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The controversial role of placental microbiome in preterm birth

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ABSTRACT

The correlation between the maternal and neonatal microbiome helps to understand the mechanisms that govern the metabolic and immune processes of the newborn. The literature of recent years brings pros and cons regarding the role of the placenta as a microbial residence and its involvement secondary to microbial colonization in premature birth. Additional multi-omics research will be needed to establish the role of this transient organ in elucidating the processes that govern premature birth. DNA sequencing has made it possible to identify microbial species in the human microbiome. Thus, the role of the maternal microbiome in pregnancy and related complications are partially elucidated. This article aimed to identify the diversity of species in the maternal placental microbiome, the possible association with the newborn microbiome, and the influence of dysbiosis in spontaneous premature birth (PTB). In pregnancy, the changes in the microbiome are multifactorial, and the analysis performed demonstrating the continuous intervention of the body in order to adapt the intestinal microbiota to have a positive maternal-fetal result.

Keywords: maternal microbiome, preterm birth, placental microbiome, pregnancy

INTRODUCTION

Premature birth (PTB) before 37 weeks of completed pregnancy continues to be an intense public health issue debated due to the high rate of morbidity and mortality of these newborns (1). The incidence of PTB is estimated at about 11.1% of all live births globally, accounting for 5% in Europe (2,3). Several studies have highlighted the role of the microbiome in the development and maintenance of a healthy pregnancy through metagenomic sequencing techniques. Disorders in the constitution of this microbiome can occur in the etiopathogenic mechanisms of premature birth (4,5). Therefore, the influence of the maternal microbiome in the etiology of premature birth requires a thorough study of the intestinal, vaginal, cervical, and placental microbiomes (6).

In the multi-omics studies in the last decade, the NIH Human Microbiome Project (HMP) was carried out, which included in the second phase the Integrative Project of human microbiomes focused on knowing the mechanisms related to the role of the microbiome in premature birth, in the etiopathogenesis of inflammatory bowel diseases and in interpreting stressors in people with prediabetes (7,8).

Research on microbial diversity has led to the idea that each person has in his constitution an individualized microbiome formed at the beginning of life and which shows rapid dynamics during it. For example,

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dysbiotic conditions due to the influence of immune mechanisms can cause side effects in the female genital tract (9,10). Franzosa et al. highlighted a series of algorithms on building the metagenomic code by connecting microbial theories with computer codes based on microbiomes and individual differentiation (9).

The Consortium achieved a role in defining the concept of a healthy microbiome for the human microbiome project by identifying maternal influences on the placental microbiome and possible modeling the neonatal microbiome. Several studies have sought to establish how the maternal microbiome can alter the microbiome in the formation of the newborn and how it can interfere with the normal course of pregnancy through the risk of premature birth (4,11).

Complementary studies are needed to see how the microbiome intervenes in metabolic and immune changes. In fact, the alteration of the microbiome has been observed in some medical conditions such as obesity, diabetes, hypertension, inflammatory bowel disease, and digestive cancer (e.g., colorectal) (4,12).

THE ROLE OF THE MICROBIOME IN PREGNANCY

Maternal adaptative physiological changes during pregnancy determine a series of morphophysiological and biochemical transformations secondary to hormonal influences and implicitly of the microbiome. The studies examined the evolution and changes of the microbiome related to pregnancy in the following sites: vagina, gut, lung, placenta, oral cavity (13,14). Over time, a continuum of microbiota has been shown in the vagina, cervix, endometrial mucosa, and fallopian tubes of women of reproductive age, demonstrating that the intrauterine environment is unsterile even before conception (15).

Hypervascularization and hyperemia of the vaginal mucosa, cellular hyper-trophy, increased volume of cervical secretions, and decreased vaginal pH by excess lactic acid produced by *Lactobacillus acidophilus* are factors will significantly influence the microbiome and the continuous transformation during pregnancy (16,17). Stout et al., in a study of a predominantly African-American population, found that early pregnancy was associated with a significant decrease in vaginal microbial diversity, being an essential moment in the chronology of events leading to premature birth (18).

The dynamics of changes in the microbiome can influence the immune status through the level of cytokines and thus increase the risk of infection. The microbiome component in pregnancy undergoes a series of transformations highlighting a decrease in species diversity, followed by the increased presence of other species such as Lactobacillus, Bacteriodales, and Clostridiales (19). The vaginal microbiome in pregnancy shows the growth of four types of Lactobacillus spp (*L. jensenii, L. gasseri, L. crispatus*, and *L. vaginalis*) and a decrease in the number of anaerobic species (19,20).

