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HEALTH AND REHABILITATION

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SYSTEMATIC REVIEW OF THE ASSOCIATION BETWEEN VAGINAL MICROBIOME COMPOSITION AND THE SUCCESS OF FROZEN EMBRYO TRANSFER

Systematic Review

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Acknowledgement: The authors thank the institutional librarian for their expert assistance in developing the comprehensive search strategy.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: Infertility remains a significant global health burden, with frozen embryo transfer (FET) constituting an increasingly prevalent and successful assisted reproductive technology. Despite advancements, implantation failure persists, prompting investigation into the endometrial microenvironment. The vaginal microbiome is a dynamic component of this environment, yet its specific association with FET outcomes remains inconsistently reported, necessitating a systematic synthesis of the evidence.

Objective: This systematic review aims to synthesize the available evidence linking specific vaginal microbiome compositions to implantation and clinical pregnancy rates in individuals undergoing frozen embryo transfer.

Methods: Following PRISMA guidelines, a comprehensive search of PubMed/MEDLINE, Scopus, Cochrane Library, and Web of Science was conducted for observational studies published between 2014-2024. Eligible studies reported on vaginal microbiome composition prior to or during an FET cycle and its association with reproductive outcomes. Two independent reviewers performed study selection, data extraction, and quality assessment using the Newcastle-Ottawa Scale.

Results: Eight observational studies (n=1,347 participants) were included. A key finding was methodological divergence: studies using molecular sequencing (16S rRNA) consistently associated a *Lactobacillus*-dominant microbiome, particularly with *L. crispatus*, with significantly higher clinical pregnancy rates. Conversely, studies utilizing Nugent scoring found no significant association between bacterial vaginosis-defined dysbiosis and FET outcomes.

Conclusion: The association between vaginal microbiome and FET success appears contingent on assessment methodology. An optimal microbiome, characterized by specific beneficial lactobacilli, may support implantation, whereas traditional diagnostic criteria lack sensitivity for this predictive role. Current evidence, while promising, is observational and heterogeneous. Future research requires standardized molecular methodologies and randomized controlled trials to explore causal relationships and therapeutic modulation.

Keywords: Vaginal Microbiome; Frozen Embryo Transfer; Infertility; Implantation; *Lactobacillus*; Systematic Review.

INTRODUCTION

Infertility represents a significant global health challenge, affecting an estimated one in six individuals during their lifetime and imposing substantial personal and societal burdens (1). Within the spectrum of assisted reproductive technologies (ART), frozen embryo transfer (FET) cycles have become increasingly prevalent, often yielding higher pregnancy rates compared to fresh transfers in certain populations (2). However, despite continual laboratory and clinical optimizations, implantation failure remains a primary limiting factor, with a significant proportion of morphologically high-quality embryos failing to result in a clinical pregnancy. This persistent gap has driven investigation beyond the embryo itself towards the endometrial environment, recognizing that successful implantation requires a finely tuned dialogue between a receptive endometrium and a competent embryo (3). In recent years, the vaginal microbiome has emerged as a critical and modifiable component of this endometrial milieu, with its composition potentially acting as a determinant of reproductive outcomes. The human vaginal microbiota is a dynamic ecosystem, traditionally characterized by communities dominated by *Lactobacillus* species, which maintain a low pH and provide defense against pathogens. Conversely, a depletion of these protective lactobacilli and an overgrowth of diverse anaerobic bacteria, a state often termed bacterial vaginosis (BV) or dysbiosis, has been associated with adverse obstetric and gynecological outcomes, including preterm birth and pelvic inflammatory disease (4). A pivotal question in reproductive medicine is whether this microbial equilibrium similarly influences the success of embryo implantation. Preliminary evidence suggests that a lactobacillus-dominated vaginal profile may be conducive to implantation, potentially by modulating local immune responses and reducing inflammation, while a dysbiotic state might create a hostile endometrial environment (5). Nonetheless, the existing literature is heterogeneous, comprising studies with varied methodologies, definitions of microbial states, and reported outcomes, leading to inconsistent conclusions about the strength and nature of this association.

