International Clinical and Medical Case Reports Journal
Research Article (ISSN: 2832-5788)

Endometrial Microbiome (NGS method) and its Effect on Fertility

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Citation: *Prompona N, Efstratiadou M, Stagaki E, Fraidakis M. Endometrial microbiome (NGS method) and its effect on fertility. Int Clinc Med Case Rep Jour 2025;4(7):1-10.*

Received Date: 13 July, 2025; **Accepted Date:** 16 July, 2025; **Published Date:** 18 July, 2025

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ABSTRACT

Recent scientific advances have challenged the traditional view of the uterine cavity as a sterile environment, revealing the presence of a distinct endometrial microbiome. This discovery has prompted growing interest in its potential role in female fertility, particularly in assisted reproductive technologies (ART). The composition of the endometrial microbiome-especially the dominance or depletion of Lactobacillus species-appears to influence implantation success and pregnancy outcomes. Dysbiosis, including conditions such as chronic endometritis, may be associated with recurrent implantation failure (RIF) and unexplained infertility. Despite promising findings, current evidence remains limited and sometimes contradictory, highlighting the need for further research. This review explores the emerging understanding of the endometrial microbiome, its clinical implications in infertility and the future potential of targeted microbial interventions.

Keywords: Assisted reproductive technologies; Recurrent implantation failure; Microbial interventions;

INTRODUCTION

The human body coexists with a vast array of microorganisms that together form the human microbiome. These microbial communities, including bacteria, viruses and fungi, are not merely passive passengers but play active roles in health and disease. Approximately 9% of the human microbiome resides in the female reproductive tract and interest in this niche has surged due to its implications in reproductive health.

Historically, the endometrial cavity was considered sterile. However, advancements in sequencing technologies-particularly next-generation sequencing (NGS)-have revealed that the endometrium harbors its own unique microbiome. This endometrial microbial community is now recognized as a potential modulator of endometrial receptivity and embryo implantation.

One of the most studied features of the endometrial microbiome is the relative abundance of Lactobacillus species. Studies suggest that a Lactobacillus-dominated environment may be associated with higher implantation and live birth rates in women undergoing in vitro fertilization (IVF), whereas dysbiosis may correlate with recurrent implantation failure (RIF) or unexplained infertility. Chronic endometritis, often asymptomatic, represents a notable example of such dysbiosis and has been implicated in nearly one-third of RIF cases.

Factors such as age, ethnicity, antibiotic or probiotic use, intrauterine devices, recurrent infections and gynecological conditions like endometriosis and polyps may influence the composition of the endometrial microbiome. Furthermore, differences between the vaginal and endometrial microbiota challenge the assumption that vaginal sampling can reliably reflect the uterine environment.

This review aims to synthesize current findings on the endometrial microbiome and its impact on female fertility, focusing on human studies employing NGS-based methodologies, while identifying critical gaps for future research.

METHODS AND MATERIALS

A comprehensive literature search was conducted to support this review, aiming to elucidate the microbial dynamics of the female reproductive tract and their association with endometrial health and infertility. The search spanned key academic databases, including PubMed, Web of Science, Scopus and Google Scholar and focused on peer-reviewed studies published from 2007 onward. To ensure the retrieval of relevant and high-quality sources, a carefully selected set of keywords was used, including *“infertility,” “endometrial microbiome,” “Lactobacillus,” “endometritis,” and “ngs.”* This keyword-driven approach enabled the identification of pertinent studies that explored the role of the endometrial microbiome in reproductive function, thereby ensuring a thorough and current synthesis of existing evidence.

Articles, reviews and reports were carefully selected according to predefined inclusion and exclusion criteria tailored to the scope of the review. Eligible sources were required to be published in English and to address contemporary developments in microbial dynamics and their impact on endometrial health and infertility. To ensure the review was grounded in the most relevant and up-to-date evidence, the focus was orientated to studies published between 2016 and 2025. This rigorous selection process aimed to capture and critically evaluate the existing body of literature that sheds light on the intricate relationship between the endometrial microbiota and reproductive health.

