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EVALUATION OF THE TAXONOMIC DIVERSITY OF MICROORGANISMS IN INFERTILITY

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ABSTRACT. *The article examines the taxonomic diversity of microorganisms in the vaginal biotope during infertility. It has been shown that the occurrence of representatives of the Lactobacillaceaye family varies from 16% to 88% in the vaginal biotope. Among the representatives of the family, L. jensenii, L. delbruyeckii, and L. amylolyticus of the Lactobacillus genus predominated. Moraxella spp. was found in large quantities in all samples, M. osloyensis was the leader among representatives of the entire community. It has been established that in infertility, the number of representatives of the Lactobacillus genus in the vaginal microbiota significantly decreases, which contributes to the excessive reproduction of conditionally pathogenic microorganisms, in particular M. osloyensis.*

KEY WORDS: *vaginal microbiota; metagenomic sequence; L. feminine; M. osloyensis; infertility.*

ОЦЕНКА ТАКСОНОМИЧЕСКОГО РАЗНООБРАЗИЯ МИКРООРГАНИЗМОВ ПРИ БЕСПЛОДИИ

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АННОТАЦИЯ. *В статье рассматривается таксономическое разнообразие микроорганизмов в вагинальном биотопе во время бесплодия. Показано, что в вагинальном биотопе встречаемость представителей семейства Lactobacillaceaye варьирует от 16 до 88%. Среди представителей семейства преобладали L. jensenii, L. delbruyeckii и L. amylolyticus рода Lactobacillus. Moraxella spp. была обнаружена в больших количествах во всех образцах, M. osloyensis был лидером среди представителей всего сообщества. Установлено, что при бесплодии количество* *представителей рода Lactobacillus в вагинальной микробиоте значительно уменьшается, что способствует избыточному размножению условно-патогенных микроорганизмов, в частности M. osloyensis.*

КЛЮЧЕВЫЕ СЛОВА: *вагинальная микробиота; метагеномическая секвенция; Л. женственный; M. osloyensis; бесплодие.*

Relevance. The question of the participation of microorganisms, especially commensal and mutualistic ones, in the development of infertility has not yet been resolved. If the participation of such sexually transmitted infectious agents as Chlamydia trachomatis and Neisseria gonorrhoeae in the pathogenesis of this disease has been described in sufficient detail [7], then many other microorganisms still remain in the field of scientific interest. Recent studies have shown a connection between conditionally pathogenic microorganisms (CPM) from the lower parts of the female genital tract and unfavorable pregnancy outcomes after both natural and artificial fertilization [8]. Moreover, it is noted that UPM during genital tract colonization typically does not cause typical symptoms of the inflammatory process, especially when localized in its upper parts [9].

There is also no unambiguous attitude towards BV as a causal factor of infertility [10]. BV refers to syndromes with a polymicrobial etiology, when a localized pro-inflammatory microenvironment is created with a decrease in the number of projective lactobacilli and an excessive growth of anaerobic UPM [11, 12]. Such disorders in the vaginal biotope provide conditions that facilitate the upward dissemination of microorganisms, which ultimately can lead to tubal infertility [2,3]. Moreover, the development of pregnancy against the background of BV is accompanied by clinical disorders, up to its termination [4].

The purpose of the study is to assess the taxonomic diversity of microorganisms in the vaginal biotope during infertility.

Materials and methods. For the study, samples of vaginal contents obtained from the posterior arch using a Folkman measuring spoon from 15 women in infertile marriages were used. Metagenome study of 16S ribosomal RNA samples was carried out on the Illumina MiSeq platform using the MiSeq Reagent Kits v3 (600-Cycle Kit) set according to manufacturer's recommendations. The libraries for sequencing the V3-V4 regions of the 16S ribosomal RNA gene were prepared according to the 16S Metagenomic Sequencing Library Preparation Illumina. During bioinformational assessment, we used Kraken Metagenomics version 2.0.0 (classifier of short nucleotide sequences) software for metagenomic analysis, using a standard database [1,5,7].

Research results. In the vaginal biotope of women with infertility, predominantly bacterial taxa were registered, the proportion of which in the total mass was 49-99% (83.9±16.02). Genetic markers of Lactobacillaceae bacteria were found in 12-84% (41.9±17.99) of samples, and in half of the cases, their content did not exceed 30% of the total bacterial mass. Among the representatives of the family, the leading position was occupied by the genus Lactobacillus, dominated by L. jensenii, L. delbrueckii, and L. amylolyticus. In isolated cases, the number of microorganisms of the Lactobacillus and Enterococcus genera was comparable. L. crispatus and L. gasseri were found in a number of samples, but their share in the genetic material was minimal. Contrary to expectations, L. iners was not detected in any patient.

