Reproductive tract microbiome in assisted reproductive technologies

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The human microbiome has gained much attention recently for its role in health and disease. This interest has come as we have begun to scratch the surface of the complexity of what has been deemed to be our "second genome" through initiatives such as the Human Microbiome Project. Microbes have been hypothesized to be involved in the physiology and pathophysiology of assisted reproduction since before the first success in IVF. Although the data supporting or refuting this hypothesis remain somewhat sparse, thanks to sequencing data from the 16S rRNA subunit, we have begun to characterize the microbiome in the male and female reproductive tracts and understand how this may play a role in reproductive competence. In this review, we discuss what is known about the microbiome of the reproductive tract as it pertains to assisted reproductive technologies. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)

Key Words: Microbiome, IVF, infertility

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Assisted reproductive technologies (ARTs) are the cornerstone of contemporary infertility treatment. Treatment success is influenced by a number of technical factors, but the true reproductive potential is defined by the quality of the oocyte, spermatozoa, and the maternal environment which supports implantation and ongoing development of the conceptus. As such, three unique physiologic environments are involved—the milieu in the testes, the follicle, and the endometrium. As more is learned about the human microbiome, it is becoming evident that it meaningfully affects the physiologic function of virtually every organ where bacteria are present.

The human body is colonized with an order of magnitude more bacteria than human cells in the body (1). The majority of published medical literature focuses on the subset of the microbiome involved in pathogenesis, and only a few publications have focused on the physiologic role that the microbiome plays. The importance of this was recognized in 2001 at the time the human genome was published (2), when scientists called for a "second human genome project" that would investigate the normal microbiome colonies at various sites to understand the synergistic interactions between the microbiome and its host (3, 4). Several initiatives commenced worldwide, and in the United States the Human Microbiome Project led by the National Institutes of Health was launched in 2007, using high-throughput sequencing technologies to characterize the human microbiome in 250 normal healthy volunteers at multiple body sites (1).

The female reproductive tract has long been known to have an active microbiome. Although the greatest focus has been on the vaginal milieu, data have been accumulating for decades demonstrating that the remainder of the female reproductive axis is not sterile. In fact, with more than 20 studies completed, virtually all of them have found that there is a small but active microbiome in the uterine cavity. Importantly, many of these studies attained their samples at the time of surgery with the use of transfundal collection techniques where there was no potential for contamination from transiting the vagina or endocervical canal. The majority of these studies were done with the use of traditional culture techniques to identify any bacteria that were present. More recently, metagenomic techniques are confirming earlier findings and providing a more comprehensive definition of the endometrial microbiome.

Interestingly, the microbiome extends above the endometrial cavity. Some studies have demonstrated bacteria in the fallopian tubes of women without obvious tubal pathology. Additional studies have demonstrated that the intrafollicular milieu may have an

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active microbiome in some patients. Finally, there are now studies showing that the microbiome of the male reproductive axis is more complex than previously appreciated. The addition of metagenomic tools allowed descriptions of much broader and more complex microbiome, even in men without evidence of acute or chronic inflammation of their reproductive tract.

As the microbiome of the female and male reproductive axis has become more clearly defined, studies evaluating the clinical impact on ART treatment have followed. Given the influence which the microbiome has in virtually every organ system, it is not surprising that subtle changes in the microbiome are associated with meaningful changes in gamete quality and ultimate clinical outcomes. In some cases, changes in the microbiome may provide insight into previously unexplained treatment failure. The present manuscript describes our current understanding of the microbiome of the reproductive axes and the potential impact on ART practice and outcomes.

CULTURE- VERSUS SEQUENCING-BASED DATA

It is important to briefly recognize that microbiome data are procured in one of two ways: culture-based or sequencing-based technology. Much of the early work describing the human microbiome comes from culture-based approaches using the 16S rRNA analysis of highly conserved genes as a way to characterize the diversity of the microbiome in a given environment (5, 6). However, data from the vaginal microbiome suggest that many organisms can not be identified with the use of culture-based techniques, which results in underestimating the diversity of the ecosystem as well as failing to identify potentially important organisms when describing their relationship to health and disease (7, 8). Thus, culture-based data, though still informative, must be interpreted within the limits of the technology.

Data presented more recently have relied on 16S rRNA gene sequencing, specifically the hypervariable regions within the gene, which serves as a molecular fingerprint down to the genus and species level (9, 10). Although to date, data that describe the microbiome of the reproductive tract have not widely used this technique, metagenomics is becoming an increasingly widespread approach to describing the microbiome (11). Using this method, also termed community genomics, analysis of microorganisms occurs by means of direct extraction and cloning of DNA from a grouping of organisms. It allows analysis that extends beyond phylogenetic descriptions and attempts to study the physiology and ecology of the microbiome.

INTERACTIONS BETWEEN THE MICROBIOME AND THE REPRODUCTIVE AXIS

The study of the microbiome and its relationship to the efficiency of conception and early pregnancy maintenance is just beginning. Although there have been efforts to distinguish between normal or favorable microbiomes and those that impair or limit clinical outcomes, early investigations are also identifying alterations in several physiologic processes. These alterations may provide insight into reproductive failure in some patients. They may also provide the foundational information to guide the development of new therapeutic interventions that could improve outcomes in previously recalcitrant clinical circumstances.

The association between clinically evident infection, inflammation, and altered reproductive function is well established. Much of this inflammation involves secretion of a number of proinflammatory cytokines and growth factors secreted by immune cells which are activated in response to the presence of apparent pathogens. In the case of small shifts in the microbiome, the resulting subtle changes in the local milieu are typically not clinically evident but may remain clinically meaningful; however, the exact molecular mechanisms are not well characterized. Accumulations of a particular interleukin or some other cytokine are described, but detailed mechanisms are still lacking.

It is possible that the influence of some components of the microbiome is not via direct interaction with the local organ system. The microbiome of the vagina is typically dominated by *Lactobacilli* (12). In fact, a normal milieu is defined by the presence of specific subspecies of *Lactobacilli* that are capable of acting as probiotics and inhibiting the overgrowth of other bacterial species. For example, *Lactobacilli* species capable of producing high levels of H_2O_2 are generally considered to be most favorable. This demonstrates an important concept that some components of the microbiome's principal function may be to alter or limit some other component of the microbiome. A direct interaction with the actual tissue may occur but is not essential.

It is becoming increasingly evident that the aggregate microbiome is not a simple accumulation of free-floating bacteria on the surface of a human tissue. In many cases, complex three-dimensional lattices are formed which may have one layer or may have an inner and an outer layer. A protective outer coating composed of polysaccharide, nucleic acid, and protein may develop. At times, these biofilms may inhibit immune detection and reduce the effectiveness of antimicrobial treatment (13). These three-dimensional structures spread across the surfaces of the tissues where they are located and are termed biofilms. Biofilms are the subject of intensive investigation and may have important physiologic and pathophysiologic roles.

Biofilms are routinely present in the vagina but commonly extend into the endometrial cavity (14) and even up into the fallopian tubes (Fig. 1). Although no definitive conclusions regarding the role of biofilms of the reproductive axis have been established, it is important to understand that the relationship between the microbiome and the müllerian system may be more complex than the simple presence or absence of various species or bacteria or even their relative concentration. The interactions that lead to different biofilms and their subsequent impact on reproduction will provide important topics for future investigation.

The influence of the microbiome, most prevalent in the müllerian system, may extend to the remainder of the reproductive axis and may even affect gametogenesis. Indeed, ovarian follicles may have an active microbiome. Some investigators have found that some bacteria may adversely