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Original article

Clinical efficacy of peripheral blood mononuclear cell secretome application in patients with repeated implantation failure

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Abstract. *Background and aim:* Recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL) are significant challenges in reproductive medicine, often linked to endometrial dysfunction and chronic endometritis (CE). Current treatments, including antibacterial therapies, are not always effective. This study aims to evaluate the clinical efficacy of peripheral blood mononuclear cell (PBMC) secretome in enhancing endometrial receptivity and improving pregnancy rates in patients with RIF associated with CE. *Methods:* At present, the research for alternative agents for the complex treatment of chronic endometritis are conducted. One of the promising options is the introduction of peripheral blood mononuclear cell (PBMC) secretome containing a variety of biologically active substances, including proteins, extracellular vesicles, antimicrobial peptides, nucleotides, lipids, interleukins and cytokines necessary for endometrial receptivity enhancement in patients with chronic endometritis and infertility. A total of 54 women with long-term infertility were enrolled and divided into two groups: the main group receiving PBMC secretome and a comparison group undergoing standard antibacterial therapy. The PBMC secretome was prepared from cultured PBMCs, and its biological activity was assessed. *Results:* In our study, the pregnancy rate in patients with long-term infertility after PBMC secretome administration was significantly higher when compared to women after antibacterial therapy. *Conclusions:* The application of PBMC secretome appears to enhance reproductive outcomes in women with RIF due to CE. These findings support the potential of PBMC secretome as a novel therapeutic option for improving endometrial health and warrant further investigation in larger clinical trials. (www.actabiomedica.it)

Key words: peripheral blood mononuclear cell secretome, chronic endometritis, recurrent implantation failure, infertility

Introduction

Recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL) represent a detrimental reason for multiple medical interventions in women desiring to conceive, especially when the etiology of infertility remains unclear. According to ESHRE (European Society of Human Reproduction and Embryology) implantation failure is believed being one of the major unresolved issues in reproductive medicine. It has been established that endometrial dysfunction and reduced endometrial receptivity, caused by inflammatory or immunological processes, are associated with both RIF and RPL.

The prevalence of chronic endometritis (CE) in women with RIF reaches 67.5%, in women with

