Do Probiotic Interventions Improve Female Unexplained Infertility? A Critical Commentary

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Keywords: microbiome, infertility, female reproductive health, microbiota, probiotic

Abstract

Disruption of the women's gut and cervicovaginal microbiota has been associated with multiple gynaecologic diseases such as endometriosis, polycystic ovary syndrome (PCOS), noncyclic pelvic pain, and infertility. Female infertility affects 12.6% of women worldwide; its aetiology is complex and multifactorial and can be underpinned by uterine pathologies, systemic diseases, and age. In addition, a new perspective has emerged on the role of the gut and vaginal microbiomes in reproductive health. Research shows that the administration of precisely selected probiotics, often in combination with prior antibiotic treatment, may facilitate the restoration of symbiotic microbiota to increase successful conception and assisted reproductive technology outcomes. However, full research clarity is currently hampered by a lack of consistency and harmonisation in clinical studies: various lactobacilli and bifidobacteria species have been delivered through both the oral and vaginal routes, in different dosages, for different treatments' durations. This commentary explores the intricate relationship between the microbiota in the cervicovaginal and gut of women, exploring their potential contribution to infertility. It highlights ongoing research on the use of probiotic formulation in improving pregnancy outcomes, critically examining the divergent findings in these studies, which complicate a conclusive assessment of the efficacy of these interventions.

Endometrial, vaginal, and gut dysbiosis in reproductive health

Roughly 80% of reproductive-age women host a vaginal microbial community predominantly composed of *Lactobacillus (L.)* species *(spp.)*, which represent a reliable biomarker for a well-balanced vaginal ecosystem (Pendharkar et al. 2023). In detail, four key species *L. crispatus*, *L. gasseri, L. iners,* and *L. jensenii*, were the most common species found in fertile asymptomatic women (Ravel et al. 2011). *Lactobacillus* colonisation of the reproductive system begins at puberty upon oestrogen exposure and then undergoes major fluctuations until menopause. Specifically, pregnancy is a period of notable hormonal shifts with consequent significant changes in the vaginal microbiota. In many cases, the dominance of the *Lactobacillus spp.* becomes even more established to increase the production of lactic acid that maintains the acidic pH of the vagina below 4.5, to favour their own survival, discourage pathogen colonisation, and thus protect against infections (Di Simone et al. 2020). Conversely, a dysbiotic vaginal microbiota (so-called vaginal dysbiosis) is characterized by reduced or no *Lactobacillus spp.* and vaginal dysbiosis was found to be correlated with bacterial vaginosis (BV) and pregnancy complications (Thanaboonyawat et al. 2023; Kyono et al. 2019).

In their systematic review, Hong et al. conducted a comprehensive analysis of vaginal microbiota data, involving 3277 women who had experienced primary and/or tubal infertility (Hong et al. 2020). Their analysis relied on categorizing the vaginal microbiota samples into two groups: low-*Lactobacillus* vaginal microbiota and high-*Lactobacillus* vaginal microbiota. Specifically, women were classified as

high-*Lactobacillus* group if they had a Nugent score of 0-3, a negative Amesel/Spiegel's test, or if the vaginal community status was dominated by *Lactobacillus spp*. via 16S rRNA gene sequencing technique. Otherwise, they were classified as low-*Lactobacillus* group. When using a fixed model to estimate the overall association, their results showed a significant negative correlation between *Lactobacillus spp.* abundance and infertility (Odds Ratio of 0.83 with 95% CI 0.77–0.90), indicating that a healthy vaginal microbiota is associated with a reduced risk of infertility (Hong et al. 2020).
Advanced sequencing techniques enabled a more detailed exploration of specific bacterial species inhabiting the vaginal tract, shedding light on variations linked to infertility. In a subset of 30 women dealing with secondary infertility, defined as the inability to conceive or carry a baby to term after previously giving birth without infertility treatments or medications, Zhao et al. found that the lower presence of *Lactobacillus* compared to healthy women resulted in a significantly increased abundance of *Atopobium*, *Aerococcus*, *Megasphaera*, and *Prevotella* (Zhao et al. 2020). Notably, *Atopobium* exhibited a particularly increase. Additionally, *Megasphaera*, *Prevotella*, and *Atopobium* species were identified in patients with BV, suggesting that the combined effects of various anaerobic bacteria might play a crucial role in infertility's pathogenesis rather than the dominance of a single species (Zhao et al. 2020).

While the composition of bacteria in the vaginal microbiota has been established, as well as its association with infertility, recent studies and discussions have focused on the endometrial microbiota, which was traditionally considered a sterile environment (Kyono et al. 2019; Moreno et al. 2022; Di Simone et al. 2020). After assessing that women physiologically carry a differentiated endometrial microbiota compared to vaginal microbiota, a potential correlation was assessed with reproductive outcomes intended as live birth, biochemical pregnancy, clinical miscarriage, or no pregnancy (Moreno et al. 2022). Specifically, they assessed the reproductive function of 342 women with infertility undergoing in vitro fertilization (IVF) through the collection of endometrial fluid (EF), endometrial biopsy (EB), or both (in 84.8% of cases). Their findings suggested that both EF and EB had *Lactobacillus* as the major genus, and this higher abundance was found to be significantly positively related to live birth. Conversely, *Lactobacillus* depletion and the presence of specific pathogenic bacteria such as *Atopobium, Bifidobacterium, Chryseobacterium, Gardnerella, Streptococcus,* and *Klebsiella* in EF, and/or *Bifidobacterium, Gardnerella, Klebsiella* and *Neisseria* in EB were significantly associated with unsuccessful pregnancies (Moreno et al. 2022).

