Microbiome and Fertility

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Abstract
The human microbiome project has shown the importance that microbiota has in reproductive health. Vaginal microbiota has been described as mostly dominated by Lactobacillus spp. and lack or diminished Lactobacillus presence is associated with bacterial vaginosis, dysbiosis, sexual transmitted infections, severe adverse gynecological and obstetric outcomes as genital infection, inflammation, and preterm birth. Microbiome is also related with infertility and this knowledge opens a new understanding for future clinical treatments.

Keywords: Microbiome; Fertility; Vaginal microbiota

Introduction
As the human genome was published in 2001 [1], scientists called for a second human genome project for investigating the normal microbiome colonies in humans at different sites to understand all interactions between microbiome and host. For that propose was created “The Human Microbiome Project” that was established in 2008 by the National Institute of Health, with the mission of generating resources that would enable the comprehensive characterization of the human microbiome and analysis of its role in human health and disease, what is known as the “second human genome”. Different sites were analyzed: nasal passages, oral cavity, skin, gastrointestinal regions, amplified and sequenced from given biological samples. 16S rRNA gene sequencing is mostly used for bacterial species identification given that different bacteria families contain different 16S rRNA gene sequences [2]. The new created field of metagenomics improved largely the traditional technique of culture and thanks to that, now it is confirmed the places traditionally thought to be sterile, as uterine cavity or placenta, are in fact colonized with an own microbiome [3].

To understand the new basic lexicon, definition of the concepts mentioned in the present review is summarized below [4].

Microbiota
The assemblage of microorganisms present in a defined environment. This microbial census is established with the analysis of 16S rRNA genes, 18S rRNA genes, or other marker genes and genomic regions, amplified and sequenced from given biological samples.

Metagenome
The collection of genomes and genes from the members of a microbiota.

Metagenomics is the process used to characterize the metagenome, from which information on the potential function of the microbiota can be gained.

Microbiome
Refer to the entire habitat, including the microorganisms, their genomes and the surrounding environmental conditions.

We focused on the reproductive axis microbiome to establish interactions that distinguish between favorable and impair microbiomes that limit clinical outcomes. Huge interest is being paid to vaginal microbiome, but endometrial, follicular and tubal microbiome is starting to gain attention regarding reproductive outcomes.

Microbiome and Health
The microorganisms harbored by the vagina are the “microbiota” that are in a mutual relationship with the host “microbiome” [4].

In reproductive age women, high levels of circulating estrogens causes glycogen deposition in the vaginal epithelium and favors the growth of species of micro-organisms glucose-fermenting as Lactobacillus species [5]. Those bacteria are able to metabolize glycogen to glucose and maltose and further to lactic acid that leads to decrease vaginal pH to 3.8 to 4.4 range, a protective acidified media, and creates an unfavorable environment for the growth of pathogen bacteria.

Human vaginal microbiota influences pregnancy, conception, mode and timing of delivery, the risk of acquiring infections, and as we know, an eubiotic vaginal microenvironment is directly related with good health. The vaginal microbiota composition is dynamic and undergoes changes with hormonal fluctuations throughout the woman’s hormonal cycle, during reproductive life and during pregnancy.

The vaginal microbiome comprises of a stratified squamous non-keratinized epithelium overlaid by a mucin-rich mucus layer and provides an attachment surface for Lactobacillus species as the most...
dominant species producing lactic acid. A number of protective Lactobacillus species dominates the healthy vaginal microbiota in most reproductive age women, and the dominant include: L. crispatus, L. gasseri, L. iners, and L. jensenii. Other anaerobes as Gardnerella, Atopobium, Mobiluncus, Pevotella, Streptococcus, Ureaplasma, Megaspheara, etc are dormant when the protective action of Lactobacillus is active, but if some situation alters the equilibrium, can cause susceptibility to infection as bacterial vaginosisis (BV), cervicovaginitis, postoperative infections, etc. It has been suggested that an L. crispatus dominant vaginal microbiome is more stable and less likely to transition to bacterial vaginosis than an L. iners or mixed Lactobacilli environment [6].

Lactic acid isomers may also play a role in determining host response and host-microbiota relationship. The both isomers that exist in the vagina are D(-) and L(+), isomers being the microbe the main supply of lactic acid for protection and the host contributing only 4 to 30% of total lactate [7].