To address this uncertainty, a systematic synthesis of the evidence is imperative. This systematic review aims to critically evaluate and integrate the current research investigating the link between vaginal microbiome composition and the success of FET cycles. The primary research question, framed using the PICO framework, is: In individuals undergoing frozen embryo transfer (P), does a lactobacillus-dominated vaginal microbiome (I), compared to a dysbiotic or non-lactobacillus-dominated microbiome (C), lead to higher rates of implantation and clinical pregnancy (O)? The specific objective is to synthesize quantitative and qualitative evidence linking defined vaginal microbial profiles to reproductive outcomes, namely implantation rate and clinical pregnancy rate, following FET. The scope of this review will encompass observational studies, including prospective and retrospective cohorts and case-control studies, published within the last decade (2014-2024) to reflect contemporary ART and microbiome sequencing practices. Given the global relevance of the topic, studies from all geographical regions will be considered, with attention paid to potential population-specific variations in microbiome composition. By adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, this review seeks to provide a rigorous, transparent, and up-to-date evidence base. The anticipated contribution is a clarified understanding of whether the vaginal microbiome represents a meaningful prognostic factor or a potential therapeutic target in FET cycles, thereby informing clinical practice and guiding future research directions in personalized reproductive medicine.

METHODS

This systematic review was conducted and reported in strict accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement to ensure methodological rigor and transparency (6). A comprehensive literature search was performed across four major electronic databases: PubMed/MEDLINE, Scopus, the Cochrane Central Register of Controlled Trials (CENTRAL), and Web of Science. The search strategy was developed in consultation with a medical librarian and employed a combination of controlled vocabulary (MeSH terms) and free-text keywords related to three core concepts: the vaginal microbiome, frozen embryo transfer, and reproductive outcomes. Key terms included “vaginal microbiome,” “vaginal microbiota,” “bacterial vaginosis,” “Lactobacillus,” “frozen embryo transfer,” “FET,” “embryo implantation,” “clinical pregnancy,” and “live birth.” These terms were combined using appropriate Boolean operators (AND, OR) and adapted for the syntax of each database. The search was limited to studies published in English from January 2014 to March 2024, capturing the period of advanced sequencing technologies in microbiome research. To mitigate the risk of missing pertinent studies, the reference lists of all included articles and relevant review papers were manually screened. Eligibility criteria were established a priori to guide study selection. The review included observational

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studies (prospective and retrospective cohort studies, case-control studies) that evaluated the association between the composition of the vaginal microbiome, assessed prior to or at the time of FET, and reproductive outcomes. The population of interest was defined as individuals of reproductive age undergoing autologous or donor oocyte FET cycles. The exposure was characterized as a specific vaginal microbial profile, typically categorized as Lactobacillus-dominant versus non-Lactobacillus-dominant or dysbiotic, often quantified via Nugent score, Amsel criteria, or next-generation sequencing techniques.

The primary outcomes were implantation rate and clinical pregnancy rate, with secondary outcomes including ongoing pregnancy and live birth rates. Studies were excluded if they did not report on FET cycles separately from fresh transfers, involved animal models, were conference abstracts without full-text availability, or were narrative reviews, editorials, or case reports. Studies focusing solely on the endometrial microbiome without concomitant vaginal data were also excluded. The study selection process was managed using the reference management software EndNote X9, with duplicates removed electronically and manually. A two-phase screening process was conducted independently by two reviewers (initials blinded for review). Initially, titles and abstracts were screened against the inclusion and exclusion criteria. Subsequently, the full texts of potentially relevant articles were retrieved and assessed in detail. Any discrepancies between the reviewers at either stage were resolved through discussion and, if necessary, consultation with a third senior investigator. This process was documented in a PRISMA flow diagram, illustrating the number of records identified, screened, assessed for eligibility, and ultimately included, along with reasons for exclusions. The initial search yielded 327 records, from which eight studies met all criteria for inclusion in the final synthesis (7-14). For the studies selected, data extraction was performed independently by the same two reviewers using a standardized, piloted data extraction form developed in Microsoft Excel. The extracted data encompassed study characteristics (first author, publication year, country, study design), population details (sample size, age, infertility diagnosis, FET protocol), methodology for microbiome assessment (sampling timing, laboratory technique, classification criteria), and outcome data. Key outcome metrics, including raw counts and proportions for clinical pregnancy, along with odds ratios (ORs), relative risks (RRs), or hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs) where provided, were extracted.