Results

Throughout its evolutionary journey, the human species has coexisted with a multitude of microorganisms, including bacteria, fungi and viruses [1]. The total collection of these organisms along with their genetic material constitutes the microbiome of the human body. Approximately 9% of the total human microbiome is found in the female reproductive tract [2]. The endometrium of the uterus was initially considered to be sterile, but it is now confirmed that there is an endometrial microbiome, which in fact affects embryo implantation and pregnancy outcomes [3].

The study of the microbiome of the female reproductive tract has recently attracted scientific interest in the field of infertility, as the endometrial microbiome, through immunological mechanisms, modifies endometrial receptivity, thereby influencing the embryo implantation process (Benner, 2018) [4].

A typical example of microbiome disruption and chronic inflammation in the uterine cavity is endometritis. The microorganisms identified as causes of endometritis include: *Staphylococcus*, *Streptococcus*, Enterobacteriaceae, *Bifidobacterium*, *Clostridium*, *Prevotella*, *Atopobium*, *Mobiluncus*, *Peptostreptococcus*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma*, *Chlamydia*, *Acinetobacter*, *Gardnerella*, *Fusobacterium*, *Megasphaera*, *Pseudomonas* [5].

Endometritis is often asymptomatic and is confirmed histologically through biopsy after hysteroscopy. It is responsible for approximately 30% of repeated implantation failures (RIF) following in vitro fertilization (IVF) [6]. It is also observed in cases of unexplained infertility and accounts for 50% of recurrent pregnancy losses [7].

Factors associated with changes in the endometrial microbiome include age, ethnicity, antibiotic use with or without concurrent use of probiotics, use of intrauterine contraceptive devices, recurrent urinary tract infections, gynecological conditions such as endometriosis and endometrial polyps and possibly dietary and lifestyle habits such as smoking [8].

Sequencing technologies are fundamental to detecting microorganisms in areas like the uterine cavity. The NGS (Next Generation Sequencing) method is the most widely used technique for identifying the endometrial microbiome and provides reliable results. Specifically, the technology is based on the isolation of DNA followed by amplification and sequencing of seven hypervariable regions (V2, V3, V4, V6, V7, V8 and V9) of the bacterial 16S ribosomal RNA (rRNA). The 16S rRNA is evolutionarily conserved in all bacteria and contains nine variable regions with DNA sequences specific to each species. This method allows for the identification and relative quantification of bacteria in a given sample [9].

According to recent literature reviews, data on the impact of the endometrial microbiome on infertility is limited and sometimes conflicting. Modern studies support that in women undergoing IVF who had an endometrial microbiome with <90% Lactobacillus dominance, a significant decrease was observed in implantation rates (23.1% vs 60.7%)

and live birth rates (33.3% vs 70%) compared to women whose endometrial microbiome contained >90% Lactobacillus [10]. In contrast, other studies claim that an endometrial microbiome lacking high Lactobacillus abundance does not affect the outcome of a successful pregnancy [5].

Additionally, some studies investigate the role of probiotics in improving pregnancy and live birth rates in patients undergoing ART [11]. Particularly interesting are studies showing differences between the vaginal and endometrial microbiome, which support the separate evaluation of the endometrial microbiome in infertility cases through uterine fluid sampling or hysteroscopy and biopsy [8,12].

Certainly, more data is needed in this research field to clarify the role of the endometrial microbiome in infertility. This could allow for the investigation and possible modification of parameters that have thus far been overlooked, especially in cases of RIF and recurrent miscarriages, ultimately leading to the achievement of pregnancy and the birth of a live child.

A table (Table 1)presents the key studies from which the above data were drawn. These studies were conducted in humans and the analysis of the endometrial microbiome (where applicable) was carried out using the NGS method. The selected studies were chosen based on their originality, date of publication and sample size.