The leading participants in the etiology of BV - Gardnerella vaginalis - were registered in almost all samples, but their proportion, as a rule, did not exceed 1%. Another causative agent of BV - Atopobium vaginae - was not detected in any sample, however, A. parvulum was present in 12 of them, also in insignificant quantities.

Notably, the specific content of anaerobic bacteria - typical BV associants: Actinomyces spp., Peptoniphilus spp., Prevotella spp., Bacteroides spp., Fusobacterium spp. and Mobiluncus curtisii - in the vaginal microbiota of infertile women turned out to be minimal.

An unexpected finding for the vaginal biotope was the discovery of a large amount of Moraxella spp genetic material. In all samples, M. osloensis occupied a leading position among representatives of the entire community.

Thus, in half of the cases, the combined presence of M. osloensis and G. vaginalis was detected, with the first symbiont predominating, and the number of gardnerelles constituting about 10% of the bacterial mass. In one-third of such cases, Enterococcus faecalis was present (8-15%). In addition, M. osloensis was more often detected in samples, when L. jensenii was the leader among lactobacilli. In one case, 20% of the genetic material was represented by the Bifidobacterium longum genome in combination with M. osloensis (19%).

The proportion of Staphylococcaceae family microorganisms in the vaginal microbiota in only 1 case was 0.5%, in the rest, trace amounts were found, while genome variants from the Staphylococcus genus predominated.

S. aureus genetic markers were found in all samples, however, in 3 cases, their number occupied the 2nd ranking position, giving way to the 1st place to S. haemolyticus. These bacteria were almost always found in association with each other. Similarly, a sparse representation is characteristic of the Enterobacteriaceae family. Only in 2 cases did their proportion in the total microbial mass reach 0.8%. In all samples, genomes of Escherichia coli, Klebsiella spp., and the

Enterobacter cloacae complex were found. In 9 cases, E. coli led, in 3 - Klebsiella spp., and in another 3 cases, these genomes were identified in comparable quantities. In 12 samples, the genomes of microorganisms of the genus Citrobacter were found, but their number was insignificant.

Discussion. One of the reasons for decreased fertility is recognized as having undergone specific urogenital infections. As our research results have shown, in infertility, noticeable changes in the composition of the vaginal biotope are also observed: the number of lactobacteria decreases, the most beneficial species with pronounced antagonistic activity - L. crispatus and L. gasseri - are practically eliminated, and the spectrum of other microorganisms, primarily UPM, also expands. Often, such events occur unnoticed by the patient. Previously, it was shown that predisposition to specific infectious-inflammatory processes in the genital tract is associated with excessive growth of bacteria, primarily associated with BV, from the genera Gardnerella, Atopobium, Prevotella, Bacteroides, Peptostreptococcus, Mobiluncus, Sneathia, Leptotrichia, Mycoplasma, and the order Clostridiales [6].

However, in this study, most of them were found, but only in minimal quantities.

With noticeable persistence, fragments of the M. osloensis coccobasilla genome were detected, which proved quite unexpected, as these bacteria are part of the respiratory microflora and belong to opportunistic microorganisms, although there is information about their detection in various inflammatory processes, including vaginitis [12]. In most cases, it is mentioned that patients infected with M. osloensis were immune-compromised. It has been described that the main metabolite of these bacteria - 4-methyl-3-hexenoic acid - participates in the formation of persistent unpleasant odors [1, 8]. Among the pathogenic factors of M. osloensis, the production of exotoxin has been described, the accumulation of which requires long-term persistence of the microorganism, as well as lipopolysaccharides, fimbria, and the participation of capsules and some adhesins [2].

When describing lactobacilli as the main participants in the vaginal microbiota, the main attention is paid to L. crispatus, which in this study were found in only one-third of the samples and in small quantities. The leading position belonged to L. jensenii, which is capable of producing a biosurfactant with antimicrobial activity against E. coli and Candida albicans and anti-adhesive action against E. coli, S. saprophyticus, and Enterobacter aerogenes [3]. There are no such data regarding moracilli. It has been shown that L. jensenii is an active producer of hydrogen peroxide [4], however, there is information that the antimicrobial activity of lactobacilli is largely determined by lactic acid and bacteriocins [9]. Moreover, the same species also produces peroxidase, which neutralizes the effect of peroxide [12].

Additionally, L. jensenii is characterized by higher activity of D-lactate dehydrogenase, which participates in the production of the D-optical isomer of lactic acid [10], for which antimicrobial activity is less characteristic than for the L-isomer. Despite L. jensenii's quite convincing immunomodulatory effects [12], this species' participation in the development of several inflammatory diseases has been described [11].