RPL – up to 67.6%. It is unclear whether these differences in prevalence are due to variations in the studied populations, the prevalence of different pathogens or methods of diagnostic evaluation and threshold values. Antibacterial or antiviral agents are used in the treatment of CE, but they are not always effective (1). Currently, the ongoing research are looking for the alternative agents that could be incorporated alongside antibacterial treatment in chronic endometritis management, as well as in the treatment of recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL). Medenica S et al report that novel cell and gene therapies, particularly mesenchymal stem cells, show great promise for the treatment of infertility and autoimmune diseases such as thyroid autoimmunity (2). Another approach involves using the secretome of peripheral blood mononuclear cells (PBMCs), which contain a variety of biomolecules including proteins, extracellular vesicles, antimicrobial peptides, nucleotides, lipids, interleukins, and cytokines necessary for improving endometrial receptivity in CE and infertility. In 2021, Gugerell et al. Reported on the use of secretome from stressed peripheral blood mononuclear cell secretome in patients with diabetic foot ulcers. The mixed extracellular secretome of peripheral blood mononuclear cells (APOSEC) and hydrogel was applied locally three times a week for 4 weeks. APOSEC is predominantly effective in tissue damage caused by hypoxia, modulating the immune system and enhancing angiogenesis, thereby positively affecting tissues through its antimicrobial and neuroregenerative abilities. This preparation also contains important cytokines: interleukin (IL)-8 (0–5214 pg/mL), epidermal growth factor (EGF; 25–226 pg/mL), and transforming growth factor β (TGF-β; 2575–21732 pg/mL) (3). The mentioned preparation turned out to be the closest analog to the medication we currently use. The secretome used in our research is of xenogeneic origin: from whole alogeneic blood. This pharmaceutical substance has an international non-patented name "Protein-peptide complex from blood leukocytes", registered in the drug registry "PN002448/01-2003" since 2010 (4). The difference in obtaining PBMC secretome lies in the means of mononuclear cell (MNC) activation and origin. For the production of APOSEC, allogeneic whole human blood is used. Centrifugation is performed using LSM 1077 (medium for lymphocyte separation, Lonza, Switzerland). LSM is removed in two stages by washing with Dulbecco's phosphate-buffered saline (Lonza, Switzerland). MNCs are suspended in CellGro GMP DC medium. The leukocyte concentration is adjusted to 25 × 10^6 cells/ml. Then irradiation with a dose of 60 Gy is performed, which induces MNC apoptosis. Cultivating apoptotic MNCs in CellGro GMP DC medium results in the secretome release. After 24 ± 2 hours of incubation, cells are removed by centrifugation, and the supernatant containing the secretome is sterile-filtered using a 0.22 μm pore size filter (5). The protein-peptide complex from alogeneic blood leukocytes is obtained as follows: peripheral blood is mixed in a 1:5 ratio with a 10% gelatin solution. The blood is allowed to settle for 30 minutes at a room temperature, followed by 30 minutes in a 37°C thermostat. Cells are washed twice with 0.9% NaCl solution, centrifuged for 10 minutes at 200g. The isolated cells are counted using a Goryaev chamber. The cell concentration is adjusted to 25×10^6 cells/ml in medium-199 containing gentamicin (40 μg/ml). To stimulate leukocytes, PHA (Phytohemagglutinin) is used at a dose of 10 – 15 μg/ml of cell suspension. Leukocyte stimulation is carried out for 3 hours at 37°C. After 3 hours, the stimulator is removed, and the cells are washed three times in 10-fold volume of 0.9% NaCl solution, followed by centrifugation (10 minutes at 200 g). The cells are then placed in clean flasks with fresh culture medium at a concentration of 25×10^6 cells/ml, cultivated at 37°C for 20 – 24 hours. After cultivation, the supernatant is separated from cells by centrifugation for 15 minutes at 400 g. Fractionation of the concentrated supernatants is carried out on a chromatographic column 2.5x100 cm in size ("Whatman", UK) filled with Sephadex G-100, equilibrated with distilled water. Elution rate – 30 ml/h, fraction collection time – 10 minutes. Elution is done in an upward flow of water. The polypeptide fraction in the area of molecular masses less than or equal to 40 kDa is collected, lyophilized. The preparation is then diluted in distilled water (100 μg/ml), subjected to biological filtration (Millipore-QS filters, USA, 0.22 μm pore size), protein concentration is determined using the Lowry method, and biological activity is determined in assays such as macrophage

migration inhibition test and macrophage chemiluminescence, and other tests of biological activity. Antiviral treatment of the final product is carried out (6). Thus, the present study aimed to evaluation of the clinical efficacy of peripheral blood mononuclear cell xenogeneic secretome application in patients with repeated implantation failure associated with uterine factor of infertility.

Patients and Methods

General study design

The study was approved by the ethics committee of "The Research Institute of Obstetrics, Gynecology and Reproductive medicine named after D. O. Ott" (protocol code 129 dated as of October 10, 2023) and performed at the Department of Assisted Reproductive Technologies. The recruitment period of participants was from February 2022 to May 2024. All participants gave informed written consent for participation. A prospective analysis of the peripheral blood mononuclear cell xenogeneic secretome clinical efficacy along with antibacterial therapy (*Moxifloxacin*) in patients with repeated implantation failure associated with uterine factor of infertility was carried out. The study included 54 women aged 29-42 y.o. Inclusion criteria were the following: history of long-term infertility (≥5 years) of uterine origin, multiple (3 or more) implantation failures in IVF cycles and/or 2 or more miscarriages, chronic endometritis diagnosed upon hysteroscopic examination, detection of microorganisms in the endometrium by Pipelle sampling, cryopreserved high-quality embryos, presence of a permanent sexual partner, absence of other significant somatic pathology affecting fertility. Exclusion criteria: couples with male or any other factor of infertility, thyroid pathology, hereditary or acquired thrombophilia, established deficiency of protein C, protein S and/or antithrombin, the presence of more than one risk factor (for women ≤ 35 years old) or one risk factor (for women > 35 years old) for the development of venous or arterial vascular thrombosis, diabetes mellitus, heart valve disease and atrial fibrillation, dyslipoproteinemia, family history of thrombosis, smoking, controlled arterial hypertension, BMI > 30 kg/m2, history of malignant tumors, history of any thrombotic event, systemic autoimmune diseases, psychiatric disorders, alcohol and/or drug abuse, HIV-positivity regardless of HAART, history of viral hepatitis B or C, low patient compliance, withdrawal of informed written consent.

