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REVIEW ARTICLE

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The Vaginal Microbiota during Pregnancy

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Abstract

Fetal development is considered to occur inside the microbiota-free intrauterine environment. Depending on recently published data resources, it is anticipated that the neonatal microbiome is established primarily throughout the amniotic membrane rupture, with extra microbiota introduced by the passage of the fetus through the vaginal birth canal. Upon delivery, the neonate has been familiarized with the maternal microbiota of the vaginal ecosystem. Since the fetus is introduced to numerous bacterial environments during the early neonatal interval such as the vaginal canal, skin, and rectum, it is important to describe the potential influence of the maternal vaginal microbial community on neonates. Dynamic changes in the vaginal microbiome during the pregnancy including decreased vaginal diversity and *Lactobacillus*-dominance contribute to diminished risk of preterm birth as well as other adverse pregnancy outcomes. This minireview summarizes the composition of the vaginal microbiota during normal pregnancy as revealed by culture-independent high-throughput sequencing-based techniques such as 16S rRNA next-generation sequencing and metagenomic shotgun sequencing techniques.

Keywords: vaginal microbiome; pregnancy; *Lactobacillus*; sequencing techniques; 16S rRNA; metagenomic; shotgun sequencing.

1. Introduction

The most incredible event in microbial ecology has been the emergence of microbiome profiling and metagenomics. These are achieved by 16S rRNA next-generation sequencing and shotgun metagenomic sequencing involve the direct analysis of entire microbiota present within an environmental sample providing relatively full access to the composition and diversity of the functional genes in the surrounding uncultured microbial communities. These culture-independent methods helped in revealing the microbial diversity within complex ecosystems, their metabolic repertoire and better understand the microbial community dynamics **(Zhou et al., 2004; Cox et al., 2013; Mendz et al., 2016).**

The evolution of the high-throughput DNA sequencing techniques increased the capability to study the microbiota inhabiting different human body sites. The vagina is one of several human body sites where bacterial communities normally exist **(Greenbaum et al., 2019)**. The human vagina together with the residing bacterial microbiota represent a balanced ecosystem **(Romero et al., 2014a).** Microbiota plays vital roles in both health and disease, including protection against pathogens, nutrient achievement, and immunity. Normal pregnancy is primarily a transient, and dynamic state of reformed anatomy and physiology. Preterm birth before 37 weeks of gestation, represents 11% of pregnancies and is the most causative agent of neonatal death **(DiGiulio et al., 2015)**.

This minireview summarizes the composition of the vaginal microbiota during normal pregnancy as revealed by culture-independent high-throughput sequencing-based techniques such as 16S rRNA next-generation sequencing and metagenomic shotgun sequencing techniques.

2. Techniques applied for analysis of the vaginal microbial ecosystem

2.1 Culture-dependent techniques

The use of culture techniques allows the detection of the phenotypic characteristics of the isolated microorganisms, like nutritional production or requirements, and the consumption of the metabolic compounds. Therefore, laboratory-growing organisms are essential for both microbial identification and characterization. These culture-dependent techniques are only restricted to those organisms which have the ability to grow under *in vitro* conditions where only the minority of the microbial species can grow. Therefore, culture-independent techniques depending on DNA, evolved to overcome the limits of culture-dependent ones **(Morgan and Huttenhower, 2012).**

2.2 Culture-independent techniques

It is estimated that about (20% - 60%) of the human microbiome, depending on the body site, is unculturable **(Zhou et al., 2004; Peterson et al., 2009).** Recently, molecular techniques and sequencing provided fast analyses with lower costs. They induced significant progress in the study of human-

associated microbial communities. These techniques do not depend on the cultured microorganisms but rather rely on the sequencing of the total DNA extracted directly from the samples of the microbial communities. These sequencing techniques are known as high-throughput sequencing. They have a significant and valuable impact on disease diagnosis, especially of human genetic diseases and cancers **(Ma and Ellis, 2013; Pinto et al., 2014; Renkema et al., 2014; Stadler et al., 2014).**

There are two approaches commonly used for the characterization of human-associated microbial communities. The first approach depends on the amplification of the highly conserved genes like the 16S ribosomal RNA (16S rRNA) gene of the small subunit of the ribosome in Bacteria and Archaea. Then, analysis of these genes using phylogenetic identification by comparison with the sequence databases. The second approach includes the isolation, sequencing, and analysis of whole DNA present in a particular environment, defined as metagenome or metagenomic shotgun sequencing (MSS). While the first strategy reveals the microbial composition depending on taxonomy, the second metagenomic approach provides information about both the taxonomy of the microbial community and the metabolic functions encoded by microbial genomes. There are modern sequencing platforms like 454, Ion Torrent, and Illumina. They depend on using barcodes to differentiate between samples that are sequenced simultaneously, providing huge volumes of data, that may reach millions of reads, and greater coverage compared to the traditional Sanger sequencing technique **(Handelsman, 2004; Hamady and Knight, 2009; Wang et al., 2015).**

