

Microflora affecting the female reproductive organ and its complication during pregnancy

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Abstract

We do not seem to be the only owner of our body; it houses a large population of microorganisms. Through countless years of coevolution, microbes and hosts have developed complex relationships. In the past few years, the impact of microbial communities on their host has received significant attention. Advanced molecular sequencing techniques have revealed a remarkable diversity of the organ-specific microbiota populations, including in the reproductive tract. Currently, the goal of researchers has shifted to generate and perceive the molecular data of those hidden travelers of our body and harness them for the betterment of human health. Recently, microbial communities of the lower and upper reproductive tract and their correlation with the implication in reproductive health and disease have been extensively studied. Many intrinsic and extrinsic factors influences the female reproductive tract microbiota (FRTM) that directly affects the reproductive health. It is now believed that FRTM dominated by Lactobacilli may play an essential role in obstetric health beyond the woman's intimate comfort and well-being. Women with altered microbiota may face numerous health-related issues. Altered microbiota can be manipulated and restored to their original shape to re-establish normal reproductive health. Female reproductive microbiota has also been suggested to affect infertility, and it may play a key role in the success of assisted reproductive technologies, such as embryo implantation and pregnancy care. The aim of the present review is to summarize the microflora that influence female reproductive health.

Keywords:

Lactobacillus; lactic acid; microbiota; probiotics; reproductive health; vagina

Introduction

Introduction of microbes: it is a living thing that is too small to be seen with the naked eye. Microbes are minute, unicellular organism that are invisible to the naked eye. The term "microbes" is used to describe several different forms with different size and characteristics. A few of these are:

Fungi, Bacteria, Prostate, Viruses, Archaea

Definition of microflora:

The community of micro-organism including algae, fungi and bacteria that live in or on another living organism or in a particular habitat.

Types of microflora:

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In most healthy women in the reproductive periods of predominant vaginally bacteria are one or more like species of *Lactobacillus*, *L. crispatus*, *L. iners* and *L. gasseri*.

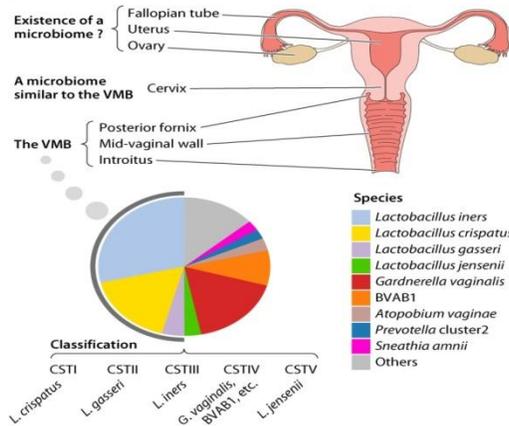


Figure 1: composition of microbiota in the reproductive tract

Female reproductive tract divided into two parts

The female reproductive tract can be divided into two connected parts: the upper and lower reproductive tracts. The former includes the ovaries, fallopian tubes, and uterus, while the latter covers the cervix and vagina.

The lower reproductive tract shows high diversity and abundance of microbiomes.

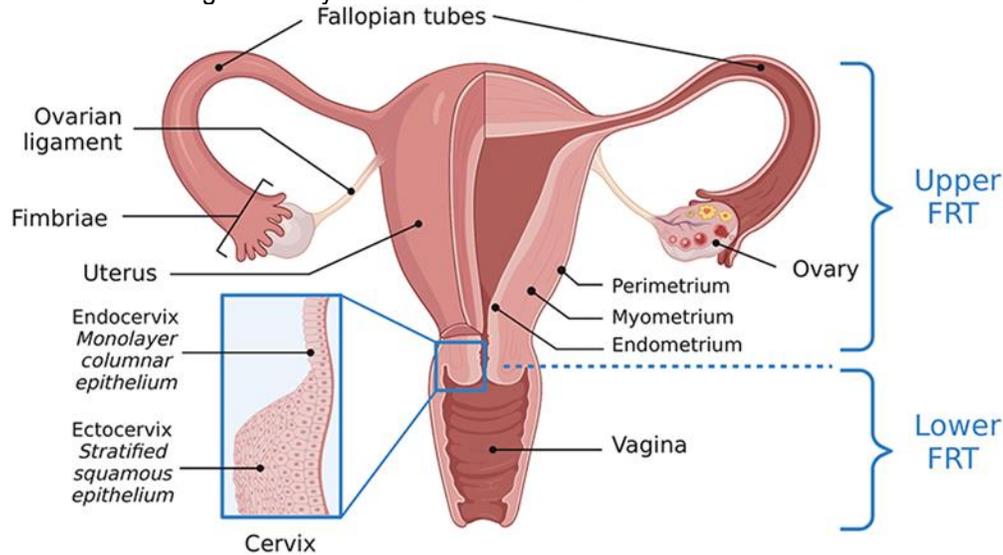


Figure 2: female reproductive tracts

Objective:

1. To investigate the normal microflora present in the female reproductive tract and understand its role in maintaining vaginal health.
2. To examine the mechanisms through which imbalance in the vaginal microflora can lead to complications such as bacterial vaginosis, yeast infections, and sexually transmitted infections during pregnancy.

Microbiota in pregnancy

In normal pregnancy, the resident vaginal flora is thought to provide protection against infection. The microbiota during pregnancy are predominantly *Lactobacillus* species. Microbiota composition can change during the course of the pregnancy. Vaginal discharge is common during pregnancy but is not an indicator of bacterial vaginosis or abnormal microbiota.

NORMAL VAGINAL FLORA

- *Lactobacillus acidophilus*
- *Lactobacillus amylovorus*
- *Lactobacillus gallinarum*
- *Lactobacillus amyolyticus*
- *Lactobacillus crispatus*
- *Lactobacillus gasseri*

- *Lactobacillus iners* *Lactobacillus jensenii*
- *Lactobacillus johnsoni*

ABNORMAL VAGINAL FLORA

Abnormal vaginal flora may occur because of a sexually transmitted infection (STI) eg trichomoniasis, colonization by an organism which is not part of the normal vaginal community e.g. *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Listeria monocytogenes*, or by overgrowth or increased virulence of an organism that is a constituent part of normal vaginal flora e.g. *Escherichia coli*. The most common disorder of vaginal flora is BV. BV is a polymicrobial condition, characterized by a decrease in the quality or quantity of *Lactobacilli* and a one thousand-fold increase in the number of other organisms as determined by cultivation-dependent techniques, particularly anaerobes *Mycoplasma hominis*, *Gardnerella vaginalis* and *Mobiluncus* species. In pregnancy, BV has been associated with early, and late miscarriage, recurrent abortion, post abortal sepsis, post partum endometritis, and preterm birth.

List of bacterial vaginosis microbiota

Bacterial vaginosis in pregnancy is an alteration of the normal vaginal microbiota of pregnancy. Intrauterine infections in pregnancy are caused by bacteria that cause inflammation. The women may experience few or no symptoms. This sometimes leads to chorioamnionitis and other negative pregnancy outcomes. Chorioamnionitis is due to the presence of bacteria such as *Ureaplasma parvum* and *Mycoplasma* species this generates the release of proinflammatory cytokines and chemokines, IL-8 which causes cervical ripening and can result in premature birth. When there are high bacterial counts in of the vagina during pregnancy is typically due to the presence of the following organisms:

<i>Gardnerella vaginalis</i>	<i>Fusobacterium nucleatum</i>
<i>Bacteroides ureolyticus</i>	<i>Atopobium vaginae</i>
<i>Staphylococci</i> species	<i>Streptococci</i> species
<i>Mobiluncus</i> species	<i>Mycoplasma</i> species

Alteration of the Microbiome During Pregnancy

Vaginal epithelial mucosa with tight junctions, cervix and thicker cervical mucus plug act as a physiological barrier against invasion of the intrauterine compartment by microorganisms during pregnancy. Throughout the reproductive career, the healthy bacteria and microbiota, predominantly, *Lactobacillus* spp., help in maintenance of a stable vaginal equilibrium and prevent infective states in the healthy reproductive tract. The vaginal microbiome is quite stable with only shifts of the subtype of *Lactobacillus* and this offers resilience and protection in pregnancy. Abundance of *Lactobacilli* in pregnancy results in reduction of vaginal pH and an increased vaginal gland secretions which act as a barrier against pathogenic microbes. The major changes in the vaginal microbiome occur during early pregnancy, while during later stages of pregnancy and the puerperium, the vaginal microbiome gets back to baseline, with an increase in diversity, decrease in *Lactobacilli*, and enrichment of bacterial associates. Any alteration in the vaginal microbiota may lead to an increase in pro-inflammatory cytokines with induction of inflammatory cascade and preterm labor. Metabolic changes during pregnancy include changes in energy homeostasis, storage of fat, and hormonal profiles leading to elevated fasting blood sugar levels, insulin resistance, glucose intolerance and weight gain. Microbiota is also influenced by changes in metabolism, as noted in obesity, metabolic syndrome, and diabetes.

