GinPolMedProject ©
1 (63) 2022 : 001-07 • REVIEW ARTICLE

Endometrial Microbiome in Health and Disease – A Brief Review

Tatyana Bodurska[1], Emiliana Konova[2], Svetlana Pachkova[1], Angel Yordanov[3]*
[1]Medical Center "Clinical Institute for Reproductive Medicine", 5800 Pleven, Bulgaria
[2]Center for Reproductive Health, Medical University Pleven, 5800 Pleven, Bulgaria
[3]Department of Gynecologic Oncology, Medical University Pleven, 5800 Pleven, Bulgaria

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection . **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

SUMMARY

Recent data from the human microbiome project show that endometrium, previously considers to be sterile, has its own unique microbiome. Although the uterine cervix serves as barrier for ascending microorganisms from the vagina new wide spread non-culturable techniques show presence of Lactobacillus-dominant environment. Rapidly accumulating data show that this microbiome is qualitatively and quantitatively different from the microbiome in lower genital tract. It plays role in embryo adhesion and development. In some conditions changes in uterine microbiome could cause disturbances in normal physiological processes and subsequent gynecological diseases.

Keywords: Microbiome; Endometrium; Lactobacilli; Gynecologic conditions

Address for correspondence:

Dr. Angel Yordanov
Department of Gynecologic Oncology, Medical University Pleven, 5800 Pleven, Bulgaria
E-mail: angel.jordanov@gmail.com

Word count: 2203 **Tables:** 00 **Figures:** 00 **References:** 57

Received: 10.01.2022, Manuscript No. gpmp-22-51437; **Editor assigned:** 11.01.2022, PreQC No. P-51437; **Reviewed:** 15.01.2022, QC No. Q-51437; **Revised:** 27.01.2022, Manuscript No. R-51437; **Published:** 29.03.2022

INTRODUCTION

Data from the human microbiome project show that body areas traditionally considered to be sterile, such as the placenta and endometrium, are inhabited by a unique microbiome [1,2]. Bacteria inhabiting the urogenital tract make up about 9% of the human microbiota [3,4] and most of them are not easy to identify and cultivate. Until recently, the uterus was considered to be sterile and most microbiological tests were based on vaginal samples. The dominant flora in reproductive age in a healthy woman consists of *Lactobacilli*, with some variations depending on age and hormonal environment [5,6]. This is an interesting characteristic of humans, as the genital tract of other mammals has a vaginal microbiome not dominated by lactobacilli [7].

During childhood, the vaginal flora is a mixture of aerobic and anaerobic bacterial populations including *Prevotella, Enterobacteria, Streptococcus*, and *Staphylococcus* species [8]. With the development of an estrogenic environment during puberty, an increase in the glycogen levels and a pH decrease, the dominance of the *Lactobacilli* types begins. Vaginal pH varies from 3.5 to 4.5 and is the second most acidic area in the human body after the stomach. This low pH created by the lactobacilli involvement significantly inhibits the development of pathogens.

What happens to the upper genital tract – does it have its own microbiome and then, eventually, what is it? For more than a century, a consensus has been established, based on Henry Tissier's 1900 work, that the uterus is a sterile environment [9]. This sterility is thought to be maintained by the cervical obstruction, compared to the Colossus of Rhodes, providing an insurmountable barrier to ascending bacteria from the vagina [10]. Isolation of pathogens from endometrial samples was initially considered to be contamination from vaginal contents or was attributed to various gynecological diseases [11,12]. The colonization of the uterine cavity is mainly due to the ascension of normal vaginal flora. Other routes of colonization by microorganisms of the upper genital tract are possible: hematogenous dissemination of microorganisms from distant body sites, retrograde infection of the uterus by peritoneal microorganisms through the fallopian tubes, direct iatrogenic inoculation, and microorganisms attached to sperm. Notably, although the endometrium can be accessed in many different ways, it has not been classified as a non-sterile medium before [13].

Data on the upper genital tract microbiome have been accumulating rapidly in recent years, with the conclusion that the upper genital tract microbiome is qualitatively and quantitatively different from that of the lower genital tract [14].

