Maternal microbiome and pregnancy outcomes

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Alterations of the human microbiome are a known characteristic of various inflammatory disease states and have been linked to spontaneous preterm birth and other adverse pregnancy outcomes. Recent advances in metagenomic research have proven that the placenta harbors its own rich diverse microbiome, even in clinically healthy pregnancies, and preterm birth may be a result of hematogenous infection rather than exclusively ascending infection as previously hypothesized. In this review, we describe the microbiome in healthy nongravid and gravid women to contrast it with the alterations of the microbiome associated with spontaneous preterm birth. We also discuss the importance of host gene-environment interactions and the potential for microbiota-specific targeted therapies to reduce the risk of adverse pregnancy outcomes. (Fertil Steril® 2015;104:1358–63. ©2015 by American Society for Reproductive Medicine.)

Key Words: Microbiome, preterm birth, inflammation, metagenomics, infection

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Following the widespread acceptance of the germ theory of disease in Europe and North America in the middle to late 1800s, the presumption that microscopic organisms are inherently pathologic has remained strongly imprinted on the human imagination. More than 150 years later, the Human Microbiome Project (HMP) was established by the National Institutes of Health in 2008 in response to two truths: 1) that the healthy human body is inhabited by a large diverse microbiota with more genetic material than the host itself; and 2) that new DNA-sequencing technologies make possible the isolation and identification of complex microbial communities previously impenetrable by traditional culture techniques (1). How this rich human microbiome is associated with both healthy pregnancy and parturition, as well as adverse pregnancy outcomes, remains incompletely understood. In this article, we review the existing literature on microbial diversity in healthy gravid women, the association between dysbiosis and spontaneous preterm birth (PTB), and potential avenues for targeted therapy and further characterization of the healthy gravid microbiome.

NONGRAVID VAGINAL MICROBIOME

Studies of the vaginal microbiome have been previously hampered because much of the vaginal flora can not be cultured in the laboratory (2). Improvements in DNA sequencing technology have vastly improved our ability to detect noncultivatable organisms with the use of culture-independent techniques such as 16S rRNA gene analysis and whole-genome shotgun (WGS) sequencing. Results from the HMP indicated the vaginal microbiome to have relatively simple biodiversity compared with the gastrointestinal and oral microbiomes (3). In addition, the vaginal flora has been shown to have both low alpha diversity (within samples) and low beta diversity (between subjects) (4). Despite having low alpha and beta diversity, the vaginal microbiome has shown a high diversity of operational taxonomic units (OTUs), species-level classifications, secondary to numerous distinct *Lactobacillus* spp. (4). The genus *Lactobacillus* encompasses >130 species, with 20 of them found in the human vagina (5–7). Healthy vaginal communities are typically dominated by only one or two species, usually *L. crispatus, L. gasseri, L. iners,* or *L. jensenii* (5). Lactobacilli are thought to protect the vaginal ecosystem from colonization by other species via several mechanisms, including the production of lactic acid (8) and competition with other pathogenic organisms for nutrients and epithelial cell receptors (9). A smaller percentage of vaginal microbiomes lack substantial numbers of *Lactobacilli* and are composed mostly of anaerobic bacteria, including *Prevotella, Megasphaera,*

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Gardnerella vaginalis, Sneathia, and *Atopbium vaginae* (5, 6). These environments are more commonly found in certain ethnic groups, including African Americans and Hispanics (5, 10). The mostly anaerobic microbiomes are also associated with higher Nugent scores, a Gram stain method used to diagnose bacterial vaginosis in research settings (11–13). Previous studies have characterized these microbiome communities into community state types (CSTs). CSTs I, II, III, and V are dominated by various species of *Lactobacillus,* and CST IV-A and IV-B have predominantly anaerobic bacteria (5, 14).

