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ASSESSMENT OF THE INTESTINAL AND VAGINA MICROBIOMA DURING EXTRACORPORAL FERTILIZATION PROGRAMS

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Abstract

It is now known that the possibility of conceiving and carrying a pregnancy is largely due to the interaction of a woman's macroorganism with microorganisms involved in the formation of the microbiome of the intestine, vagina and other organs. Attempts to treat infertility using in vitro fertilization (IVF) are not always successful. Probably, the disruption of the vaginal and intestinal microbiome can significantly reduce the effectiveness of IVF programs. The aim of our work was to study the vaginal and intestinal microbiome in women with infertility in preparation for IVF programs. Materials and research methods. 40 women suffering from infertility were examined. Primary infertility was in 12 women and secondary in 28 patients. Of these, the 1st group consisted of 20 women suffering from infertility who had 2-4 unsuccessful IVF attempts in history; Group 2 consisted of 20 women who, after infertility treatment, had a pregnancy that ended in premature birth. The control group consisted of 20 healthy women. Bacteriological studies of the vaginal and intestinal microbiome were carried out using the method of light microscopy. The level of pro-inflammatory and anti-inflammatory cytokines in the blood serum was determined by the enzyme immunoassay. Results. When conducting bacteriological examination in the 1st and 2nd groups of patients, it was found that the vaginal microbiome is characterized by a sharp decrease in normal flora (lacto-, bifidobacteria) and a significant increase in opportunistic flora (eubacteria, prevotella, peptostreptococcus). In the intestinal microbiome, a decrease in species diversity, an increase in the number of enterococci, Klebsiella, proteobacteria, characteristic of intestinal dysbiosis associated with inflammation, were found. Thus, in both groups of women, a violation of the vaginal microbiome was found in 71.7%, a violation of the intestinal microbiome - in 86.7% of patients. While in the control group, there was no violation of the vaginal microbiome, and the violation of the intestinal microbiome occurred in 10% of women. Conclusions. According to the results of the study, it can be considered that the risk factors for failed IVF attempts and premature birth are a violation of the microbiome of the vagina and intestines, as well as an imbalance of pro-inflammatory and anti-inflammatory cytokines in women with infertility. To develop individual fertility treatment programs, it is necessary to include an assessment of the vaginal and intestinal microbiome in the examination. In addition, drugs that normalize the microbiome of these organs should be included in IVF programs and for the prevention of preterm birth in women with a history of infertility, which will increase the effectiveness of IVF programs and reduce reproductive losses.

Keywords: intestinal and vaginal microbiome, cytokines, infertility, preterm labor, IVF

INTRODUCTION

The totality of all microorganisms that live in association with humans constitutes the human microbiome [1]. In dysbiotic conditions, a significant role is given to

microorganisms that inhabit the entire genital tract, and they are also potentially capable of influencing the success of conception and gestation. Environmental factors, a combination of prenatal, postnatal and intranatal factors through the microbiome, affect all stages of a woman's life [3,5,7]. The possibility of conceiving and carrying a pregnancy is largely due to the interaction of a woman's macroorganism with microorganisms involved in the formation of the microbiome of the intestines, vagina and other organs [1,2,8]. Until recently, the microbiome of the female reproductive system: as the uterine cavity, fallopian tubes, ovaries, mammary glands were considered absolutely sterile. Spanish scientists have shown that microorganisms begin to colonize the human body even in the womb, since the fetus and amniotic cavity in utero are not sterile, as was previously thought [12]. According to modern authors, it has been established that in the genital tract of a woman there is an ecological niche with a specific population of microorganisms. The microbial-tissue complex of the female genital tract, being part of a single system of mucous membranes: the gastrointestinal tract (GIT), respiratory tract, urinary system, etc., quickly responds to metabolic disorders of the body itself, as well as to dysbiotic changes, primarily, in the digestive tract as the main reservoir of the microbiome under the external influence of the ecosystem [4,8].

As you know, attempts to treat infertility using in vitro fertilization (IVF) are not always successful. Trying to determine the reasons for unsuccessful IVF attempts, many reproductive specialists began to pay great attention to the state of the endometrium and the nature of the microbiome of the uterine cavity. A number of studies have established an increase in the types of pathogenic microorganisms, such as gardnerella, streptococci, Escherichia coli, Shigella. Moreover, in the vaginal microbiome there is a microflora characteristic of the intestine (E. coli, Shigella, etc.) [3,4,10,11]. It has now been absolutely proven that the violation of the intestinal and vaginal microbiome is accompanied by a pronounced cytokine imbalance [9,13].

