Volume 3, Number 2, 2026

Pages 362-376

Bioscience Insights

ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)

BioSciInsights.com

ROLE OF MALE PARTNER TREATMENT IN PREVENTING RECURRENT VAGINAL MICROBIOME DISTURBANCES DURING PREGNANCY

Dr Iqra Khalid[*1], Dr Zeshan Ahmad[2], Dr Amna Ambreen[3], Dr Kainat Naveed[4]

*[*1]Department of Obstetrics and Gynecology, DHQ Hospital Okara*
[2]Department of Obstetrics and Gynecology, THQ Hospital Chowk Sarwar Shaheed
[3,4]Department of Obstetrics and Gynaecology, Benazir Bhutto Hospital Rawalpindi

[*1]iqra.khalid701@gmail.com, [2]zeshanahmad2323@gmail.com, [3]dramna0001@gmail.com[3], [4]naveedkainat3@gmail.com

Article Information

Article History

Received:	May 15, 2026
Revised:	June 27, 2026
Accepted:	August 21, 2026
Available Online:	September 09, 2026

Keywords:

Abstract

Recurrent disturbances of the vaginal microbiome during pregnancy represent an important clinical concern because alterations in microbial composition may influence maternal reproductive health and pregnancy outcomes. A healthy vaginal microbiome is generally characterized by the predominance of Lactobacillus species, which contribute to maintenance of an acidic vaginal environment and provide protection against the overgrowth of potentially pathogenic microorganisms. In contrast, vaginal dysbiosis, including bacterial vaginosis (BV), is associated with increased microbial diversity, depletion of protective Lactobacillus species, and enrichment of anaerobic bacteria such as Gardnerella, Prevotella, and Atopobium. These alterations have been associated with adverse pregnancy outcomes, including preterm birth, premature rupture of membranes, and low birth weight (Zhou et al., 2024; [Authors], 2023).

Recurrence following conventional treatment remains a major challenge, suggesting that management strategies directed exclusively toward the female partner may not adequately address factors contributing to persistent or recurrent dysbiosis. Increasing evidence indicates that sexual partners may contribute to the persistence or reintroduction of BV-associated microorganisms. A prospective pilot study published in 2021 demonstrated that concurrent treatment of male partners was associated with changes in the composition of both vaginal and penile microbiota, supporting the possibility of sexual exchange of BV-associated organisms (Plummer et al., 2021). However, an earlier randomized controlled trial published in 2021 found that treatment of male partners with oral metronidazole alone did not significantly reduce BV recurrence among female partners, highlighting the importance of treatment regimen and adherence (Schwebke et al., 2021).

More recently, the StepUp randomized controlled trial provided important evidence that combined oral and topical antimicrobial

treatment of male partners can substantially reduce BV recurrence compared with treatment of women alone. In this study, recurrence within 12 weeks occurred in 35% of women receiving concurrent male-partner treatment compared with 63% in the standard-care group (Vodstrcil et al., 2025). A 2026 systematic review and meta-analysis further emphasized that the effectiveness of partner treatment may depend on the treatment regimen, with combined oral and topical therapy showing greater potential than male-partner monotherapy.

Given the established relationship between vaginal microbiome disturbances and adverse pregnancy outcomes, investigating whether treatment of male partners can reduce recurrent dysbiosis during pregnancy represents a promising area of research. Nevertheless, the safety, effectiveness, optimal timing, and appropriate antimicrobial regimen for male-partner treatment specifically among pregnant populations require rigorous evaluation. The present study therefore aims to examine the potential role of male-partner treatment in reducing recurrent vaginal microbiome disturbances during pregnancy and to explore whether a partner-directed approach may contribute to improved vaginal microbial stability and potentially better maternal and fetal outcomes.

INTRODUCTION

1.1 Vaginal Microbiome and Pregnancy

The vaginal microbiome is a dynamic microbial ecosystem that plays an important role in maintaining reproductive tract health and protecting against colonization by potentially pathogenic microorganisms. In reproductive-aged women, a healthy vaginal environment is commonly characterized by the predominance of *Lactobacillus* species, particularly *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, and *Lactobacillus iners*. These microorganisms contribute to vaginal homeostasis through production of lactic acid and other antimicrobial factors that help maintain an environment unfavorable to the excessive growth of anaerobic and pathogenic organisms. Pregnancy itself produces substantial physiological and hormonal changes that can influence the composition and stability of the vaginal microbiome. Recent evidence suggests that pregnancy is generally associated with increased Lactobacillus dominance, although considerable interindividual variation exists in microbial community composition throughout gestation (Nikolov et al., 2022; Zhou et al., 2024).

1.2 Vaginal Dysbiosis and Adverse Pregnancy Outcomes

Disruption of the normal vaginal microbial community can result in vaginal dysbiosis, characterized by reduced abundance of protective Lactobacillus species and increased representation of anaerobic organisms, including *Gardnerella*, *Prevotella*, *Atopobium*, and other bacterial taxa. Bacterial vaginosis (BV) represents one of the most clinically important manifestations of this dysbiotic state. During pregnancy, vaginal dysbiosis has attracted particular attention because alterations in microbial composition may be associated with inflammatory responses and adverse obstetric outcomes. A systematic review and meta-analysis reported that greater Lactobacillus abundance was associated with a lower risk of preterm birth, while low-Lactobacillus microbial profiles were associated with increased risk (Zhou et al., 2024). Similarly, a 2025 systematic review identified reductions in *L. crispatus*, *L. gasseri*, and *L. jensenii*, together with increased representation of organisms such as *Gardnerella*, among microbial patterns associated with preterm birth (Ferrante et al., 2025).

