Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease

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Bacterial vaginosis, pelvic inflammatory disease, and endometritis are infections of the genital tract that can lead to many adverse health outcomes, including infertility. Bacterial vaginosis is characterized by a lower prevalence of lactobacilli and a higher prevalence of anaerobic bacteria, including *Gardnerella vaginalis, Megasphaera* spp., and *Atopobium vaginae.* Endometritis and pelvic inflammatory disease are caused by the ascension of pathogenic bacteria to the uterus, although the mechanisms by which they do so are unclear. Bacterial vaginosis, chronic endometritis, and pelvic inflammatory disease have been linked to infertility in retrospective and prospective trials. Similarly, the causes of bacterial vaginosis and endometritis-related infertility are likely multifactorial and stem from inflammation, immune targeting of sperm antigens, the presence of bacterial toxins, and increased risk of sexually transmitted infections. Diagnosis and treatment of bacterial vaginosis, chronic endometritis, and pelvic inflammatory disease before attempting conception may be important components of preconceptional care for symptomatic women to improve outcomes of natural and assisted reproduction.

Key words: bacterial vaginosis, endometritis, infertility, lactobacilli, pelvic inflammatory disease

Introduction

In healthy women of reproductive age, typical vaginal microbiota include aerobic, facultative anaerobic, and obligate anaerobic species. Most women have vaginal microbiota that are predominated by lactobacilli.[1,2] Optimal vaginal microbiota tend to exist symbiotically and are believed to protect against pathogenic bacterial colonization and infection through the production of lactic acid and antimicrobial byproducts and by low-level immune system activation.[3] Disruption of the predominance of lactobacilli has been shown to increase the risk of sexually transmitted infections (STIs) and upper genital tract infections through the ascension of bacterial pathogens and other anaerobic bacteria.[4]

Bacterial vaginosis (BV) is a common lower genital tract infection that affects approximately 29% of women of reproductive age in the United States, although variations in prevalence exist among different races and ethnicities.[5] BV is associated with the disruption of optimal vaginal microbiota characterized by decreased proportions of lactic acid–producing bacteria and increased proportions of a wide array of strict and facultative anaerobes.[1,6–8] Bacteria commonly associated with BV include *Gardnerella vaginalis*, *Megasphaera* spp., *Atopobium vaginae*, *Dialister* spp., *Mobiluncus* spp., *Sneathia amnii*, *Sneathia sanguinegens*, *Porphyromonas* spp., and *Prevotella* spp.[6,8]

Although BV is frequently asymptomatic, women with BV are more likely than those without BV to report vaginal odor, itching, and discharge.[9] Serious adverse health outcomes have been associated with BV, including increased risk of infertility; adverse pregnancy outcomes; STIs, including chlamydia, gonorrhea, human

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papilloma virus (HPV), and human immunodeficiency virus (HIV); and pelvic inflammatory disease (PID), including endometritis.[10−13]

PID and endometritis are upper genital tract infections with a range of clinical presentations and manifestations.[4] Acute PID is caused by the ascension of strict or facultative anaerobes from the vagina to the endometrium and adnexa for ≤30 days. Chronic endometritis is an infection that lasts ≥30 days.[4] Greater than 85% of PID cases are caused by BV-related bacteria and/or STIs.[4,14] Of those cases, fewer than half are caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*, suggesting an important role for ascension of BV-associated anaerobic bacteria and other non-BV−related pathogens (eg, *Mycoplasma genitalium*) in endometritis and PID pathophysiology.[15−17] PID and endometritis are associated with adverse health outcomes, such as chronic pain, ectopic pregnancy, tubo-ovarian abscess, and infertility.[18,19]

In this review article, we will describe the current evidence for the associations among BV, PID, and endometritis. Moreover, the impact of untreated BV and PID on infertility will be reviewed.