GRAVID VAGINAL MICROBIOME

Comparisons of the nongravid and gravid vaginal microbiome have found differences in both stability and diversity (6, 11,15–17). The composition of the nongravid vaginal microbiome has been shown to fluctuate over time in response to age, hormonal changes, infections, and sexual behaviors (6, 14). In contrast, longitudinal studies have shown the gravid vaginal microbiome to be generally more stable and less diverse throughout pregnancy (11, 15). The composition of the gravid vaginal microbiome has been shown to change during gestation, with an increase in the dominance of four *Lactobacillus* spp (*L. crispatus, L. jensenii, L. gasseri,* and *L. vaginalis*) and a decrease in the amount of anaerobic species (18). In a study by Aagaard et al., 68 samples from the introitus, midvagina, and posterior fornix were obtained from 24 gravid women to catalogue a "healthy" microbiome in pregnancy (17, 19). That study revealed that the vaginal microbiome differs with increasing gestational age and proximity to the cervix (17, 19). Interestingly, the vaginal microbiome in late gestation was found to have more similarities to the nonpregnant microbiome environment than in earlier gestation (17, 19). In a longitudinal study of vaginal microbiota in 22 parturients with subsequent uncomplicated term deliveries, during which a median of 6.5 samples per subject were collected over the course of pregnancy, Romero et al. observed occasional shifting from microbial communities dominated by one *Lactobacillus* spp. to another, but rarely did communities shift from one dominated by *Lactobacillus* spp. to one dominated by anaerobes (CST IV-A or CST IV-B) (11). In addition, CST IV-B was rarely observed in pregnant women who delivered at term (11). Numerous factors have been postulated to explain these changes, including the lack of hormone fluctuations and menstrual flow, changes in cervical and vaginal secretions, and changes in sexual activity (15). It is hypothesized that the rising estrogen levels in pregnancy result in increased vaginal glycogen deposition favoring proliferation of *Lactobacilli* (11). Additionally, it is possible that the heightened stability of the vaginal microbiome in pregnancy provides an adaptive benefit to reproductive fitness (11). The dominance of *Lactobacillus* in the gravid vaginal microbiome may play a role in establishing the neonatal upper gastrointestinal microbiota, as well as providing protection from ascending infections known to cause preterm birth (17).

PLACENTAL MICROBIOME

Traditionally, human development has been thought to occur within a "sterile" environment. Recent research has shown rich diverse placental microbiomes in clinically healthy pregnancies (20, 21). Those studies catalogued the placental microbiome and found that the taxonomic profile of the placenta actually has more similarities with the nonpregnant oral microbiome than with the urogenital tract (21). Aagaard et al. classified the microbiomes from 320 placental specimens and compared them to other microbiome environments in the human body, including the gut, oral cavity, skin, nares, and urogenital tract (21). With the use of 16S ribosomal DNA-based and WGS metagenomic technology, the placental microbiome was found to contain mostly nonpathogenic Firmicutes, Tenericutes, Proteobacteria, Bacteroides, and *Fusobacteria*. In addition, when the taxonomic profiles of each body site were compared, the placenta was found to have greatest similarity with the nonpregnant oral microbiome. Interestingly, when potential contaminants of the placenta during delivery were compared, such as stool and the vagina, no taxonomic similarities were noted (21).

THE HUMAN MICROBIOME AND PRETERM BIRTH

Although maternal inflammatory conditions have been linked to multiple adverse neonatal outcomes, including growth restriction and stillbirth, the preponderance of the research involving the microbiome in pregnancy has centered around PTB. Recent characterization of the placental microbiome and its association with the oral microbiome challenges the theory of PTB resulting from ascending lower genital tract infection (20, 21). Similarities between the oral and placental microbiome may indicate that the placental microbiome is established by means of hematogenous spread (21).

The gastrointestinal microbiome also has been shown to change throughout pregnancy (22, 23). The total bacterial load in the gut increases with advancing gestational age (22). The composition of the gut microbiome has also been shown to change as pregnancy progresses regardless of body mass index or gestational diabetes status (22). One study comparing fecal samples of 91 gravid women showed dramatic changes in the composition of stool from the first to third trimesters, with an increase in a predominance of Proteobacteria and Actinobacteria identified with advancing gestational age, as well as a decrease in alpha diversity (22). Interestingly, Proteobacteria is frequently associated with inflammatory conditions of the gut, and the significance of this microbial alteration in late gestation remains poorly understood (19, 22).

Recent studies have examined the role of the vaginal, gastrointestinal, and oral microbiome and possible associations with PTB (18, 19,24–26). Common bacterial species identified in PTB-associated infections include *Ureaplasma urealyticum, Mycoplasma hominis, Bacteroides* spp., *Gardnerella vaginalis,* and *Fusobacterium nucleatum* (25, 27, 28). These organisms typically display low virulence unless they