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DYSREGULATION OF IMMUNE RESPONSE IN THE ENDOMETRIUM OF PRIMARY INFERTILITY PATIENTS

Burac Mihaela, Assistant Professor, Department of Obstetrics and Gynecology
Nicolae Testemitanu State Medical and Pharmacy University
(Moldova, Chisinau, Blvd. Stefan cel Mare, 165)
E-mail: mihaela.burac@usmf.md

Friptu Valentin, Professor, Dr. habil., MD, Department of Obstetrics and Gynecology
Nicolae Testemitanu State Medical and Pharmacy University
(Moldova, Chisinau, Blvd. Stefan cel Mare, 165)
E-mail: valentin.friptu@usmf.md

Corolcova Natalia, MD, PhD, Associate Professor Department of Obstetrics and Gynecology
Nicolae Testemitanu State Medical and Pharmacy University
(Moldova, Chisinau, Blvd. Stefan cel Mare, 165)
E-mail: natalia.corolcova@usmf.md

Mihalcean Luminița, MD, PhD, Associate Professor Department of Obstetrics and Gynecology
Nicolae Testemitanu State Medical and Pharmacy University
(Moldova, Chisinau, Blvd. Stefan cel Mare, 165)
E-mail: luminita.mihalcean@usmf.md

Ostrofeț Constantin, MD, PhD, Associate Professor Department of Obstetrics and Gynecology
Nicolae Testemitanu State Medical and Pharmacy University
(Moldova, Chisinau, Blvd. Stefan cel Mare, 165)
E-mail: constantin.ostrofet@usmf.md

Cotelea Veronica, MD, PhD, Assistant Professor, Department of Obstetrics and Gynecology
Nicolae Testemitanu State Medical and Pharmacy University
(Moldova, Chisinau, Blvd. Stefan cel Mare, 165)
E-mail: veronica.cotelea@usmf.md

Caproș Hristiana, MD, PhD, Associate Professor Department of Obstetrics and Gynecology
Nicolae Testemitanu State Medical and Pharmacy University
(Moldova, Chisinau, Blvd. Stefan cel Mare, 165)
E-mail: hristiana.capros@usmf.md

Abstract. *In recent years, scientific works have highlighted the important role that the endometrium has in infertility. The delicate interdependence of the balance between microbial and immune agents at the endometrial level, which can lead to the disruption of its receptivity, is studied. The presence in the human endometrium of a complex immune system that acts by preventing the risk of infection, the acceptance of the blastocyst. A more extensive study of the different agents that can affect endometrial receptivity would improve both the diagnosis and treatment of infertility and obstetric complications arising from defective implantation and placentation.*

Keywords. *Endometrium, primary infertility, endometrial immunity.*

Introduction

The endometrium is the epithelial tissue that lines the inner surface of the uterus. In recent years, a growing number of scientific work has shed light on its major role in infertility, implantation, and early pregnancy development [2, 3, 10].

The endometrium is remarkable in two ways - first, by its ability to undergo regular tissue growth, desquamation, and regeneration, and second, as a mucous tissue that is capable of recognizing and responding discriminatory to foreign agents such as sperm cells, the product of conception, and sexually transmitted pathogens [1, 9].

In the human endometrium, a complex system is activated to prevent the risk of infection and, in contrast, to accept the blastocyst. The endometrium thus acts as a tertiary lymphatic organ with a central role in the immune surveillance of the uterus [15]. Cytokines and chemokines play a central role in these immune and tissue remodeling processes that are essential for the normal functioning of the endometrium. They provide intercellular communication signals that control leukocyte recruitment and regulate the induction of immune responses [7, 12, 17]. By targeting certain cell lineages other than leukocytes, including cells involved in the preimplantation period of the embryo and placenta, cytokines are also key mediators of tissue remodeling that accompanies the menstrual cycle and pregnancy [18].

Disruption of the cytokine-leukocyte axis is a primary factor in the etiology of infertility, endometriosis, and abnormal uterine bleeding, and in pregnancy causes pregnancy pathology due to superficial placentation [5, 6, 11, 16].

Cytokines were originally described based on their action on the lymphohematopoietic system, but over time their broad action on the activity of different types of cells that produce and respond to these molecules has been proven. However, cytokines are somewhat an arbitrary subdivision of the large family of growth factors, which together with neurotransmitters and hormones constitute intercellular signaling agents. Chemokines are a subset of the cytokine family that are distinguished by their chemotactic properties. They act by regulating the recruitment of leukocytes into the blood, as well as directional movements in the tissue and the exodus into the lymphatic system [7].