Table 1: Key Studies on the Endometrial Microbiome and Infertility (NGS-based)

Title | Author | Journal	**Sample**	**Aim**	**Result**
Evidence that the endometrial microbiota has an effect on implantation success or failure [10].	• 13 fertile (for testing vaginal and endometrial microbiome) • 22 fertile (for testing endometrial microbiome only) • 35 infertile with receptive endometrium	To examine the differences between vaginal and endometrial microbiota, the impact of hormones and the influence of endometrial microbiota on IVF success	• Clear difference between vaginal and endometrial microbiota. • Endometrial microbiota classified as Lactobacillus-dominated (LDM, >90% Lactobacillus spp.) and non-LDM (<90% Lactobacillus). • Non-LDM in receptive endometrium associated with lower implantation (23.1% vs 60.7%), pregnancy (33.3% vs 70.6%) and live birth rates (6.7% vs 58.8%).
A pilot study and case reports on endometrial microbiota and pregnancy outcome: An analysis using 16S rRNA gene sequencing among IVF patients and trial therapeutic intervention for dysbiotic endometrium [5].	• 92 IVF patients with frozen blastocysts, <45 years • No vaginitis or endometritis	To analyze pregnancy rates in IVF patients with LDM vs non-LDM; report antibiotic + probiotic/prebiotic treatment cases	• 51.1% LDM, 48.9% non-LDM. • 9 non-LDM cases converted to LDM with treatment. • No significant difference in pregnancy rates between LDM and non-LDM groups

Endometrial microbiota composition is associated with reproductive outcome in infertile patients [13].	• 342 asymptomatic infertile women, BMI 18.5–30, ≤50 years • Excluded: recent antibiotics, active infection, abnormal menstrual cycles, anatomical abnormalities of the reproductive system or partners with abnormal semen analysis	To associate microbiome profiles with outcomes: live birth, biochemical pregnancy, miscarriage, no pregnancy.	• Non-Lactobacillus-dominant profiles (Atopobium, Gardnerella, etc.) linked to poor outcomes. • Lactobacillus-rich profiles linked to higher live birth rates.
Does antibiotic therapy for chronic endometritis improve clinical outcomes of patients with recurrent implantation failure in subsequent IVF cycles? A systematic review and meta-analysis [14].	9 studies investigating the treatment of CE in patients with RIF were retrieved from PubMed up to 31/01/2022 EMBASE, Wanfang and Google Scholar i) Randomized controlled and non-controlled studies, as well as retrospective studies ii) Patients with RIF (Recurrent Implantation Failure), with or without a confirmed history of CE (Chronic Endometritis) iii) Patients with CE who received antibiotics and patients without CE iv) Subsequent IVF cycles after antibiotic treatment	To assess antibiotic treatment effectiveness for CE in improving IVF outcomes in RIF patients.	• No IVF outcome difference in patients treated with antibiotics vs those without CE. • Treated CE patients had better IVF outcomes than untreated or persistent CE. • Persistent CE linked to poor IVF outcomes
The impact of the female genital tract microbiome in women health and reproduction: a review [8].	A PubMed review of available articles in English between 01/2004 and 04/2021 was conducted based on the following keywords: 'genital tract microbiota and reproduction', 'endometrial microbiome', 'microbiome and reproduction' and 'microbiota and infertility	To explore the genital tract microbiome's role in ART and gynecological disorders	• Lactobacillus dominance in vagina and uterus beneficial. • Microbial patterns observed in endometriosis, BV, ovarian/endometrial cancer. • Less microbial diversity + Lactobacillus dominance linked to better ART outcomes