More recently published studies have described the human microbiome using the sequencing of the 16S rRNA gene as a metagenomic marker, like the studies of the gut microbiome showing the significant diversity in the flora, and the differences in the microbiome of obese against lean donors, then the studying the microbiome of the infants. Also, there are studies that have used the 16S rRNA gene for molecular characterization of the oral microbiome, the vaginal microbiome, and the skin microbiome **(Eckburg et al., 2005; Hyman et al., 2005; Ley et al., 2006; Gao et al., 2007; Mohamed et al., 2020; Amin et al. 2023).**

Studies of the vaginal microflora using the cultivation-independent broad-range (Polymerase Chain Reaction) “PCR” analyses of 16S rRNA gene from the microbial communities reveal richer microbiota with a much large number of taxa than those identified through culturing methods **(Ravel et al., 2011; Srinivasan et al., 2012).** In particular, the identity and the diversity of the vaginal bacterial communities during pregnancy largely remain unknown for the different racial backgrounds, health status, and lifestyle **(Gupta et al., 2020).** The most limitations to the use of high-throughput sequencing techniques are the lack of diagnostic centers for performing fast sample analyses, and the large datasets generated by these methods to be analyzed **(Mendz et al., 2016).**

3. The vagina

The vaginal opening is the opening between the urethral opening and the anus. The vagina is anatomically situated anterior to the rectum and posterior to both the urinary bladder and the urethra. It is enclosed by the Bartholin's glands or greater vestibular glands. The vagina is a fibromuscular canal from 6 to 12 cm in length. It is the exit from the uterus throughout the menses and childbirth. The outer walls of the vagina are formed of ridges. The superior part of the vagina is called the fornix, which meets the protruding cervix **(Standring, 2008; Ramírez-González et al., 2016)**.

The vagina is lined with an outer fibrous adventitia and the inner mucous membrane with transverse folds called vaginal rugae. Both the middle and the inner layers together permit vaginal expansion through intercourse and childbirth. The Bartholin's glands and the vestibular glands which are located near the clitoris secrete the mucus, which plays a vital role in keeping the moisture. The vagina is the shelter of the normal population of microbiota or the normal flora which protects against infectious diseases by pathogenic bacteria, yeast, or other organisms. In healthy women, the most predominant genus of vaginal bacteria is *Lactobacillus*. This beneficial flora secretes lactic acid, which keeps the vagina healthy by maintaining an acidic vaginal pH of < 4.5. Pathogens cannot persist in this acidic environment. Therefore, Lactic acid together with the other secretions of the vagina, make the vagina a self-cleansing organ **(Graziottin and Murina, 2011).** Meanwhile, douching or washing fluids might disrupt the normal balance of the healthy normal vaginal flora increasing the risk of diseases and irritation. Consequently, the American College of Obstetricians and Gynecologists recommends not douching, to allow the vagina to maintain its normal healthy microbial composition **(Betts et al., 2013; Rosner et al., 2020; Hoare and Khan, 2021).**

4. Human vaginal microbiota

The complicated interactions between the host cells, bacterial microbiota, and the immune components of the female reproductive tract are vital for maintaining the female reproductive tract homeostasis **(Gholiof et al., 2022).** Microbiota is defined as the microbial community found in an environment. This term was first well-defined by **Lederberg and McCray (2001).** The microbiota could be established by the evolution of the molecular methods for the analysis of 16S or 18S rRNA or any other marker genes, where taxonomic assignments is done through using multiple tools which define each sequence to the microbial taxon including bacteria, archaea, fungi, and the lower eukaryotes from phylum level to the species level **(Marchesi and Ravel, 2015; Berg et al., 2020).**

4.1 Bacterial vaginal communities

The human bacterial vaginal microbial communities are considered an important defense barrier against infectious diseases. Studies of females from various ethnicity or race backgrounds, Caucasians, Hispanics, Americans, Africans, and Asians, showed that most communities in each group are dominated by a single species of *Lactobacillus*, belonging to either *L. iners*, *L. crispatus*, *L. gasseri*, or *L. jensenii*. These results explain the possible alterations in the

metabolic pathways which may change the levels of the production of lactic acid, and subsequently, the levels of protection would vary accordingly in each ethnic group **(Zhou et al., 2007; Ravel et al., 2011; Ma et al., 2012; Stout et al., 2020).** The bacterial vaginal microbiota plays also a vital role in the colonization and the development of the neonatal microbiome throughout the maternal-offspring exchange during the delivery. Cesarian sections and antibiotics can disrupt this microbiota exchange resulting in an increased risk of different diseases like diabetes and obesity **(Mueller et al., 2015).**