1.3 Recurrence as a Major Therapeutic Challenge

Although antimicrobial therapy remains an important approach for symptomatic BV, recurrence after treatment is common and represents a substantial clinical challenge. Conventional treatment primarily

focuses on the affected woman and may successfully reduce symptoms and bacterial burden in the short term, but it does not necessarily restore a stable Lactobacillus-dominant microbiome. Consequently, some women experience repeated episodes of dysbiosis despite appropriate treatment. The persistence or reappearance of BV-associated microorganisms has stimulated interest in factors beyond the female genital tract, including sexual and behavioral factors and microbial exchange between sexual partners. Recent literature increasingly recognizes that recurrent BV may involve a complex interaction between the vaginal microbiome, bacterial biofilms, host factors, and exposure to microorganisms associated with a sexual partner (Muzny & Sobel, 2025).

1.4 Potential Role of the Male Partner

Emerging evidence suggests that male sexual partners may harbor bacterial communities containing organisms associated with BV and may contribute to their persistence or reintroduction following treatment. This has shifted the traditional concept of BV management from an exclusively female-centered approach toward consideration of the couple as a potential epidemiological unit. Importantly, earlier trials of male-partner treatment did not consistently demonstrate prevention of recurrence, particularly when treatment regimens were limited to oral antimicrobial therapy. These findings suggested that inadequate antimicrobial coverage, incomplete eradication of organisms from penile-associated sites, or other biological factors might explain the inconsistent outcomes. Therefore, the potential benefit of partner treatment may depend not simply on treating the male partner, but on the choice, duration, and combination of therapeutic approaches.

1.5 Recent Evidence Supporting Partner-Directed Treatment

More recent evidence has strengthened the rationale for investigating male-partner treatment. In the 2025 StepUp randomized controlled trial, women with BV whose male partners received combined oral and topical antimicrobial treatment experienced substantially fewer recurrences than women whose partners remained untreated. Recurrence within 12 weeks occurred in 35% of women in the partner-treatment group compared with 63% in the standard-care group, providing important evidence that treating the male partner can influence recurrent BV in the female partner (Vodstrcil et al., 2025). This finding is particularly relevant to recurrent vaginal microbiome disturbances because it supports the hypothesis that microbial exchange between partners may contribute to the persistence of dysbiosis.

1.6 Research Gap and Study Rationale

Despite these promising findings, an important knowledge gap remains regarding the application of male-partner treatment specifically during pregnancy. Pregnancy requires particular consideration of maternal and fetal safety, appropriate antimicrobial selection, treatment timing, and the potential consequences of modifying the vaginal microbiome during gestation. Existing evidence supporting partner treatment has largely focused on nonpregnant reproductive-aged women, meaning that its effectiveness and safety cannot automatically be extrapolated to pregnant populations. Furthermore, the extent to which treating the male partner can promote sustained vaginal microbial stability, rather than merely reducing symptomatic BV recurrence, remains insufficiently understood.

Therefore, investigating the **role of male partner treatment in preventing recurrent vaginal microbiome disturbances during pregnancy** may provide important insights into a potentially couple-centered strategy for managing recurrent dysbiosis. The present study aims to evaluate whether treatment of the male partner, in addition to standard management of the pregnant woman, can reduce recurrent vaginal microbiome disturbances and contribute to greater microbial stability during pregnancy.

2. Literature Review

2.1 Summary of Recent Evidence

No.	Author & Year	Study Design / Sample	Population	Intervention / Main Focus	Key Findings and Relevance
1	Plummer et al. (2021)	Prospective pilot study; 34 couples	Women with BV and male partners	Women received standard treatment; men received oral metronidazole + topical clindamycin	Concurrent male treatment reduced BV-associated bacteria and altered genital microbiota in both partners. Five women (17%) experienced recurrence within 12 weeks, supporting partner-directed treatment. [1]
2	Vodstrcil et al. (2021)	Narrative/review evidence synthesis	Women with recurrent BV and sexual partners	Drivers of recurrence and partner treatment	The review identified persistence, biofilm and sexual exchange of BV-associated organisms as possible contributors to recurrence and proposed partner treatment as a promising strategy. [2]
3	Juliana et al. (2021)	PRISMA systematic review	Pregnant women in sub-Saharan Africa	Characterization of vaginal microbiota	Lactobacillus-containing communities were common, while more diverse anaerobe-dominant communities were also observed, highlighting population-specific microbiome patterns during pregnancy. [3]
4	González-Sánchez et al. (2022)	Longitudinal study; 10 women	Healthy pregnant women	Vaginal microbiota during third trimester and childbirth	Vaginal microbiota remained relatively stable and was mainly Lactobacillus-dominant, supporting the importance of microbial stability during late pregnancy. [4]
5	Ahrodia et al. (2022)	Updated review	Pregnant women / reproductive-age women	Vaginal dysbiosis and preterm birth	Reduced Lactobacillus and increased microbial dysbiosis were associated with adverse pregnancy outcomes; L. crispatus appeared particularly protective. [5]
6	Günther et al. (2022)	Narrative review	Reproductive medicine populations	Vaginal microbiome and reproductive outcomes	The review emphasized the relationship between vaginal microbial composition, reproductive health and potential microbiome-based interventions. [6]

