

Endometrial Microbiome and Women's Reproductive Health – Review of the Problem Endometrial Microbiome and Reproductive Health

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Abstract

Currently, unlike in the past, the endometrial cavity is not considered to be sterile. The endometrium is supposed to be dominated by *Lactobacilli*, but also their deficiency can be found in the reproductive tract of asymptomatic healthy women. Sometimes the endometrial microbiome is dominated by various pathological microorganisms, and this can lead to various conditions as chronic endometritis, chorioamnionitis and preterm birth. Their presence causes uterine inflammation and infection, release of pro-inflammatory molecules, uterine contractions, disruption of cervical barrier, premature rupture of membranes. Uterine dysbiosis is associated with recurrent implantation failure and recurrent miscarriages. As the microbiome is important for maintaining immunological homeostasis at the level of gastrointestinal tract *Lactobacilli* may play a similar function at the level of uterus. The *lactobacillus*-dominated uterine microbiome is of great importance for maintaining a hostile uterine microenvironment, embryo implantation, early pregnancy development and normal pregnancy outcome.

Keywords: *Lactobacillus*, assisted reproductive techniques, pregnancy, endometritis, preeclampsia, chorioamnionitis

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Studies have established the presence of a functioning microbiome in the endometrium under defined physiological conditions.^{1,2} Pathogens isolation obtained from samples of endometria was initially considered contamination from vaginal contents or attributed to various gynecological diseases.^{3,4} Like the vaginal microbiota in healthy and asymptomatic women, *Lactobacilli* dominate the endometrium.² Non-*Lactobacillus*-dominated microbiome can be found in the reproductive tract of asymptomatic physically healthy women, which suggests it may be assumed as a norm.⁵

The colonization of the uterine cavity is mainly due to the transfer of bacteria from the vagina. This presumption is supported by the involvement of a biofilm containing *Gardnerella vaginalis* affixed to the endometrium and the fallopian tubes in women diagnosed with bacterial vaginosis.⁶ Nevertheless, in some women, other uterine conditions with different physicochemical or biological properties may cause colonization by bacteria distinct from those in the vagina.

Since the vagina is home to billions of bacteria, the cervix should be a perfect barrier to their ascension to the uterine cavity and tubes. The physical barrier provided by the cervical mucus, the high levels of antimicrobial peptides, inflammatory cytokines, immunoglobulins, and matrix-degrading enzymes are considered to act as protection from bacterial ascension.⁷⁻¹³ However, the composition and pH of cervical mucus change during monthly menstruation, which can lead to its overcoming as a barrier under certain conditions. The uterine peristaltic pump assists in the transmission of sperm from the cervical canal up to the fallopian tubes. It may be involved in the spread of bacteria in the uterus.¹⁴ During the follicular phase of the menstrual cycle, uterine contractions are at their highest frequency. Additionally, different uterine conditions can increase the spread of bacteria through hyper- and dysregulation of uterine contractions.¹⁵ In addition, assisted reproductive technology (ART) procedures can lead to dissipation of the uterine microbiota and negative reproductive and gynecological results by disrupting the local microenvironment.¹⁶

A 2017 study of the microbiome from the vagina to the peritoneal cavity confirmed the presence of an active bacterial microbiota in the urogenital tract of reproductive women,

showing that human fetal development is not a sterile event.¹⁷ Following the dynamics and composition of the microbiome in different regions of the reproductive tract, the authors found that most types of *Lactobacilli* dominating the vagina continue to dominate in the uterine cavity. The only difference was that their amount in the vagina was 4 times higher than in the endometrium. According to other authors, this difference is between 100 and 10,000 times.¹ This difference in amount means that either the cervix helps as an incomplete filter block for ascending microorganisms, or the immune response in the endometrium washes bacteria that have been able to ascend, or both. Mitchell et al. compare the vaginal and endometrial microbiome composition in healthy, non-pregnant women.¹ *Prevotella species* (76%), *Lactobacillus iners* (61%), and *Lactobacillus crispatus* [56%] were found in the vaginal microbiome, and the same basic species were found in the upper genital tract (endometrium and upper endocervix), but in different proportions – *Lactobacillus iners* (45%), *Prevotella species* (33%) and *Lactobacillus crispatus* (33%). According to the same authors, microorganisms in the upper endocervix and the endometrium can remain there even after they have disappeared from the vagina or have better development than in the vagina. The low presence of *Lactobacilli* in the uterine cavity may indicate that the vaginal microbiota might not be a constant source of *Lactobacilli* for the endometrium. Walther-Antonio et al. believe that bacterial satiation in the vagina cannot prognosticate bacterial satiation in the cervix and endometrium.¹⁸ Bacterial satiation in the cervix is related to that in the endometrium; their bacterial profiles are very similar but different from that of the vagina.¹⁹

Despite the many similarities in the cervical and endometrial profiles, there are still differences between them. Most notably, there are more *Lactobacilli* in the cervix than in the endometrium.