Lactobacillus species, *L. jensenii* and *L. crispatus*, which inhibit sexually transmitted infections by producing hydrogen peroxide with decreased pH, had an increased biological significance. *L. johnsonii* produces Lactacin F to destroy germs from the gastrointestinal tract and in the digestion of milk by infants through the neonatal microbiome (21). Another study revealed that the main role is to reduce the risk of ascending infection (chorioamnionitis) and premature birth (22).

The vaginal microbiome undergoes transformations related to gestational age and the vicinity of the cervix. Rarely a dominant species of *Lactobacillus spp.* can pass to one dominated by anaerobes (CST IV-A or CST IV-B) (20), a possible explanation would be due to the hormonal changes in pregnancy (23).

Decreased levels of lactobacilli in the vaginal microbiome cause bacterial vaginosis involved in PTB (24). This was demonstrated by Joo et al. through the role of *Lactobacillus johnsonii* HY7042 in suppressing *Gardnerella vaginalis* by inhibiting the expression of COX-2, IL-1β, and TNF-α by regulating NF-κB activation, with beneficial action on bacterial vaginosis (25).

Another important component of the microbiome is the intestinal one, whose bacterial density increases during pregnancy and whose component undergoes a continuous change (26).

Collado et al. identified an increase in Bacteroides and *Staphylococcus aureus* species in the microbiota composition related to weight gain in pregnancy. In addition, the decrease of the maternal inflammatory process and the improvement of glucose tolerance are based on the action of the Bifidobacterium species that can actively intervene in the gut microbiome of the newborn (26).

Changes in the gut microbiome in pregnancy are similar to those in associated inflammatory bowel disease dysbiosis in non-pregnant patients without being accompanied by fetal growth disorders. However, these changes are dependent on the trimester of pregnancy (19).

The interrelation of the microbiome - immune system in the intestine is under the influence of endocrine factors. Extracellular vesicles derived from bacteria have proinflammatory effects affecting the immune system (27). Koren et al., by analyzing the microbiome in pregnancy, observed significant modeling by decreasing species diversity as the pregnancy progressed independently of BMI. Analysis of faecal samples in the third trimester of pregnancy showed an increase in the concentration of Proteobacteria and Actinobacteria (28). Inflammatory bowel phenomena identified in faeces were analyzed by increasing Proteobacteria species (29) and a depleting *Faecalibacterium prausnitzii* inflammatory process (30).

The complex changes that occur at different levels during pregnancy (oral, intestinal, placental, vaginal) can play a key role in forming the neonatal microbiome. These changes are certainly influenced by the delivery mode, vaginal versus cesarean section, both in terms of component and diversity. The microbiome of infants born vaginally had similar characteristics to the maternal vaginal microbiome. Infants born vaginally will have an increased number of species of *Bifidobacterium spp.* and *Lactobacillus spp.* and a decrease in *Clostridium difficile* (31). In fact, the continued increase in the cesarean section rate will have a negative role regarding the microbiome of infants and later in finalizing their immune system.

Lack of exposure to the vaginal microbiome at birth influences the immature immune system, increasing the risk of later-life diseases such as asthma, type I and II diabetes, atopic diseases, and inflammatory bowel disease. Although in the first week of life there are differences in the microbiome of the newborn born vaginally from the one extracted by cesarean section, at 6 weeks, these differences disappear (15).

The oral microbiome includes 50-100 billion bacteria from about 700 different species of Streptococci, Spirochaetes, Bacteroidetes, Lactobacilli, Staphylococci, Actinobacteria, Chlamydiae (32). An imbalance of the oral microbiome (dysbiosis) can lead to dental diseases such as oral cavities, gingivitis, and periodontitis (33). During pregnancy, the oral microbiota variations are caused by hormonal, metabolic, and immunological changes. There is a pathogenic bacteria predominance in the oral microbiome, which declines in the post-partum period, followed by the appearance of the normal healthy microbiota (34). A comparative study between oral microorganisms in pregnant women with non-pregnant women detected a higher microbial count in the first and second trimester of pregnancy, mainly on *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, Streptococci, Staphylococci, and Candida species (35). Analyzing the bacteria of subgingival plaque and saliva during pregnancy, the predominant members were species of phyla Firmicutes, Bacteroidetes and Actinobacteria. In the subgingival plaque, the most frequently encountered were species of Prevotella, Fusobacterium, Streptococcus, Veillonella, and Terra-haemophilus (34). In addition, species like *Prevotella intermedia, Prevotella melaninogenica,* and *Porphyromonas gingivalis* are in higher quantities pregnancy gingivitis is present (35).

THE IMPLICATION OF PLACENTAL MICROBIOME

The initial concept that the placenta is a sterile environment, and its colonization comes from the rise of infections in the lower genital tract is controverselly. Several bacteries responsible for oral cavity infections were also detected in the amniotic fluid of pregnant women with PTB.

