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MECHANISMS OF IMMUNE PROTECTION OF THE VAGINAL ENVIRONMENT IN BACTERIAL VAGINOSIS

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ABSTRACT

The vaginal microbiocenosis is a complex dynamic system in which the aggregate of microorganisms is in various relationships both with each other and with the macroorganism. Their qualitative and quantitative balance is determined by endo-and exogenous factors, the most important of which is the state of the immune system. Also, variations in the immune response may affect a woman's risk of developing bacterial vaginosis, response to treatment, or risk of relapse. Activation of immune responses caused by changes in the composition of the microbiocenosis can either prevent or initiate an aggravation of vaginal dysbiosis.

KEYWORDS: *Bacterial Vaginosis; Vaginal Microbiocenosis; Mechanisms Of Immune Defense; Cytokines.*

INTRODUCTION

Along with the hormonal and metabolic mechanism of regulation of vaginal microbiocenosis, one of the fundamental causes affecting the pathogenesis of bacterial vaginosis (BV) is immune disorders at both the local and systemic levels.

Immune defense against infectious factors (bacteria, fungi, viruses) includes a whole complex of reactions of interaction between cells and mechanisms of activation of various components of the system. There are several levels of protection, including a physiological and immunological

barrier in the form of epithelial cells and a mucin layer. Further protection is provided by the components of innate and acquired (or adaptive) immunity.

Cellular immunity in bacterial vaginosis

The key role in the immune response in bacterial and fungal infections is played by macrophages, neutrophils and natural killers, which provide phagocytosis and destruction of the pathogen directly on the mucous membranes of the lower parts of the reproductive system [1]. Phagocytosis is accompanied by the synthesis of a number of cytokines in macrophages (TNF-α, IL-1P, IL-6, IL-8, etc.), signaling the introduction of pathogens and ensuring the involvement of immune system cells in the focus of inflammation. The effectiveness of phagocytosis is ensured by the involvement of CD4+ T-helper cells of type 1 (Th1-lymphocytes), which produce IFN-γ, thereby enhancing the phagocytic functions of macrophages [2].

In addition to macrophages and neutrophils, dendritic cells play an important role in the immune response to infection. Mainly, dendritic cells are able to absorb peptide fragments, move from peripheral tissue to lymph nodes, and trigger the differentiation of primitive Th0-lymphocytes by Th1-type. Pathogen recognition by dendritic cells is performed via TLR. Activated macrophages and some dendritic cells express CD68 [1].

Primitive Th0 - lymphocytes can differentiate in several directions, among which are Th1-lymphocytes (involved in cell-mediated immunity and characterized by the synthesis of cytokines such as IFN-γ, IL-2, TNF-β) and Th2-lymphocytes (involved in the humoral response, i.e., the synthesis of antibodies, as well as protection from parasitic infections and the development of allergic reactions; characterized by the synthesis of cytokines such as IL-4, IL-5, IL-6, IL-9, IL-13) [3].

Humoral component of immunity in vaginal dysbiosis

Th1 cell-mediated immunity is aimed at destroying intracellular pathogens and is provided by T-lymphocytes.

Th2 cells promote the transformation of primitive B-lymphocytes into plasma cells that produce antibodies [4]. After binding of antibodies to the antigen, the pathogen is eliminated due to the phagocytic activity of macrophages or the activation of the compliment. Unlike other mucosal surfaces: the respiratory and gastrointestinal tracts, where the dominant isotype is IgA, the secrets of the mucous genital tract equally contain both IgG and secretory sIgA. It is believed that the mucosal system is the first line of immune defense, and sIgA is the first line of mucosal immunity [5].

One of the main effects of sIgA is the obstruction of the adhesion of the infectious agent to the epithelial cells of the histohematic barrier due to the formation of intra (membrane) epithelial immune complexes. The latter not only block the adhesion of microorganisms to the epithelial cells of the barrier, but also neutralize their biological activity.

Immunoglobulins G are active against bacteria, viruses, protozoa, fix complement, and play a major role in the secondary immune response. In excess amounts, they block macrophages and stimulate T-lymphocytes-suppressors, providing suppression of primary and secondary immune responses. The amount of immunoglobulins G and A in the cervical mucus changes cyclically in accordance with the phase of the menstrual cycle (increases at the beginning and at the end). The

absolute content and ratio of various immunoglobulins can significantly change in the presence of local inflammatory processes and changes in the permeability of the vascular wall, in these cases, the proportion of immunoglobulins entering the secret by transudation from the blood serum increases, which indicates an increase in the activity of local anti-infective immunity [4].