Patient selection, examination, randomization, and therapy assignment

At the pre-screening stage, patients were selected based on their medical history and previous examination data. During the screening stage, after obtaining the signed informed consent, patients were investigated for hereditary thrombophilia (F2 G20210A, F5 G1691A), markers of antiphospholipid syndrome (total antibodies to annexin V, β2-glycoprotein, cardiolipin) and homocysteine levels. Patients negative for hereditary and/or acquired thrombophilia underwent hysteroscopy with subsequent endometrial investigation for microbial and viral agents.

Patient randomization was carried out using a random number generator. As a result, patients were divided into two groups. The first group (main group) included patients who received antibacterial or antiviral therapy followed by intrauterine infusions of peripheral blood mononuclear cell secretome for 10 days. The second group (comparison group) consisted of patients receiving antibacterial and/or antiviral therapy. The volume of examination during patients' selection is presented in Table 1.

Figure 1 contains the number of patients included in the study. Initially 111 infertile women were recruited. 28 women were excluded from the study: 9 couples suffered of male infertility, 3 women were diagnosed with thrombophilia, 4 patients had chromosomal aberrations, 4 patients had significant somatic conditions and gynecological pathology meeting the exclusion criteria was revealed in 6 women. Low patient compliance was noted in 2 women.

Thus, 83 women underwent hysteroscopy and uterine cavity examination, resulting in no signs of chronic endometritis in 21 women and lack of intrauterine microorganisms in another 8 patients. In total, 54 women meeting the inclusion criteria were

Table 1. The volume of examination during patient selection stage.

Stage	Investigation
Pre-screening	Medical history analysis Exclusion of thrombophilia and significant gynaecologic pathology
Screening	Hysteroscopy Endometrial investigation for microbial and viral agents Analysis of inclusion and exclusion criteria
Randomization	Assigning a patient number and group division
Randomized treatment period	Antibacterial/antiviral therapy followed by intrauterine infusions of mononuclear cell secretome in the main group
Follow-up period	Inclusion in the waiting list for frozen-thawed embryo transfer Patients follow-up for at least 21 weeks

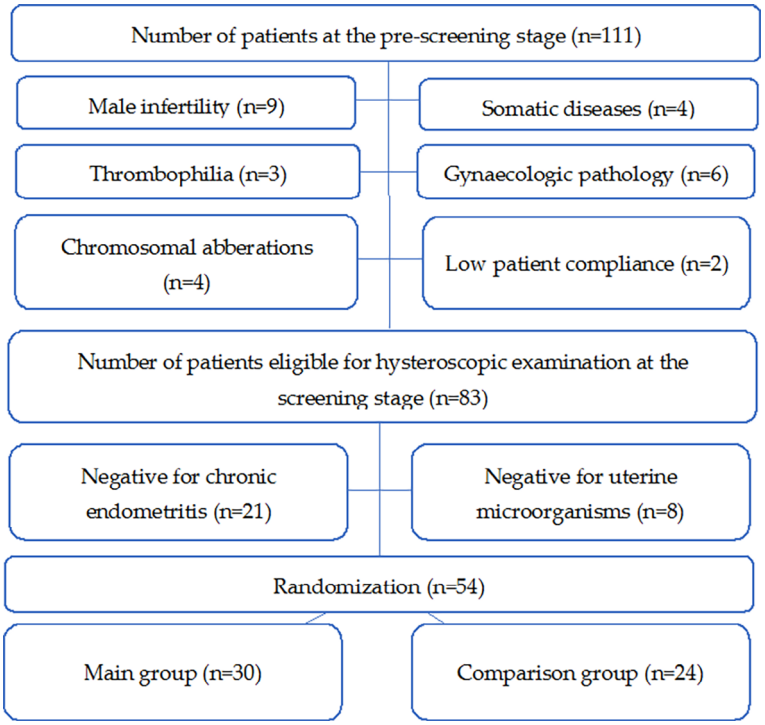

Figure 1. Number of patients included in the study.

recruited. According to randomization, 30 patients were assigned to the main group, while 24 patients comprised the comparison group.

