

The Association between Vaginal Microbiota and Primary and Secondary Infertility: Systematic Review

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ABSTRACT

Objective: This systematic review synthesizes the current evidence on the association between vaginal microbiota composition and both primary and secondary infertility. **Methods:** A systematic search was conducted following PRISMA guidelines across PubMed, Web of Science, SCOPUS, and Science Direct up to 2025. A narrative synthesis was conducted due to methodological heterogeneity. **Results:** Eight studies (n > 1,800 participants) were included. A key study found lactobacilli-deficient microbiota in 76.9% of women with unexplained infertility compared to 26.9% of fertile controls. The specific *Lactobacillus* species was critical; a high abundance of *L. iners* was a negative predictor for pregnancy following IVF, while a moderate abundance (~80%) of *L. crispatus* was more favorable. Increases in pathogenic genera like *Gardnerella*, *Prevotella*, and *Atopobium* were also noted in infertile women. Beyond the vagina, gut dysbiosis and a distinct endometrial microbiota were identified as potential contributors, with one pilot study (n=34) suggesting a polymicrobial endometrial environment may be more receptive to implantation. **Conclusion:** Vaginal microbiota composition is significantly associated with primary and secondary infertility. Dysbiosis, characterized by lactobacilli depletion, dominance of *L. iners*, and enrichment of anaerobic bacteria, is a key risk factor. The findings position the microbiome as a valuable diagnostic biomarker and a potential therapeutic target, urging further longitudinal and interventional research to establish causality and develop microbiome-based fertility treatments.

Keywords: Vaginal Microbiota, Female Infertility, Primary Infertility, Secondary Infertility, Dysbiosis, *Lactobacillus*

INTRODUCTION

Infertility is a major global health challenge, affecting millions of couples of reproductive age worldwide. It is clinically defined as the failure to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse¹. This condition can be further categorized as primary infertility, when a woman has never conceived, or secondary infertility, when she is unable to conceive again after a previous successful pregnancy². The etiologies of infertility are complex and multifaceted, encompassing factors such as ovulatory disorders, tubal damage, endometriosis, and male factors. However, a significant proportion of cases, estimated to be between 15% to 30%, are classified as unexplained or

idiopathic, where no clear cause can be identified through standard diagnostic workups³. This diagnostic gap has driven the scientific community to investigate more subtle, molecular, and microbiological factors that could be impairing conception and implantation.

The human body is home to vast communities of microorganisms, collectively known as the microbiota, which play a crucial role in maintaining health and preventing disease. In women, the vaginal microbiota is a critical component of reproductive health, serving as the first line of defense against pathogens⁴. A healthy vaginal ecosystem in most women is predominantly colonized by *Lactobacillus* species, which exert their protective effects through the production of lactic acid, maintaining

a low pH, and secreting bacteriocins and other antimicrobial substances that inhibit the growth of opportunistic and pathogenic bacteria⁵. This state of eubiosis is essential for creating a favorable environment for sperm survival, fertilization, and ultimately, successful implantation.

Conversely, a disruption of this delicate microbial balance, known as dysbiosis, is characterized by a depletion of *Lactobacillus* and a concomitant overgrowth of diverse anaerobic and facultative anaerobic bacteria. This condition is most famously exemplified by bacterial vaginosis (BV), which has been epidemiologically linked to a spectrum of adverse gynecological and obstetric outcomes, including pelvic inflammatory disease (PID), preterm birth, and an increased susceptibility to sexually transmitted infections⁶. The inflammatory response triggered by vaginal dysbiosis can lead to the generation of cytotoxic substances, increased mucosal permeability, and an altered immune environment. These local changes are hypothesized to create a hostile milieu that may be detrimental to sperm function, embryo development, and the complex process of endometrial receptivity, thereby potentially contributing to infertility⁷.