An important role in the protection of the mucous membranes of the genital tract is played by the complement produced by the mucous membrane of the cervix and vagina. The complement of the mucous membranes is able to attach to the secretory immunoglobulin A, which leads to the phenomenon of opsonization of microorganisms and their subsequent phagocytosis by mucus neutrophils. The immediate bactericidal effect is given by the lysozyme contained in the cervical and vaginal secretions, which increases the activity of neutrophils. The means of local anti-infective protection include lactoferrin, B-lysines, interferons.

Dysbiotic processes in the vagina, caused by excessive reproduction of opportunistic flora, lead to stimulation of cells of the immune system and epithelium, which is manifested in a change in the profile of cytokines expressed by cells. It is known that the interaction of microorganisms with toll-like receptors TLR-2, TLR-4 on the surface of macrophages, dendritic and other cells of innate immunity, activates the synthesis of a number of proinflammatory cytokines TNF-α, IL-1B, IL-6, IL-8, IL-12 [6, 7]. It was noted that women with bacterial vaginosis have an increased level of Th1-cytokines in the vagina [6].

The role of cytokines in the formation of the immune response

In the studies of many authors, an increase in the level of a number of cytokines in BV was noted: IL-1β, TNF-α, IL-6, IL-8, defensins, IL-2 IFN-γ, IL-12, IL-4 and IL-10, and lactoferrin [3, 6, 8, 9]. According to Budilovskaya OV, et al. (2020), there is a differential expression of local immune response genes in the vagina in response to various vaginal infections, while bacterial vaginosis was closely associated with reduced levels of IL-18 and GATA3 mRNA [10].

According to Anahtar MN, et al. (2015), bacterial communities with high diversity strongly correlate with the concentrations of proinflammatory cytokines in the genitals in both transverse and longitudinal analyses [6]. Studies by Campisciano G. et al. (2018) have confirmed that increased levels of cytokines such as IL-18, IL-2, IL-1RA (interleukin-1 receptor antagonist), MIF, RANTES (regulated on activation, normal T cell expressed and secreted), TNF-a, Mip1a, and IL-8 correlate with changes in the composition of the vagina towards dysbiosis. Other immune factors, such as IL-5, IL-13, IL-6, IL-15, IL-9, growth–related onconge-α (growth-regulating oncogene), Mip1-β, and IFN-γ, showed a correlation with the altered vaginal environment, although their number did not significantly change between the cohorts of women with normocenosis, intermediate microbiocenosis, and BV [3].

Cytokines produced by Th1- and Th2-lymphocytes negatively affect the activation of opposite subpopulations of T-helper cells. Excessive activation of any of the subpopulations of T-helper cells can direct the immune response by Th1 - or Th2-type.

In case of dysbiosis of the vaginal microflora, the balance of pro-inflammatory and immunosuppressive cytokines is very important. Proinflammatory cytokines ensure the development and full functioning of all stages of immune responses against pathogens. Anti-inflammatory cytokines restrain the excessive activity of the inflammatory process and prevent it from becoming pathological [6].

Campisciano G, et al. (2018) found that although a dysbiotic state triggers a pro-inflammatory process, the presence of a stable level of Th2 can affect the clinical manifestations of BV, i.e., a concomitant increase in the level of anti-inflammatory IL-5 and IL-13 secreted by Th2 cells in vaginal dysbiosis suggests activation of the Th2 link in countering the Th1 response and in countering the presence of clinical symptoms. The authors noted that proinflammatory mediators, usually involved in the chronic inflammatory process, synergize with cytokines involved in switching to the Th2-immune response.This reaction probably supports the dysbiotic state, regardless of the pro-inflammatory Th1 response of the macroorganism, which is usually effective in restoring the state of normocenosis. According to the authors, stimulation of the Th2 response together with a blunted Th1 response can lead to immunological tolerance, causing chronic recurrent vaginal dysbiosis [3].

The contribution to the pool of transcripts of the immune response genes is made not only by the cells of the immune system, but also by the epithelium. The epithelial cells of the vaginal mucosa not only create a mechanical barrier to infectious agents, but also participate in the presentation of foreign antigens, as well as produce a variety of immune system mediators that directly implement and regulate both innate and adaptive immunity [4]. In vitro studies of epithelial cells of the vaginal mucosa revealed a differential effect of secretory products of various microorganisms on the functional state of the mucosa of epithelial cells, manifested in an increase in the production of cytokines IL-1B, IL-6, IL-8, TNF-α [7].