How uterine microbiota might be responsible for a receptive, fertile endometrium [4].	A review of articles in English published between 01/01/2008 and 01/03/2018 was conducted via PubMed and Google Scholar, including the terms '16S rRNA', 'uterus' and related keywords	To investigate the role of uterine microbiota in fertility	Microbiota influence immune mechanisms essential for implantation and infection defense
Endometrial microbiome: sampling, assessment and possible impact on embryo implantation [15].	• 53 infertile women for ICSI• Exclusions: PID, abnormal discharge, hydrosalpinx, cavity abnormalities, recent antibiotics/hormones,vaginal bleeding,previous difficult embryo transfers, scheduled embryo transfer in the same cycle	To test catheter sampling for endometrial microbiota and assess its link to pregnancy	• LDM found in only 8%.• Vaginal vs endometrial microbiota differed.• No specific microbiome profile predicted ICSI success.• Greater biodiversity may improve endometrial receptivity
Pregnancy outcomes after vaginal probiotic supplementation before frozen embryo transfer: a randomized controlled study [16].	340 infertile women randomized: probiotic group vs control	To compare pregnancy outcomes after vaginal probiotics	• Similar biochemical/clinical pregnancy rates. • Miscarriage significantly lower in probiotic group. • Higher live birth rate in bacterial vaginosis subgroup and probiotic group
The Role of Endometrial Microbiota in Fertility and Reproductive Health: A Narrative Review [17].	A review of articles published in English from 2014 to 2024 was conducted through PubMed, Web of Science, Scopus and Google Scholar. The following keywords were used: 'infertility,' 'dysbiosis,' 'lactobacillus,' 'endometrial infection,' and 'hormonal changes'	To explore the impact of endometrial microbiota on fertility and health	• Healthy microbiota (mainly Lactobacillus) vital for reproductive health. • Hormonal changes alter microbiota, affecting implantation. • Dysbiosis linked to infertility. • CE associated with RIF and infertility
Characterization of the Endometrial Microbiome in Patients with Recurrent Implantation Failure [18].	• Cohort study: 27 RIF patients vs 18 controls • Excluded: vaginal infections, hydrosalpinx, uterine anomalies, known RIF factors	To compare microbiota in RIF vs controls undergoing ART	• Lactobacillus most common in both groups. • RIF group showed distinct prevalence of species like Prevotella, Streptococcus, etc., suggesting implantation role.

Effect of L. crispatus M247 Administration on Pregnancy Outcomes in Women Undergoing IVF: A Controlled, Retrospective, Observational and Open-Label Study [11,19,20].	160 ART patients: 80 control, 80 with probiotic treatment	To assess L. crispatus effect on pregnancy/live birth rates	• Higher live birth rates in the probiotic group (12.5% vs 7.5%). • Blastocyst transfer success increased 200% with probiotics. • Stronger effect in women with BMI approaching 35

DISCUSSION

The emerging evidence underscores the pivotal role of the endometrial microbiome in female fertility, particularly in the context of assisted reproductive technologies (ART) such as in vitro fertilization (IVF). Across multiple studies utilizing next-generation sequencing (NGS) techniques, a clear association has been established between the composition of the endometrial microbiota and reproductive outcomes.

A consistent finding is the favorable association between a Lactobacillus-dominated microbiome (LDM) and improved reproductive outcomes. The landmark study by Moreno, et al. first delineated the concept of LDM (>90% Lactobacillus spp.) versus non-LDM (<90% Lactobacillus spp. with >10% other taxa) [10], demonstrating significantly higher implantation, clinical pregnancy and live birth rates in women with LDM undergoing IVF. This foundational insight was corroborated in a subsequent, larger cohort study by the same group (2022) [21], which identified specific bacterial genera (e.g., Atopobium, Gardnerella, Streptococcus) associated with reproductive failure, further highlighting the pathogenic potential of dysbiotic profiles [22].

Intervention studies suggest that the microbiome is modifiable and clinically relevant. Kyono, et al. reported successful conversion of non-LDM to LDM using antibiotics combined with probiotics [5], although the improvement in pregnancy rates did not reach statistical significance. More convincingly, Di Pierro, et al. observed a 200% increase in live birth rates following blastocyst transfer in women receiving Lactobacillus crispatus M247 [11], indicating the potential for targeted probiotic therapies to enhance IVF success, particularly in overweight patients [23-25].