Streptococcus agalactiae was validated as the only bacteria found in the placenta before the onset of labor (36). A possible mechanism for fetal colonization immediately before birth would be the hematogenous transmission of transient bacteremia, explained by a small number of bacteria from the oral microbiome. de Goffau et al. showed that the placenta does not have a real resident microbiome (36). The same result was supported by the study of Leiby et al., which does not mention the presence of a consistent placental microbiome in term births, or spontaneous PTB (37). Theis et al. using a microbiological investigation of placental tissue, did not reveal a microbiota resident in full-term women without labor (38). A cross-sectional study Sterpu et al. conducted on a sample of 76 cases did not identify the existence of a placental microbiome by using techniques to amplify and sequence the polymerase chain reaction. The au-

thors accidentally identified bacterial species that cannot be interpreted as placental microbiome (39).

De Siena et al. enunciated the concept of colonization in utero to interpret the roles of the microbiota during pregnancy, and the modulation of the microbiota could lead to a reduction in perinatal morbidity and mortality (14). Perez-Muñoz et al. dispute the evidence of "colonization in utero", mentioning that molecular techniques used reduced biomass with limited detection, lack of control batches on contamination, and lack of evidence of bacterial viability (40).

Zhu et al. studied the microbiome composition in placenta and amniotic fluid samples by DNA extraction and showed that Enterobacteriacea has dominated phylotype (88.6% in decidual tissue and 40.3% in amniotic fluid), which means a microbiome with low diversity in the pregnant uterus. Furthermore, the Firmicutes genus was more abundant in decidual tissue. Amniotic fluid cultures were negative, while those on the fetal face of the placenta were positive in 20% of samples examined. 30 classes of bacteria and 19 fungi were identified. Among the fungi all classes belonged to the genus Candida (*Candida albicans, Candida tropicalis* and *Candida glabrata*). The most significant bacteria were *Escherichia coli* (57.71%) and *Enterococcus faecalis* (21.03%). These findings reveal that bacterial colonization occurs during a healthy pregnancy, the uterus not being a sterile cavity as has been considered (41).

Fischer et al., by analyzing observational studies have shown that pregnant women with severe periodontitis have a low birth weight and high risk of premature birth. Therapeutic procedures performed during the second trimester did not reduce these risks, a possible explanation would be that bacteria in the oral cavity colonize the placental tissue before periodontal treatment. The study of the oral microbiome in the placenta, by DNA sequencing, cannot detect important changes in the metabolic capacity of the microbiome. The pathogenic potential of the oral microbiome regarding placental colonization would be given by the number of germs and not only by their presence. These methods of detecting the presence of bacteria should be improved, and attempts should be made to eliminate the possibility of contamination of samples (42).

Seferovic et al. demonstrated by metagenomics that placental bacteria are poorly represented populations, with low biomass regardless of the age of pregnancy and mode of delivery, being distinct from bacteria resulting from contamination. They used 16S rRNA signal sequencing techniques mainly found in the syncytiotrophoblast and/ or the villous parenchyma (43).

In a large cohort of pregnancies out of a total of 400 placental samples from 256 single pregnancies, Leon et al., by sequencing 16S amplicon, analyzed the presence of contaminated operational taxonomic units without highlighting the existence of a reproducible "premature placental microbiome". Although some spontaneous premature births have an infectious intrauterine component; many sequenced reads were contaminated and not belong to DNA reads from endogenous species (44).

These controversies in the scientific world regarding the existence or not of a resident placental microbiome are not fully resolved. However, after an initial current pro the existence of the placental microbiome, current studies tend to dismantle this theory without minimizing the importance of prenatal microbiome involvement (45). The placenta colonization has multiple sources, and it shows some similarities to the oral non-pregnant microbiome within the same person. The placenta microbiome contains bacterial species from the following phyla: Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria which are Gram-negative, anaerobic, and these are encountered in the oral cavity (33).

There is a remarkable resemblance between the placenta microbiome and the oral one at high taxonomic levels (phylum, class, order), but this resemblance decreases at lower taxonomic levels mainly because the environment is different. There are high similarities between oral, gut, and placenta microbiota on Veillonella, Prevotella, and Streptococcus species. These three species have also been detected in neonates, and they persist for the first year of life (46). Thus, the oral microbiome is a possible source for the bacterial ADN of the placenta. As pregnant women have an increased risk for gingivitis and gingival bleeding, and the gut barrier is not at its highest level, both factors favor oral bacteria to enter into the blood easier and colonize the placenta (46).