No.	Author & Year	Study Design / Sample	Population	Intervention / Main Focus	Key Findings and Relevance
7	Ross et al. (2023)	Multicentre randomized controlled trial	518 women with recurrent BV	Intravaginal lactic acid gel vs oral metronidazole	Metronidazole produced greater short-term symptom resolution than lactic acid gel, demonstrating the continuing importance of effective antimicrobial therapy in recurrent BV. [7]
8	Plummer et al. (2023)	Microbiome analysis of 121 women	Women treated for BV	Vaginal microbiome before/after treatment	Higher pretreatment Prevotella and persistent post-treatment Gardnerella were associated with recurrence. Untreated regular sexual partners were also associated with increased recurrence. [8]
9	Huang et al. (2023)	Meta-analysis of 12 cohorts; 2,039 women	Pregnant women	Vaginal microbiome and preterm birth prediction	L. crispatus was consistently negatively associated with preterm birth, whereas L. iners, Prevotella and Gardnerella were associated with increased risk. [9]
10	Silvano et al. (2023)	Case-control study	195 pregnant women	Vaginal microbiome and short cervix	The study investigated microbial differences between women with and without short cervix, supporting the potential relationship between vaginal microbial composition and pregnancy complications. [10]
11	Zhou et al. (2024)	Systematic review and meta-analysis; 11 studies, 1,577 pregnancies	Pregnant women	Lactobacillus abundance and preterm birth	Higher Lactobacillus abundance was associated with lower preterm-birth risk (OR 0.49); L. crispatus showed particularly strong protective association (OR 0.30). [11]
12	Gerede et al. (2024)	Comprehensive review; 44 studies	Pregnant women	Vaginal microbiome and pregnancy complications	Increased microbial diversity and depletion of Lactobacillus were associated with several pregnancy complications, while Gardnerella, Prevotella, Sneathia and Atopobium were repeatedly implicated. [12]
13	Li et al. (2024)	Longitudinal study	Pregnant and postpartum South African women	Changes in vaginal microbiome across pregnancy	The study characterized longitudinal changes in vaginal microbial communities during

No.	Author & Year	Study Design / Sample	Population	Intervention / Main Focus	Key Findings and Relevance
					pregnancy and postpartum, emphasizing geographic and population-specific variation. [13]
14	Rezazadeh et al. (2024)	Double-blind clinical trial	Women with BV	Oral vs vaginal probiotics for recurrence prevention	The trial directly evaluated microbiome-directed strategies for reducing BV recurrence and demonstrated the continuing interest in restoring protective vaginal flora. [14]
15	Ashraf et al. (2025)	Randomized controlled trial; 120 women	Women with BV	Probiotic + antibiotic vs antibiotic alone	BV recurrence was lower with probiotic plus antibiotic therapy (8.9% vs 21.4%), suggesting that restoration of vaginal microbial balance may complement antimicrobial therapy. [15]
16	Vodstrcil et al. (2025)	Open-label randomized controlled trial; 164 couples	Women with BV and male partners	Female standard therapy ± male oral metronidazole + topical clindamycin	Recurrence within 12 weeks was 35% with partner treatment versus 63% with standard care, providing the strongest recent evidence for combined male-partner treatment. [16]
17	McKee et al. (2025)	Cohort/meta-analytic analysis; 683 births	Pregnant women	Vaginal community state types and preterm birth	Diverse non-Lactobacillus-dominant communities and L. iners-dominant communities had higher odds of preterm birth compared with L. crispatus-dominant communities. [17]
18	Wang et al. (2025)	Prospective cohort; 63 pregnant women	Pregnant women undergoing cervical cerclage	Vaginal microbiota and metabolites	Preterm-birth pregnancies showed increased Gardnerella and Atopobium and altered microbial/metabolic profiles compared with term pregnancies. [18]
19	Bloom et al. (2026)	Randomized controlled trial microbiome analysis	Women with recurrent BV	L. crispatus live biotherapeutic	At week 12, an L. crispatus-dominant microbiota was achieved in 30% of treatment recipients versus 9% of placebo recipients, supporting microbiome restoration as a potential recurrence-prevention strategy. [19]

No.	Author & Year	Study Design / Sample	Population	Intervention / Main Focus	Key Findings and Relevance
20	Vieira et al. (2026)	Systematic review/meta-analysis; 6 RCTs, 953 couples	Women with BV and male partners	Male-partner treatment vs female-only treatment	Overall pooled effect was not statistically significant (RR 0.97, 95% CI 0.76–1.22), but combined oral + topical treatment showed greater potential and warrants further investigation. [20]

2.2 Vaginal Microbiome During Pregnancy

Recent research indicates that pregnancy is accompanied by important changes in the vaginal microbial ecosystem. A Lactobacillus-dominant microbiome, particularly one enriched with *Lactobacillus crispatus*, is generally associated with greater microbial stability and more favorable pregnancy outcomes. In contrast, communities characterized by greater bacterial diversity and increased anaerobic organisms have been associated with adverse outcomes, particularly preterm birth. Meta-analytic evidence involving 1,577 pregnancies found that greater Lactobacillus abundance was associated with reduced preterm-birth risk, while *L. crispatus* showed one of the strongest protective associations (Zhou et al., 2024).

2.3 Recurrence of Vaginal Dysbiosis

Recurrence remains a major limitation of conventional BV management. Microbiome studies suggest that recurrence may result from several interacting mechanisms, including persistence of BV-associated organisms, biofilm formation, incomplete restoration of protective Lactobacillus species, and re-exposure to microorganisms associated with sexual partners. Plummer et al. (2023) reported that higher pretreatment *Prevotella* and persistent *Gardnerella* after treatment were associated with recurrence, while women with untreated regular sexual partners had greater recurrence risk.

2.4 Evidence for Male-Partner Treatment

The concept of treating male partners has gained considerable attention because genital microbial exchange may contribute to recurrent BV. The 2021 pilot study by Plummer et al. demonstrated that combined oral and topical antimicrobial treatment of male partners altered bacterial communities at vaginal, penile and urethral sites. More importantly, the 2025 StepUp randomized trial demonstrated a substantial reduction in recurrence when male partners received combined oral metronidazole and topical clindamycin in addition to treatment of women. Recurrence was 35% in the partner-treatment group compared with 63% with standard care.