Characteristic violations of the humoral link of immunity in BV are an increase in the concentration of IgG, IgM, transferrin in the blood of patients and an increase in the reaction of inhibition of leukocyte migration with phytohemagglutinin. Changes in the local immunity of the vagina are characterized by a decrease in the concentration of Iga, secretory Ig A, and C3 components of the complement with an increase in IdM and transferrin [5].

Immunomodulatory effects of vaginal lactobacilli

The vaginal microflora plays a key role in the functioning of the immune system, which promotes symbiosis between the host macroorganism and complex microbial communities [3, 6]. Toll-like receptors, cytokine production, and other components of the innate immune response are associated with *L. crispatus* , *L. iners,* and community state types (CST) [11].

The dominance of *Lactobacillus spp.* in the genital tract is important because it suppresses pathogens and maintains immune balance. Studies have shown that the concentration of pro-inflammatory factors in the vagina is very low when *L. crispatus* and *L. jensenii* are dominant [9].

The immunomodulatory effects of *L. crispatus* were noted, for example, the *L. crispatus* strain ATCC 33820 suppresses the growth of *Candida albicans* 27 in vitro by modulating the expression of TLR-2, TLR-4, IL-8, and β-defensin 2 and 3 in epithelial cells [7].

Lactic acid, as a metabolite produced mainly by *Lactobacillus spp.*, is also associated with the immunity of the reproductive tract [12]. L-lactic acid produced by *Lactobacillus spp.* it can cause an anti-inflammatory response and inhibit the production of pro-inflammatory cytokines and chemokines induced by TLR in the epithelial cells of the cervix and vagina at low pH [13].In addition, lactic acid can induce the secretion of the anti-inflammatory cytokine interleukin IL-10, reduce the production of pro-inflammatory cytokine IL-12 in dendritic cells, and reduce the

cytotoxicity of natural killer cells [14]. The anti-inflammatory activity of lactic acid also requires the presence of organic acids produced by microorganisms to maintain vaginal health, mainly by increasing the production of anti-inflammatory cytokine IL-1RA, inhibiting the pro-inflammatory signal of cytokine IL-1, while the production of pro-inflammatory cytokines IL-6 and MIP-3a is slightly reduced [13].

The association of *L. iners* dominance in the composition of lactoflora with a light proinflammatory background, similar to changes in BV, was revealed. According to a number of authors, the samples with the dominance of *L. iners* (CST III) significantly increased the levels of mRNA expression of the IL-8, TLR-4, IL-10, CD69, CD45 genes and reduced IL-18 than in the samples with the dominance of *L. crispatus*, which partly explains the increased risk of developing BV in women with this type of microbiocenosis [6]. The tendency to increase proinflammatory factors in CST IV also indicates that the formation of a proinflammatory state associated with the absence or low number of lactobacilli may be the ground for the development of BV.

Recently, a huge role in opportunistic vaginal infections has been assigned to factors affecting the macroorganism of metabolites formed by conditionally pathogenic microorganisms. The innate and adaptive immune systems of the host carry out complex interactions with microorganisms and metabolites [13, 15]. Microbial ligands bind to macroorganism receptors, producing inflammatory factors, chemokines, and antimicrobial products that regulate the immune response of the reproductive tract [16].

Vaginal dysbiosis cannot directly cause damage to the vaginal epithelium through pathogens, but indirectly causes damage to the vaginal epithelium through immune components, which in turn release metabolites into the microenvironment [17, 18]. The binding of the metabolite by bacteria leads to an increase in microbial metabolism, which has a favorable effect on the growth and reproduction of flora [18].Local competition between the host macroorganism, the pathogen, and various immune cells for metabolic precursors will also affect the ability of immune cells to respond effectively to infection, affecting the growth and immunogenicity of the pathogen and additionally affecting the host response [16, 17]. Consequently, the interaction between microorganisms, metabolites, and immunity in the host reproductive tract microenvironment plays an important role in maintaining the balance of the reproductive tract [19, 20]. An imbalance in these relationships will lead to changes in the host's phenotype, disease, and even serious complications.

