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The impact of the endometrial microbiome on IVF outcomes – current evidence

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Abstract

Background: The uterine cavity, previously considered as an aseptic physiological milieu, is now recognized as host to a distinct endometrial microbial community (EM). This analysis aims to elucidate the influence of the EM taxonomic configuration on female reproductive morbidity, with particular emphasis on its implications for Recurrent Implantation Failure (RIF). Furthermore, the study assesses the prognostic value of the EM profile in determining clinical outcomes following Assisted Reproductive Technologies (ART), specifically In Vitro Fertilization (IVF)

Aim: This systematic review was designed to elucidate the correlation between the metagenomic composition of the endometrial microbiome and female infertility.

Material and methods: The methodology centered on the comprehensive identification and rigorous critical appraisal of clinical evidence archived within the PubMed/ MEDLINE and the Cochrane Library.

Summary: The presence of a eubiotic Lactobacillus-dominated (LD) endometrial microbiome (EM) is highly correlated with optimized endometrial receptivity and

subsequently elevated rates of successful implantation, continuation of pregnancy, and live birth following assisted reproductive technology (ART) procedures. Conversely, an endometrial dysbiotic state, often characterized as non-Lactobacillus-dominated (non-LD) EM, is consistently associated with a substantial reduction in reproductive success. Studies of patients with recurrent implantation failure (RIF) identified a significantly higher relative abundance of various pathogenic bacterial genera in the EM compared with control subjects.

Although the endometrial abundance of *Lactobacillus* did not differ significantly between women with RIF and control subjects, reduced vaginal *Lactobacillus* rates in the RIF group compared to controls suggest that vaginal concentrations may represent a potential biomarker for RIF.

Keywords: microbiome; bacteria; female genital tract; vagina; endometrium; fertility; pregnancy

Introduction

Inability to achieve conception after twelve months of consistent, unprotected coitus is conventionally defined as infertility. (High-Throughput 2020), (Int. J. Mol. Sci. 2024) This condition affects approximately 8.8% of women of reproductive age worldwide, corresponding to an estimated population of 50-80 million individuals. (Int. J. Mol. Sci. 2024) While the etiology is traceable in approximately 85% of infertility diagnoses, frequently attributed to factors such as ovulatory dysfunction, tubal disease, or male factor infertility, about 15% of cases are designated as unexplained (or idiopathic) infertility. (Int. J. Mol. Sci. 2024)

The field of assisted reproductive technology (ART), specifically *in vitro fertilization* (IVF), has undergone substantial technological advancement. However, successful pregnancy outcomes are fundamentally dependent on two critical factors: the competence of the embryo and the receptivity of the endometrium. (*J. Clin. Med.* 2022) Although

these improvements have often led to higher fertilization rates, actual clinical pregnancy rates have often remained relatively static. This disparity suggests an imperative need for a more comprehensive investigation into the mechanisms governing endometrial receptivity in order to enhance therapeutic success in assisted reproduction. (*J. Clin. Med.* 2022)

Emerging research increasingly highlights a significant correlation between infertility and the microbiota. Studies suggest that infertile women possess distinct microbial compositions in their lower and/or upper reproductive tracts compared to fertile women. Consequently, the role of the microbiome must be recognized as a critical factor in reproductive health.(Int. J. Mol. Sci. 2021) The goal of analyzing the female genital tract microbiome is to enhance the understanding of factors influencing infertility, especially in the context of assisted reproductive technologies (ARTs).

A major challenge in reproductive medicine is Recurrent Implantation Failure (RIF). RIF is generally defined as the failure to achieve a clinical pregnancy after the transfer of at least four good quality embryos in at least three fresh or frozen cycles in a woman under 40 years of age. (Microorganisms 2023) Embryo implantation is a complex, spatiotemporal process involving a dynamic interaction between the mother's endometrium and the blastocyst. Failure can arise from either embryonic or uterine factors. (Microorganisms 2023)

The endometrial microbiome (EM) is characterized by having a greater species diversity (higher -diversity) than that of the lower reproductive tract. The EM can be categorized into two main community state types (CSTs) based on its composition (Acta Microbiol. Hell. 2024):

