Section 2

Original research

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INTRACELLULAR PRODUCTION OF CYTOKINES IL-1 AND IL-10 IN PATIENTS WITH THIN ENDOMETRIUM SYNDROME WITH RECURRENT IMPLANTATION FAILURE

Evaluation of endometrial cytotoxic lymphocytes using flow cytometry for “thin endome- trium” syndrome in 24 patients with recurrent implantation failure (RIF). The revealed changes in the level of immunocompetent cells indicate that the pathogenesis of recurrent unsuccessful implantation lies in a pronounced decrease in the level of CD8+ cytotoxic / suppressor lymphocytes and CD56 + lymphocytes, as well as a sharp decrease in the intracellular production of cytokines IL-1 and IL-10 by endometrial lymphocytes. Deficiency of signaling molecules and their synthesis of proteins, which oc- curs in the syndrome of “thin endometrium”, is accompanied by disruption of peri-implantation mecha- nisms, including the regulatory action of sex steroid hormones. Prospects for the treatment of failed implantation attempts lie in the selective effect on the endometrium with impaired receptivity. The treatment strategy is to eliminate the microbial-infectious agent with the use of rational antibacterial and immunomodulatory therapy at the first stage, induction of intracellular regeneration: biphasic hor- mone therapy (low doses of estrogen and progesterone). Preference is given to micronized progesterone preparations with bioidentity to endogenous progesterone, higher bioavailability and efficiency in the intravaginal route of administration.

Key words: thin endometrial syndrome, recurrent implantation failure, endometrium, endometrial cytotoxic lymphocytes, intracellular cytokines IL1, IL10.

Introduction

The problem of repeated unsuccessful implantations is considered one of the most urgent in modern reproductology. Implantation disorders are the cause of miscarriage, infertility, ineffective in vitro fertilization (IVF) cycles and other methods of assisted reproductive technologies, since only 1/3 of IVF failures are due to the quality of the embryo and 2/3 of failures are associated with pathological changes in the endometrium [1].

The thickness, morphological structure and receptivity of the endometrium are the main signs of endometrial maturity and, at the same time, criteria for predicting a successful pregnancy, which are guided in clinical practice [2]. The preimplantation endometrium is distinguished by the presence of a developed capillary network, microcirculation, tissue oxygenation, proliferative activity of epithelial and stromal cells, active metabolism and readiness of the endometrial neuroreceptor apparatus. The period of optimal receptivity of the endometrium begins on the 6th day after ovulation and lasts 4-5 days, which corresponds to 20-24 days of the menstrual cycle, and this period is called the “implantation window”.

The most frequent pathology of the endometrium, in which multiple secondary morphofunctional

changes occur, disrupting the cyclic transformation and receptivity of the mucous membrane of the uterine body, is chronic endometritis. At the same time, more and more often with this pathology, the syndrome of "thin endometrium" occurs.

The criterion for "thin endometrium" is considered to be the thickness of the endometrium less than 7 mm and the absence of a three-layer structure during the "window of implantation". The pathophysiological features of the "thin endometrium" consist in insufficient growth of the glandular epithetlium, depletion of blood vessels and impaired expression of a number of regulatory cytokines, growth factors, natural killer cells, lymphocytes, which in turn reduces the implantation capacity of embryos [3].

In addition, insufficient production of progesterone can lead to suppression of progesterone receptors in the epithelial cells of the endometrium, as a result of which there is a decrease and complete loss of its receptivity by the time of implantation, no "implantation window" is formed, implantation of the ovum does not occur, which leads to infertility, and if implantation occurs, it is ineffective and miscarriage develops [4].

In recent years, endometrial dysfunction has been associated with pathological changes in the expression of numerous factors. Among new molecular research methods, the role of cytokines IL-1, IL-6, IL-10, interferon gamma, tumor necrosis factor, growth factors (leukemia inhibiting factor - LIF, granulocyte macrophage growth factor, vascular endothelial factor, transforming growth factor beta1), expression of estrogen and progesterone receptors as markers of endometrial receptivity [5,6].

