Title

**Association Between the Microbiome and Female Infertility: A Narrative Review**

Running titles

**Microbiome and Female Infertility**

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**Abstract**

Infertility in women is influenced by a complex interplay of factors, including hormonal imbalances, infections, and lifestyle habits. Estrogen, a key hormone in female reproductive health, is pivotal in these processes. The microbiome, particularly *Lactobacillus* species, has been associated with improved outcomes in in vitro fertilization (IVF). Furthermore, alterations in both vaginal and gut microbiota can impact reproductive health and increase the risk of pregnancy complications. Recent research has highlighted the significant influence of gut microbes on behavioral, metabolic, and immune functions. This narrative review aims to explore the relationship between the microbiome and infertility in women. A comprehensive literature search was conducted using PubMed/MEDLINE, Scopus, and Embase databases, focusing on full-text original research articles published in English from 2000 to 2024. The search terms included "microbiota," "microbiome," "fertility," and "infertility."Our findings suggest that the gut microbiome and its enzymatic activity, specifically β-glucuronidase, can influence estrogen levels, potentially leading to conditions characterized by estrogen excess or deficiency. Additionally, gut microbiota may contribute to endometriosis, pelvic pain, and infertility through hormonal imbalances. The genital microbiome, particularly the abundance of *Lactobacillus* species, has also been implicated in female infertility and protection against bacterial vaginosis. The presence of *Chlamydia trachomatis* and *Gardnerella vaginalis*, as well as a deficiency of *Lactobacillus*, has been linked to infertility.

Keywords: Microbiome, Female, Infertility

**Introduction**

Infertility, defined as the inability to conceive after one year of well-timed unprotected sexual intercourse, presents a significant reproductive challenge. Research indicates that the fecundability rate, or the likelihood of becoming pregnant, is approximately 25% in the initial three months of unprotected intercourse, gradually decreasing to 15% over the subsequent nine months. A substantial majority of women (85%) are likely to conceive within a year, facilitating the understanding of normal pregnancy rates and the diagnosis of infertility(1).

Factors contributing to infertility include hormonal imbalances, infections, and lifestyle habits (2). Estrogen, a crucial hormone in female reproductive health, regulates the menstrual cycle and prepares the body for pregnancy. Abnormal estrogen levels can disrupt fertility. Fluctuations in estrogen receptor β expression and hormone concentrations are essential throughout an organism's lifespan. The intestinal microbiota also plays a significant role in estrogen metabolism, and antibiotic use can lead to decreased estrogen levels (3).

Research suggests that the microbiome influences infertility treatment, with *Lactobacillus* species being associated with improved IVF outcomes, such as implantation and pregnancy (4). Alterations in the vaginal and endometrial microbiomes have been linked to various gynecological issues (5, 6). In healthy women, the vaginal microbiota is predominantly composed of *Lactobacillus* species, which help to maintain a balanced pH, prevent the attachment of harmful microorganisms, and release antimicrobial substances (7). The endometrial microbiota differs from the vaginal microbiota (8). The endometrial microbiome exhibits lower diversity compared to the vaginal microbiome (9).

The gut microbiota functions as an endocrine organ, interacting with hormones such as estrogens, androgens, and insulin, and influencing the reproductive endocrine system in women(10). Changes in the composition of the gut microbiota can lead to pregnancy complications, adverse outcomes, polycystic ovary syndrome (PCOS), endometriosis, and certain cancers(11). While research in this area is limited, further investigation is necessary to comprehend the underlying causes and mechanisms of microbiota-related hormone-mediated diseases and develop effective treatments (12).

The human gut microbiome harbors a vast amount of genetic information, with the quantity of bacteria comparable to human cells and genetic material 150 times more abundant than the human genome (13). Recent studies suggest that gut microbes function as an additional organ, influencing physiological functions. Factors such as diet, genetics, and hormones shape the diversity of the gut microbiome(14). Sex hormones, including progesterone, estradiol, and testosterone, play a role in the interactions between microorganisms and their hosts, impacting various physiological processes (15). Commensal bacteria can influence host metabolism, immune responses, and behavior through the production of hormones(16).