It is believed that substances produced by conditionally pathogenic microorganisms reduce the protective functions of immune cells and local immunity factors. Delgado-Diaz DJ, et al. (2019) found that the prolonged action of organic acids, metabolites of the vaginal microbiocenosis associated with BV, leads to a violation of the regulation of the immune response of cervical and vaginal epithelial cells *in vitro* [13].Short-chain fatty acids can attract and activate innate immune cells of the female reproductive tract, such as neutrophils and monocytes [21]. It was found that the succinate secreted by *Bacteroides spp.* it inhibits the functions of neutrophils [22]. In addition, succinic acid produced by *Prevotella spp.* and *Mobiluncus spp.* in the genital tract, it can also suppress the chemotaxis of white blood cells and regulate the immune response [21, 23]. Sialidases produced by *Gardnerellavaginalis* and some other anaerobes stimulate some cells of

the immune system and inhibit phagocytosis [24], in addition to contributing to the destruction of mucin [25].

In the work of Burmenskaya OV, et al. (2014) analyzed the dependence of the transcription profile of the immune response genes on the type of lactobacilli. The association of *L. iners* dominance in the composition of lactoflora with a light proinflammatory background, similar to changes in vaginitis and BV, was revealed. According to the authors, the mRNA expression levels of the IL-8, TLR4, IL-10, CD69, and CD45 genes were significantly increased and IL-18 was reduced in the *L. iners*-dominated samples than in the *L. crispatus*-dominated samples [8].

Genetic basis of immune response variations

It is important to note that there is a relationship between the species composition of the normoflora, the presence of conditionally pathogenic microorganisms in the vaginal microbiocenosis and the individual genetic profile of women. According to Voroshilina E. S. et al. (2011), depending on the variants of proinflammatory and anti-inflammatory cytokine genes in women, different types of lactobacilli predominate in the vaginal fluid; the normocenosis is preserved, but has a different degree of stability [26].

The subject of this study was the polymorphism of genes associated with the system of pro-inflammatory (IL-1) and anti-inflammatory (IL-1RN, IL-4, IL-10) cytokines. As a result, a correlation was established between the presence of *L. crispatus*in the vagina and the carrier of two alleles A of the IL-10 gene (genotype AA): the presence of the "pro-inflammatory" genotype correlated with the dominance of *L. crispatus* in the vaginal lactoflora, and the "anti-inflammatory" and balanced genotype was significantly more often associated with the predominance of*L. iners* [26].

In recent years, a number of studies have confirmed the role of genetic factors in maintaining the microbiocenosis of the vagina. Studies show that genetic associations suggest the role of the innate immune system and cellular signaling in the composition of the vaginal microflora and susceptibility to the suboptimal composition of the vaginal microbiocenosis.According to Mehta SD, et al. (2020) BV was absent in CST I (dominant L. crispatus) and not often (5.1%) in CST III. 57% of women in CAT IV did not have BV [27]. This data is consistent with many other early studies: women with CST IV are much more likely to have BV, but up to 50% of women with this CST do not have BV [28, 29]. I.e., women with similar bacterial colonization of the vagina (for example, in the C STIV group) may have a different response to bacterial colonization depending on genetic characteristics [27].

Mehta SD, et al. (2020) identified single-nucleotide polymorphisms in IL1RN, IL-5, and IL-5RA. IL-1RN (the IL-1RA gene) inhibits the proinflammatory cytokines IL-1, IL-1α, and IL-1β [26]. In a study by CampiscianoG. et al. (2018), women with BV (Nugent score from 7 to 10) had elevated levels of IL-1RA, and an increase in anti-inflammatory IL-5 was associated with a decrease in the number of lactobacilli [3]. When analyzing candidate genes, Si J, et al. (2017) identifiedgeneticvariantsofIL-5, revealinganassociationwithanincreaseinthenumberof*Prevotella* [30].

These literature data confirm that the occurrence and course of the disease is determined by several factors and includes the interaction of the host organism with microorganisms, the features of the immune system and the genetic features of macro-and micro-organisms. Of great

importance is the fact that the etiological agents of the disease are conditionally pathogenic microorganisms peculiar to this biotope, therefore, genetic factors and features of their phenotypic manifestations can play a key role in the pathogenesis of the disease.

Most of the published works are devoted to the manifestation of the immune response of the genital mucosa to BV and the change in the risk of reproductive health complications, such as HIV susceptibility and preterm birth, depending on the variants of the immune response. Less is known about how variations in the immune response affect a woman's risk of developing BV, response to treatment, or risk of relapse. Activation of immune responses caused by changes in the composition of the microbiocenosis can either prevent or initiate an aggravation of vaginal dysbiosis. Although the possible associations of risk factors with recurrent BV are of considerable interest, there is too little data on mucosal immunity differences or on genetic markers of susceptibility in women with recurrent BV to provide a basis for further research.