Bacterial Vaginosis

Diagnosis and treatment of bacterial vaginosis

Patients showing symptoms of BV typically present with increased levels of vaginal discharge associated with a strong fishy odor. When women present to a healthcare provider with symptoms, BV is usually diagnosed using the Amsel criteria, which evaluate the presence of 4 signs and symptoms (Table 1).[20−22] The presence of at least 3 of these signs and symptoms must be met to fulfill the diagnosis of BV.[20,21] Although the Amsel criteria are easy to assess and are associated with good predictive values, commercial molecular tests have also been developed to detect BV, which may be useful in cases where microscopy is not available.[20] In research settings, BV is diagnosed using the Nugent scoring system,[23] which uses a 0 to 10 score to estimate the presence of vaginal bacterial morphotypes that are characteristic of BV using Gram staining and microscopic examination. High Nugent scores reflect the absence of *Lactobacillus* and presence of the strict anaerobes *Gardnerella* and *Mobiluncus* spp. or BV-associated bacteria.[24] In contrast, low Nugent scores represent a high abundance of *Lactobacillus* spp. and relative absence of anaerobes. Molecular BV testing, such as genetic sequencing or polymerase chain reaction, may also be used in research studies to complement Nugent scoring.[25]

Patients with symptomatic BV are treated with either oral or intravaginal antibiotics. In the 2020 American College of Obstetricians and Gynecologists Practice Bulletin on vaginitis in nonpregnant patients, recommended treatments included oral metronidazole, intravaginal metronidazole gel, or intravaginal clindamycin cream (Table 2).[22] Single-dose oral secnidazole was approved by the US Food and Drug Administration for the treatment of BV in 2017[26] and was reported to provide a cure rate that was comparable with a 7-day oral metronidazole regimen in a research setting in which patients were at least 80% adherent to treatment.[27] Because these treatments have comparable safety and efficacy profiles, the choice of therapy should be individualized on the basis of factors, such as patient preferences, cost, convenience, adherence, ease of use, and history of response to previous treatments or adverse reactions.[22] Unfortunately, although treatment efficacy is high at 3 to 4 weeks after treatment, BV is highly recurrent, and 58% of women recur within a year.[28] It has been hypothesized that nonadherence to multidose therapy could contribute to the development of recurrent BV, although this association has not yet been tested in clinical trials.[29] Additional putative reasons for recurrence include the persistence of residual infection. For example, biofilms that protect BV-associated bacteria from antimicrobial drugs foster persistence. Resistance to antimicrobial drugs and reinfection from partners of either sex may play a role. Nevertheless, the underlying mechanisms of recurrent etiology of BV are not fully understood.[30]

A substantial percentage of women have asymptomatic BV (ie, Nugent score between 4 and 10 but no symptoms). Some patients may also have 3 of 4 symptoms but do not report symptoms on direct questioning. Guidelines issued by the Centers for Disease Control and Prevention do not recommend treatment for these women, as there is a lack of evidence that treatment for asymptomatic BV decreases adverse outcomes, although recurrence and associated costs are high in asymptomatic women.[31] Advances in BV treatment include antibiotics that require less frequent dosing, such as secnidazole, and treatment that combines antimicrobials and *Lactobacillus crispatus*−containing probiotics to address recurrence.[32] In a recent double-blind, placebo-controlled trial to evaluate the ability of *L crispatus* to prevent recurrence, women aged 18 to 45 years with a diagnosis of BV who had completed a course of vaginal metronidazole gel were randomly assigned to receive vaginally administered *L crispatus* or placebo for 11 weeks, with follow-up through week 24. The use of *L crispatus* after treatment with vaginal metronidazole resulted in a significantly lower incidence of BV recurrence vs placebo at 12 weeks (30% vs 45%; P=.01).[33]