Treatment protocol

All patients within the treatment protocol (n=54) received 400 mg of Moxifloxacin daily for 10 days. In case of Human Herpesviruses detection 500 mg of Valacyclovir daily until the pregnancy onset was administered. Fluconazole at a dose of 150 mg daily on days 1, 4, and 7 of therapy was prescribed to women with *Candida* spp. detection within the uterus. The main group of patients received 10 intrauterine infusions of peripheral blood mononuclear cell secretome once every 3 days, excluding the period of active menstrual bleeding. Prior to intrauterine infusion, 1 vial of mononuclear cell secretome lyophilizate at a dose of

200 mg was reconstituted in 1.0 ml of sterile water for injections. The solution was administered using embryo transfer catheter. Hormone replacement cycle (HRC) protocol was implemented for endometrial preparation for frozen-thawed embryo transfer. For endometrial priming all women were administered oral estradiol 4 mg daily starting on the day 2 of menstrual cycle followed by 600 mg vaginal micronized progesterone initiation on the day 15 of menstrual cycle until the level of human chorionic gonadotropin was determined. Frozen blastocysts were transferred on the sixth day after starting vaginal progesterone.

Efficacy criteria

The onset of clinical pregnancy after frozen-thawed embryo transfer or spontaneous pregnancy during the entire observation period was considered the main efficacy criteria of the treatment.

Samples collection and the molecular-genetic testing

Prior to the treatment initiation, all patients in this study underwent Pipelle endometrial sampling on days 19-21 of the menstrual cycle. For the purpose of DNA extraction, DNA-sorb-AM kits was used ("NextBio" LLC, Moscow, Russia); DTPRIME amplifiers ("DNA-Technology" LLC, Moscow, Russia) were used for the reaction's initiation. A quantitative assessment of the total vaginal and endometrial bacterial mass was carried out using the multiplex REAL-TIME PCR Detection Kit ("DNA-Technology" LLC, Moscow, Russia). The implemented PCR method is based on amplification of a target DNA sequence using one biological sample and is expressed in genomic equivalent (GE). GE, in turn, is defined as the amount of DNA necessary to be present in a purified sample to guarantee that all genes will be present. Upon completion of the run, a quantitative analysis of total bacterial mass and genius/species-specific DNA of *Lactobacillus* spp., *Streptococcus* spp., *Streptococcus agalactiae*, *Staphylococcus* spp., *Gardnerella vaginalis*, *Atopobium vaginae*, *Anaerococcus* spp., *Bacteroides* spp./*Porphyromonas* spp./*Prevotella* spp., *Enrerococcus* spp., *Sneathia* spp./*Leptotrichia* spp./*Fusobacterium* spp., *Megasphaera* spp./*Veillonella* spp./*Dialister* spp., *Lachnobacterium* spp./*Clostridium* spp., *Peptostreptococcus* spp., *Bifidobacterium* spp., *Mobiluncus* spp./*Corynebacterium* spp., *Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Candida* spp., *Candida albicans, Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Trichomonas vaginalis,* viruses (Human Herpes Virus type 6 (HHV-6A), Human Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Herpes Simplex Virus type 1 and type 2 (HSV-1/HSV-2) was obtained.

Statistical analysis

Shapiro-Wilk test was used to evaluate the distribution of the parameters. Normally distributed measurement data were expressed as the mean ± standard deviation, while non-normally distributed parameters were presented as median (Me) with the 25th and 75th percentiles. Qualitative characteristics were presented as absolute and relative (%) data. Pearson chi-square ($\chi 2$) test was used to compare the variables. For all tests, p value of <0.05 was considered statistically significant.