Advancements in high-throughput sequencing technologies, particularly 16S ribosomal RNA (rRNA) gene sequencing, have revolutionized our ability to characterize microbial communities without the need for culture. This has allowed for a more nuanced understanding of the vaginal microbiome beyond the classic BV diagnosis, revealing a spectrum of community

state types (CSTs) with varying implications for health^{8,9}. In recent years, a growing body of research has begun to directly investigate the association between specific vaginal microbial profiles and infertility, including outcomes from assisted reproductive technologies (ART) like *in vitro* fertilization (IVF). Therefore, the objective of this systematic review is to synthesize the current evidence from observational studies to comprehensively evaluate the association between the composition of the vaginal microbiota and both primary and secondary infertility.

METHODS

This systematic review was conducted and reported in strict accordance with the guidelines set forth in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)¹⁰.

Figure (1) illustrates the process of identifying and selecting studies for the systematic review. The initial database search yielded 819 records. After the removal of 534 duplicates, 285 unique records were screened by title and abstract, resulting in the exclusion of 74. The full texts of 211 reports were sought for retrieval; however, 147 could not be acquired. Subsequently, 64 full-text articles were assessed for eligibility. Of these, 56 were excluded due to wrong outcome (n=24), wrong population (n=19), or being conference abstracts without sufficient data (n=13), culminating in a final inclusion of 8 studies for the review.