The human microbiome plays a crucial role in all aspects of female reproduction, from follicle development to pregnancy and childbirth. Imbalances in the microbiome, particularly in the gut, can impact the reproductive endocrine system and may be amenable to interventions to improve reproductive outcomes (17). Studies have identified a connection between gut microbiota and hormone levels, influencing overall health and leading to the concept of the "microgenderome." Certain intestinal bacteria may also be associated with female health issues (18).

By elucidating the intricate relationship between the microbiome and female infertility, this review aims to contribute to a deeper understanding of this complex reproductive health issue. This knowledge may ultimately lead to the development of novel therapeutic strategies tailored to the specific microbial composition of individuals, potentially improving infertility outcomes.

**Methodology**

To conduct a comprehensive literature review on the association between the microbiome and female infertility, a systematic search was performed across three major databases: PubMed/MEDLINE, Scopus, and Embase. The search strategy was carefully refined for each database to optimize results. Additionally, the references of included studies were manually screened to identify additional relevant articles.

The final search was conducted in August 2024, focusing on articles published between 2000 and 2024. Only original research articles written in English were considered eligible for inclusion. Reviews, editorials, opinions or letters, case studies, conference papers, and abstracts were excluded.

The review aimed to address the following research questions:

1. What is the role of the microbiome in female infertility?
2. How do the gut microbiome and reproductive system interact to influence women's infertility?
3. What are the mechanisms underlying the effects of genital tract microbiomes on infertility in women?

The last search was conducted in August 2024 and was limited to articles published between 2000 and 2024. Only full-text original research articles written in English were considered eligible for analysis, while reviews, editorials, opinions or letters, case studies, conference papers, and abstracts were excluded. This study was conducted to ask the following questions: 1 What is the role of the microbiome in Female infertility? 2-How do the gut microbiome and reproductive system affect women's infertility? 3-What is the mechanism of the effect of Genital Tract microbiomes on infertility in women? Methodology to conduct a comprehensive literature review on the association between the microbiome and female infertility, a systematic search was performed across three major databases: PubMed/MEDLINE, Scopus and Embase. The search strategy was carefully refined for each database to optimize results. Additionally, the references of included studies were manually screened to identify additional

The search strategy details are presented in a summarized format in Table 1.

**Table1**

|  |  |  |
| --- | --- | --- |
| Search Strategy | Number of Studies Obtained | Database |
| (Microbiota / microbiot / Gut microbiome/ Vaginal Microbiome ) AND (Infertility/fertility) | 153 | Scopus |
| (Microbiota / microbiot / Gut microbiome/ Vaginal Microbiome ) AND (Infertility/fertility) | 201 | |  | | --- | | PubMed/MEDLINE | |
| (Microbiota / microbiot / Gut microbiome/ Vaginal Microbiome ) AND (Infertility/fertility) | 68 | Embase |

**Result**

**The Microbiome and Female Infertility**

Research has identified gender-specific differences in the gut microbiome (19). The composition of the gut microbiome in women is notably distinct from that of men (20). A study on aging revealed that women exhibit greater gut microbiome diversity than men in younger adulthood, although this difference diminishes in older adulthood. This observation suggests that sex-related variations in microbiome aging may be associated with menopause (21).

The female reproductive system, consisting of the vagina, cervix, and uterus, harbors a unique and active microbial community that is believed to play a crucial role in maintaining reproductive health(22). Imbalances in this intricate ecosystem, known as dysbiosis, have been linked to various reproductive issues, including implantation difficulties, recurrent miscarriages, and infertility(23). Recent evidence suggests that microorganisms are present not only in the vagina but also throughout the upper female reproductive system, including the ovaries, Fallopian tubes, and uterus, which were previously considered sterile environments (24).