Until now, the main reasons leading to unsuccessful attempts to treat infertility using IVF are not fully understood. Probably, the disruption of the vaginal and intestinal microbiome can significantly reduce the effectiveness of IVF programs. At the same time, the study of the state of the microbiome can contribute to the pathogenetic substantiation of rational ways of ante- and intranatal protection of the fetus and prevention of gestational complications. In connection with the above, the goal of our work was to study the intestinal and vaginal microbiome in women with infertility in preparation for IVF programs.

MATERIALS AND RESEARCH METHODS

40 women suffering from infertility were examined. Primary infertility was observed in 12 and secondary in 28 patients. Of these, the 1st group consisted of 20 women suffering from infertility who had 2-4 unsuccessful IVF attempts in history; Group 2 (comparison) consisted of 20 women who, after infertility treatment, had a pregnancy that ended in premature birth. The control group consisted of 20 healthy women.

Bacteriological studies of the vaginal and intestinal microbiome were carried out using the method of light microscopy. The contents of the posterior vaginal fornix were taken with a sterile Copan innovation swab (Italy) prior to vaginal examination. The material was delivered to the laboratory within 1 hour, or placed in a buffer tube and delivered within 24 hours. In the laboratory, serial dilutions were prepared in test tubes at a rate of 1:10. Sowing was performed on a number of nutrient media, allowing to identify the maximum possible spectrum of microorganisms. Dry commercial nutrient media were used for cultivation. The grown colonies (CFU) were counted and recalculated per 1 ml of biomaterial (CFU / ml). Microorganisms were cultivated in a thermostat at 370C, yeast-like fungi at a temperature of 220C. Pure cultures were isolated on 5% blood agar, yolk-salt agar, Endo and Sabouraud media. The isolated bacteria were identified by morphological, tinctorial, cultural and biochemical characteristics using entero-, staphylo-, anaerotests (Lachema, Czech Republic).

Bacteriological examination of feces, as well as assessment of the microflora of the colon was carried out in accordance with the industry standard [6]. Feces were taken with a swab, which was placed in a sterile test tube and sent to a bacteriological laboratory no later than 1-2 hours after sampling. Grown colonies (CFU) were counted and recalculated per 1 g of biomaterial (CFU / g).

The level of pro-inflammatory interleukins was determined: interleukin-1 (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor (TNF-α) and anti-inflammatory interleukins: interleukin-4 (IL-4), interleukin-10 (IL-10) in blood serum. The studies were performed on an enzyme immunoassay analyzer from Shanghai Kehua Laboratory System Co. Ltd; KHBst-360 using a set of test systems “Vector-Best” (JSC, Russia). The technique is based on a solid-phase “sandwich” - a variant of enzyme-linked immunosorbent assay using mono- and polyclonal antibodies sorbed on the surface of the wells of a collapsible polystyrene plate.

Statistical processing of the obtained results was carried out on a Pentium-IV personal computer using the Microsoft Office Excel-2012 software package, including the use of built-in statistical processing functions.

RESULTS AND DISCUSSION

The age of the women was 27.0 ± 2.5 years. The duration of infertility was 3 to 6 years. In patients of groups 1 and 2, the frequency of chronic adnexitis was 87.5% (35), vaginal dysbiosis - 77.5% (31), and inflammatory diseases of the cervix - 47.5% (19). Also, chronic tonsillitis was observed in 37.5% (15), chronic pyelonephritis - in 57.5% (23). In patients suffering from infertility, obstruction of the fallopian tubes occurred in 27 (67.5%). Moreover, after the laparoscopic operations, patency was restored in 16 of them. In the study of the vaginal microflora, it was revealed that in women of the 1st and 2nd groups, lactobacilli (lg 3.42 ± 0.29 *) and bifidobacteria (lg 3.8 ± 0.51) predominated. But their content was significantly ($p < 0.01$) inferior to those in the control group. (Table 1).