CONCLUSION

Thus, the microbiocenosis of the vagina is a complex dynamic system in which the totality of microorganisms is in various relationships both with each other and with the macroorganism. Their qualitative and quantitative equilibrium is determined by endo-and exogenous factors. Criteria for the normocenosis of the vaginal biotope until recent years, the dominance of lactobacilli was considered. However, the results of molecular genetic research methods show that the absence of Lactobacillus spp. it is not always accompanied by the development of dysbiosis, since in addition to them, the components of the immune system and hormonal homeostasis are actively involved in maintaining the state of normocenosis.

With physiologically complete hormonal and immune regulatory systems, the composition of the vaginal microbiocenosis is stable, with random shifts (mainly due to the action of harmful external factors) – it quickly recovers without special correction. However, in the presence of serious deviations in the indicators of the state of general metabolism and with pronounced harmful exogenous effects, negative reactions from the immune and endocrine systems are inevitable, which ultimately can manifest itself in quantitative and qualitative disorders of the vaginal microbiocenosis. The persistence of such disorders and the possibility of drug correction depend on the degree of violation of the functions of the immune and endocrine systems.

REFERENCES

1. Lee SK, Kim CJ, Kim D-J, Kang J-H. Immune cells in the female reproductive tract. Immune Netw. 2015;15:16-26. doi:10.4110/in.2015.15.1.16.
2. Bondarenko KR, Ozolinya LA, Bondarenko VM. Pathogenetic aspects of vaginal dysbiosis and modern possibilities of its correction. Obstetrics and Gynecology. 2014;8:127-132.
3. Campisciano G, Zanotta N, Licastro D, De Seta F, Comar M. In vivo microbiome and associated immune markers: new insights into the pathogenesis of vaginal dysbiosis. Sci Rep. 2018;8:2307. doi:10.1038/s41598-018-20649-x.
4. Chen A, McKinley SA, Wang S, Shi F, Mucha PJ, Forest MG, Lai SK. Transient antibody-mucin interactions produce a dynamic molecular shield against viral invasion. Biophys J. 2014;106:2028–2036. doi: 10.1016/j.bpj.2014.02.038.

5. Li Y, Jin L, Chen T. The effects of secretory IgA in the mucosal immune system. Biomed Res Int. 2020;2020:2032057. doi:10.1155/2020/2032057.

6. Anahtar MN, Byrne EH, Doherty KE, Bowman BA, Yamamoto HS, Soumillon M, et al. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. Immunity. 2015;42(5):965–976. doi:10.1016/j.immuni.2015.04.019.

7. Rizzo A, Losacco A, Carratelli CR. Lactobacillus crispatus modulates epithelial cell defense against Candida albicans through Toll-like receptors 2 and 4, interleukin 8 and human beta-defensins 2 and 3. Immunol Lett. 2013;156(1-2):102-109. doi:10.1016/j.imlet.2013.08.013.

8. Burmenskaya OV, Bayramova GR, Nepsha OS, et al. Species composition of lactobacilli in non-specific vaginitis and bacterial vaginosis and its effect on local immunity. Obstetrics and Gynecology. 2014;1:41-45.

9. Kyongo JK, Jespers V, Goovaerts O, Michiels J, Menten J, Fichorova RN, et al. Searching for lower female genital tract soluble and cellular biomarkers: defining levels and predictors in a cohort of healthy Caucasian women. PLoS One. 2012;7(8):e43951. doi:10.1371/journal.pone.0043951.

10. Budilovskaya OV, Shipitsina EV, Spasibova EV, et al. Differential expression of local immune response genes in the vagina: implication for the diagnosis of vaginal infections. Bull ExpBiol Med. 2020;168:646-650. doi:10.1007/s10517-020-04771-3.

11. Nisha K, Antony B, Udayalaxmi J. Comparative analysis of virulence factors and biotypes of Gardnerellavaginalis isolated from the genital tract of women with and without bacterial vaginosis. Indian J Med Res. 2019;149(1):57-61. doi:10.4103/ijmr.IJMR_1674_16.

12. Aldunate M, Srbinovski D, Hearps AC, Latham CF, Ramsland PA, Gugasyan R, et al. Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. Front Physiol. 2015;6:164. doi:10.3389/fphys.2015.00164.