Microbiome in assisted reproduction

The vaginal microbiome is primarily assessed in the Human Microbiome Project. Some of the data emerging from this analysis are about diversity. The vaginal tract shows the smallest diversity compared to other body areas,

such as the mouth and skin. The most significant variety is found in the oral cavity. Regardless of the vaginal sample level – vaginal introitus, middle or posterior fornix, the species variation is not considerable, and *Lactobacilli* dominate at all levels. Vaginal communities in healthy people show low diversity and it is easier to identify a pathological condition.²⁰ The highest diversity in the vaginal microbiome is found in BV (bacterial vaginosis).²¹ The vaginal microbiome may serve as a predictor for outcome in Assisted reproductive techniques.^{22,23} Concerning the endometrial microbiome, until recently, upper genital tract colonization was considered nothing other than pathological.^{7-9,12,24}

The endometrial microbiome is of major interest in reproductive health studies regarding its effect on the onset and development of pregnancy. Good knowledge of what a healthy endometrial environment is and how to obtain it would favor women conducting ART (Assisted reproductive techniques) and any woman who would like to get pregnant.²⁵

The role of uterine infection is well known in infertility - the microorganisms cause inflammation and activate the immune system in the endometrium which can lead to the problems with the implantation and the beginning of a successful conception. A 2016 survey²⁶ of patients with recurrent implantation failure and recurrent miscarriages or both found dysbiosis with a shift in the ratio of *Lactobacilli*-dominant environment towards missing or reduced *Lactobacilli* environment. Other studies of patients with subfertility confirm the above.²⁷ In these patients, the dominant bacteria in the endometrium were the *Firmicutes*, *Bacteroides*, and *Proteobacteria* types.

The endometrial microbiome can be classified as *Lactobacillus*-dominant or non-*Lactobacillus*-dominant according to the structure and relative predominance of bacteria in the endometrial fluids, with a cut-off value of more than 90% *Lactobacilli* as the only significant predictors of reproductive effectiveness. Hence, the non-*Lactobacillus*-dominant (less than 90%) endometrial microbiome is associated with poor achievement of fertility intentions measured by implantation, pregnancy, current pregnancy, and miscarriage, compared to the *Lactobacillus*-

dominant endometrial microbiome.²⁸ It all comes together to show the importance of *Lactobacilli* for reproductive health.²⁹⁻³²

At the gastrointestinal tract level, the microbiome is essential for maintaining immunological homeostasis, stimulating mucosal immunity, and averting excessive inflammation.^{33,34} Mucosal T-regulatory cells maintain a tolerance environment and are selected by interactions with the commensal intestinal microbiome.³⁵ T-regulatory cells are essential for embryo implantation and early placental development.^{36,37} Moore et al. established a higher percentage of live births in cases of *Lactobacilli* present at the tip of the embryo transfer catheter,³⁸ which suggests that intrauterine commensal bacteria have a similar role in the selection of intrauterine T-regulatory cells.

Microbiome and pregnancy

The high consistency of the vaginal microbiota during pregnancy is due to high estrogen concentrations, lack of menstruation, changes in the cervical and vaginal environment.¹⁸ *Lactobacilli* may have a preventive effect against the ascension of pathological microorganisms from the vagina to the maternal-fetal interface, preventing pregnancy damage.³⁹ Numerous studies have shown that bacteria and viruses can colonize the maternal-fetal interface and amniotic fluid even in healthy pregnancies in women at term.⁴⁰⁻⁴³ Three different mechanisms responsible for the colonization of the fetus by microorganisms have been suggested. The first two mechanisms are distinctly described: advancement from the vagina to the uterus or hematogenous spread from the oral cavity to the placenta. The third hypothesis originates in recent studies connecting bacterial populations present in the endometrial and gastrointestinal microbiome.⁴⁴

Irrespective of the pathway of colonization, bacterial transmission from mother to fetus may play a substantive role in maintaining pregnancy, fetal development, preparing the fetal microbiota for optimal postnatal health.

Placental microbiome

Bacterial isolation from normal pregnancy at term was first successful in 1988 using culture-dependent techniques.⁴⁵ The morphological evidence of sterile placenta harvested from preterm and at-term pregnancies demonstrated

the representation of intracellular bacteria with diverse morphology in the placenta's basal part.⁴⁶ These data have been recently confirmed by low biomass sequencing of the placental microbiome, which is established to be unique and composed of *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroides*, *Tenericutes*, and phylum *Fusobacteria*.⁴⁷ As bacterial colonization of the placenta happens in physiological conditions, microorganisms may play a favorable role in pregnancy and fetus development. Commensal colonization of the fetus by commensal bacteria may be involved in the induction of endotoxin tolerance in future bacterial exposure, prevent subsequent pathogenic access to host cells, and prepare the neonatal gastrointestinal tract for feeding.³⁴