Ethics

The research was conducted in full compliance with the principles of the Helsinki Declaration of the World Medical Association "Ethical Principles for Medical Research Involving Human Subjects", current regulations and standards for providing medical care, as well as other regulatory requirements for conducting clinical research and observational programs in the Russian Federation. When considering the benefits and risks of the intrauterine PBMC secretome administration, the overall benefit for the study participants with long-term infertility was taken into account, based on the determination of the uterine microbiota, as well as previously demonstrated efficacy of the peripheral blood mononuclear cells secretome in the treatment of chronic endometritis.

Results

The study included 54 patients with history of long-term infertility. Table 2 contains general

Table 2. Comparative analysis of age, infertility duration and social status within the groups.

Parameter	All patients	Groups (%)	
		Main group	Comparison group
Number of patients (n)	**54**	**30**	24
Age			
Mean [min; max], y.o	35.2 ± 0.5 (3.3)* [29 – 42]	35.5 ± 0.8 (3.8) [29 – 41]	34.9 ± 0.7 (3.4) [29 – 42]
Median [25*th *percentile; 75*th *percentile], y.o.	34.5 [33; 38]	35 [33,25; 38]	34 [33; 38.75]
W-criterian (p)	0.955 (0.065)	0.96 (0.437)	0.938 (0.144)
Duration of infertility			
Mean [min; max], years	7.4 ± 0.3 (1.8) [5 – 12]	7.3 ± 0.4 (1.8) [5 – 11]	7.5 ± 0.3 (1.7) [5 – 12]
Median [25*th *percentile; 75*th *percentile], years	7 [6; 8]	7 [6; 8]	7 [6; 8.75]
W-criterian (p)	0.889 (0.012)	0.936 (0.131)	0.918 (0.002)
Social status			
Married	42 (77.8%)	23 (76.7%)	19 (79.2%)
Not married (permanent partner)	12 (22.2%)	7 (23.3%)	5 (20.8%)

Abbreviations: *- mean values are presented as μ±SE (SD), SE – standard error, SD – standard deviation.

characteristics of the patients. Women of both groups were comparable by age, duration of infertility and social status.

Thus, the majority of patients corresponded to the early reproductive age and had high chances of achieving pregnancy. However, based on the results of patient's examination, the prolonged infertility was caused by endometrial insufficiency. Analysis of the endometrial microbiota revealed the predominance of *Lactobacillus* spp. (n=39; 81.3%), *Staphylococcus* spp. (n=35; 72.9%), *Gardnerella vaginalis/Prevotellabivia/ Porphyromonas* (n=10; 20.3%), *Atopobium vaginae* (n=8; 16.7%), *Eubacterium* (n=7; 14.6%), *Candida* spp. (n=6; 12.5%). Figure 2 demonstrates no significant differences in the microorganisms detection rate between the groups.

Among the viral agents identified in the uterine cavity, Herpesviridae viruses were found in 6 patients: Human Cytomegalovirus (CMV) was detected in two women, Human Herpes Virus type 6 (HHV-6A) - in two women, Epstein-Barr virus (EBV) - in one woman, and Herpes Simplex Virus type 1 (HSV-1) - in one woman. Upon randomization, there were no significant differences in the detection rate of uterine viruses (Figure 3).

Out of 54 patients, 6 women conceived spontaneously during the prospective observation before frozen-thawed embryo transfer (FET): 5 pregnancies occurred in the main group and 1 pregnancy took place in the comparison group. The remaining 48 women underwent FET (main group - 25 patients, comparison group - 23 women). The clinical pregnancy rate (CPR) per embryo transfer reached 72% (18/25) in the main group and 39.13% (9/23) in the comparison group (p=0.022). Therefore, the overall CPR among all patients included was 61.11% (33/54) (main group - 76.66% (23/30), comparison group - 41.7% (10/23), p=0.009). These data are summarized in Table 3.

Thus, the intrauterine administration of the peripheral blood mononuclear cells secretome demonstrated high efficacy in the complex treatment of infertility associated with bacterial or viral chronic endometritis. The clinical pregnancy rate in patients with long-term infertility after PBMC secretome application was significantly higher relatively to that in the comparison group of women (76.66% vs. 41.7%, p = 0.009).