Ravel et al. [8] demonstrated that there are five main grade of microbial taxa: Grade I characterized by dominance of Lactobacillus crispatus, identified in 26.2% of studied population, Grade II with dominance of L. gasseri present in 6.3%, Grade III with dominance of L. iners with a 34.1%, Grade V with dominance of L. jensei with a 5.3% of frequency. All this four grades are present in white and Asian women and the Grade IV mainly present in black and Hispanic women and characterized by a non Lactobacillus dominated microbiota including Gardnerella, Prevotella, Corynebacterium, Atopobium, Megaspheara and Sneathia. This confirms that the quantity and proportion of specific vaginal microorganisms may vary between women of different ethnic origins. Lactobacilli and lactic acid in multiple mechanisms can promote antimicrobial defense without inducing immune-mediated inflammation unlike the pathogenic anaerobes. Lactic acid lyse bacteria different than Lactobacillus causing bacterial cell death by acidifying the cytosol, disrupting intracellular function, increasing the membrane permeability to H2O2, diacetyl, etc, potentiating the antimicrobial effect of other substances [9].

One important concept to have in account is to understand that these bacteria are not simply floating free on the surface of the tissue, but are forming a "biofilm" in a three dimensional way [3] with an inner and an outer layer and alterations in the composition of the vaginal microbial community affects the integrity of protective mucosal surface layer. These biofilms exist from the vagina to the fallopian tubes and allows complex and dynamic interactions between the gametes and embryo, as well as the maternal tissue interface.

In addition, metagenomic used in several recent studies has lighted the knowledge that endometrial has a resident population of microorganisms that reaches a 30% of concordance with those of the cervical-vaginal flora [10].

**Microbiome and Pathology**

It is clearly known that microbial dysbiosis can result in disease by giving the opportunity for pathogens to grow and threaten the host health.

Dysbiosis in obstetrics is related with inflammatory states that can result in preterm birth or other adverse obstetric outcomes.

It is observed in postmenopausal women, especially in those with vaginal dryness or atrophy, a decrease in Lactobacilli, especially in L. crispatus, as well as a decrease in bacterial diversity.

As we know, stress may influence immune response and ultimately stimulate cortisol secretion that inhibits the estrogen associated vaginal epithelial maturation and accumulation of glycogen, reducing Lactobacillus dominance and exacerbating the susceptibility and severity of vaginal infection. Stress also induces an increase in norepinephrine, and finally, a lowered Lactobacilli abundance will promote proliferation of other bacteria and the increase in norepinephrine would potentiate the ability of these microorganisms to induce an inflammatory immune response [11].

The most common vaginal infection is bacterial vaginosisis (BV), characterized by a reduction of Lactobacilli and an increase of anaerobes (Gardnerella, Prevotella, Atopobium, Mobiluncus, Ureaplasma, Streptococcus, Mycoplasma, Didister, Bacteroides, etc.) potentially pathogenic.

The Amstel criteria [12] are still being used to diagnose BV. The criteria include:

(I) thin, homogeneous vaginal discharge; (II) vaginal pH >4.5; (III) release of a fishy odor from the vaginal discharge on alkalization with 10% potassium hydroxide; (IV) presence of abnormal amines in vaginal fluid; (V) vaginal epithelial cells heavily coated with bacilli (chue cells). When combined together, the presence of at least three criteria significantly increases the likelihood of making an accurate diagnosis of BV.

Using next generation sequencing of the vaginal microbiota, women with idiopathic infertility were found to have a microbiota profile consistent with BV compared to healthy women [13]. But most BV positive women can be asymptomatic or symptoms could appear in the form of a non itchy but irritating, vaginal discharge creamy with a fishy odor that can be more intense during menstruation or after sexual intercourse. Nevertheless, BV is associated with increased risk of acquisition of sexual transmitted infections such as N. gonorrhoea, T. vaginalis, C. trachomatis, HSV, HPV, HIV, pelvic inflammatory disease, chorioamnionitis, endometritis and amniotic fluid infection [14].

Other dysbiosis is vaginal dysbiosis that can manifest as aerobic vaginitis (AV), also characterized by a diminished Lactobacillus microbiota causing inflammation, leukocyte and parabasal cell infiltration and proliferation of enteric aerobic bacterial organism as E.coli, Enterococci, Staphylococcus aureus and B. Streptococcus. As BV does, AV also is associated with sexual transmitted infections and consequently with severe adverse gynecological and obstetric outcomes as genital infection, inflammation, and preterm birth.

The presence of an endometrial infection has been pointed as possible etiologic factor of chronic endometritis, with most common bacteria presence E.coli, Enterococcus faecalis, Streptococcus agalactiae, followed by Mycoplasmae, Ureaplasma and Chlamidiya trachomatis. The prevalence of chronic endometritis in general population is 0.8 to 19%, but in infertile patients rises to 30 to 45%, mainly with recurrent implantation failure and recurrent pregnancy loss [15]. In fact, the endometrium, previously considered as sterile, currently shows to be characterized by meaningful microbe populations [16].