It should be noted that today the focus of scientific interest is intracellular cytokines and growth factors responsible for the receptivity of the endometrium. Intracellular production of proinflammatory cytokines by endometrial lymphocytes, in particular IL-1, are considered as biologically active factors that improve the decidualization process. At the same time, the intracellular production of anti- inflamematory cytokines, in particular IL-10, improves the receptivity of the endometrium. Integral assessment of pro- and anti-inflammatory cytokines serves as a marker of the functional state of the endometrium and a prognostic criterion for the effectiveness of therapy [7].

Thus, the aim of the present study was to study the clinical and immunological parallels in the syndrome of "thin" endometrium in patients with recurrent implantation failure.

Materials and Methods

The study included 24 patients with recurrent implantation failure and thin endometrial syndrome. The comparison group consisted of 20 patients without reproductive losses and the presence of normal endometrial thickness on days 20-24 of the cycle. For each woman, an individual observation card was drawn up, including the results of the examination with the study of complaints, somatic and obstetric-gynecological anamnesis, data of general and gynecological status, generally accepted laboratory, as well as special research methods according to indications: ultrasound of the pelvic organs, determination of the level of hormones in the blood, ELISA for infections, determination of lupus coagulant and anti-hCG in the blood, genetic consultation and karyotyping.

The criterion for "thin endometrium" was the thickness of the endometrium less than 7 mm on the 20-24 day m.c. with transvaginal echography.

The material for the immunological study was biopsy specimens of the uterine endometrium obtained by Pipel biopsy using a Goldstein catheter. Isolation of immunocompetent cells from endometrial tissue was performed using an enzyme- free method. The endometrial fragments were placed in a Medicon container (Becton Dickenson / USA), phosphate buffer was added, and ground in a Medimachine homogenizer (Becton Dickenson / USA) for several minutes. The resulting cell suspension was centrifuged for 30 min in a ficoll-verographin density gradient (d = 1.078). The content of endometrial lymphocytes CD3+, CD8+, CD16+, CD56+ (stained with phycoerythrin) and the level of intracellular production of cytokines IL-1 and IL-10 (stained with FITC after permeabilization of the cell membrane) were determined on a flow cytometer using the CELLQuest program.

Statistical analysis of the obtained results was carried out using Student's t-cryteria. Differences between the compared groups and numbers were considered significant when the probability of error was $P \leq 0.05$.

Results and Discussion

In the main group, the average age of the patients was 31.3 ± 3.0 years, in the comparison group - 32.8 ± 3.6 years. The analysis of reproductive function revealed the following. All patients in the main group had a history of two or more episodes of implantation failure. Recurrences of implantation failure manifested themselves in the form of spontaneous miscarriages,

missed pregnancies, or unsuccessful IVF attempts. The history of the patients noted the presence of chronic endometritis, endometriosis / endometriotic cyst, uterine fibroids, hydro / saktosalpinx, polycystic ovary, endometrial polyp. During karyotyping, a normal karyotype was revealed in all examined patients. The study of the hemostasiogram revealed thrombophilia in 20% of cases. The examination revealed CMV, HSV, mycoureaplasma.

In the comparison group, 80% of patients had repeated pregnancies, and 60% had repeated births. In three cases, the male factor of infertility (azoospermia), the presence of STIs was the reason for contacting a reproductologist. When examining all applicants of the comparison group, the thickness of the endometrium was 19-22 m.s. was more than 8 mm.

The data characterizing the peculiarities of the subpopulation composition of endometrial cytotoxic lymphocytes in patients with recurrent implantation failure and thin endometrial syndrome are presented in the table 1.

Table 1. Cytotoxic endometrial lymphocytes in patients with recurrent implantation failure and thin endometrial syndrome.

Indices, %	Comparison group, n = 20	main group, n = 24
total CD8+	8,6±1,7	0,6±0,4*
total CD16+	2,3±0,6	1,8±0,6
total CD56+	3,8±0,9	1,3±0,3*
total IL1+	8,3±3,3	0,7±0,8*
total IL10+	5,6±0,8	0,13±0,16*
IL1+/ IL10+	1,56±0,68	0,43±0,38

* Differences with comparison group are significant, P <0.05.

It was found that in patients with recurrent implantation failure and thin endometrial syndrome, a decrease in endometrial receptivity was observed, which was expressed in a significant (18-fold) decrease in the level of CD8 + cytotoxic / suppressor lymphocytes (P <0.001), as well as a 3-fold decrease in the level CD56 + cells. The level of natural killer cells with the CD16 + phenotype tended to decrease.

