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The influence of vaginal microbiome on the development of cervical incompetence and preterm birth

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ABSTRACT

There is a hypothesis that the state of a woman's cervicovaginal microbiome can correlate with the possibility of such obstetric complications as cervical incompetence and preterm birth. This review summarizes the information about the connection between microbiome and cervical incompetence. It turned out that the dominance of *Lactobacillus crispatus* and possibly *Lactobacillus gasseri* in the microbiome is associated with full-term pregnancy while the predominance of other types of *Lactobacillus* and anaerobic bacterias (*Gardnerella, Atopobium, Prevotella, Streptococcus, Ureaplasma., Megasphaera, Escherichia, Shigella, etc.*) leads to preterm rupture of fetal membranes and preterm birth. We should pay attention to the fact that high concentration of antimicrobial peptides β-defensin-2 even without dominance of *L. crispatus* is also associated with full-term pregnancy. When studying cervicovaginal and amniotic fluid of woman who had preterm birth, the rise in concentration of inflammatory-related cytokin, such as IL-2, IL-8, IL-10 etc., is discovered. The change of components of microbiome and the rise of mother's immune reaction leads to preterm remodeling and softening of cervix. Thus, the early diagnostics of the changes in cervicovaginal microbiome, cervicovaginal and amniotic fluid may predict cervical incompetence and preterm birth.

1. Introduction

Preterm labor is delivery before 37 weeks of gestation. Currently, premature birth is an ordeal for obstetricians and gynecologists around the world. For instance, every tenth child in the United States is born prematurely. The tendency to premature birth is increasing every year and, surprisingly, its number among African American women is about 50% more than among representatives of the Caucasian race and Latin American women [1]. Preterm labor is the polyethological pathology that may be caused by extragenital diseases, pathologies of the reproductive system, stress and be influenced by the race, age, etc. The research carried on by [2] has shown that women aged 30-34 years have the lowest risk of preterm delivery, while women over 40 years have high risks. In addition, the causes of premature birth are a violation of the

normal vaginal microbiome (and, consequently, ascending vaginal infection through the cervix into the uterine cavity) and cervical insufficiency [3]. In this review, we will study the connection between these two causes of premature delivery.

2. Findings and discussion

Cervical incompetence is characterized by shortening of the cervix < 25 mm and/or dilation of the cervical canal > 10 mm. Its common feature is rapid and painless shortening of the cervix in the second or early third trimester of pregnancy, followed by late miscarriage or premature birth. Prolapse of the fetal bladder into the cervical canal and premature rupture of the fetal membranes are possible. The most optimal method of diagnosing cervical incompetence during pregnancy is the transvaginal ultrasound investigation of the cervix (ultrasound cervicometry) [4]. The shorter the gestation period (16-22 weeks) and the shorter the cervix when diagnosing cervical insufficiency, the higher the risk (50%) of early premature birth (<32 weeks). This risk decreases to 15% at later gestational age [5].

Microbiota is a collection of microorganisms that inhabit a particular niche in the human body. The concept of microbiome includes both the microbiota itself and the totality of the genetic material of these microorganisms in conjunction with the conditions of their habitat [6]. New-generation DNA sequencing methods are applied for studying the composition of the microbiota: the 16S ribosomal RNA gene comprising the most variable regions V1-V3 and V3-V5 is specific for a bacterial cell. Applied methods allow assessing the qualitative and quantitative composition of the microbiome [7], [8]. Different *Lactobacillus* species can dominate in vaginal microbiome; therefore, five types of vaginal bacterial communities (community state types) have been selected: *L. crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III) and *L. jensenii* (CST V); a variant with a predominance of pathogenic anaerobes (*Gardnerella, Atopobium, Prevotella, Streptococcus, Ureaplasma., Megasphaera, Escherichia, Shigella, etc.*) in the microbiome and a decrease in the *Lactobacillus* amount leading to bacterial vaginosis (CST IV) is also possible [9]. Menarche is associated with an increase in circulating estrogen levels which induce proliferation of epithelial cells in the vagina and the deposition of glycogen metabolized to lactic acid by alpha-amylase from lactobacilli. *Lactobacillus* protect the vaginal epithelium against invasion and colonization of pathogenic and opportunistic microorganisms by means of production of hydrogen peroxide and bacteriocins increasing the permeability of target cells, as well as biosurfactants and lactic acid reducing the pH of the environment, which creates unfavorable conditions for the growth and reproduction of pathogens; lactic acid also induces lysis of damaged epithelial cells of the vaginal mucosa with the production of glycogen, and the cycle closes. Mucins, β-defensins, antibodies, etc. also perform a protective function [10], [11]. The lower the pH in the vagina (<4), the greater the dominance of *Lactobacillus* and, conversely, the higher the pH, the lower the content of *Lactobacillus* and the greater the predominance of anaerobic bacteria [9], [12]. During pregnancy, the estrogen content is constant, there is no menstrual bleeding and endometrial exfoliation, which creates favorable conditions for the growth and reproduction of lactobacilli [7], [13]. The preterm delivery is characterized by the high diversity of the microbiome components in combination with a decrease in the dominance of *Lactobacillus* variants (especially *L. crispatus*). As for the normal delivery on time, there is a low diversity of the microbiome, represented mainly by *L. crispatus*. Consequently, the predominance of *L. crispatus* in the microbiome is connected with full-term pregnancy [14- 18]. However, full-term pregnancy and delivery on time are possible both in the absence of *L. crispatus* dominance and with the predominance of another bacterial community [19].