◦ Lactobacillus-dominant (LD): Characterized by a *Lactobacillus* spp. abundance percentage greater than 90%. (Acta Microbiol. Hell. 2024) (Int. J. Mol. Sci. 2024)

◦ Non-Lactobacillus-dominant (non-LD): Characterized by a *Lactobacillus* spp. abundance percentage smaller than 90%. Other species frequently identified include *Bifidobacterium*, *Gardnerella*, *Prevotella*, and *Streptococcus*. (Acta Microbiol. Hell. 2024) (Int. J. Mol. Sci. 2024)

Depending on the percentage of Lactobacillus bacteria, the endometrial microbiome is generally classified into two community categories, both of which serve as key indicators for successful pregnancy outcomes:

• Eubiosis (Lactobacillus-Dominant, LD): This state is characterized by a Lactobacillus spp. abundance percentage greater than 90%. (Acta Microbiol. Hell. 2024),(Front. Endocrinol. 2023) Eubiosis appears to be best for maximizing endometrial receptivity and is associated with higher chances of successful implantation, full-term pregnancy, and live birth following IVF. (Acta Microbiol. Hell. 2024)

• Dysbiosis (Non-Lactobacillus-Dominant, non-LD): This state occurs when Lactobacillus spp. Abundance is smaller than 90%. Dysbiosis involves a disruption where pathogenic bacteria predominate over endogenous bacteria. The non-LD EM is associated with a significant decrease in implantation, pregnancy, and live birth rates in infertile women undergoing Assisted Reproductive Technology (ART). Dysbiosis in the female genital tract is generally linked to infertility, implantation failure, and adverse pregnancy complications. (Int. J. Mol. Sci. 2024) (Int. J. Mol. Sci. 2022) (Microbiome 2022) In the total population studied, Lactobacillus abundance was positively correlated with the levels of anti-inflammatory factors IL-10 and IGF-1 and negatively correlated with pro-inflammatory molecules (IL-1β, IL-6, HIF-1α, and COX-2). (J. Clin. Med. 2022)

The role of Lactobacillus dominance in the endometrium as a definitive biomarker for implantation success is complex and debated.

Supporting Evidence (LD is essential): LD-type EM (≥90% Lactobacillus abundance) is strongly associated with good pregnancy prognoses after IVF. (Int. J. Mol. Sci. 2024) Conversely, low levels of Lactobacillus and the presence of a dysbiotic microbiome are linked to a less receptive endometrium. (J. Clin. Med. 2022)

In the total population studied, Lactobacillus abundance was positively correlated with the levels of anti-inflammatory factors IL-10 and IGF-1 and negatively correlated with pro-inflammatory molecules (IL-1β, IL-6, HIF-1α, and COX-2). (J. Clin. Med. 2022)

Some studies report that while a high abundance of pathogenic genera (e.g., the 14 RIF-associated genera) was significantly higher in RIF patients, the endometrial Lactobacillus abundance did not significantly differ between the RIF (56.2% ± 36.4%) and control groups (58.8% ± 37.0%). (*Reprod. Med. Biol.* 2021)

Only six women (28.6%) in one control group had ≥90% Lactobacillus abundance, suggesting that ≥90% Lactobacillus may not be an absolute biomarker for implantation failure. *(Reprod. Med. Biol.* 2021)

In some cases, successful pregnancies occurred in women with very low or complete absence of Lactobacillus in the endometrium. One study showed a higher number of pregnancies in women with a complete absence of Lactobacillus and higher failure rates in women with LD-type microbiomes.

An alternative hypothesis suggests that the presence of specific pathogens (e.g., Gardnerella or Prevotella) may be more predictive of unsuccessful IVF outcomes than the exact percentage of Lactobacillus. *(J. Clin. Med.* 2022) This complexity suggests that a comprehensive assessment must consider the specific composition of the non-Lactobacillus fraction and its associated inflammatory profile, rather than relying solely on the percentage of Lactobacillus. Endometrial dysbiosis, defined primarily by a non-Lactobacillus-dominant (non-LD) microbiota (Lactobacillus abundance), is intrinsically linked to inflammation-related endometrial changes that severely affect the process of embryo implantation. (J. Clin. Med. 2022)