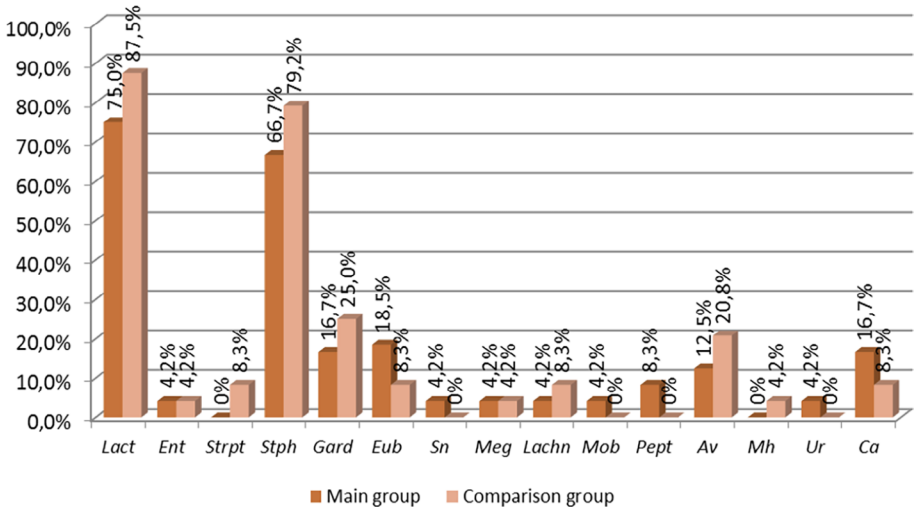

Figure 2. The detection rate of uterine microorganisms in patients with long-term infertility.

Abbreviations: Lact - *Lactobacillus* spp.; Ent - *Enterobactariaceae;* Strpt - *Streptococcus* spp.; Stph - *Staphylococcus* spp.; Gard – *Gardnerella vaginalis/Prevotella bivia/Porphyromonas* spp.; Eub - *Eubacterium* spp.; Sn - *Snethia* spp./*Leptotrichia* spp./*Fusobacterium* spp.; Meg - *Megasphaera* spp./*Veillonella* spp./*Dialister* spp.; Lachn - *Lachnobacterium* spp./*Clostridium* spp.; Mob - *Mobilincus* spp./*Corynebacterium* spp.; Pept - *Peptostreptococcus* spp.; Av - *Atopobium vaginae*; Ca - *Candida* spp.; Mh - *Mycoplasma hominis*; Ur - *Ureaplasma* (*urealiticum+parvum*).

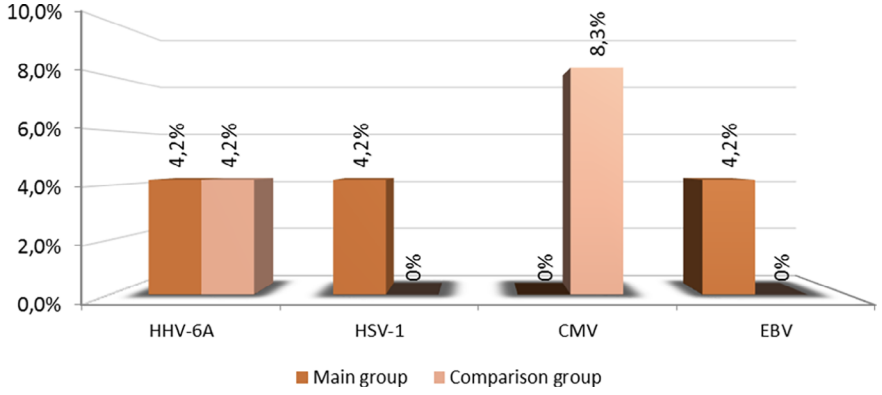

Figure 3. The detection rate of uterine viruses in patients with long-term infertility.

Abbreviations: CMV - Human Cytomegalovirus; HHV-6A - Human Herpes Virus type 6; EBV - Epstein-Barr virus; HSV-1 - Herpes Simplex Virus type 1.