The endometrial immune axis is the key to solving major problems in female reproductive health, including infertility, many pathologies of pregnancy, and sexually transmitted diseases. The molecular determinants of tolerance and immunity in the reproductive tract are now being studied and identified, and the basic principles are similar to those in other mucosal tissues. Cytokines are implicated as pivotal regulators of immune response processes. However, the flexibility to respond appropriately to such a wide range of antigens as bacterial microorganisms, spermatozoa, and the product of conception is attributable to the sensitization of the cytokine network in initiating the immune response [1, 4, 13].

Th1 cells are characterized by the transcription factor T-beta and the transcription activator STAT 4. Th1 cells produce proinflammatory cytokines such as TNF-α, TNF-β, IFN-γ, IL-1, IL-2, which activate cytotoxic T cells and macrophages to stimulate cellular immunity and inflammation. They are involved in the process of cellular immunity as well as the rejection process. In contrast, the mediators of humoral immunity, Th2 cells produce anti-inflammatory cytokines namely: IL-4, IL-5, IL-6, IL-10, IL-12, etc., which stimulate the production of antibodies by B cells and act as antagonists of Th1 cytokines [7, 19]. Since the blastocyst represents a semi allograft for maternal tissues, even subtle alteration of the endometrial immune environment represents a potential with a mega active impact on the implantation process. The effects of these growth factors and cytokines on the preimplantation period have been studied. It has been shown that Th2 cytokines, particularly IL-10 and IL-4, are essential for implantation and trophoblast formation. Proinflammatory Th1 cytokines are responsible for inhibiting Th2 cytokines, and the balance of Th1 - Th2 cytokines is responsible for the manifestation of inflammation [10, 12, 20]. In fact, successful implantation of the embryo and maintenance of pregnancy are the result of the fine balance between the embryo and the endometrium, and both seem to be dependent on the prevalence of the Th2 versus Th1 cytokine profile at the endometrial level. Thus, any condition that leads to a disruption of this balance results in impaired receptivity and infertility, respectively [8, 14].

Material and Methods

We provided a prospective study, which included 96 patients divided into 2 groups. The study group included 48 patients with primary infertility and the control group: 48 fertile patients. The inclusion criteria for the study group were: patients suffering from primary infertility with indications for laparoscopy and hysteroscopy, age 20 - 40 years, lack of hormonal therapy and antibiotic therapy in the last 6 months, lack of intrauterine manipulations in anamnesis, research participation agreement. The inclusion criteria for the control group were: patients who have had a delivery with a living fetus in the last 2 years and who do not

brestfeed, patients without a complicated reproductive gynecological history (infertility, spontaneous or missed abortion), lack of hormonal treatment, and antibiotic therapy over the past 6 months, research participation agreement.

Exclusion criteria from the research were: patients with acute genital infection, age <20 years and > 40 years, patients suffering from congenital malformations of the uterus, patients who had prior intrauterine surgical manipulations, atypical endometrial hyperplasia, patients refusal for voluntary participation in research.

The study was approved by the Research Ethics Committee of the State University of Medicine and Pharmacy "Nicolae Testemitanu", Chisinau, Republic of Moldova (No. 79/62 of 26.04.2017). Patients have signed informed consent to participate in the research.

In both groups we performed endometrial biopsy in the proliferative phase with endometrial suction curette „Pipelle de Cornier" (Cooper Surgical). The endometrial microbiome was assessed using the "Femoflor - 16" reagent set ("DNA – Technology", Moscow, Russian Federation), pro- and anti-inflammatory cytokines IL1β, IL8, IL10, IL4 and morphohistological study of the endometrium.

Statistical data processing was performed using Microsoft Excel 2016 and SPSS 20. The results are expressed as mean values ± standard deviation for parametric variables, and for categorical ones in percentages. The Pearson test was applied for correlation analysis. P values <0.05 were considered statistically significant.

Results

The study included 48 patients in each group who met the inclusion criteria, the control study - patients with the diagnosis of primary infertility and the study group - fertile patients. Both groups were homogeneous according to such criteria as age, age of partners, duration of menstrual cycle, body mass index (Table 1).