Impairment of Mucosal Integrity: Dysbiosis, caused by pathogenic or non-commensal bacterial populations, may compromise the integrity of the endometrial mucosal barrier. This can occur by disrupting epithelial tight junctions and reducing the secretion of antimicrobial peptides and mucins. (Int. J. Mol. Sci. 2024)

Chronic Endometritis (CE): The dysbiotic state often leads to Chronic Endometritis (CE), a persistent inflammation of the endometrial lining. CE is reported in up to 40% of patients with RIF. This condition is characterized by immune activation in the endometrium, which subsequently impairs endometrial receptivity. (J. Clin. Med. 2022)

Pro-inflammatory Overexpression: The invasion of pathogen species, such as *Streptococcus* and *Gardnerella*, elicits a strong immune response. Patients with endometrial dysbiosis exhibit a marked increase in the levels of key pro-inflammatory factors.

A Lactobacillus-dominant (LD) EM is considered pivotal to maintain a physiologically non-inflamed microenvironment. This state of eubiosis is associated with successful reproductive outcomes, while dysbiosis is associated with implantation failure.

(Microorganisms 2023) In the total population studied, Lactobacillus abundance was inversely related to the concentrations of all tested inflammatory molecules: IL-1, IL-6, HIF-1, and COX-2. (J. Clin. Med. 2022) Conversely, *Lactobacillus* abundance was positively related to markers indicating endometrial wellness and anti-inflammatory action. *Lactobacillus* contributes to uterine homeostasis. It prevents pathogen entry by secreting lactic acid, which creates an acidic environment that inhibits pathogen growth. Furthermore, *Lactobacillus* can regulate the local immune response necessary for embryo implantation by acting on Pattern Recognition Receptors (PRRs) of mucosal cells. (J. Clin. Med. 2022)

Because the inflammatory cascade links dysbiosis to implantation failure, treating the bacterial imbalance may improve IVF outcomes. Successful antibiotic treatment of Chronic Endometritis (CE) has been shown to improve pregnancy and live birth rates. (Acta Microbiol. Hell. 2024) In one study, patients treated with antibiotics and probiotic supplementation to restore a non-LD EM to an LD EM subsequently achieved clinical pregnancy in their next IVF attempt. (J. Clin. Med. 2022)

Research materials and methods

This work represents a structured narrative review that included a systematic search of the literature. Electronic databases, including PubMed/MEDLINE and the Cochrane Library, were screened to identify relevant publications. To ensure comprehensive coverage, the reference lists of the most relevant articles were also examined manually. Both controlled vocabulary (MeSH terms) and free-text keywords were applied in the search strategy.

Research results

Clinical strategies primarily focus on eradicating pathogenic microorganisms to re-establish a state of eubiosis—characterized by a Lactobacillus-dominant (LD) environment—throughout the female reproductive tract. (Acta Microbiol. Hell. 2024), (Int. J. Mol. Sci.2022), (*Moreno et al. Microbiome 2022)*

This microbiological shift is intended to optimize endometrial receptivity, thereby enhancing the physiological potential for successful embryo implantation and ongoing pregnancy. (J. Clin. Med. 2022), (Acta Microbiol. Hell. 2024)

Management of Microbiological Dysbiosis and Chronic Endometritis (CE)

• Antimicrobial Pharmacotherapy: The administration of targeted antibiotics is a fundamental requirement for addressing Chronic Endometritis (CE), a pathology defined by the presence of persistent, underlying bacterial colonization.(Int. J. Mol. Sci. 2024)(J. Clin. Med. 2022) Research indicates that in populations experiencing repeated implantation failure (RIF), the successful cure of CE through antibiotic intervention is significantly associated with superior live birth and clinical pregnancy rates compared to those with unresovled infections. (J. Clin. Med. 2022), (Int. J. Mol. Sci) Furthermore, the conversion of a non-Lactobacillus-dominant (NLD) endometrial profile into a eubiotic state through antibiotics and adjuvant probiotics has been shown to markedly improve outcomes in assisted reproduction. (Int. J. Mol. Sci. 2024)