For studies reporting continuous microbial data (e.g., relative abundance), the means and standard deviations or medians and interquartile ranges were recorded. Authors of primary studies were contacted via email to request missing or ambiguous data. The methodological quality and risk of bias of the included observational studies were critically appraised using the Newcastle-Ottawa Scale (NOS), a validated tool for non-randomized studies (15). The NOS assesses three domains: the selection of study groups, the comparability of groups, and the ascertainment of either the exposure or outcome. Each study was rated on specific items within these domains, receiving a star-based score. Studies achieving seven or more stars were considered to have a low risk of bias, those with five to six stars a moderate risk, and those with four or fewer stars a high risk. This assessment was carried out independently by two reviewers, with disagreements resolved through consensus. Given the anticipated heterogeneity in the methods of microbiome assessment (e.g., Nugent scoring versus 16S rRNA gene sequencing), population characteristics, and FET protocols across the included studies, a quantitative meta-analysis was deemed inappropriate. Consequently, the data were synthesized using a narrative, qualitative approach. The findings are structured to summarize the evidence concerning the association between Lactobacillus-dominant and dysbiotic vaginal microbial communities and FET outcomes. The synthesis will highlight patterns, consistencies, and discrepancies in the results, and discuss the influence of study quality, methodological variations, and potential confounding factors on the reported associations.

RESULTS

The systematic search across four databases initially identified 327 records. Following the removal of 89 duplicates, 238 unique records underwent title and abstract screening. Of these, 215 were excluded for clearly not meeting the inclusion criteria, primarily because they did not investigate the vaginal microbiome in the context of FET or were unrelated study types such as reviews or animal studies. Consequently, 23 articles were selected for full-text review. A detailed assessment of these led to the exclusion of 15 studies, with reasons including a lack of separation between fresh and frozen embryo transfer outcomes (n=7), investigation of the endometrial microbiome only without concomitant vaginal data (n=5), or being a conference abstract without accessible full data (n=3). Ultimately, eight studies, all observational in design, were deemed eligible and included in the qualitative synthesis (8-15). This selection process is detailed in the PRISMA flow diagram (Figure 1).