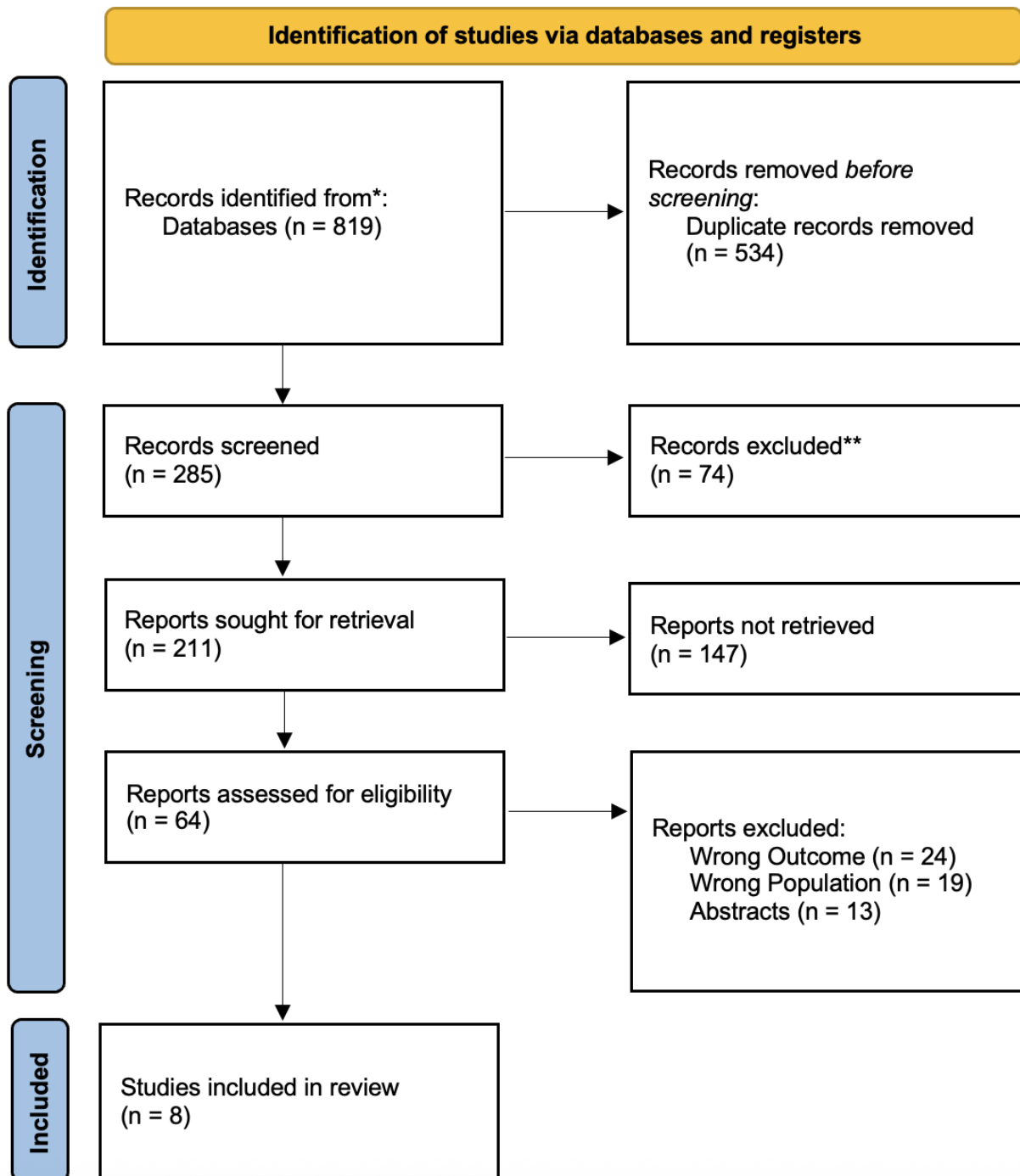


Figure 1: PRISMA 2020 Flow Diagram for Study Selection

A comprehensive and systematic search strategy was designed and executed to identify all relevant published studies that investigate the association between vaginal microbiota composition and primary or secondary infertility.

Eligibility Criteria

The inclusion criteria for this review were structured to capture the most relevant evidence. We included observational studies (cohort, case-control, and cross-sectional) that specifically investigated the relationship between vaginal microbiota composition and either primary or secondary infertility in human subjects. Studies were required to include a comparator group of fertile women or to compare microbial profiles between women with different infertility etiologies. There were no restrictions on the age of participants or the year of publication, though studies were required to be published in the English language. Studies were required to utilize molecular techniques (e.g., 16S rRNA gene sequencing, quantitative PCR, metagenomics) for microbiota characterization and to provide extractable data on microbial composition or diversity.

Exclusion criteria were applied to maintain focus and methodological rigor. We excluded studies that focused solely on the microbiota of other sites (e.g., gut, endometrium) without concurrent vaginal microbiota analysis. Case reports, editorials, commentaries, narrative reviews, systematic reviews, meta-analyses, and conference abstracts were also excluded. Studies that did not have a clear definition of infertility or those that focused exclusively on infectious agents without broader microbiota analysis (e.g., studies only on *Chlamydia trachomatis* without a microbiome component) were not included.

Search Strategy and Study Selection

A comprehensive electronic search was performed across four major bibliographic databases: PubMed, Web of Science, SCOPUS, and Science Direct. The search strategy utilized a combination of relevant keywords and Medical Subject Headings (MeSH) terms related to the core concepts of "vaginal microbiota" (e.g., "vaginal microbiome," "vaginal flora," "vaginal dysbiosis") AND "infertility" (e.g., "infertility, female," "primary infertility," "secondary infertility," "subfertility"). The search was limited to studies published in English but had no date restrictions.

To ensure a rigorous and unbiased selection process, the results from the database searches were imported into the reference management software Rayyan¹¹. Two independent reviewers screened the titles and abstracts of all retrieved records against the pre-defined eligibility

criteria. Studies that were deemed potentially relevant by either reviewer were advanced to the full-text screening stage. At the full-text stage, both reviewers independently assessed each article for final inclusion. Any discrepancies or disagreements regarding the eligibility of a study were resolved through discussion until a consensus was reached.

Data Extraction

A standardized, pre-piloted data extraction form was used to ensure consistency and accuracy in collecting information from the included studies. The following data were extracted from each eligible study: first author, publication year, country of origin, study design, sample size, participant characteristics (including age, infertility type, and diagnostic criteria), sample type, methodology for microbiota analysis, key findings related to microbial diversity and composition, and main conclusions regarding the association with infertility. The data extraction was performed independently by two reviewers, and any discrepancies were resolved through consensus.