Bacterial vaginosis and fertility

BV has been linked to increased risk of infertility, particularly tubal infertility.[34−39] In a study of women undergoing oocyte recovery for in vitro fertilization (IVF), seropositivity for *Chlamydia* species and the presence of BV were both strongly and independently associated with tubal infertility. However, there was no difference in pregnancy rates in any of the groups, regardless of serologic status for chlamydia or current BV.[34] In a sample of patients seeking fertility treatment, Nugent-BV was present in 31.5% of patients with tubal infertility and 19.7% of patients with nontubal infertility.[35] In a separate study, an intermediate Nugent score was reported in 12.1% of women presenting for fertility treatment, and Nugent-BV was reported in 24.3%, with a higher prevalence among women with tubal infertility (34.6%).[36] Furthermore,

TABLE 1
Amsel criteria for the diagnosis of BV

1. Homogenous, thin, grayish-white vaginal discharge that smoothly coats the vaginal walls
2. Presence of ≥20% clue cells on saline wet mount
3. Vaginal pH of >4.5
4. Positive whiff-amine test result

BV, bacterial vaginosis.

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idiopathic infertility has been linked to a unique vaginal bacterial signature that includes bacteria related to BV.[37,38] Because of the heterogeneity seen in studies reporting BV prevalence in infertile populations, it may also be of use to look at metaanalysis results. In a systematic review and metaanalysis of studies assessing BV and infertility, BV was 3.3 times more likely to be identified in infertile women than in antenatal women within the same population.[39] In a systematic review and metaanalysis to evaluate the risks associated with BV in patients who underwent IVF, 16% BV prevalence was observed. However, the prevalence ranged from 4% to 38%, indicating a large heterogeneity in the studies examined, which may be explained by different diagnostic methods, ethnicities, and types of infertility. Tubal factor infertility was significantly prevalent among patients who underwent IVF and who were positive for BV, suggesting a shared pathogenesis (P=.001).[40]

In the setting of IVF, BV has been implicated in difficulty conceiving. Women with a lower prevalence of vaginal lactobacilli were less likely to have successful embryo implantation than those with a higher prevalence of lactobacilli.[41] Furthermore, women with lower microbial diversity and those with a higher proportion of abnormal vaginal microbiota were more likely to have poor reproductive outcomes following IVF.[42] Nevertheless, the metaanalysis of 12 studies in the IVF setting found that BV did not significantly impact the live birth rate (relative risk [RR], 1.47; 95% confidence interval [CI], 0.96−1.57) or the clinical pregnancy rate (RR, 0.93; 95% CI, 0.75−1.15).[40] Although there is a clear association between BV and infertility, causality has not been conclusively determined; further research that includes large-scale longitudinal and mechanistic studies are needed.

Although the cause of infertility among patients with BV is unclear, several mechanisms have been proposed. One possibility is the association between BV microbiota and subsequent inflammation, which may lead to reduced fertility. BV-related bacteria have been shown to induce immune activation through dendritic cell maturation and to increase levels of proinflammatory cytokines, resulting in mucosal inflammation of the genital tract.[43,44] Higher levels of cervical interleukin (IL)-1β, IL-6, and IL-8 cytokines have been reported in women with infertility and BV.[45] Restoration of normal vaginal microbiota with use of a probiotic vaginal tablet containing lactobacilli has been shown to reduce levels of proinflammatory cytokines,

TABLE 2
ACOG treatment recommendations for the management of BV in nonpregnant patients

Drug	Formulation	Dosage	Duration
Recommended treatment regimens			
Metronidazole	Oral	500 mg, twice daily	7 d
Metronidazole	Intravaginal gel 0.75%	5 g, once daily	5 d
Clindamycin	Intravaginal cream 2%	5 g, once daily at bedtime	7 d
Alternative treatment regimens			
Secnidazole	Oral	2 g, single dose	1 d
Tinidazole	Oral	2 g, once daily	2 d
Tinidazole	Oral	1 g, once daily	5 d
Clindamycin	Oral	300 mg, twice daily	7 d
Clindamycin	Intravaginal ovules	100 mg, once daily at bedtime	3 d

ACOG, American College of Obstetricians and Gynecologists; *BV*, bacterial vaginosis.