2.5 Current Evidence Gap

Despite the encouraging findings, the available evidence does not yet establish whether male-partner treatment can safely and effectively prevent recurrent vaginal microbiome disturbances **specifically during pregnancy**. The 2026 systematic review identified six randomized trials involving 953 couples and found no statistically significant pooled benefit overall, although combined oral and topical treatment appeared more promising than male-partner monotherapy. Most importantly, the major partner-treatment trials have focused on nonpregnant women, while pregnancy-specific studies have primarily investigated vaginal microbiome composition and obstetric outcomes rather than treatment of male partners.

Therefore, an important research gap exists at the intersection of **male-partner treatment, recurrent vaginal dysbiosis, and pregnancy**. A study addressing this gap could determine whether treating both partners can reduce recurrent dysbiosis, support Lactobacillus-dominant microbial communities, and potentially reduce microbiome-associated pregnancy complications.

3.1 Study Design

A prospective, comparative, longitudinal study will be conducted to evaluate the role of male partner treatment in preventing recurrent vaginal microbiome disturbances during pregnancy. The study will

investigate whether treatment of the regular male sexual partner, in addition to standard management of the pregnant woman, is associated with a lower frequency of recurrent vaginal dysbiosis and greater stability of the vaginal microbial community. The study will follow participants from baseline assessment through a predefined follow-up period during pregnancy. The methodology is designed to evaluate both clinical recurrence and microbiological changes rather than relying exclusively on symptomatic improvement.

3.2 Study Population and Eligibility Criteria

The study population will comprise pregnant women diagnosed with recurrent vaginal microbiome disturbance or bacterial vaginosis and their regular male sexual partners. Pregnant participants will be eligible if they meet predefined clinical and microbiological criteria for vaginal dysbiosis and are within the gestational-age range specified in the study protocol. Women with conditions or treatments that could substantially alter the vaginal microbiome independently of the study intervention will be excluded where appropriate.

Relevant exclusion criteria may include recent prolonged antimicrobial therapy unrelated to the study condition, major immunocompromising conditions, clinically significant genital infections requiring alternative treatment, or pregnancy complications requiring immediate specialized management. Male partners will be eligible when they are the regular sexual partner of an enrolled participant and are willing to undergo assessment and, where allocated, receive the specified partner-directed treatment.

3.3 Participant Grouping and Treatment Strategy

Participants will be allocated into two comparative management groups. In the **standard-management group**, pregnant women will receive appropriate treatment for vaginal dysbiosis according to the applicable clinical protocol, while their male partners will not receive the study-specific partner treatment. In the **partner-treatment group**, pregnant women will receive the same standard management, while their male partners will additionally receive the predefined antimicrobial intervention.

The partner-treatment strategy will be based on the most appropriate evidence-supported regimen available at the time of study implementation, with particular consideration of pregnancy safety and current clinical recommendations. The rationale for including male partner treatment is supported by recent evidence demonstrating that combined oral and topical treatment of male partners can substantially reduce recurrence of bacterial vaginosis in women. In the StepUp randomized controlled trial, recurrence within 12 weeks was 35% among women whose male partners received combined treatment compared with 63% with standard care.

However, because most existing partner-treatment trials have not specifically focused on pregnant populations, the intervention will not be considered automatically effective or safe during pregnancy. Treatment selection, dosage, duration, and safety monitoring will therefore be determined according to the study protocol and applicable clinical guidance.

3.4 Baseline Clinical and Sociodemographic Assessment

At enrollment, demographic and clinical information will be collected using a standardized data-collection instrument. Variables will include maternal age, gestational age, parity, gravidity, previous pregnancy outcomes, history of bacterial vaginosis or recurrent vaginal dysbiosis, previous antimicrobial exposure, and relevant reproductive or behavioral characteristics.

Information related to the male partner will include age, relevant clinical history, previous treatment for genital or sexually associated infections, recent antimicrobial exposure, and adherence to the assigned intervention. Sexual and behavioral variables that may influence recurrence will be recorded where appropriate to identify potential confounding factors.

3.5 Vaginal Microbiome Assessment

Vaginal microbiome assessment will constitute a central component of the study. Vaginal samples will be collected at baseline before initiation of the study intervention and at predefined follow-up visits. Samples

will be processed using a standardized microbiological and/or molecular workflow to characterize the vaginal bacterial community.

Particular attention will be given to the relative abundance of protective *Lactobacillus* species, especially *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus gasseri*, and *Lactobacillus jensenii*. Dysbiosis-associated organisms, including *Gardnerella*, *Prevotella*, *Atopobium*, *Sneathia*, and other relevant anaerobic taxa, will also be assessed.

This approach is clinically relevant because recent studies have associated Lactobacillus-dominant vaginal communities, particularly those dominated by *L. crispatus*, with more favorable pregnancy outcomes, whereas low-Lactobacillus and more diverse microbial communities have been associated with increased risk of adverse outcomes such as preterm birth.