Table 1. Characterization of the vaginal microbiome

Microorganisms	First group n =20		Second group, n=20		Control group, n=20	
	%	lg M±m КОЕ/мл	%	lg M±m КОЕ/мл	%	lg M±m КОЕ/мл

Bifidobacterium bifidum	50,0	2,6±0,4	62,5	3,8±0,51	85	4,60±0,47
Corynebacterium sp.	15,0	1,11±0,21	20,0	1,21±0,41	25	1,35±0,54
Lactobacillus	60,0	3,08±0,23*	70,0	3,42±0,29*	95	5,46±0,33
E.coli LP	40,0	1,76±0,35	27,5	1,68±0,45	25	1,63±0,65
E.coli LN	45,0	2,6±0,28*	40,0	2,16 ±0,39*	10	0,76±0,52
S. aureus	25,0	1,26±0,16*	20,0	1,16±0,36*	-	-
Eubacterium sp.	45,0	1,48±0,32	40,0	1,44±0,36	35	1,63±0,58
Prevatellus sp.	50,0	3,3±0,54*	47,5	3,03±0,44*	20	1,44±0,58
Peptococcus sp.	30,0	2,46±0,44	25,0	1,76±0,39	25	1,37±0,54

However, the highest seeding rates in women of these groups were distinguished by E. coli LP (lg 1.76 ± 0.35), E. coli LP (lg 2, 6 ± 0.28) and S. aureus (lg 1.26 ± 0 16), which significantly exceeded the values in healthy women, while S. aureus was not detected in the control group at all.

From the Enterobacteriaceae family, only E. coli was recorded in the vagina (20%, lg 3.3 ± 2.3 CFU / ml). Indicators of E. coli LN with a detection rate of 10% to 45% had a tendency to replace opportunistic flora. Eubacteria, as well as peptostreptococci, were in reduced numbers (p <0.05). Thus, according to our research, the laboratory picture fits into a moderate vaginal dysbiosis.

Similar studies of the intestinal microflora in patients with infertility (Table 2) showed that there is a slight decrease in the detection rate of Lactobacillus pp. (75%) and Bifidobacterium (70%), with a simultaneous increase in Peptostreptococcus sp. (45%) and Bacteroides sp. (95%) compared to the control group of women.

Table 2. Characterization of the gut microbiome

Microorganisms	First group n =20		Second group, n=20		Control group, n=20	
	%	lg M±m KOE/r	%	lg M±m KOE/r	%	lg M±m KOE/r
Lactobacillus sp.	60,0	3,22±0,42*	75,0	5,44±0,6*	100,0	7,89±0,12
Bifidobacterium sp.	60,0	3,27±0,47*	70,0	4,37±0,6*	100,0	7,82±0,15
Bacteroides sp.	95,0	3,07±0,79*	90,0	2,17±0,6*	85,0	1,16±0,63
Peptostreptococcus sp.	45,0	6,37±0,19	40,0	4,16±0,8	15,0	7,53±0,58
E. coli LP	80,0	6,26±0,02*	85,0	7,96±0,1*	100,0	8,72±0,19
E. coli LN	65,0	4,94±0,35*	50,0	3,76±0,2*	20,0	7,38±0,56
Proteus sp.	40,0	4,98±0,86*	35,0	3,76±0,6*	15,0	1,49±0,68
Klebsiella sp.	15,0	2,46±0,70*	15,0	2,46±0,7*	10,0	0,99±0,50
S. epidermidis	30,0	0,70±0,40	35,0	0,90±0,50	75,0	0,49±0,34
S. aureus	45,0	1,85±0,52*	30,0	1,60±0,3*	10,0	3,19±0,54
Streptococcus gr.A	40,0	3,20±0,63*	30,0	1,60±0,3*	10,0	0,55±0,27
Streptococcus gr.D	60,0	0,86±0,38*	65,0	0,72±0,2*	90,0	0,48±0,01
Candida albicans	45,0	3,01±0,47*	40,0	3,72±0,*	20,0	5,64±0,36

There was also a significant increase in the seeding rate of E. coli LN (65%) and Proteus sp. (40%), Staphylococcus aureus (45%).

In the control group of women, during bacteriological examination of feces, the intestinal microbiome was disturbed only in 10% (2). The content of bifidobacteria and lactobacilli was significantly reduced (p <0.05) in comparison with the formally normative ones. Among all representatives of the facultative microflora, Klebsiella and Protea significantly exceeded the norm (p <0.05). Other representatives of the optional and obligatory groups in terms of detection frequency, quantitative characteristics also corresponded to the norm. The data of our study indicate the presence of intestinal dysbiosis in the patients of the studied groups.

Thus, the results of bacteriological examination of patients with infertility showed that the vaginal microbiome is characterized by a sharp decrease in normal flora (lacto-, bifid bacteria) and a significant increase in opportunistic flora (eubacteria, prevotella, peptostreptococcus).