Five vaginal bacterial community state types (CSTs) have been defined **(Ravel et al., 2011).** Four of these CSTs are dominated by *Lactobacillus*, *L. crispatus* (CST I), *L. gasseri* (CST II), *L. iners* (CST III), and *L. jensenii* (CST V). CST IV is characterized by low levels of *Lactobacillus* and increased diversity of anaerobic bacteria involving *Prevotella*, *Atopobium vaginae*, *Dialister*, *Gardnerella vaginalis*, *Peptoniphilus*, *Megasphaera*, *Sneathia*, *Mobiluncus*, and *Finegoldia*. These species are mainly associated with bacterial vaginosis, a clinical syndrome of vaginal discharge. This is usually associated with a characteristic vaginal odor characterized by polymicrobial overgrowth. CST IV has been correlated with the increased risk of preterm birth (PTB) **(Hillier et al., 1995; Flynn et al., 1999)**, and the histological chorioamnionitis **(Martius and Eschenbach, 1990; Gibbs, 1993; Takei and Ruiz, 2006).**

Interestingly, in the American populations, the vaginal bacterial communities are dominated *Lactobacillus* (CST I, II, III, and V) are most observed in Asian and White women, while a diverse microbiome (CST IV) is more frequently observed in the Black and Hispanic populations suggesting that the composition and the structure of the vaginal microbiome may be shaped by the genetic differences between hosts and by the cultural and behavioral factors **(Zhou et al., 2007; Ravel et al., 2011).** These findings have been recently confirmed and extended by Fettweis and colleagues who identified clear ethnic-related differences in the vaginal microbiome of a large population of healthy Black and White Northern American women **(Fettweis et al., 2014).**

4.1.1 Vaginal microbiome during pregnancy

4.1.1.1 Vaginal microbiome during the uncomplicated pregnancy

The phylum *Firmicutes* was reported to be the most prevailing member of the vaginal microbiota throughout pregnancy **(Li et al., 2020; Mohamed et al., 2020; Sroka-Oleksiak et al., 2020).** Meanwhile, at the genus level, *Lactobacillus*, previously called Döderlein Bacillus, is defined as the main commensal of the human vagina **(Thomas, 1928).** Pregnancy is associated with increased levels of circulating placental estrogen. These high levels of estradiol enhance glycogen deposition in the vaginal epithelium. Then, α-amylase of host vaginal mucosa breaks glycogen into products involving maltose, maltotriose, as well as maltotetraose which support *Lactobacillus* proliferation **(MacIntyre et al., 2015; Juliana et al., 2021).** This dominance of *Lactobacillus* is accompanied by the health status of the vagina, and it is thought to be

protective against the invasion of non-indigenous pathogens **(Mirmonsef et al., 2011; Miller et al., 2016; Gupta et al., 2020)**. This is mainly achieved by maintaining a vaginal pH less than 4.5 through lactic acid production. Moreover, these lactobacilli act as a protective barrier to microbes by competing for adhesion sites of the vaginal epithelial cells and the production of antimicrobial substances like, hydrogen peroxide, and bacteriocin-like compounds. The aptitude of lactobacilli to inhibit infectious microbes without prompting inflammation may enhance fecundity as well as successful pregnancy outcomes **(Zhou et al., 2004; O'Hanlon et al., 2013; Borges et al., 2014**; **Miller et al., 2016; Witkin and Linhares, 2017**; **Pino et al., 2019; Grewal et al., 2021).**

Under the normal physiological conditions of pregnancy, the most frequently reported *Lactobacillus* species of a healthy vagina include *L. crispatus*, *L. iners*, *L. gasseri*, and *L. jensenii*. The vaginal microbiome is relatively more stable during pregnancy and is characterized by a significant decrease in richness and diversity compared to non-pregnant state. **(Ravel et al., 2011; Aagaard et al., 2012; Romero et al., 2014a; Amir et al., 2020).** It is noteworthy that *L. crispatus* is most prevalent among European pregnant populations while *L. iners* is most prevalent among African pregnant populations **(Ravel et al., 2011; Juliana et al., 2021; Zheng et al., 2021; Shabayek et al., 2022, Amin et al., 2023).** According to **Sroka-Oleksiak et al. (2020),** *L. iners* was the most predominant *Lactobacillus* among healthy Caucasian pregnant women as well.