In a study by Mitchell CM, et al. [14] using next-generation sequencing of the 16s rRNA gene, bacterial cultures from endometrial samples in healthy women were detected in 100%, with lactobacilli being dominant, followed by *Gardnerella, Prevotella, Atopobium, and Sneathia*, which were also found in vaginal samples. Moreno I, et al. [15] performed a simultaneous comparison of the vaginal and endometrial microbiome. They found that the endometrial microbiome was not equal to the vaginal one. Vaginal *lactobacilli* produce lactic acid and short-chain fatty acids causing lower pH levels. However, this is not the case in the uterine cavity, the pH in the endometrium is 6.6 – 8.51. These data suggest other biochemical processes in the endometrium, where the embryo adheres and develops. Non-Lactobacillus dominant microbiome triggers an inflammatory process in the endometrium affecting embryo implantation because inflammatory mediators are tightly regulated in the blastocyst adhesion to the endometrial wall [16].

The female reproductive system is an open system. During ovulation, the mature egg released from the ovary passes into the peritoneal cavity, where it is seized by the fimbriae of the fallopian tubes. In the fallopian tube, the egg is fertilized developing into an embryo, which is moved by the peristaltic waves and the oscillations of the ciliated epithelium of the fallopian tube into the uterine cavity where it implants. The vagina is home to billions of bacteria and 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 is considered to be protection from ascending bacteria [17-23]. During the menstrual cycle, the composition and pH of cervical mucus change, which under certain conditions, can lead to its overcoming as a barrier. The uterine peristaltic pump aids in the transport of sperm to the fallopian tubes and may be involved in the spread of bacteria in the uterus [24]. During the follicular phase of the menstrual cycle, uterine contractions are at their highest frequency. Additionally, different uterine conditions can lead to hyper- and dysregulation of uterine contractions [25].

METHODS

In current review 57 articles identified from Pubmed using the following search criteria: "microbiome", "endometrium", "lactobacilli", "gynecologic conditions" are included. Gynecologic conditions as chronic endometritis, endometriosis, endometrial polyps, dysfunctional menstrual bleeding and others are discussed in relation to endometrial microbiome.

CHRONIC ENDOMETRITIS

The most telling example of pathology as a result of damaged endometrial microbiota is chronic endometritis. It is characterized by prolonged inflammation of the endometrial mucosa produced by the colonization of the uterus by common bacteria: *Enterococcus faecalis, Escherichia coli, Gardnerella vaginalis, Klebsiella pneumoniae, Proteus spp., Pseudomonas aeruginosa, Staphylococcus heptacuscopa, Streptococcus spp. Mycoplasma* and *Ureaplasma* spp., and fungi such as Saccharomyces cerevisiae and Candida spp. [26,27]. The incidence of chronic endometritis in the general population is estimated at 19% [28] and 45% in the infertile population [29]. It should be noted that this high incidence is more highly associated with recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL) than with other causes of infertility [30-33]. As chronic endometritis is asymptomatic and undetectable by vaginal ultrasound, it is rarely suspected and diagnosed. Current diagnosis of chronic endometritis is based on histopathological identification of plasma cells by conventional staining or immunohistochemical examination of CD 138 in endometrial stroma obtained by in-office endometrial biopsy. In addition to this gold standard for the diagnosis of chronic endometritis, some authors suggest hysteroscopy as a reliable method of diagnosis [34,35]. Hysteroscopic diagnosis is based on the detection of micropolyps, stromal edema, focal or diffuse hyperemia [36,37]. Endometrial tissue culture testing obtained by endometrial biopsy is not a routine procedure as it takes a long time and because not all microorganisms causing chronic endometritis can be cultivated.

ENDOMETRIOSIS

Endometriosis is a condition characterized by the growth of endometrial epithelial and stromal tissue outside the uterine cavity. It affects about 10% of women of reproductive age and is clinically manifested by dysmenorrhea, pelvic pain, dyspareunia, infertility, impaired quality of life of patients [38]. Despite extensive research on endometriosis, its origin remains unclear. Recent results from some studies indicate bacterial contamination of the endometrium as a potential new factor in the development of endometriosis. This theory comes from a study showing that the menstrual blood of women with endometriosis is highly contaminated with *E. coli* [39] with correspondingly higher levels of endotoxins in menstrual blood and peritoneal fluid as a result of reflux to the small pelvis. In addition to the traditional transplantation and coelomic metaplasia theory, the authors propose a novel hypothesis of bacterial contamination causing the development and maintenance of endometriosis. The same authors found that pathogenic genera such as *Gardnerella, Enterococcus, Streptococcus*, and *Staphylococcus* were the main ones identified in endometrial samples from women diagnosed with endometriosis, followed by other taxonomic species such as *Actinomyces*, *Corynebacterium*, *Fusobacterium*, and *Prevotellapion*. For comparison, mainly *lactobacilli* were found in the control group [40].