Conclusions

Hysteroscopy can potentially serve as a screening tool for chronic endometritis (CE). Common hysteroscopic findings indicative of CE includes the presence of endometrial micropolyposis and strawberry-like appearances. These distinct findings show a strong positive correlation (sensitivity ranging from 16% to 54% and specificity between 60% and 94%) with chronic endometritis (7-9).

CD138 identification during immunohistochemical (ICH) examination of endometrium has been

Table 3. Comparative analysis of clinical pregnancy in patients with long-term infertility.

Parameter	All patients n=54 (%)	Main group n=30 (%)	Comparison group n=24 (%)	χ^2 (p)*
Spontaneous pregnancy	6 (11.1%)	5 (16.66%)	1 (4.16%)	2.109 (0.147)
Frozen-thawed ET	48 (88.8%)	25 (83.33%)	23 (95.8%)	2.109 (0.147)
Clinical pregnancy rate per ET	27 (56.25%)	18 (72%)	9 (39.13%)	5.259 (0.022)
Total pregnancies	33/54 (61.11%)	23/30 (76.66%)	10/24 (41.7%)	6.873 (0.009)

* - as compared between the main and comparison groups

found to enhance the sensitivity of plasma cells detection, however, the technique lacks standardization (10). Laboratory tests and quality control procedures (11), sample dilution (11, 12), incubation time and temperature, the thickness of endometrial section as well as the number of sections examined influence the final result. Based on these substantial considerations, we made a decision to exclude this method for diagnosing chronic endometritis in our study. Under normal conditions the uterine cavity is known to harbor various microorganisms, primarily of the Lactobacilli species, indicating on the fact that the endometrial microorganisms detection may not always signify endometrial inflammation (13, 14). Buzzaccarini et al. proposed an inflammatory pathway which involves an interaction between inflammatory substances such as microbial products, interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), toll-like receptors (TLRs), IL-1 receptor (IL-1R), IL-6 receptor (IL-6R) and TNF receptor (TNFR) (15). Our previous study also demonstrated a reliable decline in endometrial TGFβ1 and bFGF2 and increase in DEFa1 in women with idiopathic infertility when compared to fertile patients (16). A key component of the outer membrane of gram-negative bacteria – Lipopolysaccharide - serves as the ligand for toll-like receptor 4 on host cells, potentially triggering and mediating chronic endometritis. According to the current available literature, the broad use of antibiotics in patients with CE is not justified. A varying cure rates after antibiotic administration were demonstrated in a recent meta-analysis, which is possibly explained by non-uniform medication and treatment courses in addition to the absence of a single CE recovery definition among the studies (17). However, we believe that infection triggers more complex changes in the reproductive system's function. Disturbed cytokine and chemokine secretion results in altered recruitment of white blood cells, all together negatively affecting uterine contractility, decidualization, receptivity, and vascularization (15). A recent study examining women with reproductive failure and diagnosed with CE found elevated levels of specific immune cells in endometrium of patients with CE, regardless of whether they suffered recurrent implantation failure or recurrent pregnancy loss. These immunological changes linked to CE may contribute to poor endometrial receptivity. The immune profile was normalized after antibiotic treatment, potentially enhancing endometrial receptivity and improving reproductive outcome (18). While some studies suggest alternative treatment approaches for chronic endometritis, the data on their efficactiveness and safety are insufficient (19,20). Cellular secretomes with their mix of biologically active substances, including PBMC secretome, have shown anti-inflammatory, cytoprotective, and pro-angiogenic activities (21). It was demonstrated that the administration of peripheral blood mononuclear cell secretome, regardless of embryo's developmental stage or FET type, may enhance clinical pregnancy and live birth rates in patients with recurrent implantation failure (22). Current therapeutic approaches for endometrial pathology involve intrauterine autologous platelet-rich plasma (PRP), peripheral blood mononuclear cells (PBMC), and mesenchymal stem cells (MSC) administration. The possible benefit is explained by enhanced production of cytokines, growth factors and other biologically active substances, resulting in reduced natural killer (NK) cell activity, decreased number of Th17 and Th1 cells, and increased