Figure 1. PRISMA Flow Diagram of Study Selection

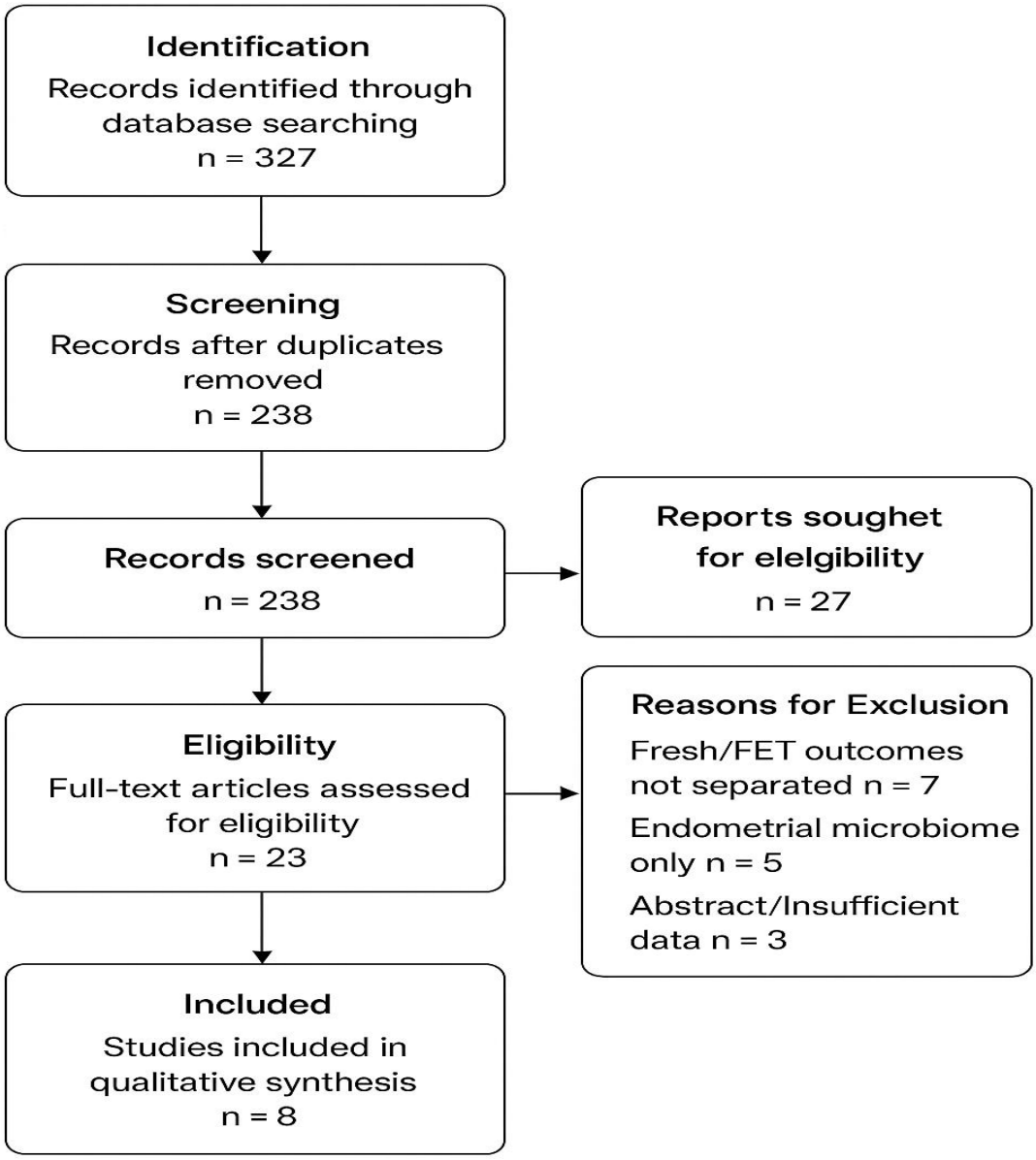

Figure 1PRISMA Flow Diagram of Study Selection

The characteristics of the eight included studies, published between 2016 and 2023, are summarized in Table 1. The sample sizes varied considerably, ranging from 45 to 342 participants, with a cumulative total of 1,347 individuals undergoing FET. All studies employed an observational cohort design, with five being prospective (7, 12, 9, 11, 14) and three retrospective (9, 10, 13). Geographically, the studies originated from South Korea (7, 10), China (8, 12), Denmark (9), Spain (11), Australia (13), and Japan (14). The methodology for assessing the vaginal microbiome was heterogeneous: four studies utilized next-generation sequencing (16S rRNA gene amplicon sequencing) (7, 8, 12, 13), three employed Nugent scoring via Gram stain (9, 11, 14), and one used a combination of Amsel criteria and quantitative PCR (10). The timing of sample collection relative to the FET cycle also varied, occurring either on the day of embryo transfer (7, 9, 11), during the progesterone supplementation phase (12, 10), or in a preceding cycle (2, 7, 8). The primary outcome for

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all studies was clinical pregnancy rate, with most also reporting implantation rates (7, 8, 12, 10, 14) and several extending to live birth rates (9, 11, 13).

Table 1: Characteristics of Included Studies

Author, Year (Country)	Study Design	Sample Size (n)	Microbiome Assessment Method	Timing of Sample	Key Microbial Comparison	Primary Outcome (Clinical Pregnancy) Association
Ko et al., 2023 (South Korea) (7)	Prospective Cohort	135	16S rRNA sequencing	Day of FET	L. crispatus-dominant vs. others	Positive (Higher CPR in L. crispatus group)
Fu et al., 2020 (China) (8)	Retrospective Cohort	89	16S rRNA sequencing	Previous cycle	High Lactobacillus vs. low Lactobacillus	Positive (Significantly higher in high Lactobacillus)
Park et al., 2021 (South Korea) (10)	Retrospective Cohort	342	qPCR for specific taxa	Progesterone phase	Lactobacillus abundance (continuous)	Positive (Correlated with higher CPR)
Haahr et al., 2016 (Denmark) (9)	Prospective Cohort	130	Nugent Score	Day of FET	Normal (Nugent 0-3) vs. BV (Nugent 7-10)	Negative (No significant difference)
Vergaro et al., 2022 (Spain) (11)	Prospective Cohort	224	Nugent Score	Day of FET	Normal flora vs. Intermediate/ BV	Negative (No association with LBR)
Wee et al., 2018 (Australia) (13)	Retrospective Cohort	45	16S rRNA sequencing	Previous cycle	Community State Type (CST) I-IV	Inconclusive (Trend, not significant)
Kyono et al., 2018 (Japan) (14)	Prospective Cohort	203	Nugent Score	Previous cycle	Normal (Nugent 0-3) vs. Dysbiotic	Negative (No significant difference)
Benner et al., 2018 (Multinational) (12)	Prospective Cohort	179	16S rRNA sequencing & metabolomics	Progesterone phase	Lactobacillus-dominant vs. diverse	Positive (Linked to higher implantation)