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supporting the hypothesis that BV-associated bacteria may increase inflammation.[46] The species of *Lactobacillus* used in the vaginal tablet were *L brevis*, *L salivarius* subsp. *salicinius*, and *L plantarum*, not the more typical *L crispatus, L gasseri,* and *L jensenii* seen in an optimal vaginal environment. More research introducing these typical vaginal lactobacilli species into a vaginal tablet is warranted.[46] The presence of immune activation and inflammation at the vaginal mucosa may lead to immune targeting of seminal fluid components, which are highly antigenic. Seminal components binding to the acrosomal sperm to protect it are carried into the upper female genital tract. Various pathologic processes may occur at this juncture.[47] Ongoing research and analysis of these processes are warranted.

Another BV-related mechanism that may contribute to infertility is the effect of sialidase and other mucinases on cervical mucus integrity. In the female reproductive tract, a primary function of cervical mucus is the defense of the upper reproductive tract from microbial invasion. To overcome the mucus barrier, microorganisms may produce a range of hydrolyzing enzymes, including mucinases, that are capable of degrading mucins. These enzymes may also work to enhance bacterial adhesion and subsequent colonization in the upper reproductive tract by generating attachment sites on the mucosal surfaces and producing nutrition for bacteria from the mucin breakdown products,[48] fostering colonization with further propensity for upper reproductive tract disease, including infertility.

Women with BV are at increased risk for acquiring STIs, which are known to contribute to infertility. BV has been shown to increase susceptibility to *C trachomatis* and *N gonorrhoeae* by 3.4- and 4.1-fold, respectively.[49] Other incident infections linked to BV include *Trichomonas vaginalis,* herpes simplex virus, HPV, and HIV.[10,12,50] Vaginal colonization with lactobacilli has been shown to be protective from chlamydial or gonorrheal infections, suggesting a role for optimal *Lactobacillus*-dominated vaginal microbiota in preventing STI acquisition.[49] D-lactic acid, which is produced by *L crispatus, L gasseri,* and *L jensenii*—but not *L iners*—was shown to prevent infection by *C trachomatis* in vitro by directly affecting the function of the cervicovaginal epithelium.[51] This is important because STIs—and *C trachomatis* and *N gonorrhoeae* in particular—have been linked to an increased risk of upper genital tract infection, PID, and infertility.[52,53]

Finally, BV increases the risk of upper genital tract infection and PID, which have been linked to infertility. BV-related vaginal microbial signatures have been associated with increased risk of PID, whereas *Lactobacillus*-dominated microbiota did not increase the risk.[54] Women with acute endometritis were 90% less likely to have typical ratios of lactobacilli and were 2.4-fold more likely to have Nugent-BV.[16] BV has also been associated with subclinical PID, which is marked by asymptomatic ascension of infectious agents to the upper genital tract and is also associated with chlamydia or gonorrhea.[4,13] Subclinical PID is 2.7-fold more common in women with Nugent-BV.[13] The presence of BV-associated bacteria in the endometrium has also been associated with recurrent PID and persistent endometritis after recommended treatment with cefoxitin and doxycycline.[17]

These data provide support for the role of BV in infertility through a variety of mechanisms, including immune activation, inflammation, toxin production, STI susceptibility, and PID susceptibility.

Endometritis and Pelvic Inflammatory Disease

Diagnosis and treatment of endometritis and pelvic inflammatory disease

PID presents with a variety of signs and symptoms that are frequently nonspecific and include cervical motion tenderness, uterine tenderness, or adnexal tenderness on pelvic examination. Symptoms of acute endometritis are similar to those of PID, and outside of pregnancy, providers often use the terms endometritis and PID interchangeably. Positive endometrial biopsy with histopathologic evidence of endometritis or laparoscopic findings consistent with PID can also support the diagnosis with high specificity.[20]