Bacteria from the Streptococcaceae and Staphylococcaceae families are significantly elevated

in the cystic fluid obtained from women with ovarian endometrioma compared to control groups [41]. A similar finding with significant changes in the composition of the microbiome has been found in patients with adenomyosis [42]. Changes in the microbiome can trigger endometriosis by changing the microenvironment. Significantly lower levels of *lactobacilli* in the endometrial microbiome were found in women with endometriosis after the administration of GnRH agonist treatment compared to women who did not receive such a treatment [41]. Endometriosis is associated with higher levels of the proinflammatory cytokine IL-6 in follicular fluid [43] and IL-1 beta, which causes inflammation in the peritoneum [44]. The link between endometriosis and inflammation is also evidenced by a Danish study showing that women with endometriosis had a significantly higher risk of developing inflammatory bowel disease, ulcerative colitis, and Crohn's disease compared to healthy controls [45]. Even 20 years after initial hospitalization for endometriosis, these patients remain at increased risk of developing ulcerative colitis and Crohn's disease.

Cicinelli E, et al. found that patients with endometriosis treated with antibiotics before implantation had a better pregnancy outcome than patients who were not treated, suggesting that partially the negative impact of endometriosis on fertility may be associated with bacteria in the uterus [33]. These results support the association of chronic endometritis with endometriosis. It can be explained by the examination of cultures of ectopic endometrial cells in response to inflammatory-induced dysperistalsis and impairment of uterine contractility in patients with chronic endometritis [46,47].

ENDOMETRIAL POLYPS

Endometrial polyps are a common gynecological disease characterized by local overgrowth of the endometrial mucosa, associated with chronic endometritis. Long-term stimulation by biological inflammatory factors is thought to contribute to the disease [48,49]. Elevated *Lactobacillus*, *Bifidobacterium*, *Gardnerella*, *Streptococcus*, *Alteromonas*, and *Euryarchaeota* (Archaea) and decreased *Pseudomonas* and *Enterobacteriaceae* were found in endometrial samples of women with endometrial polyps compared to healthy women [50].

DYSFUNCTIONAL MENSTRUAL BLEEDING

Certain microbial species are thought to play a role in gynecological pathologies such as menorrhagia and dysmenorrhea. In nulipari and virgo intacta undergoing surgical treatment for menorrhagia, Pelzer et al. found obligate anaerobic microorganisms – *Fusobacterium* spp., *Jonquetella* spp., which can be considered members of the spectrum of bacteria associated with bacterial vaginosis, instead of the expected *lactobacilli* [13]. The authors found no evidence of inflammation and suggested that the microorganisms lead to dysbiosis presented with excessive menstrual bleeding. It is the result of damaged angiogenic mediators known to be upregulated in conditions of infection. On this occasion, the authors suggest the use of probiotics or antibiotics in cases of menstrual abnormalities when no organic cause is identified.

OTHER GYNECOLOGICAL CONDITIONS

Bacterial vaginosis has been shown to affect the onset of pelvic inflammatory disease [51] and cervical intraepithelial neoplasia [52]. Walther-António MR, et al. [53] investigated the importance of the microbiome in genital cancer by performing sequencing of bacterial 16S rRNA gene. For this, they examined vaginal, cervical, tubal, ovarian, peritoneal samples, and urine in 31 women who underwent hysteroscopy for endometrial carcinoma, endometrial hyperplasia, or other benign conditions. An abundance of taxa, such as Anaerostipes, Dialister, Peptoniphilus, Ruminococcus, Anaerotruncus, Atopobium, Bacteroides, and Porphyromonas, has been found in the reproductive tract of patients with endometrial hyperplasia and carcinoma compared to patients with benign conditions. This suggests an infectious/inflammatory role of bacteria in the occurrence of endometrial cancer [53]. The microbiota can activate a malignant process through various mechanisms: suppression of apoptosis, stimulation of proliferation, or by causing genomic instability [54]. The effect of microorganisms on triggering a pathological process may not only be expressed in inflammation and secretion of cytokines by the host cells, but may also be influenced by the hormonal status of the host. Sex hormones, in particular estrogens, are important factors in certain cancers.