Composition of the Vaginal Microbiota

The vaginal microbiota is a complex interplay of host cells, symbionts, pathogens with mucosal, endocrinological and immunological factors; and hence it keeps changing throughout a women's life cycle. During childhood, due to low estrogen and thin mucosa, it is mostly dominated by Gram-negative anaerobic bacteria (*Bacteroides*, *Veillonella*, *Fusobacterium*), Gram-positive anaerobic bacteria (*Peptococcus*, *Peptostreptococcus*, *Actinomyces*, *Bifidobacterium* and *Propionibacterium*) and certain aerobic bacteria (*Staphylococcus aureus*, *S. epidermidis*, *Streptococcus viridans*, and *Enterococcus faecalis*). The vaginal microbiome of prepubertal girls is characterized by less abundance of *Lactobacilli*, *Gardnerella vaginalis*, and *Prevotella*. Under the estrogenic effect of puberty, the vaginal epithelium thickens and becomes suitable for glucose-fermenting micro-organisms, hence the microbiome in puberty resembles that of adult women being dominated by *Lactobacillus* spp. Other inhabitants include *Escherichia*, *Staphylococcus*, *Corynebacterium*, *Gardnerella*, *Streptococcus*, *Mycoplasma*, *Mobiluncus*, *Prevotella*, and *Atopobium*. Lactic acid produced by *Lactobacilli* acidifies the vaginal pH (<4.5) thus creating a hostile environment for colonization of pathogenic bacteria and fungi. Molecular diagnostic techniques have enabled detection of uncultivated bacteria

that were earlier missed by conventional culture techniques. On the basis of the composition of bacterial species in reproductive age women, certain unique microbial community types have also been identified.

During pregnancy, the presence of normal microbiota helps to protect the genital tract against infection. The vaginal microbiome patterns of pregnant women delivering at term gestation is different from those of non-pregnant women.

Recent studies have used techniques like RNA gene sequencing to compare the vaginal microbiome of women with Preterm birth (PTB) with those delivering at term and have revealed higher microbial richness and diversity with decreased *Lactobacilli*, abundance of *Gardnerella* and other organisms causing BV. An alteration in microbiome composition also called dysbiosis, may make a woman susceptible to genital tract infections which can result in adverse gestational outcomes like preterm onset of labor, preterm prelabour rupture of membranes (pPROM), pre-eclampsia, miscarriage, fetal growth restriction, low birth weight, stillbirth, and neonatal sepsis.

Mycobiome and Virome

Researchers have traditionally focussed mainly on the bacteriological aspect of the vaginal microbiome. However, viruses and fungi also contribute to the microbiome and the metagenomics. Hence, authors have termed this as the 'Mycobiome' and 'virome'. Zheng et al. have studied the vaginal mycobiome and found *Candida* and *Saccharomyces* as the predominant species with alterations in the mycobiome with diabetes and pregnancy. The vaginal virome has been poorly identified due to difficulties in isolation owing to small viral genomic material and ongoing mutations. However, *Herpesviridae*, *Papillomaviridae*, *Polomaviridae* and *Parvoviridae* have been isolated.

Other Genital Microbiota

The vaginal microbiota is the most comprehensively studied microbiome with significant changes in pregnancy and associated with adverse pregnancy outcomes in dysbiotic states. The cervix also has its distinct microbiome. While the ectocervix has stratified epithelial cells resembling the vagina, the pattern of microbiome is also similar and heavily laden; often called cervico-vaginal microbiome. Presence of mucins in the cervical mucus offer antimicrobial activity to prevent ascending infections to the upper genital tract. The endocervix is lined by a single layer of columnar cells with cell junctions and is generally considered sterile, though this has been challenged by identification of microbes using NGS. Distinct microbiota have also been isolated from the endometrium, ovaries and fallopian tubes. The association of these microbiota have been studied in women undergoing assisted reproductive techniques.