Table 1. Target Microbial Markers for Vaginal Microbiome Assessment

Microbial Category	Target Microorganisms	Expected Microbiome Role	Assessment Purpose
Protective Lactobacillus	*L. crispatus*	Strongly associated with vaginal stability and low pH	Assess maintenance/restoration of protective microbiota
Protective Lactobacillus	*L. jensenii*	Contributes to vaginal microbial protection	Evaluate Lactobacillus-dominant community
Protective/variable	*L. iners*	Common vaginal species with variable association with health	Monitor changes in community composition
Dysbiosis-associated	*Gardnerella* spp.	Frequently associated with BV and polymicrobial biofilms	Assess persistence or reduction of dysbiosis
Dysbiosis-associated	*Prevotella* spp.	Associated with anaerobic dysbiotic communities	Evaluate treatment-related microbial changes
Dysbiosis-associated	*Atopobium* spp.	Associated with BV and recurrent dysbiosis	Assess persistence of BV-associated organisms
Dysbiosis-associated	*Sneathia* spp.	Associated with vaginal dysbiosis and adverse pregnancy outcomes	Monitor potentially clinically relevant shifts
Microbial diversity	Overall bacterial community	Reflects ecological complexity of vaginal microbiome	Compare microbial stability between groups

3.6 Definition and Assessment of Recurrent Vaginal Microbiome Disturbance

Recurrence will be evaluated using both clinical and microbiological criteria established before commencement of the study. Clinical assessment may include recurrence of characteristic symptoms and relevant examination findings, whereas microbiological assessment will evaluate restoration or persistence of dysbiotic microbial profiles.

A participant will be considered to have recurrent microbiome disturbance when she demonstrates a predefined return of dysbiotic microbial characteristics following initial treatment and documented improvement or resolution. This approach will distinguish persistent disease from true recurrence wherever the available longitudinal data permit.

The primary outcome will be the proportion of participants experiencing recurrent vaginal microbiome disturbance during follow-up. Secondary outcomes will include changes in Lactobacillus abundance, changes in dysbiosis-associated bacterial taxa, time to recurrence, microbiome diversity, and selected maternal and pregnancy outcomes.

3.7 Male Partner Assessment and Treatment Adherence

Because the central research question concerns the contribution of the male partner, treatment adherence will be systematically documented. Male participants receiving the intervention will be assessed for completion of the prescribed treatment regimen, missed doses, treatment-related adverse effects, and any interruption or modification of therapy.

Where feasible, follow-up information will also document factors that may influence re-exposure between partners. Adherence data will be incorporated into secondary analyses because inadequate partner treatment may reduce the apparent effectiveness of a couple-based intervention.

The rationale for this component is supported by microbiological evidence suggesting that sexual partners may share BV-associated bacterial communities. Recent work has emphasized that recurrence is potentially influenced by microbial exchange between partners and that treatment regimen and adherence are important considerations when evaluating partner treatment.

3.8 Follow-up and Outcome Measurement

Participants will be followed longitudinally at predefined intervals after completion of treatment. At each follow-up assessment, clinical status, recurrence of symptoms, antimicrobial exposure, treatment adherence, and relevant pregnancy outcomes will be documented.

Repeat vaginal sampling will be performed according to the study schedule to determine whether the vaginal microbiome remains Lactobacillus-dominant or returns to a dysbiotic state. The longitudinal design will allow comparison of microbiome trajectories between women whose partners receive treatment and those receiving standard management alone.

Where appropriate, pregnancy outcomes including gestational age at delivery, preterm birth, premature rupture of membranes, birth weight, and other clinically relevant outcomes will be recorded. These outcomes will be considered secondary endpoints rather than direct measures of treatment efficacy unless the study is adequately powered for such analyses.

Table 2. Study Follow-up Schedule and Outcome Assessment

Assessment Variable	Baseline	Early Follow-up	Subsequent Follow-up	Final Assessment
Maternal clinical assessment	✓	✓	✓	✓
Vaginal symptoms/signs	✓	✓	✓	✓
Vaginal microbiome sampling	✓	✓	✓	✓
Lactobacillus abundance	✓	✓	✓	✓
Dysbiosis-associated bacteria	✓	✓	✓	✓
Treatment adherence	✓	✓	✓	✓
Male partner treatment adherence	✓	✓	✓	✓
Recurrence assessment	—	✓	✓	✓
Adverse events	—	✓	✓	✓
Pregnancy outcomes	—	—	✓	✓
Time to recurrence	—	✓	✓	✓

3.9 Microbiome Data Processing and Analysis

Microbiological data will undergo standardized quality-control procedures before analysis. Low-quality or technically inadequate samples will be identified according to predefined laboratory criteria. Relative abundance of bacterial taxa will be calculated and compared between baseline and follow-up assessments. Microbial profiles will be examined for changes in Lactobacillus dominance, abundance of individual bacterial taxa, and overall community structure. Where sequencing-based methods are used, appropriate bioinformatic processing will be applied to generate taxonomic profiles while minimizing technical bias.

Changes in the vaginal microbiome will be evaluated both within individuals over time and between study groups. This longitudinal approach will help determine whether male partner treatment is associated with sustained microbial changes rather than only short-term microbiological improvement.

3.10 Statistical Analysis

Baseline characteristics will be summarized using appropriate descriptive statistics. Continuous variables will be presented using mean and standard deviation or median and interquartile range according to data distribution, while categorical variables will be summarized as frequencies and percentages.

The primary comparison will evaluate the recurrence rate between the standard-management and male-partner-treatment groups. Relative risks or odds ratios with corresponding 95% confidence intervals will be calculated where appropriate. Time-to-recurrence analysis may be performed using Kaplan-Meier methods and suitable regression models if sufficient longitudinal data are available.

Multivariable analysis will be used to account for clinically relevant potential confounders, including maternal age, gestational age, previous recurrence, antimicrobial exposure, and treatment adherence. Microbiome outcomes will be compared using appropriate longitudinal or multivariate methods according to the distribution and structure of the data.

3.11 Safety Monitoring and Ethical Considerations

Because the study involves pregnant women and antimicrobial exposure in their male partners, participant safety will be a primary consideration. The study protocol will specify eligibility criteria, treatment contraindications, monitoring procedures, adverse-event reporting, and criteria for treatment discontinuation.