number of Treg cells and Th2 cells (23). Intrauterine infusion of autologous platelet-rich plasma is considered a promising therapeutic option for women diagnosed with chronic endometritis who are resistant to antibacterial treatment, as higher implantation rate of donated embryos was demonstrated after the treatment (24). Regardless of the agents used (PRP, MSC, PBMC) the restoring effect is provided indirectly by the products of cell secretion. Since 2010, an expression of regulatory proteins secreted into the extracellular space has been collectively referred to as the "Secretome" (25). The secretome is involved in intercellular communication and includes microvesicles, exosomes, growth factors, chemokines, cytokines, immune regulatory factors, adhesion molecules, proteases, nucleic acids, antimicrobial peptides, non-protein components such as lipids, microRNAs, coding RNAs and others, playing a crucial role in key biological processes regulation (26). Giordano et al (2023) emphasize the importance of the intrauterine environment to pregnancy outcome, specifically examining how transplacental permeability of heavy metals affects fetal development in a sex-dependent manner. Although this study focused on environmental factors rather than direct therapeutic interventions, it revealed complex interactions between maternal health, placental function, and fetal development, which has implications for understanding how therapies aimed at improving endometrial receptivity, such as PBMC secretion, may also affect pregnancy success (27). Pregnancies in patients undergoing Assisted Reproductive Technology (ART) are considered high-risk. Gullo et al. (2022) investigated the neuropsychomotor development of newborns conceived through ART, finding that while these methods significantly increase the likelihood of pregnancy in women with infertility, they may also carry risks that can impact the child's development. This underscores the critical importance of evaluating the safety and efficacy of treatments both during pregnancy and in the preconception phase. Moreover, Gullo et al. (2022) reviewed non-invasive prenatal testing (NIPT), highlighting the crucial role of early gestational health in preventing chromosomal abnormalities (28,29). This is particularly important in frozen embryo transfer (FET), where endometrial receptivity plays a crucial role in improving success rates (30,31). The therapeutic administration of cell-free secretome has emerged as a new direction in regenerative medicine. This approach allows avoiding the potential risks associated with traditional cell therapy. The technological process for isolation can be standardized, and methods similar to traditional medicine can be applied for its efficacy and safety evaluation (32). Austria possesses the technology for cell-free lyophilized PBMCs production in a formulated drug called APOSEC (apoptotic Secretome). The maximum therapeutic effect was achieved only when all the secreted factors of PMNC were present simultaneously compared to individually isolated biomolecules from the cell-free PMNC (33). A clinical trial conducted in 2015 (ClinicalTrials.gov: NCT02284360) on APOSEC therapy was dubbed a "breakthrough in regenerative medicine"(34). Currently, this field of research is actively investigated in other countries (35). The xenogeneic secretome was shown to exhibit similar therapeutic effects on regenerative processes. There is a high degree of homology of antigenic and cytokine structure between humans and some animals (36), which makes it possible to use them as a source of the PMNC lyophilizate we are using. The secretome we use also contains the progerin peptide (PG) which exert a broad activity against gram-positive and gram-negative bacteria, including *E. coli*, *P. aeruginosa*, *N. gonorrhoeae* as well as fungal agent such as *C. albicans* (37-39). As a conclusion, we believe that two-stage therapy should be recommended to patients with chronic endometritis and repeated implantation failure for endometrium rehabilitation, consisting of antibacterial therapy and intrauterine agents promoting endometrial regeneration. This approach appears to restore receptivity in patients with a history of repeated implantation failures and improve the reproductive outcome.

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Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity

interest, patent/licensing arrangement, etc.) that might pose a conflict of interest in connection with the submitted article.

Authors Contribution: Conceptualization, N.I.T and A.M.S.; formal analysis, O.V.B., J.R.R.; investigation, N.I.T., K.V.S., V.V.K., K.V.O.; writing—original draft preparation, K.V.O., G.K.S. and A.A.K.; writing—review and editing, A.M.S., N.I.T and A.M.G.; funding acquisition, O.N.B. All authors have read and agreed to the published version of the manuscript.

Ethic Approval: Ins The study was conducted in accordance with the Declaration of Helsinki, and approved by the ethics committee of the D.O. Ott Institute of Obstetrics, Gynecology, and Reproductive medicine.

Declaration on the Use of AI: None.

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