The endometrium has a unique microbiome comprising *Bacteroidetes* esp *Flavobacterium* spp. and *Firmicutes* (*Lactobacillus* spp). Ovarian follicular fluid and fallopian tubes have shown presence of *Propionibacterium*, *Streptococcus* and *Lactobacillus*. Alteration in these microbiomes have been associated with recurrent implantation failures.

Common Genital Infections in Pregnancy

Normal vaginal microbiome comprises of both aerobic and anaerobic bacteria, with *Lactobacillus* being amongst the predominant microbiota. *Lactobacilli* provide defense against infections by maintenance of an acidic vaginal pH and ensuring the presence hydrogen peroxide. Decreased concentration of *Lactobacillus* with a concomitant increase in pathogenic microbes may alter the genital tract microbiome and may lead to various infections resulting due to dysbiotic states. Genital infective states often remain inconspicuous during pregnancy, as their signs and symptoms are overlooked as normal symptoms of pregnancy. Infections like Vulvovaginal candidiasis (VVC), BV (Bacterial vaginosis), TV (*Trichomonas vaginalis*), HPV (*Human Papilloma Virus*), HIV (*Human Immunodeficiency Virus*), HSV (*Herpes Simplex Virus*), *Neisseria gonorrhoeae*, *Chlamydia trachomatis* etc may be consequent to dysbiotic states. HPV is one such infection which infects the basal layer of cervical epithelium and is now believed to be linked to alterations in the vaginal microbiome. The most commonly encountered dysbiotic conditions during pregnancy namely VVC, BV and TV will be highlighted here.

Vulvovaginal Candidiasis in Pregnancy

Vulvovaginal candidiasis is a frequent dysbiosis affecting upto 75% of women at least once in their lifetime and 40–45% will have two or more episodes. Risk factors include recent sexual activity, antibiotic intake, pregnancy, and immunosuppressive states resulting from conditions such as HIV or diabetes. The risk for developing VVC among healthy women is ~20%, it increases by 30% during the last trimester of pregnancy. VVC is caused mainly by *Candida albicans*; however, other species of *Candida* such as *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* may be implicated. In pregnancy, VVC may often be prolonged and associated with more severity in symptoms requiring longer courses of treatment for resolution of symptoms. Although pregnant women are more frequently infected by VVC, they appear to be less symptomatic for vulvovaginitis.

Bacterial Vaginosis in Pregnancy

It is the most common cause of lower genital tract infection in both pregnant and non-pregnant women . Bacterial vaginosis is a dysbiotic state in which the vaginal microbiome that is dominated by Lactobacilli is replaced by an overgrowth of certain anaerobic and facultative bacteria. Majority of the women with such an altered microbiome do not elicit any signs or symptoms apart from elevated vaginal pH and microscopic presence of Clue cells studded with bacteria. There is no single microorganism implicated in the diagnosis of BV, but rather the alteration of microbiome and presence of different bacteria which characterize this. The bacterial composition of BV include *G. vaginalis*, *Mycoplasma*, *Atopobiumvaginalis*, and species of *Clostridiales*, *Mobilincus*, *Prevotella*, and *Leptotrichia*, but may vary with ethnic groups . Bacterial vaginosis may lead to increased susceptibility to STIs and complications after gynecological surgeries. BV during pregnancy has been associated with different obstetric complications such as preterm labor and PTB, pPROM, spontaneous miscarriages, chorioamnionitis, puerperal sepsis, Cesarean wound infections, and gynaecologic complications like postoperative infections and subclinical pelvic inflammatory disease .

Microbiome Changes in Pregnancy Disorders

Hypertensive Disorders

Hypertensive pregnancy disorders (HPDs) complicate up to 10% of pregnancies worldwide; if untreated, these conditions can cause adverse effects on both the mother and child, such as preeclampsia, fetal growth restriction (FGR), and preterm birth . Gestational hypertension is defined as pressure values persistently $\geq 140/90$ mmHg in outpatient controls after the 20th week of gestation in normotensive women before pregnancy .