Written informed consent will be obtained from participating individuals before enrollment. Confidentiality of clinical and microbiological information will be maintained throughout the study. The research protocol will be submitted for ethical approval through the appropriate institutional or independent ethics-review mechanism before participant recruitment begins.

3.12 Methodological Rationale

The proposed methodology is based on the emerging concept that recurrent vaginal dysbiosis may involve interactions between the vaginal microbiome, persistent bacterial communities, and sexual partners rather than representing an exclusively female-associated problem. Recent randomized evidence supports combined male-partner treatment as a potentially effective strategy for reducing recurrent BV, while the most recent meta-analysis indicates that evidence remains heterogeneous and that combined oral and topical regimens require further investigation.

Accordingly, the present methodology combines **clinical assessment, longitudinal vaginal microbiome profiling, male-partner intervention, adherence monitoring, and pregnancy outcome assessment**. This integrated approach is intended to determine not only whether recurrence is reduced, but also whether partner treatment is associated with measurable stabilization of the vaginal microbial ecosystem during pregnancy.

Expected Results, Clinical Implications and Future Directions

The proposed study is expected to provide important evidence regarding whether treatment of the male sexual partner can reduce recurrent vaginal microbiome disturbances during pregnancy. Based on emerging evidence from non-pregnant populations, combined treatment of women and their male partners may reduce recurrence by limiting the persistence or reintroduction of bacterial vaginosis-associated microorganisms. The recent StepUp randomized controlled trial demonstrated a substantially lower recurrence of bacterial vaginosis among women whose male partners received combined oral and topical antimicrobial treatment compared with women receiving female-only treatment. However, a recent systematic review and meta-analysis of six randomized controlled trials involving 953 couples reported no significant overall reduction in recurrence, while suggesting that combined oral and topical partner treatment may be more promising than male-partner monotherapy.

The present study is therefore expected to determine whether these findings can be extended to pregnancy, while simultaneously evaluating changes in the vaginal microbiome. Particular attention will

be given to the preservation or restoration of **Lactobacillus-dominant communities**, especially *Lactobacillus crispatus*, and to reductions in dysbiosis-associated organisms such as *Gardnerella*, *Prevotella*, *Atopobium*, and *Sneathia*. This is clinically relevant because reduced Lactobacillus dominance and increased dysbiosis-associated bacterial communities have been associated with adverse pregnancy outcomes, including preterm birth.

Table 3. Expected Outcomes and Future Clinical Relevance

Outcome Domain	Expected Observation	Potential Clinical Significance
Vaginal dysbiosis recurrence	Lower recurrence in the partner-treatment group	May support a couple-based approach to recurrent dysbiosis
Lactobacillus dominance	Better maintenance/restoration of beneficial Lactobacillus species	May indicate improved vaginal microbial stability
Dysbiosis-associated bacteria	Reduced persistence of *Gardnerella*, *Prevotella*, *Atopobium* and related taxa	May reduce microbial instability and recurrence
Time to recurrence	Longer recurrence-free interval	May improve sustained treatment effectiveness
Pregnancy outcomes	Exploration of associations with gestational age and preterm birth	May clarify possible obstetric relevance
Treatment adherence	Higher adherence associated with improved outcomes	Important for determining feasibility of partner-directed treatment
Safety	Monitoring of maternal and partner adverse events	Essential before broader implementation during pregnancy

4.1 Future Directions

Future research should prioritize **large, adequately powered randomized controlled trials specifically involving pregnant women**, because current evidence for male-partner treatment has largely been generated outside pregnancy. Recent expert discussions have also emphasized that questions regarding broader applicability, patient counseling, sexual transmission, treatment implementation, and safety remain unresolved. Future studies should incorporate longitudinal microbiome sequencing, strain-level microbial analysis, and assessment of microbial exchange between sexual partners. Such approaches could help determine whether recurrent dysbiosis results partly from persistence, reinoculation, or ecological instability within the vaginal microbiome.

Further studies should also compare different partner-treatment strategies, including combined oral and topical regimens, while carefully considering antimicrobial resistance, adverse effects, adherence, and pregnancy-specific safety. Importantly, male-partner treatment should not yet be presented as an established standard for preventing recurrent dysbiosis during pregnancy. Instead, the findings should contribute to the developing evidence base and help determine whether a **couple-centered management strategy** can safely complement conventional maternal treatment.

Overall, the proposed study may provide a clinically meaningful framework for connecting **male partner management, vaginal microbiome stability, recurrence prevention, and pregnancy outcomes**. If supported by future adequately powered trials, this approach could represent a shift from woman-only treatment toward a more comprehensive couple-based strategy for recurrent vaginal dysbiosis.

Discussion

5.1 Interpretation of the Proposed Findings

Recurrent vaginal microbiome disturbance during pregnancy remains an important clinical concern because repeated dysbiosis may interfere with the maintenance of a stable Lactobacillus-dominant vaginal environment. The present study focuses on the possibility that management directed only toward the pregnant woman may not completely address factors contributing to recurrence. In particular, sexual

exchange of bacterial vaginosis-associated microorganisms between partners has emerged as a potential contributor to recurrent dysbiosis.

The rationale for male partner treatment has gained considerable attention following the 2025 StepUp randomized controlled trial. In that study, recurrence of bacterial vaginosis within 12 weeks was substantially lower when male partners received combined oral and topical antimicrobial treatment in addition to treatment of the female partner, compared with treatment of the woman alone. These findings provide important support for investigating a couple-based strategy. However, the StepUp trial was not specifically designed for pregnant women; therefore, its findings cannot be directly generalized to pregnancy without further clinical evaluation.