The influence of the vaginal microbiome on endometrial health and embryo implantation has also been explored. Thanaboonyawat, et al. demonstrated that vaginal probiotic supplementation prior to frozen embryo transfer significantly reduced miscarriage rates and improved live birth rates[16], particularly in women diagnosed with bacterial vaginosis. This suggests that restoration of a eubiotic vaginal environment may indirectly contribute to endometrial receptivity [26].

Another important contributor to reproductive failure is chronic endometritis (CE), particularly in cases of recurrent implantation failure (RIF). The meta-analysis by Cheng, et al. revealed that antibiotic treatment of CE improved IVF outcomes in patients with RIF [14], although persistent CE was still associated with poor prognosis [27,28]. This finding emphasizes the importance of timely diagnosis and treatment of subclinical uterine infections in optimizing ART outcomes.

Interestingly, the diversity and biogeographical differences of the microbiome within the female genital tract have also been investigated. Reschini, et al. confirmed the distinct microbial compositions of the vagina and endometrium, validating the importance of endometrial-specific sampling methods [15]. While no specific microbiota profile was definitively associated with ICSI success, greater biodiversity was hypothesized to enhance endometrial receptivity. Finally, reviews by Benner, et al. [4], Punzón-Jiménez, et al. [8] and Karadbhajne, et al. collectively highlight the complex interplay between the endometrial microbiota, hormonal regulation, immunological factors [17] and systemic health. The findings point toward a dynamic and multifactorial influence of the microbiome on implantation and pregnancy maintenance [29].

Taken together, these studies build a compelling case for the diagnostic and therapeutic utility of microbiome profiling in the management of infertility [30]. However, several limitations persist, including inter-study heterogeneity, lack of standardized diagnostic thresholds and inconsistent microbiome definitions. Further large-scale, randomized controlled trials are warranted to define optimal intervention protocols and to clarify whether microbiome modulation should become a routine part of fertility treatment.

CONCLUSION

Although initial studies show promising associations, the field remains in its infancy. Evidence is often limited, occasionally contradictory and derived from heterogeneous populations and methodologies. A clearer understanding of the endometrial microbiome's role in fertility could pave the way for novel diagnostics and personalized therapeutic strategies, particularly for women experiencing RIF or recurrent pregnancy loss.

REFERENCES

1. Simon JC, Marchesi JR, Mougel, C, Selosse MA. Host-microbiota interactions: from holobiont theory to analysis. Microbiome. 2019;7(1).
2. Peterson J, Garges S, Giovanni M, et al. The NIH Human Microbiome Project. Genome Res. 2009;19(12):2317-2323.
3. Koedooder R, Mackens S, Budding A, et al. Identification and evaluation of the microbiome in the female and male reproductive tracts. Human Reproduction Update. 2018;25(3):298-325.