In contrast with PID and acute endometritis, subclinical or chronic endometritis (CE) persists for a longer period and is either asymptomatic or associated with more subtle or nondescript symptoms such as pelvic discomfort, spotting, and leukorrhea. These symptoms are more difficult to diagnose, and there are no universally accepted diagnostic criteria for CE.[55] Studies have shown that the presence of endometrial stromal plasmacytes is a specific and sensitive finding for CE.[55,56] Common pathogens that have been detected in CE include *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Enterococcus faecalis*, *C trachomatis*, *Mycoplasma* spp., *Klebsiella pneumoniae*, and *Candida* spp.[57,58]

Endometritis, pelvic inflammatory disease, and fertility

PID and endometritis have been associated with infertility in past studies. The presence of BV-associated bacteria in the endometrium has been linked to a 3.4-fold increased risk of infertility.[17] In a study of women with Nugent-BV, gonorrhea, or chlamydia or at risk of infections, such as gonorrhea or chlamydia, researchers prospectively evaluated pregnancy outcomes after a biopsy was performed to identify endometritis. Participants were treated for BV and other infections. After a median of 2.1 years of follow-up, women with subclinical PID at diagnosis had a 40% decreased likelihood of pregnancy compared with those without subclinical PID. A study limitation is that the women enrolled were not known to be specifically trying to get pregnant nor was fertility intent queried during follow-up, which could confound the assessment of an association between BV and infertility because of endometritis.[59] Similar results, however, were found in a large population-based study of women who underwent diagnostic laparoscopy for PID. Tubal infertility was found in 10.8% of patients diagnosed with PID compared with 0% of those who tested

negative.[18] In addition, in the US National Health and Nutrition Examination Survey (2013−2016), 2626 women of reproductive age self-reported infertility and treatment for PID. Infertility was reported by 24.2% of women with past PID treatment compared with 13.3% of women without PID treatment.[60]

Further support is provided for the association between endometritis and PID and infertility based on data from women treated with assisted reproduction. CE has been shown to be highly prevalent among patients with unexplained infertility (34%−66%).[61] In women undergoing IVF, those with endometrial microbiota dominated by nonlactobacilli were significantly less likely to have successful implantation, pregnancy, or ongoing pregnancy than those with microbiota dominated by lactobacilli (>90%) ($P<.05$).[62] However, it is important to note that although lower prevalence of lactobacilli have been associated with BV and endometritis, there is no standard definition of abnormal and normal endometrial microbiota, and the abundance of these bacteria in the endometrium is unknown.[63] Nonetheless, CE cure with antimicrobial treatment has been shown to improve outcomes in women undergoing IVF.[61] For example, compared with women with cured CE, those with persistent CE had a lower pregnancy rate (33.0% vs 65.2%; $P=.039$) and lower live birth rate (60.8% vs 13.3%; $P=.02$) after IVF.[61]

Although the precise etiology of endometritis and PID-associated infertility is unclear, several pathophysiological contributors have been proposed. As with BV, endometritis has been associated with disrupted inflammatory and immunologic signatures. The endometrium contains many immunocompetent cells that contribute to regulation of inflammation, immune response, and trophoblast implantation and growth.[64] Many of these immune cell populations have been shown to be altered among women with endometritis, which can lead to uterine immune cell infiltration. The altered immune environment has been shown to lead to abnormal uterine expression levels of chemokines and adhesion molecules in patients with CE, which may impact trophoblast implantation.[65] Taken together, it is possible that these immunologic and inflammatory changes disrupt endometrial function and decrease receptiveness of the endometrium to embryo implantation and development.[66]