In the intestinal microbiome, a decrease in species diversity, an increase in the number of enterococci, Klebsiella, antibacterial, characteristic of intestinal dysbiosis associated with inflammation, were found. Thus, in patients of the 1st and 2nd groups, a violation of the vaginal microbiome was found in 71.7%, the intestinal microbiome - in 86.7% of patients. However, changes in the gut microbiome in healthy women occurred in 10% of women.

When studying the level of cytokines in the blood serum, it was found (Table 3) that in women of the control group the level of cytokine IL-1β in the blood serum was 2.35 ± 0.18 pg / ml, IL-2 - 7.54 ± 0.64 pg / ml, IL-4 -5.76 ± 0.44 pg / ml.

In the blood serum, the IL-6 content was 2.25 ± 0.17 pg / ml, IL-8 - 6.36 ± 0.58 pg / ml, IL-10 - 23.14 ± 1.57 pg / ml, and the level TNF-α was in the range of 1.68 ± 0.13 pg / ml.

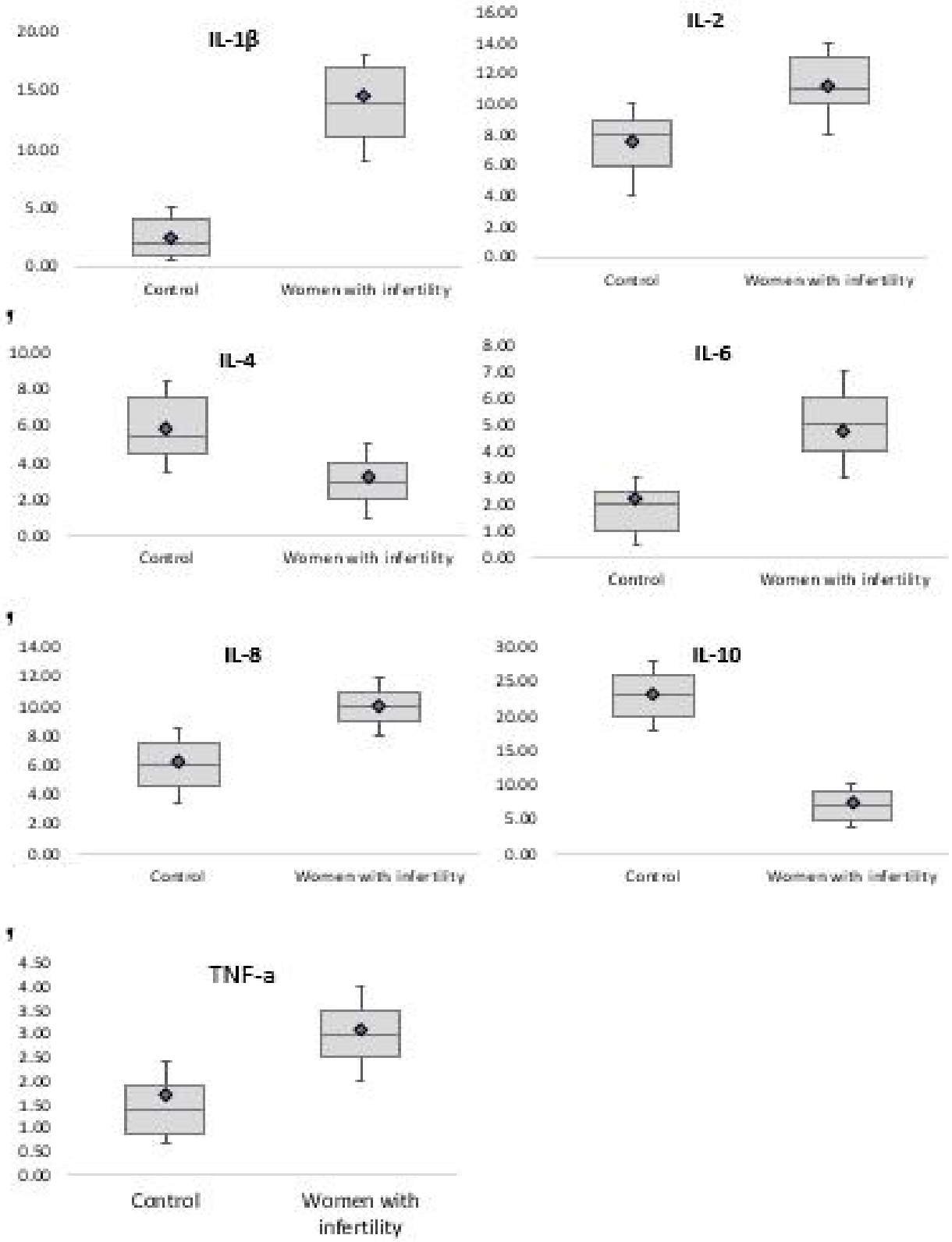

Figure 3. Indicators of the level of cytokines (pg / ml) in blood serum in patients with infertility