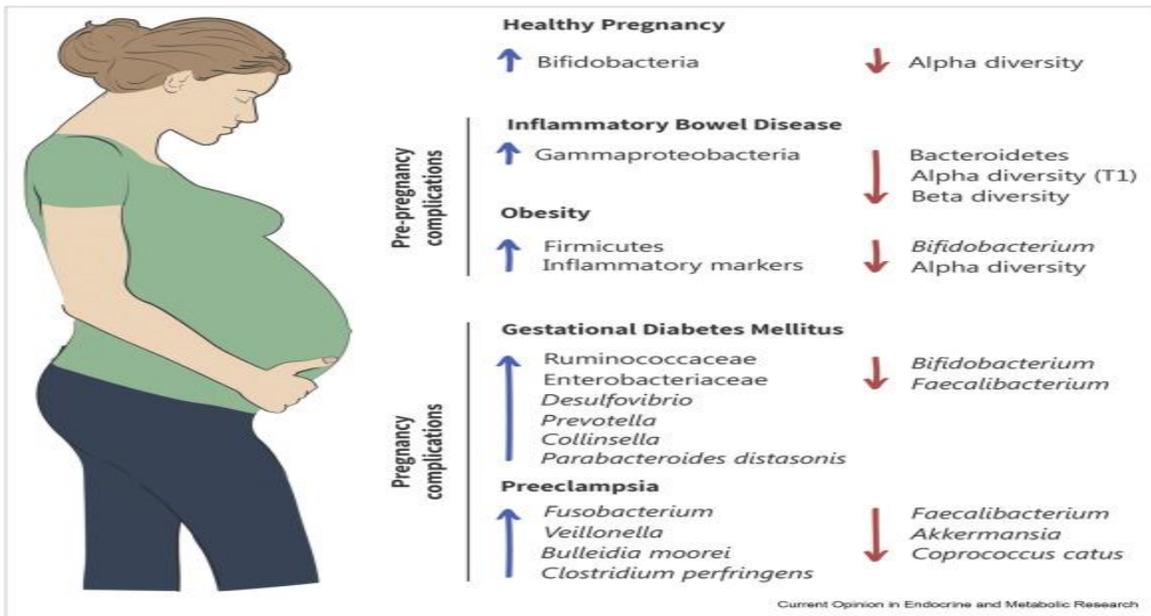


Figure:3 microbiome changes during pregnancy

A significant reduction in the abundance of Prevotella, Porphyromonas, Varibaculum, and Lactobacillus was observed in women with preeclampsia compared to pregnant women without this complication. Prevotella is a bacterium that exerts many functions in the human gastrointestinal tract .Prevotella’s use of fiber and polysaccharides in the production of short-chain fatty acids (SCFAs) such as butyrate has been proven in recent studies and publications. Various fatty acids are beneficial for the functioning of the body . One of them is butyrate, which lowers the maternal blood pressure during pregnancy . Butyrate is the main source of energy for cells building the intestinal epithelium, and is involved in the differentiation of T lymphocytes and affects the functioning of the immune system. The presence of Prevotella in the digestive tract serves to counteract microbial infections .

A reduction in the genus Firmicutes in pregnant women with preeclampsia was shown, which includes the species: Bulleidiamoorei, Clostridium perfringens, and Coprococcusatus. Clostridium perfringens are involved in the metabolism of carbohydrates and proteins.

4. Fetal Growth Restriction

Fetal growth restriction (FGR) is a common obstetric complication and may also be known as intrauterine growth restriction (IUGR). The factors involved in the pathogenesis of FGR include: infections, maternal age, malnutrition, genetic disorders, and insufficient placenta to supply the fetus with nutrients.

5. Gestational Diabetes Mellitus

There is an increasing trend towards the occurrence of GDM worldwide, contributing to an increased risk of obesity, T2DM, and metabolic syndrome. GDM is one of the most common metabolic complications of pregnancy, with an incidence ranging from 1.8% to 22%. The intestinal microbiota is involved in metabolic changes that affect the blood glucose level. The influence of intestinal dysbiosis on the development of GDM is a contentious issue for many scientists. Changes in various taxa are shown, including types, genera, and species, especially in mid- and late gestation. Cortez et al. showed an increase in Firmicutes and a decrease in Bacteroidetes in GDM patients, as well as an increase in the Firmicutes/Bacteroidetes (F/B ratio) during the third trimester of pregnancy. The F/B ratio is considered to be a marker of low-grade systemic inflammation in obesity and insulin resistance. Furthermore, Sililas et al. observed that F/B in the third trimester of pregnancy was higher in patients with GDM compared to the control group.

CONCLUSION-The human microbiota plays an important role in health and female morbidity. It is well established that human microbiota, "the forgotten organ", is not an invader but a beneficial colonizer. The FRTM maintains a healthy environment by dominating infectious microorganisms and is accountable for the normal functioning of the entire reproductive system. An abnormal and more diverse microbiota can adversely affect reproductive health. Maternal microbiomes that affect offspring are mainly those in the vagina.