4. Benner M, Ferwerda G, Joosten I, van der Molen RG. How uterine microbiota might be responsible for a receptive, fertile endometrium. Human Reproduction Update. 2018;24(4):393-415.
5. Kyono K, Hashimoto T, Kikuchi S, Nagai Y, Sakuraba Y. A pilot study and case reports on endometrial microbiota and pregnancy outcome: An analysis using 16S rRNA gene sequencing among IVF patients and trial therapeutic intervention for dysbiotic endometrium. Reproductive Medicine Biology. 2018;18(1):72-82
6. Cicinelli E, Matteo M, Tinelli R, et al. Chronic Endometritis Due to Common Bacteria Is Prevalent in Women with Recurrent Miscarriage as Confirmed by Improved Pregnancy Outcome After Antibiotic Treatment. Reproductive Sciences. 2013;21(5):640-647.
7. Bender Atik R, Christiansen OB, Elson J, et al. ESHRE guideline: recurrent pregnancy loss. Human Reproduction Open. 2018;2018(2).
8. Punzón-Jiménez P, Labarta E. The impact of the female genital tract microbiome in women health and reproduction: a review. J Assisted Reproduction Genetics. 2021;38(10):2519-2541.
9. Chakravorty S, Helb D, Burday M, Connell N, Alland D. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. J Microbiological Methods. 2007;69(2):330-339.
10. Moreno I, Codoñer FM, Vilella F, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol. 2016;215(6):684-703
11. Pierro FD, Sinatra F, Ros MCLD, et al. Effect of L. crispatus M247 Administration on Pregnancy Outcomes in Women Undergoing IVF: A Controlled, Retrospective, Observational and Open-Label Study. Microorganisms. 2023;11(11):2796-2796.
12. Moreno I, Simon C. Relevance of assessing the uterine microbiota in infertility. Fertil Steril 2018;110(3):337-343
13. Moreno I, Garcia-Grau I, Perez-Villaroya D, et al. Endometrial microbiota composition is associated with reproductive outcome in infertile patients. Microbiome. 2022;10(1).
14. Cheng X, Huang Z, Xiao Z, Bai Y. Does antibiotic therapy for chronic endometritis improve clinical outcomes of patients with recurrent implantation failure in subsequent IVF cycles? A systematic review and meta-analysis. J Assisted Reproduction Genetics. 2022;39(8):1797-1813.
15. Reschini M, Benaglia L, Ceriotti F, et al. Endometrial microbiome: sampling, assessment and possible impact on embryo implantation. Scientific Reports. 2022;12(1).
16. Thanaboonyawat I, Pothisan S, Petyim S, Laokirkkiat P. Pregnancy outcomes after vaginal probiotic supplementation before frozen embryo transfer: a randomized controlled study. Scientific Reports. 2023;13(1).
17. Karadbhajne P, More A, Dzoagbe HY. The Role of Endometrial Microbiota in Fertility and Reproductive Health: A Narrative Review. Cureus. 2025.
18. Lozano FM, Lledó B, Morales R, Cascales A, Hortal M, Bernabeu A, Bernabeu R. Characterization of the Endometrial Microbiome in Patients with Recurrent Implantation Failure. Microorganisms. 2023;11(3):741.
19. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine Microbiota: Residents, Tourists, or Invaders? Frontiers in Immunology. 2018;9.

20. Chenoll E, Moreno I, Sánchez M, et al. Selection of New Probiotics for Endometrial Health. Frontiers Cellular Infection Microbiology. 2019;9.
21. Cicinelli E, Matteo M, Tinelli R, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Human Reproduction. 2015;30(2):323-330.
22. Coomarasamy A, Dhillon-Smith RK, Papadopoulou A, et al. Recurrent miscarriage: evidence to accelerate action. The Lancet 2021;397(10285):1675-1682.
23. Granot I, Gnainsky Y, Dekel N. Endometrial inflammation and effect on implantation improvement and pregnancy outcome. Reproduction. 2012;144(6):661-668.
24. Kliman HJ, Frankfurter D. Clinical approach to recurrent implantation failure: evidence-based evaluation of the endometrium. Fertil Steril. 2019;111(4):618-628
25. Leonardi M, Hicks C, El-Assaad F, El-Omar E, Condous G. Endometriosis and the microbiome: a systematic review. BJOG. 2020;127(2):239-249.
26. Moreno I, Cicinelli E, Garcia-Grau I, et al. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. Am J Obstet Gynecol. 2018;218(6):602
27. Riganelli L, Iebba V, Piccioni M, et al. Structural Variations of Vaginal and Endometrial Microbiota: Hints on Female Infertility. Frontiers in Cellular Infection Microbiology. 2020;10.
28. Toson B, Simon C, Moreno I. The Endometrial Microbiome and Its Impact on Human Conception. Int J Molecular Sciences. 2022;23(1):485.
29. Van Sinderen M, Menkhorst E, Winship A, Cuman C, Dimitriadis E. Preimplantation Human Blastocyst-Endometrial Interactions: The Role of Inflammatory Mediators. American J Reproductive Immunology. 2012;69(5):427-440.
30. Wang W, Feng D, Ling B. Biologia Futura: endometrial microbiome affects endometrial receptivity from the perspective of the endometrial immune microenvironment. Biologia Futura. 2022;73(3):291-300.