4.1.1.2 Vaginal microbiome of pregnant women with vaginal infections

Preterm birth (PTB) at < 37 weeks of gestation is a major cause of neonatal mortality. The risk of PTB is inversely related to gestational age **(Witkin, 2015).** Increased vaginal microbiome diversity with a low abundance of *lactobacilli* contribute as a risk factor of PTB. Interestingly, *L. crispatus* dominant vaginal communities were reported as protective against PTB **(Shi et al., 2020; Gudnadottir et al., 2022).**

In a study of 374 pregnant women, a culture-independent technique using the 16S rRNA genes of 12 bacterial taxa was carried out on fluid collected from the upper part of the vagina. This study revealed that the vaginal bacterial community in the second trimester of pregnancy was correlated with the birth outcome but this correlation depended on the race or the ethnicity of the mother. *Mycoplasma* wwas positively correlated with the PTB in both black and Hispanic groups of participants, while this association was not observed in white participants. Although a specific Group B *Streptococcus* lineage was associated with bacterial vaginosis, it was showing a negative correlation with PTB **(Wen et al., 2014).** Another study of 88 pregnant women from different racial groups using the 16S rRNA gene amplification, displayed that the vaginal microbiome diversity in human pregnancy was correlated with PTB. Race, ethnicity, and the sampling site were also important factors **(Hyman et al., 2014)**. Preterm pre-labour rupture of the fetal membranes (PPROM) represents 30% of PTB, it is strongly correlated with vaginal infections as well as prophylactic antibiotics used. Likewise, the vaginal microbiota

composition associated with *Lactobacillus* depletion is an important factor for PPROM **(Brown et al., 2018). Romero and coworkers (Romero et al., 2014b)** conducted a case-control study, using pyrosequencing of the 16S rRNA gene to investigate the differences in the vaginal microbiome of pregnant women giving birth at term or PTB. The study included 18 women with pregnancy complicated by the spontaneous PTB and 72 controls with an uncomplicated healthy pregnancy. No differences were found in the relative abundance of the vaginal microbial phylotypes. Likewise, there were no differences in the frequency of the CSTs between the groups.

4.2 Viral vaginal communities

Most of the studies of the vaginal microbiome focus on bacterial communities. One reason for this is that the sequencing techniques used for research of the bacterial microbiome do not work well for viruses and fungi. Another reason is that viruses are a group of highly variable microbes which, unlike bacteria, do not have the conserved gene which can be used for the amplicon-based characterization of the viral community. Besides, viruses have very diverse genomic structures of DNA or RNA and could be as single-stranded or double-stranded. Most importantly, the abundance of the viral genome within samples is very low relative to bacterial and host genomes **(Knipe and Howley, 2013; Stout et al., 2020).**

According to the human microbiome project (HMP), viruses could be detected in the vaginal samples of asymptomatic, healthy, reproductive-aged women **(Wylie et al., 2014).** The viral vaginal communities were found to include different types of herpesviruses and alpha papillomaviruses. These were found in about 37% of subjects. Furthermore, the presence of the viral community in the vaginal sample was correlated with the high-diversity of bacterial communities and the presence of anaerobic bacterial taxa. This bacterial-viral relationship has been detected in several studies and appears to be susceptible to viral infections compared to women who have vaginal bacterial communities rich in *Lactobacillus*. Although the women sampled were asymptomatic, the presence of these viruses might affect future health **(Mao et al., 2003; Gillet et al., 2011).**

4.3 Fungal vaginal communities

The fungal component of the vaginal mycobiome is not well characterized compared to the bacterial communities. The vaginal mycobiome, and the resources available to characterize it, are more limited. The fungal analysis is difficult because the samples are exceedingly susceptible to environmental contamination **(Drell et al., 2013; Bradford and Ravel, 2017).**

However, *Candida* is a common fungus in the vagina. Some studies showed that the *C. albicans* represented about 20–70% of samples tested by both the culture-dependent techniques and the amplicon-based sequencing. These fungi are frequently carried asymptomatically but also can cause symptomatic infectious diseases **(Beigi et al., 2004; Drell et al., 2013; Bradford and Ravel, 2017). Previous literature demonstrated a significant association of** *C. albicans* with the high-diversity and

Lactobacillus-poor anaerobic bacterial communities **(Liu et al., 2013; Pramanick et al., 2019)** as well as the vaginal colonization of group B *Streptococcus*, *Trichomonas vaginalis*, and *Escherichia coli* **(Cotch et al., 1998; Beigi et al., 2004; Cools et al., 2016).**

5. Conclusion

Vaginal microbiota plays vital roles in both health and disease, including protection against pathogens and nutrient achievement. Pregnancy is associated with augmented levels of circulating placental estrogen which support *Lactobacillus* proliferation. These lactobacilli act as a protective barrier to microbes by competing for adhesion sites of the vaginal epithelial cells and the production of antimicrobial substances. Recently, molecular techniques and sequencing ones provide professional analyses of more microbiota without culturing. They induce significant progress in the study of pregnancy-associated microbial communities.

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