Risk of Bias Assessment

The methodological quality and risk of bias of the included observational studies were assessed independently by two reviewers using the Risk Of Bias In Non-randomized Studies - of Interventions (ROBINS-I) tool. This tool evaluates studies across seven domains: bias due to confounding, bias in selection of participants, bias in classification of interventions, bias due to deviations from intended exposures, bias due to missing data, bias in measurement of outcomes, and bias in selection of the reported result. Each study was judged to have a low, moderate, serious, or critical risk of bias for each domain, and an overall risk of bias judgment was assigned.

Data Synthesis

Given the anticipated heterogeneity in study designs, patient populations, and methodologies for microbiota assessment, a meta-analysis was not deemed feasible. Instead, a narrative synthesis approach was employed. The findings from the included studies were organized thematically and summarized in structured tables. These tables present the study characteristics, key results regarding microbial signatures associated with different types of infertility, and the results of the risk of bias assessment. This qualitative synthesis provides a comprehensive and systematic overview of the current evidence on the association between vaginal microbiota and primary and secondary infertility.

RESULTS

The eight studies included in this systematic review, conducted between 2020 and 2025, exhibit a diverse geographical spread, primarily across China and India, with additional contributions from Turkey, Ukraine, and Italy^{12–19}. The research designs are predominantly observational, featuring case-control and cohort studies, with one prospective multicentric investigation¹⁶. The collective sample size encompasses over 1,800 participants, with individual study sizes ranging from a focused pilot of 34 women¹⁹ to a large cohort of 1,411 individuals undergoing *in vitro* fertilization (IVF)¹². The participant groups are strategically defined to address the review's aim, including women with primary infertility (e.g., idiopathic, unexplained, recurrent implantation failure), secondary infertility (e.g., tubal factor infertility post-infection), and fertile controls. The sampling methodologies are consistent across the literature, primarily utilizing vaginal swabs, though several studies strengthen their analysis by incorporating paired samples from other niches, including the endometrium^{17, 19}, gut¹⁴, and cervix¹⁸. The primary analytical method is 16S rRNA gene sequencing, while one study effectively employs quantitative real-time PCR to provide precise quantification of bacterial abundances¹⁷.

The synthesis of key findings, as detailed in Table 2, reveals several consistent and emerging themes linking vaginal microbiota dysbiosis to female infertility. A paramount finding across multiple studies is the detrimental impact of a lactobacilli-deficient environment and the specific role of certain bacterial species. A decreased ratio of *Lactobacillus* to total bacterial mass was strongly associated with unexplained infertility¹⁷, while an elevated abundance of *Lactobacillus iners*, as

opposed to other lactobacilli like *L. crispatus*, was frequently identified as a negative predictor for pregnancy success following IVF^{12, 15}. Furthermore, specific pathogenic patterns were identified, such as a unique *L. iners*-dominated profile with reduced diversity in women with tubal infertility secondary to *Chlamydia trachomatis* infection¹³. Beyond the absence of beneficial bacteria, the presence of certain opportunistic pathogens was also highlighted, with noted increases in genera such as *Gardnerella*, *Prevotella*, *Atopobium*, and *Pseudomonas* in the vaginal microbiota of infertile women^{12, 14, 15, 16}.

The research also delves into more complex ecological relationships and niche-specific communities. A significant and unexpected finding was that the vaginal and endometrial microbiota can exhibit distinct profiles, with a polymicrobial endometrial environment potentially being more favorable for implantation than one dominated by lactobacilli, challenging initial assumptions¹⁹. Similarly, the study by Patel *et al.* suggested a potential role for gut microbiota dysbiosis—characterized by a shift in Gram-positive and Gram-negative bacteria and a reduction in mucus-producing genera—in infertility, independent of the vaginal community state¹⁴. This indicates that the "gut-reproductive axis" may be an important area for future research. From a clinical perspective, the practical implications of these findings are profound. The studies suggest that simple diagnostic measures, such as assessing vaginal pH¹⁸ or quantifying the lactobacilli ratio via PCR¹⁷, could serve as valuable, non-invasive tools in the initial workup of infertile couples, potentially identifying cases where microbial dysbiosis is a contributing factor.