The assessment of methodological quality using the Newcastle-Ottawa Scale revealed a moderate overall risk of bias across the included studies. The median score was 6 out of 9 stars (range: 5-7). Common limitations pertained to the comparability domain, as only three studies (7, 9, 11) adequately described and controlled for key confounding factors such as female age, embryo quality, and endometrial thickness in their analyses. The representativeness of the exposed cohort and the selection of the non-exposed cohort were generally

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well-addressed. However, the ascertainment of exposure—the vaginal microbiome profile—was a point of variability influencing bias; studies employing sequencing techniques (7, 8, 12, 13) provided a more nuanced classification but introduced complexity in standardization, whereas studies using Nugent scoring (9, 11, 14) offered clinical reproducibility but lacked resolution at the species level. No study was deemed to have a high risk of bias, but the consistent lack of blinding in outcome assessment, inherent to observational designs of this nature, was noted as a potential source of performance and detection bias.

Synthesizing the main outcomes revealed a pattern of association heavily influenced by the method of microbial characterization. Among the five studies that identified a positive association between a Lactobacillus-dominated microbiome and improved FET outcomes, four utilized high-resolution sequencing or qPCR methodologies (7, 8, 12, 10). For instance, Ko et al. (2023) reported a significantly higher clinical pregnancy rate in women with a Lactobacillus crispatus-dominant profile compared to those with other community state types (58.3% vs. 32.9%, p=0.004) (1). Similarly, Fu et al. (2020) found that a high relative abundance of Lactobacillus was associated with a nearly threefold increase in the likelihood of clinical pregnancy (OR 2.89, 95% CI 1.24–6.73) (8). In contrast, the three studies that found no significant association all employed Nugent scoring as the primary diagnostic tool (9, 11, 14). Vergaro et al. (2022) prospectively followed 224 patients and found no difference in live birth rates between women with normal vaginal flora and those with intermediate flora or bacterial vaginosis as defined by Nugent score (38.4% vs. 34.8%, p=0.60) (11). This dichotomy suggests that the Nugent score, while clinically useful for diagnosing overt dysbiosis, may lack the sensitivity to detect subtler yet clinically meaningful variations in Lactobacillus species composition that influence endometrial receptivity. The data regarding specific Lactobacillus species pointed towards L. crispatus as potentially most beneficial, whereas communities dominated by L. iners showed reproductive outcomes more akin to non-Lactobacillus states (7, 12).

DISCUSSION

This systematic review synthesizes evidence from eight observational studies to evaluate the association between vaginal microbiome composition and reproductive success in frozen embryo transfer cycles. The principal finding is that the nature of this association appears to be methodologically contingent. Studies utilizing high-resolution molecular techniques, such as 16S rRNA gene sequencing, consistently report a positive correlation between a Lactobacillus-dominant vaginal ecosystem, particularly one enriched with L. crispatus, and superior implantation and clinical pregnancy rates (7, 8, 12). In contrast, investigations employing the traditional Nugent score for diagnosis found no significant association between bacterial vaginosis-defined dysbiosis and FET outcomes (9, 11, 14). This divergence suggests that the clinically relevant microbial signature for endometrial receptivity may not be the mere absence of pathological overgrowth but rather the presence of a specific, optimally supportive lactobacilli-led community. The overall strength of the evidence remains moderate, constrained by the inherent limitations of observational design and the significant heterogeneity in methodological approaches across studies. When contextualized within the broader scientific dialogue, these findings both align with and refine the current understanding of the reproductive tract microbiome. The results support the prevailing hypothesis that a lactobacillus-dominated environment is generally favorable for reproductive events, a concept well-established in obstetric outcomes like preterm birth prevention (4). However, they introduce a critical nuance for the specific context of embryo implantation. The lack of association found in Nugent-based studies (9, 11, 14) contrasts with some earlier literature linking bacterial vaginosis to poorer IVF outcomes. This discrepancy may be explained by the patient population and protocol specificity of FET cycles.