Table 1

Demographic criteria of the pacients included in the study

Evaluation Criteria		**Study group L_1 n=48 %(n)**	**Control group L_0 n=48 %(n)**	**p**
Age, years	20 – 24	12,5 (6)	18,8 (9)	0.07
	25 – 29	41,7 (20)	27,1 (13)	
	30 – 34	31,3 (15)	50,0 (24)	
	35 – 40	14,6 (7)	4,2 (2)	
Matrimonial status	married	100 (48)	97,9 (47)	0.5
	unmarried	0 (0)	2,1 (1)	
Place of residence	urban	64,6 (31)	75,0 (36)	0.18
	rural	35,4 (17)	25,0 (12)	
Studies degree	highschool	8,3 (4)	12,5 (6)	0.5
	college	37,5 (18)	43,8 (21)	
	university	54,2 (26)	43,8 (21)	
Workplace	unemployed	18,8 (9)	22,9 (11)	0.4
	unqualified work	29,2 (14)	37,5 (18)	
	skilled work	52,1 (25)	39,6 (19)	

Evaluating the study groups according to the criteria of general anamnesis we can conclude that the groups were homogeneous. The age of the patients included in the study group L_1 was between 22 and 39 years with an average of 29.00 ± 4.58 years and in the control group L_0 was between 20 and 35 years with an average of 29.23 ± 4.29 years p = 0.80. The duration of the menstrual cycle was on average 35.23 ± 12.54 days in L_1 versus 28.33 ± 3.09 days in L_0, p<0.001. Endometrial biopsy samples were collected in the proliferative phase of the menstrual cycle in both groups, so that in L_1 the day of collection was 11.06 ± 2.01 and in L_0 10.58 ± 2.20, p = 0.26. The level of interleukin IL1B was considerably increased in the L_1 study group with an average of 1044.67 ± 125.79 pg/ml and in the L_0 control group it was 354.32 ± 70.98 pg/ml, p<0.001 (Figure 1). The average level of the anti-inflammatory interleukin IL10 in L_1 was 186.06 ± 30.71 pg/ml and in L_0 186.93 ± 34.26 pg/ml, p = 0.897. A more detailed analysis of cytokine levels in endometrial fluid collected from infertile patients showed a direct correlation between IL1B and IL10: Pearson correlation index r=0.318, p = 0.027.

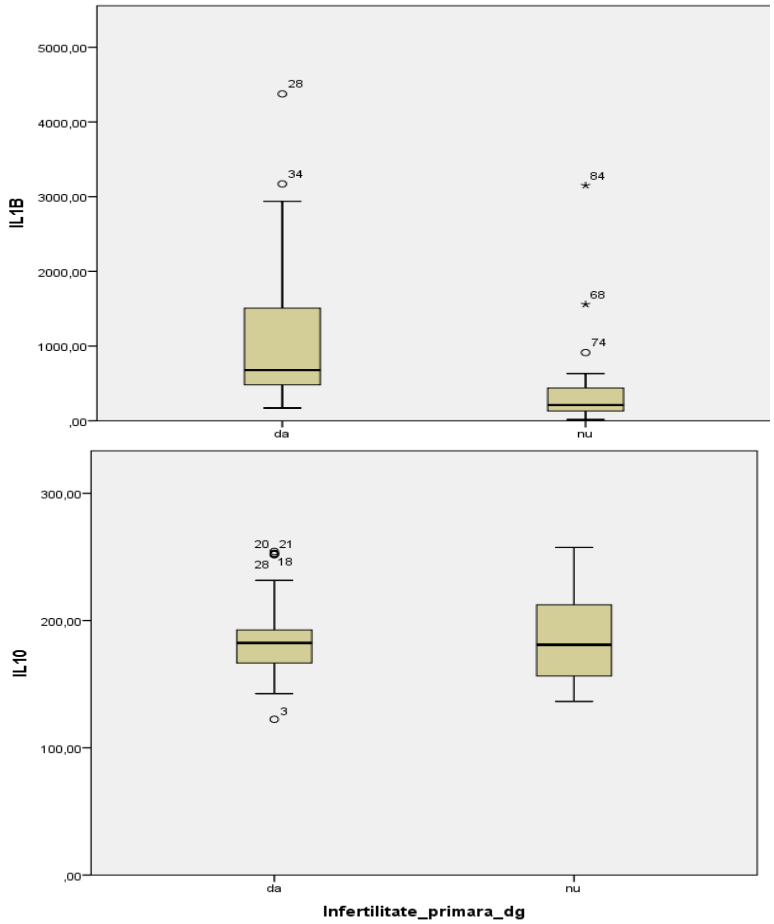

Figure 1. The average levels of interleukin IL1B and IL10 in the endometrial fluid

Discussions

A challenge in the field of reproductive immunology regarding embryo implantation is to define more precise approaches to better understand the immunology of pregnancy. Our study demonstrated that patients with primary infertility have altered parameters of endometrial immunology compared to fertile patients, in particular IL1B levels are increased several-fold compared to fertile patients. In particular, we found a positive correlation between the level of the proinflammatory cytokine IL1B and the anti-inflammatory cytokine IL10.