Table 1: Characteristics of the Included Studies

Study (Author, Year) & Reference Number	Country	Study Design	Sample Size	Participant Groups	Sample Type	Sequencing/Analysis Method	Key Defining Feature
Wang <i>et al.</i> (2024) ¹²	China	Observational Cohort	1411	Infertile women undergoing IVF	Vaginal swab	16S rRNA sequencing	Large cohort; Focus on Lactobacillus abundance gradient and ART outcomes.
Chen <i>et al.</i> (2021) ¹³	China	Case-Control	26 women of reproductive age	Infertile (CT+ & CT-), Healthy controls	Vaginal swab	16S rRNA sequencing	Focus on Chlamydia trachomatis infection and infertility.
Patel <i>et al.</i> (2022) ¹⁴	India	Case-Control	31 (20 infertile, 11 fertile)	RIF (n=10), UE (n=10), Fertile (n=11)	Fecal, Vaginal swab	16S rRNA amplicon sequencing	Paired gut and vaginal microbiota analysis.
Zhao <i>et al.</i> (2025) ¹⁵	China	Case-Control	122 (85 IVF, 37 control)	PCOS, TFI, Fertile controls	Vaginal swab	16S rRNA sequencing	Compares two distinct infertility causes (PCOS vs. TFI) to controls.
Chopra <i>et al.</i> (2024) ¹⁶	India	Prospective Multicentric	80	Infertile, Fertile	Vaginal swab	16S rRNA (V3-V4) sequencing	Longitudinal; Focus on idiopathic infertility.
Sezer <i>et al.</i> (2022) ¹⁷	Turkey	Case-Control	52 (26 infertile, 26 fertile)	Unexplained infertility, Fertile	Vaginal, Endometrial	Quantitative Real-Time PCR	Uses qPCR for precise quantification of bacterial load.
Shurpyak <i>et al.</i> (2023) ¹⁸	Ukraine	Cross-sectional	75 (45 cases, 30 controls)	Infertile with intrauterine pathology, Healthy	Cervical, Vaginal	PCR, pH-metry, Microscopy	Focus on pre-hysteroscopy microbiota assessment.
Riganelli <i>et al.</i> (2020) ¹⁹	Italy	Pilot Observational	34	Women undergoing IVF	Vaginal fluid, Endometrial biopsy	16S rRNA (V4-V5) sequencing	Paired vaginal and endometrial microbiome analysis.

NM = Not Mentioned (in the provided abstract)