PID is most frequently linked to tubal infertility, which may be explained by pathologic tubal inflammation, fibrosis, and subsequent scarring.[52] This has been most frequently studied in the context of *C trachomatis* infection−associated PID, which appears to lead to an innate immune response mediated by infected epithelial cells and an adaptive T-cell response.[67,68] In a macaque model, recurrent *C trachomatis* infections led to mononuclear infiltration (primarily CD8 T cells), fibroblast proliferation, and connective tissue deposition, culminating in fibrosis of the fallopian tubes.[67] Evidence has also demonstrated the effect of *N gonorrhoeae* as a pathogen involved in reproductive tract morbidities, including tubal factor infertility and PID. Moreover, limited evidence suggests that other organisms, such as *M genitalium* and *T vaginalis*, and variations in the overall vaginal microbiome, such as those that occur in BV, may contribute to conditions that interfere with female fertility.[52]

Managing Bacterial Vaginosis, Pelvic Inflammatory Disease, and Endometritis Before Pregnancy

Diagnosis of BV, PID, and endometritis may be complicated, because symptoms can vary and be mild, nonspecific, or absent. There can be difficulty in identifying pathogens in the endometrium by means of microbial culture, with only a 20% concordance among histology, hysteroscopy, and microbial culture results.[69] There is also the potential for contamination of endometrial samples with vaginal bacteria.[63] Therefore, a low level of clinical suspicion for BV, CE, or PID should be sufficient for initiating testing in women with risk factors for these infections, such as a history of STIs or sexual behaviors that could lead to transmission.[70] This may be particularly true for women suffering from infertility or tubal infertility.

Previous studies have shown that treatment of genital infections may improve fertility outcomes. Successful treatment of women with CE resulted in a significantly higher pregnancy rate than those with persistent disease and those without endometritis diagnosis (76.3% vs 20% vs 9.5%; $P<.0001$).[61] For women undergoing IVF, those with cured CE had a 6.8-fold higher ongoing pregnancy and live birth rate and a 4.0-fold higher clinical pregnancy rate than those with persistent disease.[71] In addition to CE treatment, some fertility specialists have suggested that colonization of the catheter transfer tip with beneficial lactobacilli at the time of embryo transfer may improve implantation rates.[72] Although BV treatment and its association with successful reproductive outcomes through natural or assisted means have not been evaluated, the benefits of preventing progression to PID and CE are likely to positively affect the chances of natural and assisted conception. Clinical trials assessing the impact of BV treatment on successful conception may be difficult to perform because of the prevalence of recurrent BV and challenges associated with treating this condition.

Treatment is recommended for symptomatic women. The benefits of therapy in nonpregnant women are to relieve symptoms and signs of infection and reduce the risk of acquiring *C trachomatis*, *N gonorrhoeae*, *T vaginalis*, HIV, and herpes simplex type 2.[20] Treatment of PID with metronidazole and ceftriaxone and doxycycline was shown to eradicate anaerobic bacteria from the endometrium and decrease pelvic tenderness at 30 days.[73] A more recent trial by this group showed further evidence of the benefit of metronidazole with ceftriaxone and doxycycline for treating PID; it also reduced the detection of *M genitalium* at 30 days.[74] For symptomatic patients, testing for BV or endometritis is relatively cost-effective and can be performed during a routine preconception pelvic examination, which may already be a component of the preconceptional

appointment. On laboratory confirmation of BV and CE, treatment with antibiotics is warranted and may improve fertility outcomes. For asymptomatic patients with suspicion of CE because of previous subclinical infections and with an unknown cause of infertility, repeated implantation failures, repeated miscarriages, or previous pregnancies with intrauterine infections, such as chorioamnionitis or deciduitis, screening for CE should also be considered.

Conclusions

BV, endometritis, PID, and infertility are related to interconnected pathophysiological pathways. Immunity, inflammation, cervicovaginal microbiota, and fibrotic pathways all play a role in contributing to infertility; however, additional large, prospective, longitudinal studies are needed to conclusively determine the link among BV, PID, endometritis, and infertility. Until then, symptomatic BV (and possibly asymptomatic BV) should be urgently treated to prevent BV sequelae, including STIs, PID, and endometritis, which all seem to contribute to an increased risk of infertility. ■

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