Endometrial microbiota—new player in town

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Detection of bacteria with molecular techniques has enabled the study of low biomass microbiomes in tissues and organs previously considered sterile, such as the endometrium. Subsequently, an abnormal endometrial microbiota has been associated with implantation failure, pregnancy loss, and other gynecological and obstetrical conditions. Further investigation of the reproductive tract microbiome will allow for a better understanding of bacterial communities' role in both physiology and pathophysiology, which in turn impacts the ability to achieve pregnancy and maintain a healthy pregnancy. Here we review the current literature that surrounds the endometrial microbiome and highlight the importance of assessing it as a future tool for improving reproductive outcomes in infertile patients. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)

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THE REPRODUCTIVE TRACT MICROBIOME

The Human Microbiome

The definition of the term human microbiome—the totality of microorganisms and their collective genetic material present in or on the human body—was attributed to the American molecular biologist Joshua Lederberg in 2001 as discussed by Mor et al. (1). Although this symbiotic relationship is long-standing, our understanding of the physiologic and pathophysiologic role of the microbiome remains limited. The use of culture-based technologies has limitations, as many microorganisms are not readily detected by traditional cultivative techniques. As many as 50% of pathogens classified as "dominant" and 85% of "major" pathogens in wound infections are not identified by standard culture techniques (2). However, new technologies have changed the way that we think of the microbiome.

Historically, microbiome research focused on pathology rather than physiology; however, this is changing with data from comprehensive studies such as the Human Microbiome Project (HMP) led by the National Institutes of Health. This project was launched in 2007 and used high-throughput sequencing technologies to characterize the human microbiome in normal, healthy volunteers at numerous different body sites (3). The HMP and other large projects focus on characterizing the physiologic interactions between the microbiome and its host.

New investigative techniques including DNA fingerprinting, microarrays, and targeted or whole genome sequencing have empowered the study of metagenomics by analyzing the bacterial communities contained in samples based on their genetic information. Data from the HMP and other studies using these techniques have revealed that sites in the body historically thoughts to be sterile, such as the uterine cavity and the placenta, are in fact colonized with their own unique microbiome (4, 5). These molecular techniques take advantage of the 16S rRNA gene that is unique to bacteria and contains a number of hypervariable regions that serve as unique identifiers for a genus or species of bacterium. As the technology has evolved, so has our understanding of the role of the microbiome in human health.

Technical Assessment of the Microbiome

When interpreting data in the literature, it is important to understand how metagenomic samples were obtained and analyzed. In general, microbiome data are procured in one of two ways: culture-based or sequencing-based technology. Much of the early work describing the human microbiome came from culture-based approaches using the 16S rRNA analysis of highly conserved genes as a way to identify organisms in mixtures (6, 7).

Data from cultivation-independent techniques demonstrate that culture-based techniques vastly underestimate microbiome diversity (8, 9). Thus,

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culture-based data, while still foundational and often informative, must be interpreted cautiously.

With this in mind, projects such as the HMP used high-throughput sequencing of the 16S rRNA gene. Specifically, the sequencing focuses on hypervariable regions within the 16S rRNA gene, which serves as a molecular fingerprint specific to individual genus and species (10, 11). This approach to characterization of the reproductive tract is becoming widespread (12). Using this method, also termed "community genomics," allows for analysis that extends beyond phylogenetic descriptions and attempts to study the physiology and ecology of the microbiome.

Biological samples for metagenomic analysis with high-throughput sequencing can be simply collected, followed by DNA extraction and microbial DNA purification. Subsequently, one of several molecular genetics techniques is applied; most commonly used is DNA fingerprinting, DNA microarrays, targeted sequencing, and whole genome sequencing, each with strengths and weaknesses. Fingerprinting, which uses the 16S rRNA gene to cluster bacterial communities, is relatively inexpensive but lacks specificity. Targeted sequencing and microarray data allow for greater specificity down to the genus and species level by focusing on the hypervariable regions of the 16S rRNA. However, this technique relies on bioinformatics processing, which maps reads to a known or reference genome of a previously identified sequence or species. Although costly, whole genome sequencing allows for full discovery of an organism genome and may yield information about functional differences of bacteria in a community.