The results of our study show high levels of proinflammatory cytokines in infertile patients versus fertile patients. This seems to be a reflection of the Th1/Th2 imbalance at the endometrial level, where a similar microenvironment still occurs in such inflammatory diseases as rheumatoid arthritis or irritable bowel disease. The prevalence of the Th1 profile, in fact, increases the recruitment and activation of endometrial macrophages with an increase in the release of IL-1, TNF-α and Il-6, which act by maintaining the chronic inflammatory process.

Conclusions

This study provides evidence that in patients with primary infertility the endometrial microenvironment is altered, characterized by a marked predominance of the Th1 cytokine profile, supporting significant levels of proinflammatory cytokines. The results of this study may represent a first step in the development of diagnostic tests for pro- and anti-inflammatory cytokines in patients with infertility.

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ДИСРЕГУЛЯЦИЯ ИММУННОГО ОТВЕТА В ЭНДОМЕТРИИ У БОЛЬНЫХ С ПЕРВИЧНЫМ БЕСПЛОДИЕМ

Бурак Михаэла, асистент кафедры акушерства и гинекологии
Государственный медицинский и фармацевтический университет имени Николая Тестемицану
(Молдова, Кишинёв, бул. Штефан чел Маре, 165)
E-mail: mihaela.burac@usmf.md

Фрипту Валентин, профессор, доктор медицинских наук, кафедра акушерства и гинекологии
Государственный медицинский и фармацевтический университет имени Николая Тестемицану
(Молдова, Кишинёв, бул. Штефан чел Маре, 165)
E-mail: valentin.friptu@usmf.md

Королкова Наталья, кандидат медицинских наук, доцент кафедры акушерства и гинекологии
Государственный медицинский и фармацевтический университет имени Николая Тестемицану
(Молдова, Кишинёв, бул. Штефан чел Маре, 165)
E-mail: natalia.corolcova@usmf.md

Михалчян Луминица, кандидат медицинских наук, доцент кафедры акушерства и гинекологии
Государственный медицинский и фармацевтический университет имени Николая Тестемицану
(Молдова, Кишинёв, бул. Штефан чел Маре, 165)
E-mail: luminita.mihalcean@usmf.md

Острофец Константин, кандидат медицинских наук, доцент кафедры акушерства и гинекологии
Государственный медицинский и фармацевтический университет имени Николая Тестемицану
(Молдова, Кишинёв, бул. Штефан чел Маре, 165)
E-mail: constantin.ostrofet@usmf.md

Котелеа Вероника, кандидат медицинских наук, асистент кафедры акушерства и гинекологии
Государственный медицинский и фармацевтический университет имени Николая Тестемицану
(Молдова, Кишинёв, бул. Штефан чел Маре, 165)
E-mail: veronica.cotelea@usmf.md

Капрош Христиана, кандидат медицинских наук, доцент кафедры акушерства и гинекологии
Государственный медицинский и фармацевтический университет имени Николая Тестемицану
(Молдова, Кишинёв, бул. Штефан чел Маре, 165)
E-mail: hristiana.capros@usmf.md

Аннотация. *В последние годы научные работы подчеркивают важную роль эндометрия в развитии бесплодия. Изучается тонкая взаимозависимость баланса между микробными и иммунными агентами на уровне эндометрия, которая может привести к нарушению его рецептивности. Обнаружено наличие в эндометрии человека сложной иммунной системы, которая действует, предотвращая риск инфицирования и обеспечивая принятие бластоцисты. Более глубокое изучение различных агентов, влияющих на рецептивность эндометрия, улучшило бы как диагностику, так и лечение бесплодия и акушерских осложнений, возникающих вследствие дефектов имплантации и плацентации.*

Ключевые слова: *эндометрий, первичное бесплодие, иммунитет эндометрия.*