Preterm birth

Premature birth is a primary cause of fetal and neonatal morbidity and mortality worldwide.⁴⁸ Intrauterine microbial infection, which affects the choriodecidual space, amnion, chorion, placenta, amniotic fluid, umbilical cord, or fetus, causes 25 - 40% of preterm births.^{49,50} The change in the normal endometrial microbiome with an increase in *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Escherichia coli*, *Bacteroides species*, *Gardnerella vaginalis*, *Sneathia sanguinegens*, *Streptococcus species*, *Fusobacterium nucleatum*, accompanied by a decrease in *Lactobacillus crispatus*, is associated with preterm birth.⁵¹⁻⁵⁴ Underlying causes of infection-induced preterm birth include the release of pro-inflammatory molecules like IL-1b, IL-6, IL-8, MCP-1, TNF- α , and prostaglandins, which induce uterine contractions with simultaneous bacterial activation of matrix metalloproteinases and hyaluronidases disrupting the cervical epithelial barrier and causing preterm birth.⁵⁵⁻⁵⁸ For the reasons listed above and to prevent premature birth, attempts have been made to use antibiotics during the second and third trimesters of pregnancy. The only result has been a reduction in maternal infection but not premature birth itself. A possible explanation for this result is the negative effect of antibiotics on pathogenic microorganisms and beneficial bacteria in the genital tract.⁵⁹

Chorioamnionitis

Chorioamnionitis is a complication generated by inflamed fetal membranes

due to bacterial infection. This intraamniotic infection is polymicrobial, most often including *Streptococcus agalactiae*, *Fusobacterium nucleatum* and *Ureaplasma parvum*. Severe chorioamnionitis is found on the fetal side of the placenta with colonization by *Corynebacterium species*, *Escherichia coli*, *Peptostreptococcus magnus*, *Prevotella bivia*, *Streptococcus species* and genital *mycoplasmas*.^{41,54,60-62} These bacteria may result from the ascension of microorganisms from the vagina and uterine colonization.⁶³ Their development on chorion and amnion induces immunological and inflammatory changes which can cause an early rupture of the membranes. However, in this case, antibiotic therapy is highly recommended because it reduces chorioamnionitis, prolongs the time to birth at full term, and reduces neonatal infections.⁶⁴

Preeclampsia

Despite decades of research, the modes by which pregnancy causes or exacerbates hypertension remain unclear, and hypertensive conditions persist to be an essential factor in maternal morbidity and mortality worldwide. Preeclampsia is more probable to develop in women who first come in contact with chorionic villi (nulliparous); who are genetically predisposed to the development of hypertensive conditions during pregnancy; who have pre-existing conditions correlated with endothelial cells induction or inflammation such as diabetes, cardiovascular or kidney disease, or immunological disorders; or females who are susceptible to an increased amount of chorionic villi (multiple pregnancies, molar pregnancy). Currently, four main assumptions for the development of preeclampsia have been accepted: immunological intolerance of either maternal, paternal, or fetal tissues; defective trophoblastic invasion; oxidative stress, leading to endothelial cell disruption; genetic predisposition, and epigenetic factors.⁶⁵

An association between preeclampsia and bacterial infection, premised on microbial cultures and targeted PCR for the bacterial 16S rRNA gene in patients with preeclampsia, has recently been proposed.^{66,67} The authors examined the placenta of patients with preeclampsia and found bacterial species typically found in the oral cavity – *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas*

gingivalis, *Prevotella intermedia*, *Tannerella forsythensis* and *Treponema denticola*. Another study found *Lactobacillus iners* and bacteria of the genera *Leptotrichia*, *Sneathia*, *Streptococcus* and *Ureaplasma* in the amniotic fluid of patients with preeclampsia.⁶⁶ A correlation between the quantitative increase of bacteria in the placenta of women with preeclampsia and normotensive women has been established. The 16S rRNA metagenomics analysis of placentas from women with preeclampsia found bacteria routinely related to gastrointestinal infections (*Bacillus*, *Escherichia*, *Listeria* and *Salmonella*), respiratory tract infections (*Anoxybacillus*) and periodontal infections (*Dialister*, *Porphyromonas*, *Prevotella* and *Variovorax*).⁶⁸ The variety of bacterial species present in preeclampsia conditions suggests more likely the presence of more infectious agents than a specific pathogen. The association of polymicrobial communities and preeclampsia is based on the triggering inflammatory and antiangiogenic activity with subsequent impaired trophoblastic and endothelial function and increased blood pressure.

CONCLUSION

Pregnancy is a complex process of fertilization, implantation of fertilized ovum, embryonic and fetal tissue growth and differentiation. A lot of changes in maternal organism are developed in order to promote this process and also a lot of factors, internal and external, may complicate it. With rapidly developing new technologies more information is added but more data is still needed to fully understand these complex relationships. Although some authors cannot confirm presence of endometrial microbiome or its impact on pregnancy a lot of data is almost available acknowledging its influence both in normal and pathologic conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

TB conceptualize, investigate and visualize the study and drafted the manuscript. EK, SP designed the methodology. SP performed formal analysis. TB, EK collected the resources. AY, EK wrote, review and edited the manuscript. AY did the supervision.

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DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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