• Probiotic Supplementation: Since antibiotics are non-specific and reduce beneficial *Lactobacillus* populations along with pathogens, the simultaneous use of antibiotics and probiotics (containing *Lactobacillus* spp.) may represent an optimal therapy for pathogen eradication and microbiome restoration. (Int. J. Mol. Sci. 2024)

The goal is to restore a non-LD EM to an LD EM (Lactobacillus abundance). In one pilot study, patients who underwent antibiotic treatment followed by probiotic supplementation to modify their altered uterine composition achieved a clinical pregnancy in their subsequent IVF attempt, suggesting dysbiosis was the cause of their RIF. (J.Clin. Med. 2022)

• Lactic Acid Products: Intravaginal application of lactic acid products has been found to alleviate symptoms associated with BV, promoting the recolonization of the vagina by *Lactobacillus* species. (Int. J. Mol. Sci. 2024)

• Microbiome Transplantation: Strategies like microbiome transplantation (similar to fecal transplantation) are proposed as a promising therapeutic approach for gynecological conditions, especially when patients do not respond to standard treatments. (Int. J. Mol. Sci. 2024) (Front. Cell. Infect. Microbiol. 2024)

However, little is currently known about microbiome transplantation in the female genital tract, and it requires further research to determine key parameters (dosage, administration

method, optimal composition) and rigorous donor screening to prevent transmission of pathogens. (Int. J. Mol. Sci. 2024) (Front. Cell. Infect. Microbiol. 2024)

Discussion

Future research should focus on refining understanding and closing knowledge gaps:

• Large Cohort Studies: A greater number of studies utilizing larger patient cohorts is required to clarify contradictory results and establish certain biomarkers.

• Species-Level Specificity: Research should move towards taxa-level resolution (species or strain level) to better understand the specific effects of different *Lactobacillus* strains (e.g., L. *iners* versus L. *crispatus*) and truly pathogenic bacteria.(Int. J. Mol. Sci. 2024)

• Multi-Omics Integration: The combination of metagenomic investigations with other multi-omics analyses (proteomic, metabolomic, and transcriptomic analyses) is necessary to understand the functional potential of the microbiome and decipher pathogenic signatures linked to disease. (Int. J. Mol. Sci. 2024)

• Personalized Therapies: The goal is to develop advanced diagnostic tools and identify specific biomarkers to allow for the development of personalized therapeutic protocols (e.g., nutritional, microbiotic, or pharmaceutical interventions). (Microorganisms 2023)

Conclusion

Understanding of the endometrial microenvironment has fundamentally shifted, as the uterine cavity, once considered sterile, is now recognized to harbor a distinct microbiome. This emerging knowledge addresses the critical challenge in reproductive medicine: Recurrent Implantation Failure (RIF), which remains difficult to treat even with high-quality embryos. The composition of the endometrial microbiome (EM) is strictly associated with reproductive success. Despite the overall association, the field faces several uncertainties: evidence is contradictory regarding whether EM Lactobacillus abundance alone is a definitive biomarker, as some studies have found no significant difference between RIF patients and controls. Furthermore, successful pregnancies have been reported even with low or absent *Lactobacillus* in the endometrium.

The EM typically exhibits higher diversity than the vaginal microbiome (VM). While EM diversity often does not differ significantly between RIF and control groups, changes in diversity (compositional shift) show a tendency toward separation between the cohorts.

Analyzing the vaginal microbiome (VM) is a promising diagnostic route, as sampling is non-invasive and repeatable. Some findings suggest that vaginal *Lactobacillus* rates, unlike endometrial rates, may be a relevant biomarker for RIF.

Since dysbiosis-related inflammation affects IVF outcomes, preliminary studies suggest that restoring eubiosis, typically via antibiotics combined with probiotic supplementation, may improve success rates. Successful antibiotic treatment for CE is also associated with improved pregnancy rates. However, the field is limited by small sample sizes and a lack of standardized protocols across sampling methods (e.g., fluid vs. tissue), sequencing, and bioinformatics. Therefore, a definitive microbial biomarker has not yet been identified. Further large-scale studies and the standardization of methodologies are critical to clarifying the contradictions, developing accurate diagnostic tools, and establishing personalized therapeutic strategies.

Disclosure Section

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