REFERENCES-

- Anahtar, M. N., Gootenberg, D. B., Mitchell, C. M., & Kwon, D. S. (2018). Cervicovaginal Microbiota and Reproductive Health: The Virtue of Simplicity. *Cell host & microbe*, 23(2), 159–168.
- Africa, Charlene; Nel, Janske; Stemmet, Megan (2014). "Anaerobes and Bacterial Vaginosis in Pregnancy: Virulence Factors Contributing to Vaginal Colonisation". *International Journal of Environmental Research and Public Health*. 11 (7): 6979–7000.
- fig.3-The gut microbiome in pregnancy and pregnancy complications Sondra Turjeman¹, Maria Carmen Collado², OmryKoren¹.
- Fig-1&2.ASM Journals, Microbiology and Molecular Biology Reviews, Vol. 86, No. 4, Roles of the Microbiota of the Female Reproductive Tract in Gynecological and Reproductive Health.
- Goldenberg, R.L.; Culhane, J.F.; Iams, J.D.; Romero, R. Epidemiology and causes of preterm birth. *Lancet* 2008, 371, 75–84.
- Kroon SJ, Ravel J, Huston WM. Cervicovaginal microbiota, women's health, and reproductive outcomes. *Fertil Steril*. (2018) 110:327–36. doi: 10.1016/j.fertnstert.2018.06.036
- Lamont, RF; Sobel, JD; Akins, RA; Hassan, SS; Chaiworapongsa, T; Kusanovic, JP; Romero, R (2011). "The vaginal microbiome: new information about genital tract flora using molecular based techniques". *BJOG: An International Journal of Obstetrics & Gynaecology*. 118 (5): 533–549
- Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. *Am J Obstet Gynecol*. (2016) 215:684–703. doi: 10.1016/j.ajog.2016.09.075
- Moreno I, Simon C. Deciphering the effect of reproductive tract microbiota on human reproduction. *Reprod Med Biol*. (2019) 18:40–50. doi: 10.1002/rmb2.12249
- Moreno I, Simon C (2019) Deciphering the effect of reproductive tract microbiota on human reproduction. *Reprod Med Biol* 18:40–50
- Moosa Y, Kwon D, de Oliveira T, Wong EB. Determinants of vaginal microbiota composition. *Front Cell Infect Microbiol*. (2020) 10:467. doi: 10.3389/fcimb.2020.00467
- Nuriel-Ohayon, M.; Neuman, H.; Koren, O. Microbial Changes during Pregnancy, Birth, and Infancy. *Front. Microbiol.* **2016**, 7, 1031.

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- Petricevic, L., Domig, K. J., Nierscher, F. J., Sandhofer, M. J., Fidesser, M., Krondorfer, I., Husslein, P., Kneifel, W., & Kiss, H. (2014). Characterisation of the vaginal Lactobacillus microbiota associated with preterm delivery. *Scientific reports*, 4, 5136.
- Peric, A., Weiss, J., Vulliemoz, N., Baud, D., & Stojanov, M. (2019). Bacterial Colonization of the Female Upper Genital Tract. *International journal of molecular sciences*, 20(14), 3405.
- Pararas MV, Skevaki CL, Kafetzis DA. Preterm birth due to maternal infection: Causative pathogens and modes of prevention. *Eur J ClinMicrobiol Infect Dis*. 2006;25(9):562–569.
- Schoenmakers S., Steegers-Theunissen R., Faas M. The matter of the reproductive microbiome. *Obstet Med*. 2019;12(3):107–115.
- Schwebke JR, Richey CM, Weiss HL. Correlation of behaviors with microbiological changes in vaginal flora. *J Infect Dis*. (1999) 180:1632–6. doi: 10.1086/31506.
- Srinivasan, S., & Fredricks, D. N. (2008). The human vaginal bacterial biota and bacterial vaginosis. *Interdisciplinary perspectives on infectious diseases*, 2008, 750479.
- Turnbaugh, P.J.; Ley, R.E.; Hamady, M.; Fraser-Liggett, C.M.; Knight, R.; Gordon, J.I. The human microbiome project. *Nature* 2007, 449, 804–810.
- van de Wijert JHHM, Jaspers V. The global health impact of vaginal dysbiosis. *Res Microbiol*. (2017) 168:859–64. doi: 10.1016/j.resmic.2017.02.003