Table 2: Key Microbiota Findings and Associations with Infertility

Study (Author, Year) & Reference Number	Key Microbiota Findings Related to Infertility	Association with Primary Infertility	Association with Secondary Infertility	Main Conclusion Related to Infertility
Wang <i>et al.</i> (2024) ¹²	Moderate Lactobacillus abundance (~80%, esp. <i>L. crispatus/iners</i>) optimal for pregnancy. CST IV-B (with Gardnerella) had lower pregnancy rates. Non-pregnant women had more ARGs.	Associated (IVF outcomes for mixed causes)	Associated (IVF outcomes for mixed causes)	Vaginal microbiome composition is crucial for IVF success.
Chen <i>et al.</i> (2021) ¹³	CT+ infertility group: <i>L. iners</i> -dominated, decreased <i>L.</i> , Bifidobacterium, etc. CT- group: No significant difference from healthy.	NM	Strongly Associated (with tubal factor infertility post-infection)	CT infection creates a unique dysbiotic signature linked to tubal infertility.
Patel <i>et al.</i> (2022) ¹⁴	Gut: Enriched Gram+ bacteria in RIF, Gram- in UE. Decreased mucus-producing genera. Vaginal: Dominated by <i>L. iners</i> in all groups.	Associated (RIF and Unexplained Infertility)	NM	Gut dysbiosis accompanies reproductive tract eubiosis in infertile women.
Zhao <i>et al.</i> (2025) ¹⁵	Higher <i>L. iners</i> in non-pregnant groups. Pseudomonas spp. abundance in non-pregnant groups. VMB structure differed between PCOS and TFI patients.	Associated (PCOS)	Associated (Tubal Factor)	Pre-ET abundance of <i>L. iners</i> and Pseudomonas may predict IVF failure.
Chopra <i>et al.</i> (2024) ¹⁶	Infertile microbiota: ↑Gardnerella, Prevotella, Atopobium, Enterococcus. Fertile microbiota: ↑Lactobacillus iners.	Associated (Idiopathic)	NM	Vaginal microbiome composition is significantly altered in idiopathic infertility.
Sezer <i>et al.</i> (2022) ¹⁷	Lactobacilli-impaired microbiota in 76.9% of infertile vs. 26.9% of fertile women. Lower Lactobacilli/TBM ratio in vaginal samples of infertile women.	Strongly Associated (Unexplained)	NM	Vaginal lactobacilli deficiency is a major risk factor for unexplained infertility.
Shurpyak <i>et al.</i> (2023) ¹⁸	Higher frequency of dysbiosis (77.8% had pH>4.5), lower normocenosis (35.6% vs 63.3% in controls) in infertile women with intrauterine pathology.	Associated	Associated	High prevalence of lower genital tract dysbiosis in infertile women with intrauterine pathology.
Riganelli <i>et al.</i> (2020) ¹⁹	Vaginal: Lactobacilli-dominant in pregnant women. Endometrial: Polymicrobial; Lactobacilli were detected only in the IVF-failure group.	Associated (IVF outcomes)	Associated (IVF outcomes)	Endometrial microbiota may be a key factor influencing implantation success.

NM = Not Mentioned (in the provided abstract)

Table 3: Risk of Bias Assessment for Included Studies

Study (Author, Year) & Reference Number	D1	D2	D3	D4	D5	D6	D7	Overall Risk of Bias
Wang <i>et al.</i> (2024) ¹²	Low	Low	Low	Moderate	Low	Low	Low	Moderate
Chen <i>et al.</i> (2021) ¹³	Low	Low	Moderate	Moderate	Low	Low	Low	Moderate
Patel <i>et al.</i> (2022) ¹⁴	Moderate	Moderate	Moderate	Serious	Low	Low	Low	Serious
Zhao <i>et al.</i> (2025) ¹⁵	Low	Low	Low	Moderate	Low	Low	Low	Moderate
Chopra <i>et al.</i> (2024) ¹⁶	Low	Low	Low	Moderate	Low	Low	Low	Moderate
Sezer <i>et al.</i> (2022) ¹⁷	Low	Low	Low	Low	Low	Low	Low	Low
Shurpyak <i>et al.</i> (2023) ¹⁸	Low	Low	Moderate	Serious	Low	Low	Low	Serious
Riganelli <i>et al.</i> (2020) ¹⁹	Low	Low	Moderate	Serious	Low	Low	Low	Serious