13. Delgado-Diaz DJ, Tyssen D, Hayward JA, Gugasyan R, Hearps AC, Tachedjian G. Distinct immune responses elicited from cervicovaginal epithelial cells by lactic acid and short chain fatty acids associated with optimal and non-optimal vaginal microbiota. Front Cell Infect Microbiol. 2020;9:446. doi:10.3389/fcimb.2019.00446.

14. Ilhan ZE, Laniewski P, Thomas N, Roe DJ, Chase DM, Herbst-Kralovetz MM. Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. EBioMedicine. 2019;44:675-690. doi:10.1016/j.ebiom.2019.04.028.

15. Agostinis C, Mangogna A, Bossi F, Ricci G, Kishore U, Bulla R. Uterine immunity and microbiota: a shifting paradigm. Front Immunol. 2019;10:2387. doi: 10.3389/fimmu.2019.02387.

16. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012;336(6086):1268-73. doi:10.1126/science.1223490.

17. Olive AJ, Sassetti CM. Metabolic crosstalk between host and pathogen: sensing, adapting and competing. Nat Rev Microbiol. 2016;14(4):221-34. doi:10.1038/nrmicro.2016.12.

18. Serrano MG, Parikh HI, Brooks JP, et al. Racioethnic diversity in the dynamics of the vaginal microbiome during pregnancy. Nat Med. 2019;25(6):1001-1011. doi:10.1038/s41591-019-0465-8.

19. Pruski P, Lewis HV, Lee YS, Marchesi JR, Bennett PR, Takats Z, et al. Assessment of microbiota: host interactions at the vaginal mucosa interface. Methods. 2018;149:74-84. doi:10.1016/j.ymeth.2018.04.022.

20. Rakhmatullaeva MM. Types of Vaginal Lactobacilli as Biomarkers of the Physiological State of Microbiocenosis. American Journal of Medicine and Medical Sciences. 2020;10(11):837-841. doi:10.5923/j.ajmms.20201011.02.

21. Vitali B, Cruciani F, Picone G, Parolin C, Donders G, Laghi L. Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. Eur J ClinMicrobiol Infect Dis. 2015;34(12):2367-2376. doi:10.1007/s10096-015-2490-y.

22. Mirmonsef P, Gilbert D, Zariffard MR, Hamaker BR, Kaur A, Landay AL, Spear GT. The effects of commensal bacteria on innate immune responses in the female genital tract. American journal of reproductive immunology. 2011;65(3):190-195. doi:10.1111/j.1600-0897.2010.00943.x.

23. McMillan A, Rulisa S, Sumarah M, Macklaim JM, Renaud J, Bisanz JE, et al. A multi-platform metabolomics approach identifies highly specific biomarkers of bacterial diversity in the vagina of pregnant and non-pregnant women. Sci Rep. 2015;5:14174. doi:10.1038/srep14174.

24. Santos-Greatti MMV, da Silva MG, Ferreira CST, Marconi C. Cervicovaginal cytokines, sialidase activity and bacterial load in reproductive-aged women with intermediate vaginal flora. J ReprodImmunol. 2016;118:36-41. doi:10.1016/j.jri.2016.08.005.

25. Moncla BJ, Chappell CA, Debo BM, Meyn LA. The effects of hormones and vaginal microflora on the glycome of the female genital tract: cervical-vaginal fluid. PLoS One. 2016;11(7):e0158687. doi:10.1371/journal.pone.0158687.

26. Voroshilina ES, Sergeev AG, Tumbinskaya LV. Gene polymorphism of the cytokine system and the species composition of vaginal lactobacilli in women of reproductive age with preserved normoflora. Obstetrics and Gynecology. 2011;3(81):58-63.

27. Mehta SD, Nannini DR, Otieno F, Green SJ, Agingu W, Landay A, et al. Host genetic factors associated with vaginal microbiome composition in kenyan women. mSystems. 2020;5(4):e00502-20. doi:10.1128/mSystems.00502-20.

28. Brotman RM, Shardell MD, Gajer P, Fadrosh D, Chang K, Silver MI, et al. Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. Menopause. 2014;21:450-458. doi:10.1097/GME.0b013e3182a4690b.

29. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl AcadSci USA. 2011;108:4680-4687. doi:10.1073/pnas.1002611107.

30. Si J, You HJ, Yu J, Sung J, Ko G. Prevotella as a hub for vaginal microbiota under the influence of host genetics and their association with obesity. Cell Host Microbe. 2017;21:97-105. doi:10.1016/j.chom.2016.11.010.