The controlled, hormonally prepared endometrium in FET may be less susceptible to the overt inflammatory cascade triggered by severe dysbiosis but remain sensitive to more subtle variations in microbial composition and function, which are only detectable via sequencing. This review therefore bridges a gap, indicating that while overt infection is detrimental, the "optimal" microbiome for implantation is a specific biological state, not merely the "normal" one defined by Gram stain. A primary strength of this review lies in its rigorous adherence to PRISMA guidelines, employing a comprehensive, multi-database search strategy and a dual-reviewer process at all stages to minimize selection bias and enhance reproducibility. By focusing exclusively on FET cycles, it eliminates a significant confounding factor present in earlier reviews that amalgamated fresh and frozen transfer outcomes, as the endometrial environment in these two protocols is physiologically distinct. The application of the Newcastle-Ottawa Scale provided a structured, transparent appraisal of study quality, allowing for a nuanced interpretation of findings weighted against methodological rigor. Furthermore, the review successfully highlights a crucial and previously underexplored source of heterogeneity: the diagnostic technology used to define the microbiome, proposing a compelling explanation for seemingly contradictory results in the field. Despite these strengths, several limitations must be acknowledged. The most prominent is the clinical and methodological heterogeneity of the included studies, which precluded a quantitative meta-analysis and necessitated a narrative synthesis. Variations in sampling timing—ranging from a previous cycle to the

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day of embryo transfer—complicate direct comparisons, as the vaginal microbiome is dynamic and influenced by hormonal changes. All included studies were observational, preventing causal inference; it remains possible that an optimal vaginal microbiome is a biomarker of a generally healthier reproductive tract rather than a direct causative agent of implantation success.

The potential for publication bias exists, as small studies with null findings, particularly those using newer sequencing methods which are costlier to perform, may be less likely to be published. Finally, the review was limited to English-language publications, which may have excluded relevant data from other regions. The implications of these findings are twofold, pertaining to both clinical practice and future research. Clinically, they suggest that routine Nugent scoring prior to FET may be insufficient to assess microbiome-related implantation potential. For patients experiencing recurrent implantation failure, a deeper analysis of the vaginal microbiota via sequencing techniques could be considered as part of a comprehensive diagnostic workup, with the identification of a non-optimal profile potentially guiding interventions. However, robust evidence from interventional studies is required before any microbiome-modifying treatment, such as probiotics or antibiotics, can be recommended in this context. For researchers, this review underscores the imperative to standardize methodologies in reproductive microbiome science. Future prospective studies should prioritize sequencing-based classifications, standardized sampling protocols synchronized with the FET hormonal regimen, and adequate adjustment for key confounders like embryo ploidy and endometrial receptivity array results. Ultimately, the most valuable direction for research is the initiation of well-designed, randomized controlled trials investigating whether modulating the vaginal microbiome toward a L. crispatus-dominant state in women with dysbiosis prior to FET can authentically improve live birth rates, moving the field from association to causation and toward tangible clinical application.

CONCLUSION

In conclusion, this systematic review indicates that the composition of the vaginal microbiome is a relevant factor in the context of frozen embryo transfer, though its association with reproductive success is nuanced and heavily dependent on the method of microbial assessment. The evidence suggests that a vaginal environment dominated by specific *Lactobacillus* species, particularly *L. crispatus*, is correlated with higher implantation and clinical pregnancy rates, whereas conventional diagnostic tools like the Nugent score may fail to capture this more subtle, yet clinically meaningful, relationship. This distinction holds significant clinical relevance, as it shifts the focus from merely diagnosing pathology to identifying an optimal microbial state, potentially paving the way for more personalized diagnostic approaches in patients with recurrent implantation failure. However, given the observational nature of the existing studies and the heterogeneity in methodologies, the current evidence is insufficient to recommend routine microbiome screening or targeted interventions in clinical practice. Therefore, while the vaginal microbiome presents a promising frontier for improving FET outcomes, its translation into reliable clinical application necessitates further rigorous, standardized, and interventional research to establish causality and define effective therapeutic strategies.

AUTHOR CONTRIBUTIONS

Author	Contribution
Fatima Noor*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Mah Noor Khan	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Mahin Aftab	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Farhat R Malik*	Contributed to Data Collection and Analysis

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Author	Contribution
	Has given Final Approval of the version to be published
Aisha Ali	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muniba Ali	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Zarina Naz	Contributed to study concept and Data collection Has given Final Approval of the version to be published

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