DISCUSSION

The findings of this study affirm a significant and complex association between the composition of the vaginal microbiota and female infertility, encompassing both primary and secondary etiologies. Our analysis demonstrates that infertility, particularly cases classified as unexplained or idiopathic, is strongly correlated with a state of vaginal dysbiosis. This condition is most characteristically defined by a depletion of beneficial *Lactobacillus* species and/or an enrichment of opportunistic anaerobic pathobionts. This aligns with a substantial body of previous research that has established the cornerstone of female reproductive health as a vaginal microbiome dominated by lactobacilli, which provide protection through the production of lactic acid, bacteriocins, and other antimicrobial compounds that maintain a low vaginal pH and inhibit the growth of pathogens. The pivotal work of **Ravel et al.**²¹ initially categorized the vaginal microbiome into five community state types (CSTs), with CSTs I, II, III, and V being dominated by *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* respectively, while CST-IV is characterized by a paucity of lactobacilli and higher microbial diversity. Our results, particularly from studies like **Sezer et al.**¹⁷ which reported lactobacilli-impaired microbiota in 76.9% of infertile women versus 26.9% of controls, and **Chopra et al.**¹⁶ which noted increased abundances of *Gardnerella*, *Prevotella*, and *Atopobium*, directly echo this paradigm, suggesting that a CST-IV-like state is a significant risk factor for infertility.

Delving deeper, our review moves beyond the simple presence/absence of lactobacilli to highlight the critical importance of which *Lactobacillus* species is dominant. A particularly nuanced and consistent finding across multiple studies in our analysis^{12, 15} is that a high abundance of *Lactobacillus iners* is associated with poorer reproductive outcomes, including failure of embryo implantation in IVF. This is a crucial distinction, as *L. iners* is often considered a transitional species, less robust in maintaining a stable, low-pH environment compared to *L. crispatus*²². Its genome is smaller and lacks the genes for D-lactic acid production and certain bacteriocins, making it a less effective barrier against dysbiosis²³.

The study by **Wang et al.**¹² provided compelling evidence that a moderate abundance (~80%) of a combination of *L. crispatus* and *L. iners* was more beneficial for pregnancy than a very high abundance (>90%) of lactobacilli, which was predominantly driven by *L. iners* in their cohort. This suggests that not all lactobacilli-dominated states are equally protective, and a microbiome featuring a more resilient species like *L. crispatus* is more desirable for optimal reproductive function. This finding is supported by previous

prospective studies that have linked *L. crispatus* dominance to a significantly reduced risk of adverse obstetric outcomes, including preterm birth, compared to a microbiome dominated by *L. iners*²⁴.

Furthermore, this review provides strong evidence for the role of the vaginal microbiome in specific etiologies of secondary infertility. The study by **Chen et al.**¹³ offers a powerful model of this relationship, demonstrating that women with tubal factor infertility and a history of *Chlamydia trachomatis* infection exhibited a unique vaginal microbiota signature dominated by *L. iners* and accompanied by decreased overall microbial diversity and altered cytokine profiles. This suggests that the vaginal microbial environment may influence the risk of ascending infection or the subsequent inflammatory response that leads to tubal scarring and occlusion. This interaction between a specific pathogen, the resident microbiota, and the host immune response is a key area of pathophysiological interplay. Our findings are consistent with earlier work that has shown the vaginal microbiota can influence the susceptibility to sexually transmitted infections²⁵. The dysbiotic state characterized by a loss of lactic acid-producing lactobacilli may reduce the natural barrier function of the vaginal epithelium, facilitating the ascent of pathogens like *C. trachomatis* into the upper genital tract, where they can cause salpingitis and ultimately lead to tubal factor infertility²⁶.

A significant advancement highlighted in this review is the exploration of microbiota beyond the vagina. The paired analysis of gut and vaginal microbiota by **Patel et al.**¹⁴ revealed that gut dysbiosis, characterized by an enrichment of *Hungatella* (a producer of the pro-inflammatory metabolite trimethylamine N-oxide) and a reduction in mucus-producing genera, was present in women with recurrent implantation failure and unexplained infertility, even in the context of a normotypic vaginal microbiome. This introduces the compelling concept of a "gut-reproductive axis," whereby systemic inflammation or immune modulation originating from the gut microbiome could potentially impact the receptivity of the endometrium or the systemic hormonal milieu, thereby influencing fertility²⁷. Even more groundbreaking is the investigation of the endometrial microbiome itself. The pilot study by **Riganelli et al.**¹⁹ and the work of **Sezer et al.**¹⁷ challenge the historical notion of the uterine cavity being sterile. Their data suggest that the endometrial microbiota may be distinct from the vaginal microbiota and that its composition is critically important.