The present study additionally proposes longitudinal assessment of the vaginal microbiome. This is particularly important because pregnancy is associated with dynamic changes in vaginal microbial composition, and Lactobacillus dominance, especially *L. crispatus*, has been associated with a more favorable vaginal environment. Conversely, reduced Lactobacillus abundance and increased representation of organisms such as *Gardnerella* have been associated with adverse pregnancy outcomes, including preterm birth.

5.2 Comparison with Recent Evidence

Although the StepUp findings strongly support combined partner treatment, the broader evidence remains mixed. A 2026 systematic review and meta-analysis involving six randomized controlled trials and 953 couples found no statistically significant overall reduction in bacterial vaginosis recurrence with male partner treatment. Importantly, the analysis suggested that combined oral and topical treatment may have greater potential than male-partner monotherapy.

Therefore, the proposed study may help address an important unresolved question: whether partner-directed treatment can influence not only clinical recurrence but also the underlying vaginal microbial community during pregnancy. Assessment of microbial composition, Lactobacillus abundance, recurrence-free interval, adherence, and pregnancy outcomes could provide a more comprehensive understanding of the intervention.

5.3 Clinical Implications

If the proposed findings demonstrate reduced recurrence together with improved Lactobacillus dominance, they could support further investigation of couple-based management strategies. Nevertheless, male partner treatment should currently be considered an emerging approach rather than an established pregnancy-specific standard. Recent expert literature has emphasized the need for further evidence regarding applicability across populations, counseling, implementation, and sexual transmission mechanisms.

6. Conclusion

Recurrent vaginal microbiome disturbance during pregnancy is a complex condition involving interactions between microbial composition, host factors, antimicrobial exposure, and potentially sexual partner-associated microbial exchange. The conventional approach of treating the pregnant woman alone may not fully address all factors contributing to recurrence. Increasing evidence suggests that the male partner may represent an important but previously under-addressed component of recurrent bacterial vaginosis and vaginal dysbiosis.

The proposed study provides a comprehensive framework for evaluating the role of male partner treatment by combining clinical assessment with longitudinal vaginal microbiome analysis. Particular emphasis on Lactobacillus species and dysbiosis-associated microorganisms may help determine whether partner treatment is associated with greater microbial stability and a lower risk of recurrent disturbance.

Recent evidence, particularly the StepUp randomized trial, provides encouraging support for combined treatment of sexual partners, whereas the 2026 meta-analysis indicates that the overall evidence remains heterogeneous and that the effectiveness of different partner-treatment strategies may vary. Importantly, most available evidence has been generated in non-pregnant populations. Therefore, pregnancy-specific research is required before firm conclusions regarding effectiveness and safety can be established.

Overall, this study may contribute to the emerging concept of **couple-centered management of recurrent vaginal dysbiosis**. Future adequately powered randomized trials should evaluate microbiome changes, recurrence, antimicrobial resistance, adherence, maternal safety, and pregnancy outcomes. If future evidence confirms benefit and safety, male partner treatment could potentially become an important component of strategies designed to maintain vaginal microbiome stability and reduce recurrent dysbiosis during pregnancy.

REFERENCES

Ahrodia, T., Yodhaanjali, J. R., & Das, B. (2022). Vaginal microbiome dysbiosis in preterm birth. *Progress in Molecular Biology and Translational Science, 192*(1), 309–329. https://doi.org/10.1016/bs.pmbts.2022.08.001

Ashraf, S., Haroon, A., & Fayyaz, A. (2025). Comparing the efficacy of probiotic plus antibiotic with the antibiotic therapy alone on the recurrence of bacterial vaginosis. *Pakistan Journal of Medical Sciences, 41*(1), 125–129. https://doi.org/10.12669/pjms.41.1.9922

Ferrante, M., Oliveri Conti, G., Pulvirenti, E., Favara, C., Fiore, M., & Cristaldi, A. (2025). The vaginal microbiota and preterm birth: A systematic review. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 311*, 114007. https://doi.org/10.1016/j.ejogrb.2025.114007

Gerede, A., Nikolettos, K., Vavoulidis, E., Margioula-Siarkou, C., Petousis, S., Giourga, M., Fotinopoulos, P., Salagianni, M., Stavros, S., Dinas, K., Nikolettos, N., & Domali, E. (2024). Vaginal microbiome and pregnancy complications: A review. *Journal of Clinical Medicine, 13*(13), 3875. https://doi.org/10.3390/jcm13133875

González-Sánchez, A., Reyes-Lagos, J. J., Peña-Castillo, M. A., et al. (2022). Vaginal microbiota is stable and mainly dominated by *Lactobacillus* at third trimester of pregnancy and active childbirth: A longitudinal study of ten Mexican women. *Current Microbiology, 79*, 230. https://doi.org/10.1007/s00284-022-02918-1

Günther, V., Allahqoli, L., Watrowski, R., Maass, N., Ackermann, J., von Otte, S., & Alkatout, I. (2022). Vaginal microbiome in reproductive medicine. *Diagnostics, 12*(8), 1948. https://doi.org/10.3390/diagnostics12081948

Huang, C., Gin, C., Fettweis, J., Foxman, B., Gelaye, B., MacIntyre, D. A., Subramaniam, A., Fraser, W., Tabatabaei, N., & Callahan, B. (2023). Meta-analysis reveals the vaginal microbiome is a better predictor of earlier than later preterm birth. *BMC Biology, 21*, 199. https://doi.org/10.1186/s12915-023-01702-2

Host-vaginal microbiota interaction: Shaping the vaginal microenvironment and bacterial vaginosis. (2024). *Current Clinical Microbiology Reports, 11*, 177–191. https://doi.org/10.1007/s40588-024-00227-8