Findings indicate that changes in the microbiome of the female reproductive system may contribute significantly to the development of infertility (25). *Lactobacillus* has been identified as the dominant genus present throughout the female reproductive system (26). These resident microorganisms play a vital role in maintaining health, and alterations in their composition have been associated with various gynecological disorders (27). Research suggests that *Lactobacilli* provide protection against pathogen invasion, while dysbiosis has been linked to conditions such as chronic endometritis, endometriosis, pelvic inflammatory disease, and gynecological cancers (28). Furthermore, multiple studies have established a positive association between the presence of certain bacteria in the uterus and the development of pelvic inflammatory disease, an inflammation of the upper genital tract that may lead to infertility (29). Elna et al. found that the microbiome of the reproductive tract in women with endometriosis differs from that of women experiencing infertility due to other factors (30).

**The Role of the Gut Microbiome in Estrogen Regulation and Infertility**

The gut microbiota is influenced by circadian rhythms regulated by environmental cues, including day and night cycles. This regulation affects the microbiota's behavior and metabolite secretion, impacting host homeostasis(31). Recent studies suggest that the gut microbiota also contributes to the development of the reproductive system, affecting sexual maturation in both males and females(32). Metabolites like secondary bile acids and indole, as well as estrogenic properties of soybean, play a role in this process (33).

The gut microbiota is influenced by estrogens and also plays a role in modulating estrogen levels. Estrogens serve as a key regulator of the gut microbiome, and the collection of genes within the gut microbiota that can metabolize estrogens is referred to as the "estrobolome" (34). Estrogen receptor β (ERβ) and serum levels of steroid hormones, particularly estradiol, fluctuate throughout a person's life. The regulation of estrogen is crucial for women's health(35).

Intestinal microbiota significantly influences estrogen metabolism. Antibiotic use can reduce estrogen levels(36). Microbial β-glucuronidase converts estrogens from their conjugated forms to their active deconjugated forms. Dysbiosis and decreased gut microbiota diversity can diminish β-glucuronidase activity, resulting in lower deconjugation of estrogen and phytoestrogens into their active circulating forms (37). Reduced estrogen levels can disrupt estrogen receptor activation, potentially leading to conditions associated with excess estrogen. An increase in β-glucuronidase-producing bacteria may elevate circulating estrogen levels, contributing to diseases like endometriosis and cancer (38). Estrogen levels can influence various health conditions, including polycystic ovary syndrome (PCOS), endometrial hyperplasia, and fertility. Research indicates that certain bacterial orders and specific phyla vary according to ERβ status, highlighting the potential impact of steroid nuclear receptor status and dietary complexity on microbiota composition (39). Additionally, a negative correlation exists between alpha diversity and estradiol concentrations. New research suggests that the gut microbiome is important for 17β-estradiol's protective effects against metabolic endotoxemia and chronic inflammation (40).

Changes in gut microbial composition and β-glucuronidase activity may lead to disruptions in estrogen levels, potentially causing hyper- or hypo-estrogenic states and conditions influenced by estrogen. The microbiota could contribute to the onset of endometriosis, chronic pelvic pain, and infertility by promoting inflammation and hormonal imbalances through the estrobolome. The estrobolome, which affects various cellular processes, remains relatively unexplored, but researching it could provide insights into future strategies and treatments for endometriosis and estrogen-related conditions (41).

Uterus Microbiome

Various studies have demonstrated the presence of distinct microbial communities in female reproductive organs, which are crucial for normal function and the prevention of infections (42, 43). More recent research has recognized the existence of a unique uterine microbiome. Although the uterine microbiome is less dense than the vaginal microbiome, it is believed to play a critical role in reproductive outcomes, such as implantation rates and preterm birth(44). The human microbiome is the diverse community of microorganisms in our bodies. The Human Microbiome Project has advanced our understanding of microbiota in different body sites, but the criteria for a healthy bacterial composition in the uterus remain a subject of debate(45) .

Traditional beliefs that the uterine cavity was devoid of microorganisms have been challenged by advanced technologies. Studies suggest that the uterus may be colonized from the gut, oral cavity, bloodstream, or through vaginal ascent. Additionally, procedures like assisted reproductive technology and contraceptive device insertion may contribute to uterine microbiome seeding (46). The uterine microbiome is less populated compared to the lower genital tract, and its complete composition is not fully understood(47).

