at enhancing endometrial receptivity and improving pregnancy outcomes.

DISCUSSION

In reproductive medicine, the challenge of recurrent implantation failure and early miscarriages presents significant clinical implications, often linked to underlying endometrial abnormalities such as thin endometrium syndrome, characterized by a tissue thickness below 7 mm. This condition, though infrequent, profoundly impacts pregnancy outcomes and embryo implantation. The authors of the study highlight a notable trend: diminished immunogenic activity of cytotoxic lymphocytes and cytokine synthesis in individuals experiencing RPL and RIF. Immunophenotyping revealed decreased endometrial receptivity marked by structural abnormalities and reduced production of inflammatory and anti-inflammatory cytokines among the study participants.

According to Gamliel *et al.* [32], the CD56 receptor is universal for both uterine and peripheral NK cells, whereas CD16 is a marker for peripheral NK cells, monocytes, and macrophages also present in the uterus. The results the authors obtained are consistent with those obtained earlier. The works of Lui *et al.* [33] and Babayeva *et al.* [34] show that the number of CD56 and CD16-lymphocytes is lower in patients with recurrent miscarriage and RIF compared to the control group. However, some works prove the opposite fact [35, 36]. This discrepancy may be due to the specificity of the research methods. The elevated quantity of cytotoxic cells observed in the aforementioned instances may potentially lead to foetal rejection. The number of peripheral and uterine NK cells correlates with each other [37].

Regarding the secretion of γ -IFN, there is also disagreement in the literature. In particular, Li *et al.* [38] traced an increase in the synthesis of this cytokine in patients with recurrent miscarriages. Moreover, the level of mRNA expression appears to be activated in decidual NK cells but not in tro-

phoblast cells (under the condition of co-culture). Li *et al.* [38] prove in *in vitro* experiments that uterine NK cells in cases of recurrent miscarriage are more active and cytotoxic than uterine NK cells in women without pregnancy disorders. Regarding active compounds, they synthesize perforin and granzyme B.

At the same time, the findings of this study are similar to those of Fukui *et al.* [39], showed a decrease in gamma-interferon production by lymphocytes in patients with RM. This discrepancy is probably due to the use of different detection methods (enzyme-linked immunosorbent assay and real-time polymerase chain reaction (ELISA)) and the examination of different tissue types. γ -IFN, which is produced by uterine NK cells, together with other angiogenic cytokines (AngI, Ang2, and VEGF-C), is involved in spiral artery remodelling. Throughout a typical pregnancy, the levels of these cytokines see an elevation. Therefore, its decrease in the case of this study may be one of the causes of foetal rejection.

The interruption of vascular reorganisation frequently corresponds to an elevation in the quantity of uterine NK cells and smooth myocytes that construct the vascular walls. Increased angiogenic activity can lead to excessive blood supply even before implantation of the trophoblast is complete, resulting in oxidative stress and the destruction of foetal cells by reactive oxygen species. This process potentially elucidates the augmented quantity of uterine NK cells observed in instances of recurrent rejection [33]. Angiogenesis is often inhibited in patients with RIF due to a lack of cytokine activators, in particular decreased IL-6 synthesis. Deficiency of IL-6 results in an augmented cytotoxic response of CD16 and CD56 cells [40]. These two factors, as well as poor invasion of the trophoblast due to an underdeveloped vasculature, may explain the cause of rejection during repeated failed implantations. Thus, maintaining NK cell titers within reference values is important for normal vascular rearrangement because both deficiency and excess can account for foetal non-implantation.

Data obtained by Huang *et al.* [41] suggest an important regulatory role for microRNA-30e expressed by NK cells. In trophoblast cells, this RNA molecule stops HLA-G from being expressed. It also stops lymphocytes from killing K562-bearing target cells, increases the production of substances that help blood vessels form (like IL-4, IL-10, VEGF, and Ang2), and lowers the levels of inflammatory

cytokines (like alpha and others). This data presents an opportunity for the advancement of genetically modified therapy for recurrent miscarriage. According to Gharibeh *et al.* [3], there are several effective therapies for Asherman syndrome. One of them is platelet-rich plasma (PRP) transfusion. The method is based on the transfusion of autologous blood obtained from the peripheral vein. PRP contains many cytokines, chemokines, and growth factors accumulated in platelet granules. These compounds facilitate the regeneration of endometrial tissue, stimulate the development of new blood vessels, and modify the composition of the extracellular matrix. Additionally, they influence the development and growth of stem cells. Due to the autologous origin of blood, conditions are created to avoid undesirable immunogenic reactions. The consequence of this procedure is a thickening of the endometrial layer, a restoration of the level of expression of endometrial signalling compounds, and an increase in the chances of successful foetal delivery by up to 83% [42].

Natural compounds and growth factors are signalling chemicals found within cells that can promote cell growth, specialisation, and the healing of wounds. The research conducted by Gleicher *et al.* [43] has shown the efficacy of granulocyte-colony-stimulating growth factor G-CSF in increasing the growth of the endometrium. After administering granulocyte colony-stimulating factor (G-CSF) through intrauterine injection, persons diagnosed with thin endometrium syndrome had endometrial thickening, leading to a 19% higher chance of healthy foetal birth. The use of this growth factor at a concentration of 300 µg/ml proved effective for endometrial thickening in RIF cases as well [44]. Thus, this is another treatment option for Asherman syndrome, especially relevant for patients resistant to sildenafil and oestradiol.