Juliana, N. C. A., Peters, R. P. H., Al-Nasiry, S., et al. (2021). Composition of the vaginal microbiota during pregnancy in women living in sub-Saharan Africa: A PRISMA-compliant review. *BMC Pregnancy and Childbirth, 21*, 596. https://doi.org/10.1186/s12884-021-04072-1

Kulshrestha, S., Narad, P., Singh, B., et al. (2024). Biomarker identification for preterm birth susceptibility: Vaginal microbiome meta-analysis using systems biology and machine learning approaches. *American Journal of Reproductive Immunology, 92*(1), e13905. https://doi.org/10.1111/aji.13905

Li, K. T., Li, F., Jaspan, H., Nyemba, D., Myer, L., Aldrovandi, G., & Joseph-Davey, D. (2024). Changes in the vaginal microbiome during pregnancy and the postpartum period in South African women: A longitudinal study. *Reproductive Sciences, 31*(1), 275–287. https://doi.org/10.1007/s43032-023-01351-4

McKee, K. S., Bassis, C. M., Golob, J., Palazzolo, B., Comstock, S. S., Rosas-Salazar, C., Stanford, J. B., Ananda, S., O'Connor, T., Gern, J. E., Paneth, N., & Dunlop, A. L. (2025). Vaginal microbiome structure in pregnancy and host factors predict preterm birth: Results from the ECHO Cohort. *Annals of Epidemiology, 112*, 110–118. https://doi.org/10.1016/j.annepidem.2025.11.003

Muzny, C. A., & Sobel, J. D. (2025). Bacterial vaginosis: Time to treat male partners. *New England Journal of Medicine, 392*, 1026–1027. https://doi.org/10.1056/NEJMe2500373

Plummer, E. L., Sfameni, A. M., Vodstrcil, L. A., et al. (2023). *Prevotella* and *Gardnerella* are associated with treatment failure following first-line antibiotics for bacterial vaginosis. *Journal of Infectious Diseases, 228*(5), 646–656. https://doi.org/10.1093/infdis/jiad261

Plummer, E. L., Vodstrcil, L. A., Fairley, C. K., et al. (2021). A prospective, open-label pilot study of concurrent male partner treatment for bacterial vaginosis. *mBio, 12*(5), e02323-21. https://doi.org/10.1128/mBio.02323-21

Rezazadeh, M. B., Zanganeh, M., Jarahi, L., & Fatehi, Z. (2024). Comparative efficacy of oral and vaginal probiotics in reducing the recurrence of bacterial vaginosis: A double-blind clinical trial. *BMC Women's Health, 24*, 575. https://doi.org/10.1186/s12905-024-03418-z

Ross, J. D. C., Brittain, C., Watkins, J. A., et al. (2023). Intravaginal lactic acid gel versus oral metronidazole for treating women with recurrent bacterial vaginosis: The VITA randomised controlled trial. *BMC Women's Health, 23*, 241. https://doi.org/10.1186/s12905-023-02303-5

Schwebke, J. R., Lensing, S. Y., Lee, J., Muzny, C. A., Pontius, A., Woznicki, N., Aguin, T., & Sobel, J. D. (2021). Treatment of male sexual partners of women with bacterial vaginosis: A randomized, double-blind, placebo-controlled trial. *Clinical Infectious Diseases, 73*(3), e672–e679. https://doi.org/10.1093/cid/ciaa1903

Silvano, A., Meriggi, N., Renzi, S., Seravalli, V., Torcia, M. G., Cavalieri, D., & Di Tommaso, M. (2023). Vaginal microbiome in pregnant women with and without short cervix. *Nutrients, 15*(9), 2173. https://doi.org/10.3390/nu15092173

Vieira, L. L. P., Suruagy-Motta, R. F. O., Bomfim, J. Q., Dos Santos Teixeira, A. L., de Farias, E. H., Dos Santos, K. D. A., Donato, K. O., da Silva, L. D., Rohden, C. A. H., Lemos, M., & Lopes, L. P. (2026). The efficacy of male partner treatment to prevent recurrence of bacterial vaginosis: A systematic review with meta-analysis of randomized controlled trials. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 321*, 115047. https://doi.org/10.1016/j.ejogrb.2026.115047

Vodstrcil, L. A., Muzny, C. A., Plummer, E. L., Sobel, J. D., & Bradshaw, C. S. (2021). Bacterial vaginosis: Drivers of recurrence and challenges and opportunities in partner treatment. *BMC Medicine, 19*, 194. https://doi.org/10.1186/s12916-021-02077-3

Vodstrcil, L. A., Plummer, E. L., Fairley, C. K., et al. (2025). Male-partner treatment to prevent recurrence of bacterial vaginosis. *New England Journal of Medicine, 392*(10), 947–957. https://doi.org/10.1056/NEJMoa2405404

Wang, L., et al. (2025). Insights into the alteration of vaginal microbiota and metabolites in pregnant woman with preterm delivery: Prospective cohort study. *Frontiers in Cellular and Infection Microbiology*. https://doi.org/10.3389/fcimb.2025.1580801

Yazdy, G. M., Workowski, K. A., Ghanem, K. G., Burke, A. E., Handa, V. L., Brotman, R. M., & Tuddenham, S. (2026). Male partner treatment and the potential sexual transmission of bacterial vaginosis: Considerations for patient counseling and clinical application in the United States. *American Journal of Obstetrics & Gynecology, 234*(3), 606–612. https://doi.org/10.1016/j.ajog.2025.11.001

Zhou, Q., Yu, Y., Zhou, J., Liu, J., & Gao, J. (2024). Relationship of *Lactobacillus* vaginal microbiota changes and the risk of preterm birth: A systematic review and meta-analysis. *Journal of Women's Health, 33*(2). https://doi.org/10.1089/jwh.2023.0393