Concerning the reproductive tract, the question is whether they can affect the uterine microbiome like the vaginal microbiome. The administration of a GnRH agonist has been associated with a change in the composition of the uterine microbiome, indicating that it can be hormonally regulated [41]. The intestinal microbiota facilitates estrogen reuptake, thus being related to estrogen-dependent cancer [55]. In support of this, both intestinal microbiota composition and systemic estrogen levels have been found to be significantly different in breast cancer patients compared to healthy controls [56, 57].

CONCLUSION

A lot of data is available nowadays showing the uterine cavity is as non-sterile environmentcompartment. Current studies on the microbiome provide basic point for future research to understand its role in uterine physiology in health and determinationdisease. Determination and qualification of its microbiome plays a great role in development of some gynaecologicgynecologic diseases and also in maintaining of women's health. On the other hand, it is unclear whether described gynecological conditions may provoke changes in otherwise normal endometrial microbiome and consequent uterine dysbiosis. More

data is needed to clarify the relationship between uterine normo- and dysbiosis and gynecological diseases as this research area is new and lot of questions is steadily arising.

FUNDING

This research received no external funding.

CONFLICTS OF INTEREST

The authors declare no competing interests.

All authors declare that the material has not been published elsewhere, or has not been submitted to another publisher.

DATA AVAILABILITY

Authors declare that all related data are available concerning researchers by the corresponding author's email.

ACKNOWLEDGMENTS

None.