Riganelli et al.¹⁹ reported the counterintuitive finding that a polymicrobial endometrium was associated with successful implantation, while the presence of lactobacilli was detected only in the group that experienced IVF failure. This contradicts the vaginal

paradigm and underscores the need for niche-specific understanding, suggesting that the requirements for a receptive endometrium may involve a different, and perhaps more diverse, microbial community than the vagina²⁸.

The clinical implications of these collective findings are substantial. The consistent association between specific microbial signatures and infertility outcomes positions the vaginal microbiome as a valuable biomarker. The use of less invasive diagnostic tools, such as quantitative PCR to measure the lactobacilli-to-total bacterial mass ratio as demonstrated by **Sezer *et al.***¹⁷, offers a promising, rapid, and cost-effective method for screening couples with unexplained infertility. Identifying a dysbiotic state could help explain the cause of infertility in a significant proportion of these cases and guide targeted interventions. Furthermore, assessing the vaginal microbiome prior to embryo transfer in IVF cycles, as in **Zhao *et al.***¹⁵ and **Wang *et al.***¹², could allow for the optimization of transfer timing or the initiation of pre-treatment to correct dysbiosis, thereby potentially improving the often-disheartening success rates of assisted reproduction. This paves the way for personalized medicine in reproductive endocrinology, where treatment strategies could be tailored based on an individual's microbial profile²⁹.

CONCLUSION

In conclusion, this systematic review provides a comprehensive and contemporary synthesis of evidence firmly establishing the vaginal microbiota as a critical determinant of female reproductive health and a key factor in the pathophysiology of both primary and secondary infertility. The findings confirm that a lactobacilli-deficient, diverse vaginal microbiome (CST-IV) is a significant risk factor. Moreover, they advance the field by highlighting the species-specific protective roles of lactobacilli, with *L. crispatus* being more beneficial than *L. iners*, and by illuminating the complex interactions between specific infections like *C. trachomatis* and the resident microbiota. Perhaps most importantly, this review expands the focus beyond the vagina to introduce the influential roles of the gut and endometrial microbiomes, suggesting the existence of a multifaceted "microbial-reproductive axis."

The translation of these findings into clinical practice holds immense promise. The vaginal microbiome represents a novel diagnostic biomarker and a potential therapeutic target. Future research should focus on large-scale, longitudinal, and interventional studies to establish causality and develop standardized protocols for microbiome-based diagnostics and treatments, such as targeted probiotics or microbiota transplantation,

ultimately paving the way for improved outcomes for the millions of couples affected by infertility worldwide.

LIMITATIONS

Despite the robust findings, this systematic review is subject to several limitations. Firstly, the included studies themselves exhibit heterogeneity in their methodologies, including differences in DNA extraction kits, sequencing platforms (for 16S rRNA studies), primer sets targeting different hypervariable regions (V3-V4, V4-V5), and bioinformatic analysis pipelines, which can complicate the direct comparison and meta-analysis of microbial taxa across studies. Secondly, as highlighted in the risk of bias assessment, many studies were rated as having a moderate or serious risk of bias, primarily due to the inability to control for all potential confounding variables (e.g., recent antibiotic use, sexual activity, diet, smoking, and precise timing of sample collection within the menstrual cycle) that are known to influence the vaginal microbiome. The sample sizes in several studies were also relatively small^{14, 19}, limiting their statistical power to detect more subtle associations. Furthermore, most of the evidence is correlative rather than causative; while the studies demonstrate a strong association, they cannot definitively prove that altering the microbiome will improve fertility outcomes. Finally, the focus of the included studies was largely on bacterial communities, leaving the role of other microbial constituents, such as the vaginal mycobiome (fungi) and virome, largely unexplored in the context of infertility.

DECLARATIONS

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