An evaluation of the upper genital tract (UGT) using endometrial swabs from 58 women who had hysterectomies for non-cancer reasons found that 95% had UGT colonization. High levels of L. iners, Prevotella spp., and L. crispatus were predominant. Endometrial fluid (EF) samples from fertile women showed a significant presence of Lactobacillus. Two distinct bacterial compositions were proposed: Lactobacillus-dominant (LD) and non-LD (NLD), with other genera identified, including Bifidobacterium, Gardnerella, Prevotella, and Streptococcus(48).

The significance of the endometrial microbiota at the beginning of pregnancy is of great interest in reproductive medicine(49). Understanding what constitutes a healthy uterine environment and how to achieve it would benefit women undergoing IVF as well as those trying to conceive naturally(50) . Research suggests that changes in the endometrial microbiome can affect the fertility of infertile individuals, and treating microbial imbalances could improve success rates. Uterine infections can contribute to infertility by causing inflammation and immune responses that interfere with embryo implantation and a successful pregnancy. Recognizing endometrial dysbiosis as a potential cause of infertility highlights the importance of evaluating uterine microbiota in infertile patients and aiming to restore a healthy balance to improve care for those facing infertility challenges(51).

**Vaginal Microbiome**

Maintaining a healthy female genital tract requires a delicate balance of microbiota, metabolites, and immune factors. The vaginal microbiota is primarily composed of *Lactobacillus* species, with four main types: *L. crispatus*, *L. iners*, *L. jensenii*, and *L. gasseri* (52). A dysbiotic vaginal microbiome (VMB) is characterized by a decrease in *Lactobacillus* prevalence and an increase in anaerobic bacteria such as *Prevotella*, *Mobiluncus*, *Gardnerella*, *Ureaplasma*, and *Mycoplasma*(53) .

This dysbiotic state poses a significant risk for the development of bacterial vaginosis, an inflammatory condition that can negatively affect fertility through various mechanisms. These mechanisms include increased susceptibility to sexually transmitted infections (STIs) like *Chlamydia*, *Neisseria*, and *Trichomonas*, as well as viral infections such as human papillomavirus (HPV) and human immunodeficiency virus (HIV) (54). A dysbiotic VMB is also linked to adverse pregnancy outcomes, such as preterm delivery, maternal infectious morbidity, and late miscarriage (55).

VMB dysbiosis affects IVF success. Patients without bacterial contamination have higher pregnancy rates. Low *Lactobacillus* levels predict poor outcomes, while *Lactobacillus iners* is associated with successful IVF and vaginal health (56).

**Conclusion**

Alterations in the composition of gut microbes and the activity of β-glucuronidase can disrupt estrogen levels, leading to conditions characterized by either estrogen excess or deficiency. The gut microbiota may contribute to the development of endometriosis, chronic pelvic pain, and infertility by promoting inflammation and hormonal imbalances through the estrobolome.

Research indicates that the genital microbiome plays a significant role in women experiencing infertility. The interplay among different *Lactobacillus* species is crucial for maintaining a healthy vaginal microbiome. Adequate levels of *Lactobacillus crispatus* serve as a protective factor against asymptomatic bacterial vaginosis (BV), which is often caused by *Ureaplasma* and *Gardnerella vaginalis* and can negatively affect fertility. Furthermore, the presence of Gram-negative bacteria, such as *Chlamydia trachomatis* and *Gardnerella vaginalis*, along with a lack of *Lactobacillus* in the cervical flora, is associated with infertility. Interestingly, *Gardnerella vaginalis* has also been found in the endometrium of infertile women.

**Funding**

The work was not supported by any fund/grant.

**Institutional Review Board Statement**

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

**Informed Consent Statement**

Informed consent is not required for this type of study.

**Data Availability Statement**

The data presented in this study are available on request from the corresponding author.

**Conflicts of Interest**

The authors declare that they have no conflict of interest.

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