REFERENCES

1. **Franasiak JM, Scott RT Jr.** Reproductive tract microbiome in assisted reproductive technologies. *Fertil Steril.* 2015;104(6):1364-1371.
2. **Franasiak JM, Werner MD, Juneau CR, et al.** Endometrial microbiome at the time of embryo transfer: next-generation sequencing of the 16S ribosomal subunit. *J Assist Reprod Genet.* 2016;33(1):129-136.
3. **Sirota I, Zarek SM, Segars JH.** Potential influence of the microbiome on infertility and assisted reproductive technology. *Semin Reprod Med.* 2014;32(1):35-42.
4. **González A, Vázquez-Baeza Y, Knight R.** Snapshot: The human microbiome. Cell. 2014;158(3):690-690.e1.
5. **Ravel J, Gajer P, Abdo Z, et al.** Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A.* 2011;108 Suppl 1(Suppl 1):4680-4687.
6. **Gajer P, Brotman RM, Bai G, et al.** Temporal dynamics of the human vaginal microbiota. *Sci Transl Med*. 2012;4(132):132-152.
7. **Miller EA, Beasley DE, Dunn RR, et al.** Lactobacilli dominance and Vaginal pH: Why is THE human vaginal microbiome unique? *Front Microbiol.* 2016;7:1936.
8. **Huang B, Fettweis JM, Brooks JP, et al.** The changing landscape of the vaginal microbiome. *Clin Lab Med.* 2014;34(4):747-761.
9. **Tissier H.** Recherches sur la Flore Intestinale des Nourrissons (e□tat Normal et Pathologique). *G Carre and C Nau.* Paris; 1900.
10. **Quayle AJ.** The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. *J Reprod Immunol.* 2002;57(1-2):61-79.
11. **Møller BR, Kristiansen FV, Thorsen P, et al.** Sterility of the uterine cavity. *Acta Obstet Gynecol Scand.* 1995;74(3):216-219.
12. **Hemsell DL, Obregon VL, Heard MC, et al.** Endometrial bacteria in asymptomatic, non-pregnant women. *J Reprod Med.* 1989;34(11):872-874.
13. **Pelzer ES, Willner D, Buttini M, et al.** A role for the endometrial microbiome in dysfunctional menstrual bleeding. *Antonie Van Leeuwenhoek.* 2018;111(6):933-943.
14. **Mitchell CM, Haick A, Nkwopara E, et al.** Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. *Am J Obstet Gynecol.* 2015;212(5):611.e1-9
15. **Moreno I, Codoñer FM, Vilella F, et al.** Evidence that the endometrial microbiota has an effect on implantation success or failure. *Am J Obstet Gynecol.* 2016;215(6):684-703.
16. **Dominguez F, Gadea B, Mercader A, et al.** Embryologic outcome and secretome profile of implanted blastocysts obtained after coculture in human endometrial epithelial cells *vs.* the sequential system. *Fertil Steril.* 2010;93(3):774-782.e1.
17. **Ulcova-Gallova Z.** Immunological and physicochemical properties of cervical ovulatory mucus. *J Reprod Immunol.* 2010;86(2):115-121.
18. **Lieberman JA, Moscicki AB, Sumerel JL, et al.** Determination of cytokine protein levels in cervical mucus samples from young women by a multiplex immunoassay method and assessment of correlates. *Clin Vaccine Immunol.* 2008;15(1):49-54.
19. **Ming L, Xiaoling P, Yan L, et al.** Purification of antimicrobial factors from human cervical mucus. *Hum Reprod.* 2007;22(7):1810-1815.
20. **Hein M, Helmig RB, Schønheyder HC, et al.** An *in vitro* study of antibacterial properties of the cervical mucus plug in pregnancy. *Am J Obstet Gynecol.* 2001;185(3):586-592.
21. **HHein M, Valore EV, Helmig RB, et al.** Antimicrobial factors in the cervical mucus plug. *Am J Obstet Gynecol.* 2002;187(1):137-144.
22. **Hein M, Petersen AC, Helmig RB, et al.** Immunoglobulin levels and phagocytes in the cervical mucus plug at term of pregnancy. *Acta Obstet Gynecol Scand*. 2005;84(8):734-742.
23. **Becher N, Hein M, Danielsen CC, et al.** Matrix metalloproteinases in the cervical mucus plug in relation to gestational age, plug compartment, and preterm labor. *Reprod Biol Endocrinol*. 2010;8:113.
24. **Kunz G, Beil D, Deiniger H, et al.** The uterine peristaltic pump. Normal and impeded sperm transport within the female genital tract. *Adv Exp Med Biol.* 1997;424:267-77.
25. **Kunz G, Leyendecker G.** Uterine peristaltic activity during the menstrual cycle: Characterization, regulation, function and dysfunction. *Reprod Biomed Online.* 2002;4 Suppl 3:5-9.
26. **Cicinelli E, De Ziegler D, Nicoletti R, et al.** Poor reliability of vaginal and endocervical cultures for evaluating microbiology of endometrial cavity in women with chronic endometritis. *Gynecol Obstet Invest*. 2009;68(2):108-115.
27. **Greenwood SM, Moran JJ.** Chronic endometritis: morphologic and clinical observations. *Obstet Gynecol.* 1981;58(2):176-184.
28. **Yoshii N, Hamatani T, Inagaki N, et al**. Successful implantation after reducing matrix metalloproteinase activity in the uterine cavity. *Reprod Biol Endocrinol.* 2013;11:37.
29. **Kushnir VA, Solouki S, Sarig-Meth T, et al.** Systemic inflammation and autoimmunity in women with chronic endometritis. *Am J Reprod Immunol*. 2016;75(6):672-677.
30. **Johnston-MacAnanny EB, Hartnett J, Engmann LL, et al.** Chronic endometritis is a frequent finding in women with recurrent implantation failure after *in vitro* fertilization. *Fertil Steril.* 2010;93(2):437-441.
31. **Yang R, Du X, Wang Y, et al.** The hysteroscopy and histological diagnosis and treatment value of chronic endometritis in recurrent implantation failure patients. *Arch Gynecol Obstet.* 2014;289(6):1363-1369.
32. **Cicinelli E, Matteo M, Tinelli R, et al.** Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Hum Reprod.* 2015;30(2):323-330.
33. **Cicinelli E, Matteo M, Tinelli R, et al.** Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reprod Sci.* 2014;21(5):640-647.
34. **Cicinelli E, De Ziegler D, Nicoletti R, et al.** Chronic endometritis: Correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. *Fertil Steril.* 2008;89(3):677-684.
35. **Bayer-Garner IB, Nickell JA, Korourian S**. Routine syndecan-1 immunohistochemistry aids in the diagnosis of chronic endometritis. *Arch Pathol Lab Med*. 2004;128(9):1000-1003.

36. **Park HJ, Kim YS, Yoon TK, et al.** Chronic endometritis and infertility. *Clin Exp Reprod Med*. 2016;43(4):185-192.