**Microbiome and Reproduction**

Several factors as well as vaginal microbiota are associated with adverse reproductive and obstetric results. A meta-analysis [17] revealed that women with microbiota of Bacterial Vaginosis (BV) are significantly more prevalent in tubal infertility compared with other...
infertility causes, but is not associated with decreased conception rates. But the immune response to pathogens can trigger cascades of signals that can lead ultimately to miscarriage, intrauterine infection, preterm labor and tubal and ectopic pregnancy [7]. It is clear that a vaginal Lactobacillus dominance is linked to in vitro fertilization and miscarriage. Microbial composition of the cervicovaginal space influences the percentage of women that ends with a delivery of a live birth after an embryo transfer.

In general, lower vaginal tract microbiome is associated with good pregnancy and obstetrical outcomes, while upper reproductive tract remains poorly studied, but a research conducted by Franasiak [3], showed that endometrial microbiome can be studied at the time of embryo transfer without altering clinical procedure. A poor Lactobacillus dominated endometrial microbiota has a negative effect and causes poor reproductive outcomes, implantation failure and loss of pregnancy. This opens a field of determining the microbiome associated with pregnancy outcome and the pathophysiologic microbiota.

It is clear the association of Lactobacillus sp. with vaginal health in reproductive age women as it gives a direct and indirect protection by the Lactobacillus products, such as lactic acid and bacteriocin and against inhibition of pathogens and mucus degradation.

A Lactobacilli rich vaginal microbiota appears to favor the establishment of tolerance that would diminish the likelihood of development of antisperm immunity inhibiting sperm antibody formation. In addition, women with diminished Lactobacilli in vagina develop antisperm antibodies and have failure in in vitro fertilization.

Moreno et al. [18] investigated the reproductive impact of endometrial microbiota and found a Lactobacillus dominated (>90% Lactobacillus spp) and non-Lactobacillus dominated (<90% Lactobacillus with >10% of other bacteria) microbiota. Women with non-Lactobacillus dominated microbiota in a receptive endometrium had significantly lower rate of implantation, pregnancy, ongoing pregnancy, and live birth compared with women with a Lactobacillus dominated microbiota. In addition, a study conducted by Woe BA et al. [19] showed a trend of Gardinerella in the cervix and Ureaplasma in the vagina associated with women with a history of infertility, adding support to the idea that microbiota has to be evaluated as a relevant subject for fertility and assisted reproduction.

Early and late miscarriages have long been associated with BV or the presence of specific flora in the vagina [20]. Also, lower abundance of Lactobacillus in the specific flora in the vagina as well as the presence of Mycoplasma or Ureaplasma, have been found to be more frequent in vagina of women experiencing preterm premature rupture of membrane. Clearly, an unbalanced microbiome has been associated with multiple pathologic conditions, such as infertility, susceptibility to infections, cancers, autoimmune diseases and also neuro-psychiatric disorders, highlighting the importance of a healthy microbiome.

One benefit of metagenomic knowledge is the clinical attitude and as antimicrobial use have not shown clear benefits, other ways are open, instead of eliminating pathogens, perhaps beneficial bacteria could be replaced [3]. Vaginally delivered live biotherapeutics are safe and can be used in combination therapy after antibiotic treatment [20]. Probiotic has been proven beneficial using Lactobacillus rhamnosus supplementation, especially in restoring microbiota after antibiotic therapy [21]. As well, to provide either exogenous lactic acid, a prebiotic that selectively promotes the growth of lactic acid producing bacteria, or lactic acid producing bacteria that colonizes the vagina, can be an alternative to antibiotic treatment and might improve pregnancy outcomes in women with a sub optimal vaginal microbiota.

Interactions between the microbiome and the human reproductive axis knowledge are increasing rapidly and in the next future can provide meaningful enhancements in clinical care.

**Conclusion**

New technology has showed a comprehensive characterization of human microbiome. Species of Lactobacillus have been historically associated with vaginal health due to direct and indirect protective nature related with acid lactic production and the pH regulation in the 3.8 to 4.4 range. A deeper understanding of normal physiology, identification of different disposes and knowledge of microbiome’s impact on reproductive outcomes promises a powerful way of future clinical treatments. An early detection of pathophysiologic processes and intervention with a proven probiotic treatment will give us a standard tool of treatment and prevention in the next decade.

**References**