Metagenomic sample sequencing produces read lengths of variable size depending on what sequencing platform is used. Read lengths and read depth are important when it comes to accurate characterization. The data generated by the sequencing must be processed and organized into clusters termed operational taxonomic units (OTUs) by mapping the 16S sequence to publicly available taxonomic databases. OTUs are then used to determine sample composition and diversity. Several open sourced software packages assist with the bioinformatics processing and analysis.

The resultant mapped reads will allow for a determination of presence or absence of microbial genetic material; importantly, this does not inform on the viability of the organisms present. Further, although read counts can be helpful in this regard, quantification of a particular organism in a sample can be challenging. This read count clustering, also known as "binning," can be performed when known sequences exist; it becomes much more challenging and less accurate when analyzing novel species (13).

Further limitations of microbiome sequencing are related to the clinical utility of the results. For example, while sequencing can give insight into the makeup of the microbiome, it does not give information about its biologic function, such as antibiotic susceptibility testing. And what of the physical structure of the microbiome? There are growing data suggesting these microorganisms form their own three-dimensional biofilms with inner and outer layers; this adds complexity that has been very little explored. The fact that these biofilms exist from the vagina to the fallopian tubes allows complex and dynamic interactions between the gametes and embryo as well as the maternal tissue interface (14, 15).

Characterization of the Microbiome in the Reproductive Tract

Historically, the uterus was assumed to be free of bacteria, and most of the data on the reproductive tract are based on vaginal samples. The normal vaginal microbiome in healthy women is generally dominated by *Lactobacilli* species (16), although variation due to age and hormonal milieu is evident (17). For example, the vaginal flora during infancy is a mixture of aerobic and anaerobic bacterial populations including *Prevotella*, *Enterobacteria*, *Streptococcus,* and *Staphylococcus* species (18), while the estrogenic environment brought on by puberty causes glycogen to rise and pH to decrease with subsequent domination by *Lactobacilli* species.

In an effort to categorize the vaginal flora, it has been further classified into five community state types (CSTs). More than 70% of women demonstrated vaginal microbiota dominated by *L. crispatus*, *L. gasseri*, *L. iners,* or *L. jensenii*, corresponding to CST-I, -II, -III, and -V. A smaller proportion of women exhibit CST-IV, characterized by lower percentage of *Lactobacilli* and dominance of anaerobic bacteria including *Aerococcus*, *Atopobium*, *Dialister*, *Gardnerella*, *Megasphaera*, *Prevotella*, and *Sneathia* (16).

Data on the normal upper genital tract microbiome are not as prevalent (19). However, the upper genital tract microbiome has been characterized by quantitative polymerase chain reaction (qPCR) detection of bacteria in 95% of endometrial samples in asymptomatic women undergoing hysterectomy (20). Of note, the upper genital tract microbiome is quantitatively and qualitatively different from that of the lower genital tract; however, the qPCR data targeted only a limited number of bacteria (20).

A study using next-generation sequencing of the 16S rRNA gene has compared the vaginal and endometrial microbiota of asymptomatic and fertile nonpregnant women (21). Consistent with the work by Mitchell and coworkers (20), bacterial communities were detected in the endometrial samples of 100% of the subjects analyzed, with *Lactobacillus* being most represented followed by *Gardnerella*, *Prevotella*, *Atopobium,* and *Sneathia*. In approximately one fifth of the women analyzed, the bacteria community identified in the endometrium varied greatly from that in the vagina, suggesting that the endometrial and vaginal microbiota are not identical (21).

ENDOMETRIAL MICROBIOTA IN HEALTH AND DISEASE

Physiological Endometrial Microbiome in Reproductive-Age Women

Several studies have now reported that the endometrium possesses a functional microbiome in physiological conditions (20, 21). Originally, the isolation of pathogens from endometrial samples had been linked to contamination of the samples with vaginal microorganisms and to different