Moreover, there are some limitations of the methodology techniques for detecting bacteria in the placenta. In oral, intestinal, and vaginal mucosa, the bacteria quantities are higher, and in the placenta, there is low biomass of bacteria. This imposes the use of low sensibility techniques and take into account

that the possibility of reagents contamination is high (47).

The potential biological relevance of the similarities of the placenta and oral microbiome still raises questions. The bacteria of the placental microbiome play different functional roles when compared to other microbiomes. The roles of oral bacteria are in connection with carbohydrate, and amino acid metabolism, and the placental bacteria are enriched with genes regulating tryptophan, fatty acid metabolism, and benzoate degradation. Tryptophan metabolism is involved in the neurodevelopment in the fetus and the maintenance of feto-maternal tolerance, placental circulation, and antimicrobial activity. The fatty acids supply the energy for the fetus, and benzoate is considered a carbon source for many microorganisms (46).

Amos et al. proposed standardization of microbiome analysis methodologies as extremely important for the knowledge and correct description of microbial populations in the microbiome. To differentiate the microbial population of the host organism from the degree of exogenous bacterial contamination, they proposed a series of reference reagents produced by the National Institute for Biological Standards and Control (NIBSC) for microbiome analysis by DNA sequencing, as international reference reagents of the World Health Organization (48).

PRETERM BIRTH AND MATERNAL MICROBIOME

Premature birth continues to be an important element of public health due to the high rate of perinatal morbidity and mortality (5). However, the etiopathogenic mechanisms underlying PTB have not been elucidated, with approximately 50% being idiopathic and 30% associated with premature rupture of membranes (PPROM) (49).

Chorioamnionitis is frequently associated with premature birth and is most likely caused by an increase in the germs of the vaginal flora with a placental pro-inflammatory effect. The inflammatory reaction caused by prostaglandins and cytokines in chorioamnionitis is the trigger for uterine contractions and local changes in cervical collagen. Steel et al. revealed that the presence of bacteria is common in most preterm tissues and not sufficient to cause preterm labor or PPROM, unrelated to delivery mode (50).

In the cervix, the production of cytokines IL-6 and IL-8 can be reduced by the use of corticosteroids, without influence on soluble E-cadherin level (51). Currently, the role of the microbiome is related to the inflammatory aspects that can occur in addition to PTB, intrauterine growth restriction, and fetal deaths in utero (52).

Romero et al., in a case-control study showed by techniques based on 16S RNA gene sequencing on the composition of vaginal microbiome taxa that there were no differences between pregnant women who delivery at term and prematurely (20). Kacerovsky et al. presented a possible explanation in PPROM of the association of high levels of L. crispatus in the cervical microbiota with a low risk of intraamniotic complications and a low rate of early neonatal sepsis (53).

Arboleya et al. revealed that the microbiome of the premature newborn is less diversified compared to that of the full-term newborn. These differences may be due to lack of exclusive breastfeeding, admission to the NICU and sterile isolators, and antibiotic treatment (54).

Baker et al., in case of preterm infants, identified low levels of strict anaerobes (Bifidobacterium, Bacteroides, and Atopobium) and elevated levels of facultative anaerobic microorganisms. In addition, larger amounts of pathogenic bacteria such as Klebsiella, Enterobacteriaceae, Clostridium, Streptococcaceae have been identified in the microbiota of the premature newborn (55). Other studies showed a significant similarity between the placental microbiota and that of the newborn meconium (26,56).

Another possible influence in PTB can be given by the microbiome determined by the oral, gastrointestinal, vaginal triangle (20).

The dissemination of oral pathogenic bacteria in periodontitis is a factor causing adverse outcomes of pregnancy (fetal loss, preeclampsia, preterm birth, low birth weight, fetal growth restriction). The dissemination is done by a hematogenous spread of the bacteria or the inflammation mediators or colonization from the vaginal microbiome (33). The amniotic fluid of women with preterm birth includes disseminated oral bacteria, and the presence of periodontal disease carries a 7-fold increased risk of preterm birth (57).

Several bacteria have been identified in PTB-associated infections, such as: *Ureaplasma urealyticum, Mycoplasma hominis, Bacteroides spp.* and *Fu-*

sobacterium nucleatum, the latter being a cofactor in the colonization of other potentially pathogenic organisms (*Escherichia coli*) (58).

CONCLUSIONS

The microbe of the newborn is acquired during birth and postpartum through a possible uterine colonization in which the involvement of the placental microbiome has not yet been proven to have a predominant role. The connection between the maternal microbiome at all levels (oral, intestinal, vaginal, and possibly placental) and the immune system is different depending on the mode of birth, the time of birth; many studies demonstrating the role of the maternal microbiome in the mechanisms of premature birth.

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