It should be noted that in patients with recurrent implantation failure and thin endometrial syndrome, there was a sharp decrease in intracellular cytokines by endometrial lymphocytes - interleukin-1 by 11 times and interleukin-10 by 4 times compared to the same parameters in the comparison group. The IL1 + / IL10 + index tended to decrease, but no significant differences were found, as there was a decrease in both pro-inflammatory IL-1 and anti-inflammatory IL-10 cytokines.

Thus, the revealed changes in the level of immunocompetent cells indicate that the pathogenesis of relapses of unsuccessful implantation is a pronounced decrease in the level of CD8 + cytotoxic / suppressor lymphocytes and CD56 + lymphocytes, as well as a sharp decrease in the intracellular production of cytokines - IL-1 and IL-10 by endometrial lymphocytes. It is known, the implantation process can be thought of as an inflammatory reaction that promotes attachment and invasion of the embryo into the endometrium, providing the necessary interaction with the maternal vascular system. Deficiency of signaling molecules and their synthesis of proteins, which occurs in the syndrome of «thin endometrium», is accompanied by disruption of periimplantation mechanisms, including the regulatory action of sex steroid hormones [8].

The decisive role in the effect on the endometrium is not played by the steroid hormones themselves circulating in the peripheral bloodstream, but is determined by their interaction with functionally complete receptors of the endometrial tissue for the corresponding steroid hormones. Due to the presence of receptors - «recognition molecules», the target cell is able to accurately distinguish the smallest concentration of tropic hormones in the extracellular fluid [9].

Prospects for the treatment of failed implantation attempts lie in the selective effect on the endometrium with impaired receptivity.

Considering that the leading role in the gen- esis of miscarriage is assigned to an infectious fac- tor, and according to V.M. Sidelnikova [8], chronic endometritis is histologically verified in 73% of cases, and in 87% there is persistence of opportunistic microorganisms in the endometrium, and taking into account the decision of the World Congress of Obstetricians and Gynecologists (FIGO, Kuala Lumpur, 2007) that all without exception, cases of undeveloped pregnancy should be associated with the presence of chronic endometritis - an important stage in the treatment strategy is to eliminate the microbial-infectious agent and includes rational antibacterial and immunomodulatory therapy.

In addition, when studying the pathomorphogenesis of habitual miscarriage, I.O. et al. [9] demonstrated the disruption of the processes of cellular and intracellular regeneration of endometrial epithelial cells, which leads to insufficient expression of receptors for both progesterone and estrogens and underlies a decrease in the receptivity of the endometrium with a “closed window of implantation”. The treatment strategy is the induction of intracellular regeneration: biphasic hormone therapy (low doses of estrogen and progesterone). For the intensification of regenerative reactions in endometrial cells, great importance is attached to both hormonal and mechanical influences with the restoration of endometrial receptivity.

Conclusion

Thus, the preparation of the endometrium for pregnancy should be carried out in stages. The preliminary stage includes antibiotic therapy of chronic endometritis with the use of immunomodulators, as well as other means, including physiotherapy, that can potentially restore the receptivity of the endometrium. Impaired endometrial receptivity, based on the lack of expression of receptors for both progesterone and estrogens, formed the basis for the development of a therapy strategy with the induction of intracellular regeneration: biphasic hormone therapy (low doses of estrogens and pro- gesterone), which aims to induce the regenerative activity of endometrial epithelial cells with subse- quent differentiation under the influence of proges- terone [11-14]. In this case, preference is given to intravaginal routes of administration of micronized progesterone with a proven higher bioavailability and effectiveness. In addition, scientific studies of the pharmacokinetics of progesterone have shown that with the vaginal route of administration, the concentration of progesterone in the endometrium is significantly higher than with intramuscular administration [15].

From these positions, the drug of choice for hormone therapy with gestagens is Luteina - this is the latest generation of gestagen, which is completely bioidentical to endogenous progesterone: by the formula, by the mechanism of action in the body and by the effect. When using vaginal tablets Luteina, due to absorption through an extensive network of venous and lymphatic vessels of the vagina, a «first-pass effect through the uterus» is created, providing high concentrations in the endometrium and high clinical efficacy of Luteina.

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