[20] in their study “Motherhood and Microbiome” (M&M) found out that *Mobiluncus curtsii/mulieris* related to CST IV are most strongly associated with preterm birth, especially at less than 34 weeks of gestation. If the number of *Lactobacillus spp.* is low, the risk of premature delivery in presence of

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Mobiluncus curtsii/mulieris increases. If the number of *Lactobacillus spp.* is high, this risk disappears. This proves that the presence of *Lactobacillus spp.* in the microbiome eliminates the risk of preterm delivery even in the presence of representatives of pathological flora. African American women do not tend to have the predominance of *L. crispatus* in the cervicovaginal microbiome. This phenomenon leads to unfavourable pregnancy outcomes. As for the European women, on the contrary, there is a predominance of this type of *Lactobacillus.* The risk of preterm birth among African American women against this background is twice as high. Besides, they have a higher risk of bacterial vaginosis [21], [22]. However, the high content of *L. crispatus* does not guarantee delivery on time for neither African American nor European women. As a result of the research on the effect of the local immune response, namely, the role of β–defensin-2 (an antimicrobial peptide that is used by immune cells to destroy phagocytized antigen) on the outcome of pregnancy, scientists concluded that its high content eliminates the risk of preterm delivery associated with a lack of *Lactobacillus spp*. However, the low content of this antimicrobial peptide even in case of the predominance of *Lactobacillus spp.* may lead to premature birth [20]. Increased psychoemotional stress of a pregnant woman may be a reason for a decrease in β-defensin-2 content, and the combination of these two factors drastically increases the risk of premature birth [23].

[24] conducted a secondary analysis of the study "Motherhood and microbiome" and found out that the frequency of CST IV detection in the cervical smear among women with spontaneous preterm birth was almost 45%. A short cervix was observed more often in CST IV than in other types of vaginal bacterial communities. In addition, the frequency of premature rupture of the fetal membranes caused by preterm delivery was slightly more often observed in patients with CST IV. As a result, women with a short cervix and CST IV in a cervical smear have a higher risk of preterm delivery. In addition, most of the women who were found to have these parameters were African American.

Interestingly, the predominance of *L. iners* in the microbiome at the 16th week also results in premature shortening of the cervix and preterm delivery before the 34th week of gestation, whereas *L. crispatus*-dominant microbiota at the same gestational age is associated with normal delivery on time. *L. crispatus* in the microbiome prevails in European women, and *L. iners* and CST IV – in African American women [3]. Perhaps this is due to the fact that *L. crispatus* provides synthesis of D- and L-lactic acid, while *L. iners* is able to form only its L-isomer, which has lower protective properties against pathogenic bacteria in contrast to its D-isomer [10], [25]. [26] have shown that L. iners-dominant microbiota (among all Lactobacillus species - dominant microbiotas) is often associated with an extremely short cervix (< 10 mm) and, consequently, leads to cervical remodeling and cervical incompetence as well as the microbiota represented by anaerobes (CST IV). Besides, L. jensenii-dominant microbiota can also be associated with preterm birth, while the predominance of L. gasseri has a similar to L. crispatus protective effect and is related to full-term pregnancy [27]. L. iners and G. vaginalis lead to an increase in the permeability of cervical epithelial cells, excessive hydration of the cervical stroma and softening of the cervix, that leads to its remodeling and further preterm birth. L. crispatus, on the contrary, provides the powerful protection of the epithelial barrier of the cervix from the impact of pathological flora [28].