In general, it can be assumed that during the development of infertility, a decrease in the number of lactobacilli and a change in their leading species occur. In the case of replacement with L. jensenii, it is possible to expect the manifestation of antagonistic properties in relation to a number of PPMs, including the main causative agents of BV, however, due to their small number and changes in functional activity, the effectiveness of the lactoflora effect decreases, and microorganisms such as M. osloensis can successfully persist in the vaginal biotope.

In this case, gradual colonization of the upper parts of the genital tract is possible, which leads to reproductive problems.

Conclusions. In the women included in the study with infertility, asymptomatic dysbiotic processes were observed, characterized by a number of features. In vaginal microbiota, the number of representatives of the Lactobacillus genus significantly decreases. Among the latter, L. jensenii dominates, whose functional activity does not fully ensure the colonization resistance of the vaginal biotope, allowing excessive reproduction of the vaginal biotope, in particular M. osloensis. Observed persistent disorders in the vaginal microbiota, combined with changes in local immune reactivity, can cause decreased fertility, and in acute cases, loss of reproductive function.

LITERATURE:

1.Chen C., Song X., Wei W., Zhong H., Dai J., Lan Z., et al. The continuum of microbiota along the female reproductive tract and its relationship to uterine diseases. Nat. Commun. 2017; 8 (1): 875. DOI: http://doi.org/10.1038/s41467-017-00901-0

2. Stone L. Infection: Vaginal microbiota and infectious infertility. Nat. Rev. Urol. 2018; 15 (3): 136. DOI: http://doi.org/10.1038/nrurol.2018.11

3. Ding T., Schloss P.D. Dynamics and associations of microbial community types across the human body. Nature. 2014; 509 (7500): 357-60. DOI: http://doi.org/10.1038/nature13178

4. Shishkova Yu.S., Dolgushina V.F., Grafova E.D., Zavyalova S.A., Kurnosenko I.V., Evstigneeva N.P. et al. Interrelation of the functional status of cervical secretion

neutrophils in pregnant women with the species composition of lactoflora. Journal of Microbiology, Epidemiology and Immunobiology. 2018; 95 (4): 51-6. DOI: http://doi.org/10.36233/0372-9311-2018-4-51-56

5.Sycheva M.V., Peshkova Yu.I., Kartashova O.L., Andreeva A.V. Regulation by antimicrobial peptides of microorganisms' sensitivity to antagonistically active representatives of mutualistic microflora. Journal of Microbiology, Epidemiology and Immunobiology. 2017; 94 (6): 21-5.

DOI: http://doi.org/10.36233/0372-9311-2017-6-21-25

6.Franasiak J.M., Werner M.D., Juneau C.R., Tao X., Landis J., Zhan Y., et al. Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit. J. Assist. Reprod. Genet. 2016; 33 (1): 129-36.

DOI: http://doi.org/10.1007/s10815-015-0614-z

7. Campisciano G., Florian F., D'Eustacchio A., Stanković D., Ricci G., De Seta F., et al. Subclinical alteration of the cervical-vaginal microbiome in women with idiopathic infertility. J. Cell Physiol. 2017; 232 (7): 1681-8.

DOI: http://doi.org/10.1002/jcp.25806

8. Mikhailova N.A., Voevodin D.A., Poddubikov A.V. Correction of dysbiosis - the basis of regenerative medicine. Journal of Microbiology, Epidemiology and Immunobiology. 2018; 95 (5): 107-13.

9.Bogun A.G., Kislichkina A.A., Galkina E.V., Mayskaya N.V., Solomentsev V.I., Mukhina T.N. et al. Using modern methods for identifying bacteria in the activities of the state collection of pathogenic microorganisms and cell cultures (GKPM-Obolensk). Infection and immunity. 2016; 6 (3): 8.

10.Onderdonk A.B., Delaney M.L., Fichorova R.N. The human microbiome during bacterial vaginosis. Clin. Microbiol. Rev. 2016; 29 (2): 223-38.

DOI: http://doi.org/10.1128/CMR.00075-1511.

11.Lewis F.M., Bernstein K.T., Aral S.O. Vaginal microbiome and its relationship to behavior, sexual health, and sexually transmitted diseases. Obstet. Gynecol. 2017; 129(4): 643-54. DOI: http://doi.org/10.1097/AOG.0000000000001932

12.Gómez-Camarasa C., Fernández-Parra J., Navarro-Marí J.M., Gutiérrez-Fernández J. Moraxella osloensis emerging infection. Visiting to genital infection. Rev. Esp. Quimioter. 2018; 31(2): 178-81. (in Spanish)