Analysis of cytokine parameters in infertile patients revealed a significant increase in serum IL-1β production by 6.2 times (14.6 ± 0.87 pg / ml), p <0.05. IL-1 is an inducible protein, the synthesis of which is required for an acute phase response. The main producer cells are monocytes, macrophages, endothelium and other cells. An excessively high level of IL-1 indicates the possibility of undesirable immunopathological processes. IL-1 is characterized by the ability to stimulate the production of prostaglandins. Maintaining this cytokine at a low level is one of the factors contributing to the maintenance of pregnancy.

Also, in patients with infertility, the level of IL-8 was 1.6 times increased (9.98 ± 0.63 pg / ml) compared with the same indicator in the control group (p <0.05). A high level of spontaneous production of IL-8 may indicate a significant activation of mononuclear phagocytes - producers of

proinflammatory cytokines, which play an important role in the development of immunopathological processes.

The data obtained on the increase in IL-1β and IL-8 are a reflection of the activity of the inflammatory process. An increase in the concentration of pro-inflammatory cytokines indicates that the inflammatory response in this group of patients is systemic.

As the results of our studies show, patients with infertility have an increase in the serum IL-6 content by 2.1 times (4.83 ± 0.39 pg / ml) compared with the data of healthy women (P <0.05) ...

In addition, in women with infertility, the serum TNF-α level increases 1.9 times (3.12 ± 0.28 pg / ml) compared with the control data (P <0.05)). TNF-α is produced by tissue macrophages, monocytes and lymphocytes in the area of acute inflammation, enhances the basic functions of leukocytes, stimulates the release of histamine by basophils and mast cells, causes the activation of fibroblasts, smooth myocytes and vascular endothelium in the focus of inflammation, induces the synthesis of proteins of the acute phase of inflammation. Hypersecretion of TNF-α leads to a significant increase in the number of apoptotic trophoblast cells, which may serve as one of the factors contributing to miscarriage.

In our study, anti-inflammatory cytokines were: IL-4 - 3.15 ± 0.23 pg / ml (P <0.05), IL-10 - 7.36 ± 0.62 pg / ml (P <0.001), which, respectively reliably 1.5 times and 3.1 times lower than those of the control group.

We have determined the sensitivity and specificity of cytokines. It was found that the most pronounced sensitivity and specificity were in IL-6 and IL-10. Thus, the sensitivity of IL-6 was 82.6%, IL-10 was 89.6% and the specificity was 88.9% and 90.0%, respectively.

Thus, our research results suggest that the study of the cytokine balance is significant for assessing the direction of the immune response. Perhaps this may affect the ability of women to conceive.

It should be noted that the studies carried out indicate a certain relationship between the state of the vaginal and intestinal microbiome. Disruption of the vaginal and intestinal microbiome is a factor affecting the balance of pro-inflammatory and anti-inflammatory cytokines, which indicates the development of a systemic inflammatory response in a woman's body and, of course, is a factor that disrupts the processes of conception.

CONCLUSIONS

Thus, our research results suggest that the risk factors for unsuccessful IVF attempts and premature birth is a violation of the vaginal microbiome (71.7%) and intestines (86.7%)% in women with infertility.

An increase in the level of the proinflammatory cytokine IL-6 by 2.1 times and a decrease in the anti-inflammatory cytokine IL-10 by 3.1 times indicate the development of a systemic inflammatory response, which may affect the possibility of conception and gestation.

For the development of individual programs for the treatment of infertility, it is necessary to include in the examination an assessment of the microbiome of the vagina and intestines and, accordingly, to correct these disorders.

When preparing patients for IVF programs, as well as during pregravid preparation, patients with infertility and preterm birth in history should be included in the treatment regimens for pre- and probiotics, which will increase the effectiveness of IVF programs and reduce reproductive losses.

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