Vaginal dysbiosis results in an increase in the production of proinflammatory cytokines [29]. [30] in their research found out that colonization of the cervicovaginal zone with *G. vaginalis* leads to increased mucin secretion and initiates the synthesis of interleukin-6 (IL-6) in both cervicovaginal and amniotic fluids, despite the absence of ascending infection of the fetal membranes, placenta and uterine cavity. This indicates that the pathological flora is able to cause a local inflammatory reaction in the cervicovaginal space that probably leads to a decrease in the elasticity of the cervix, its remodeling and premature delivery in the future [31]. Women with a very short cervix (< 15 mm) have a higher concentration of IL-6 and other

pro-inflammatory mediators in amniotic fluid than women with a short cervix < 25 mm, but > 15 mm. The concentration of IL-2 was increased among women with a very short cervix who subsequently gave birth prematurely, but not among women who gave birth on time [32]. [5] determined that the patients with a short cervix at 16-22 weeks of gestation had a high concentration of proinflammatory proteins in the amniotic fluid at amniocentesis, especially IL-8, MIP-1β, IL-6 and IL-10; at 22-26 weeks - a high concentration of IL–8, MIP-1β and IL-6; at 26-31 weeks – only IL-8. Consequently, the shorter the cervix and the shorter the gestational age, the higher the concentration of proinflammatory proteins in amniotic fluid and the higher the risk of early preterm delivery (< 32 weeks). These proteins are produced by macrophages and lymphocytes. They are cytokines that due to chemotaxis attract neutrophils and other granulocytes to the area of inflammation. Women with full-term pregnancy had no markers of bacterial invasion in the amniotic fluid [33].

The content of D-lactic acid is maximal in CST I (*L. crispatus*) and slightly less in CST V (*L. jenseni*). The level of TIMP-1 (endogenous metalloproteinase inhibitor) in these communities is minimal. The parameters above are associated with the normal length of the cervix. The content of L-lactic acid is the highest in CST III (*L. iners*) and the level of TIMP-1 in this community and in CST IV, represented by *G. vaginalis*, are maximal. In this case shortening of the cervix and unfavourable pregnancy outcomes in the form of preterm delivery are possible. [34]. [35] carried on a research and found out that, in addition to TIMP-1, the high levels of vitamin D binding protein (VDBP) and Dickkopf family protein 3 (DDK 3) were present in the cervicovaginal fluid of the patients diagnosed with cervical insufficiency (cervical canal > 10 mm) and a shortened cervix (<25 mm). The combination of these indicators is a more accurate predictor of premature birth than their levels individually. The level of matrix metalloproteinase-8 (MMP-8) was also increased among women with further preterm delivery [36]. Thus, these indicators can be used in order to predict preterm delivery.

The decrease in the content of *L. crispatus* and the dominance of anaerobic flora (*Bacteroides, Fusobacteriales and Clostridiales*) may lead to premature rupture of fetal membranes and to preterm birth in the future. [35]. At 24-28 weeks of gestation, about 9 days pass since premature rupture of fetal membranes till delivery, at 31 weeks – about 5 days [36]. Treatment while having premature rupture of fetal membranes is currently aimed at accelerating the maturation of fetal lungs (formation of surfactant) with the intake of glucocorticosteroids. The oral administration of 250 mg of erythromycin by the mother for 10 days is also necessary to prevent ascending infection [37]. However, [16] found out that among women who got therapy with erythromycin and whose microbiota was initially *Lactobacillus* dominant, vaginal dysbiosis occurs with a decrease in the number of *Lactobacillus*. This process may lead to the development of chorioamnioitis, whereas the erythromycin treatment of women with a microbiome, represented mainly by pathological flora, led to a decrease in its diversity and to an increase in the content of *Lactobacillus*. Thus, the erythromycin therapy is impractical for the first group of women, and acceptable for the second group.

3. Conclusion

The data shown above confirm the hypothesis that the woman's vaginal microbiome state may correlate with the likelihood of development of obstetric complications such as cervical incompetence and premature birth. *L. crispatus* provides protection for cervix' and vagina's epithelial barrier, that leads to delivery on time. But the presence of the other vaginal communities is associated with cervical insufficiency and preterm delivery. The activation of the mother's immune reaction in response to the impact of pathological flora, characterized with an increase in the content of proinflammatory cytokines in cervicovaginal and amniotic fluids, is also the risk factor for the development of cervical incompetence and premature birth.

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However, further study of this field is required for the prevention of cervical insufficiency, preterm delivery and for the selection of rational therapy for these obstetric complications.

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