Today, there are prospects for the latest therapies for thin endometrium syndrome, in particular treatment with stem cells. Stem cells divide with the generation of multipotent and pluripotent lines. In their study, Vitale *et al.* [45] performed a network meta-analysis to assess the efficacy of mechanical strategies in preventing the recurrence of intrauterine adhesions after hysteroscopic adhesiolysis and to evaluate their impact on subsequent fertility. They found that a copper intrauterine device (IUD) together with an intrauterine balloon or cross-linked hyaluronic acid gel was effective

in preventing adhesion recurrence. Hyaluronic acid gel demonstrated the highest pregnancy rates, and the combination of hyaluronic acid gel and an IUD showed the greatest improvement in adhesion scores and menstrual pattern. The authors concluded that cross-linked hyaluronic acid gel, with or without an IUD, appears to be the most effective approach.

In particular, Zhao *et al.* [46] describe an effective intrauterine injection of mesenchymal stem cells in an animal study that led to the thickening of the endometrial wall. Other scholars emphasise the utilisation of exosomes released by stem cells. Exosomes contain bioactive paracrine components like proteins, mRNA, and cytokines that help cells grow and heal and encourage angiogenesis [47]. Therefore, they are a possible therapy option for the thin endometrial syndrome. However, such studies are currently in the developmental stage. Stem cells originate from human amniotic epithelial cells found in the amniotic membrane. They have the ability to transform into cells from various tissues. Moreover, they function as suppressors of B-lymphocyte proliferation and the generation of inflammatory cytokines. They also hinder the migration of neutrophils and macrophages. A study by Zhou *et al.* [48] showed that they synthesize specific osteogen receptors (nuclear transcription factor) and growth factor VEGF, which stimulate the proliferation, metabolism, and regeneration processes of the endometrium in mice. Therefore, amniotic cells can be used for clinical research from the perspective of Asherman syndrome treatment.

Thus, CD56 and CD16 markers on NK cells show consistent patterns of expression across studies, although discrepancies in their levels among patient groups suggest variations in research methodologies. Cytokine profiles, particularly γ -IFN, vary widely, with some studies indicating elevated synthesis associated with RM while others report decreased levels. These inconsistencies may stem from different assay techniques and tissue types analysed. The role of NK cells in angiogenesis regulation, especially through cytokine modulation, underscores their potential impact on vascular remodelling crucial for successful pregnancy. Emerging therapies like microRNA-30e and growth factors such as G-CSF and stem cells offer promising avenues for treating conditions like Asherman syndrome, addressing endometrial thickness, and enhancing fertility outcomes.

CONCLUSIONS

A significant issue in reproductive medicine is the failure of repeat implantation during *in vitro* fertilization or spontaneous miscarriages in the first trimester. Disruption of implantation mechanisms is associated with endometrial abnormalities. Thin endometrium syndrome is determined when a tissue layer thickness of less than 7 mm is found. Although it is rare (the frequency is 1.5%), this pathology has critical consequences for pregnancy and embryo implantation. These processes involve multifunctional regulatory components of the immune response, including cytotoxic lymphocytes and their synthesized cytokines. This study demonstrates a trend in which the immunogenic activity of these cells diminishes in individuals who experience recurrent miscarriages and repeated failed implantations. All individuals in the study sample were diagnosed with 6.4 mm thick, thin endometrium syndrome with structural abnormalities: oedemas, polyps, endometritis, and uterine adhesions. Immunophenotyping by flow cytometry proved a decrease in endometrial receptivity in the RPL and RIF subgroups due to a statistically significant decrease in lymphocytes carrying CD8, CD16, and CD56 markers. The cells demonstrated reduced production of both inflammatory and anti-inflammatory cytokines compared to the control group. More precisely, the production of IL-1 reduced by a factor of 11, whereas the production of IL-10 decreased by a factor of 5 on average. The predominance of IL-10 over IL-1 may indicate the generation of an anti-inflammatory defence mechanism, which is markedly evident in the group with repeated failed implantations. A decrease in total γ IFN concentration within one order of magnitude has been shown for RIF and RPL cases. In healthy individuals, CD56 NK cells are the primary synthesisers of gamma interferon. However, in the patients in the major group, CD16-marker lymphocytes are responsible for the highest production of γ IFN. The results obtained allow us to predict the risk of rejection due to changes in the number of tested compounds and receptors. In order to mitigate these potential hazards in the future, it is recommended to carry out a sequence of clinical trials to assess the efficacy of stem cells from different sources in regulating the implantation processes in cases of thin endometrial syndrome. Strengths of the study include its detailed characterization of thin endometrium syndrome and

its immunological underpinnings through flow cytometry, providing robust evidence linking decreased lymphocyte activity and cytokine production to recurrent miscarriages and failed implantations. However, limitations include the small sample size and the retrospective nature of the analysis, which may limit generalizability. Prospects for future studies lie in prospective clinical trials assessing the therapeutic potential of stem cells from various sources to enhance endometrial function and improve implantation success rates, potentially paving the way for targeted treatments in reproductive medicine.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contribution

G.A.: Conceptualization. A.K., G.K., N.M.: Data curation, formal analysis, writing – review & editing. G.A., A.K.: Investigation, project administration, visualization. N.M., S.B.: Methodology. G.K.: Supervision, validation. G.A., S.B.: Writing – original draft.

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Study registration

N/A.

Disclosure of interests

The authors declare that they have no conflict of interests.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. A study was approved by Ethics Commission of the Al-Farabi Kazakh National University (approval code IRBA400/IRB 00010790).

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