37. **De Ziegler D, Pirtea P, Galliano D, Et Al.** Optimal Uterine anatomy and physiology necessary for normal implantation and placentation. *Fertil Steril.* 2016;105(4):844-854.

38. **Fuldeore MJ, Soliman AM**. Prevalence and symptomatic burden of diagnosed endometriosis in the United States: National estimates from a cross-sectional survey of 59,411 women. *Gynecol Obstet Invest.* 2017;82(5):453-461.

39. **Khan KN, Kitajima M, Hiraki K, et al.** *Escherichia coli* contamination of menstrual blood and effect of bacterial endotoxin on endometriosis. *Fertil Steril.* 2010;94(7):2860-3.e1-3.

40. **Khan KN, Fujishita A, Kitajima M, et al.** Intra-uterine microbial colonization and occurrence of endometritis in women with endometriosis. *Hum Reprod.* 2014;29(11):2446-56.

41. **Khan KN, Fujishita A, Masumoto H, et al.** Molecular detection of intrauterine microbial colonization in women with endometriosis. *Eur J Obstet Gynecol Reprod Biol.* 2016;199:69-75.

42. **Hernandes C, Silveira P, Rodrigues Sereia AF, et al.** Microbiome profile of deep endometriosis Patients: Comparison of Vaginal fluid, endometrium and lesion. *Diagnostics (Basel).* 2020;10(3):163.

43. **Tseng JF, Ryan IP, Milam TD, et al.** Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. *J Clin Endocrinol Metab.* 1996;81(3):1118-1122.

44. **Sikora J, Mielczarek-Palacz A, Kondera-Anasz Z.** Association of the precursor of interleukin-1 and peritoneal inflammation-role in pathogenesis of endometriosis. *J Clin Lab Anal.* 2016;30(6):831-837.

45. **Jess T, Frisch M, Jørgensen KT, et al.** Increased risk of inflammatory bowel disease in women with endometriosis: A nationwide Danish cohort study. *Gut.* 2012;61(9):1279-1283.

46. **Takebayashi A, Kimura F, Kishi Y, et al.** The association between endometriosis and chronic endometritis. *PLoS One.* 2014;9(2):e88354.

47. **Pinto V, Matteo M, Tinelli R, et al.** Altered uterine contractility in women with chronic endometritis. *Fertil Steril.* 2015;103(4):1049-1052.

48. **Al-Jefout M, Black K, Schulke L, et al.** Novel finding of high density of activated mast cells in endometrial polyps. *Fertil Steril.* 2009;92(3):1104-1106.

49. **El-Hamarneh T, Hey-Cunningham AJ, Berbic M, et al.** Cellular immune environment in endometrial polyps. *Fertil Steril.* 2013;100(5):1364-1372.

50. **Fang RL, Chen LX, Shu WS, et al.** Barcoded sequencing reveals diverse intrauterine microbiomes in patients suffering with endometrial polyps. *Am J Transl Res*. 2016 Mar 15;8(3):1581-92.

51. **Sweet RL.** Gynecologic conditions and bacterial vaginosis: implications for the non-pregnant patient. *Infect Dis Obstet Gynecol.* 2000;8(3-4):184-90.

52. **Gillet E, Meys JF, Verstraelen H, et al.** Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. *PLoS One.* 2012;7(10):e45201.

53. **Walther-António MR, Chen J, Multinu F, et al.** Potential contribution of the uterine microbiome in the development of endometrial cancer. *Genome Med.* 2016;8(1):122.

54. **Rajagopala SV, Vashee S, Oldfield LM, et al.** The human microbiome and cancer. *Cancer Prev Res (Phila).* 2017;10(4):226-234.

55. **Baker JM, Al-Nakkash L, Herbst-Kralovetz MM.** Estrogen-gut microbiome axis: Physiological and clinical implications. *Maturitas.* 2017;103:45-53.

56. **Bard JM, Luu HT, Dravet F, et al.** Relationship between intestinal microbiota and clinical characteristics of patients with early stage breast cancer. *Faseb J*. 2015;29(914):2.

57. **Flores R, Shi J, Fuhrman B, et al.** Fecal microbial determinants of fecal and systemic estrogens and estrogen metabolites: A cross-sectional study. *J Transl Med.* 2012;10:253.