Unlike the vaginal tract microbiota, the gut microbiota hosts a diverse microbial community, with *Lactobacillus spp*. accounting for a small proportion of the total bacterial count (Cho and Blaser 2012). Research on the direct correlation between intestinal microbiota and infertility is limited: Komiya et al. observed an abundance of the phylum Verrucomicrobiota in infertile females compared to the fertile control group (Komiya et al. 2020). However, it is important to acknowledge that the gut microbiota significantly impacts the endocrine reproductive system by interacting with hormones like oestrogen, androgen and insulin; impaired levels of oestrogen and androgen may affect polycystic ovary syndrome (PCOS) and endometriosis, both of which are endocrine pathologies associated with a higher risk of infertility (Qi et al. 2021). Thus, both the gut and reproductive tract microbiota may be related to reproductive health issues, although research hasn't been able so far to explain whether bacteria in the gut can translocate to the reproductive tract.

Despite the health benefits of probiotics and prebiotics, when it comes to unexplained infertility, there is still uncertainty regarding the efficacy of live biotherapeutic products in resolving reproductive health challenges.

Do probiotics improve fertility?

The clinical studies outlined in , represent the only research efforts that have specifically examined the pregnancy rates when probiotics or prebiotics are administered to infertile subjects, for both natural pregnancy and IVF procedures (Fernández et al. 2021; Kyono et al. 2019; Komiya et al. 2020; Gilboa et al. 2005; Thanaboonyawat et al. 2023).

In a recent study, Thanaboonyawat et al. supplemented intravaginal *Lactobacillus acidophilus* for 6 days to infertile women who underwent IVF at the end of the probiotic treatment: the biochemical and clinical pregnancy rates were comparable in both groups (39.9 and 34.2% in the study group vs. 41.8 and 31.7% in the control group) (Thanaboonyawat et al. 2023). However, infertile women who were diagnosed with BV experienced higher clinical pregnancy rates and statistically significant higher live births meaning that the probiotic product may have helped restore a healthier vaginal microbiome and reduce BV-related reproductive complications. Additionally, *Lactobacillus* supplementation significantly reduced the miscarriage rate, defined as the spontaneous loss that occurs before 20 completed weeks of gestation, in the study group compared to the control group (9.5% vs. 19.1%) (Thanaboonyawat et al. 2023).
Four weeks of intravaginal *Lactobacillus* supplementation, after one week of antibiotic treatment, managed to also shift the endometrial microbiome from non-*Lactobacillus* dominant to *Lactobacillus* dominant group when measuring the mucus of the endometrium via 16S rRNA gene sequencing (Kyono et al. 2019). The therapy may have helped increase the implantation and pregnancy rates caused by BV: 58.9% success per patient in the *Lactobacillus* dominant group, compared to 47.2% success per patient in the non-dominant group. However, as the observed difference was not statistically significant it was not possible to assess the treatment's effectiveness in affecting pregnancy rates (Kyono et al. 2019).
However, not all intravaginal interventions have been shown to increase pregnancy rates, or positively influence microbial composition. A total of 117 women who underwent ovarian stimulation and IVF were equally assigned to the study group that received a single intravaginal administration of the probiotics *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Bifidobacterium longum*, and the control group. For instance, the intravaginal administration of Lactobacilli following oocyte retrieval did not manage to successfully colonise the vaginal tract, and neither managed to improve the pregnancy rate (Gilboa et al. 2005).

In addition to exploring intravaginal probiotics, researchers have also ventured into investigating the potential of oral probiotics for addressing infertility. For example, a group of 18 infertile women were administered an oral supplementation of 10g/day of partially hydrolysed guar gum, a prebiotic dietary fibre, to improve gut dysbiosis and pregnancy outcomes (Komiya et al. 2020). The faecal samples collected from the infertile subjects exhibited structural differences in microbial β-diversity compared to 18 fertile females, with the Verrucomicrobiota phylum being more abundant in the infertile group. Among the infertile participants, 12 patients received assisted reproductive technology (ART) combined with oral supplementation of guar gum fibre for one month, resulting in a pregnancy

success rate of 58.3%. The screening of their gut microbiota composition showed that the administration of the dietary fibre resulted in an increased abundance of *Bifidobacterium*, a great producer of lactic and acetic acids. Interestingly, the group who achieved successful pregnancies maintained stable levels of *Bifidobacterium* even after four weeks, whereas the group with failed pregnancies experienced a decrease in abundance. However, this study is a single-arm intervention study with guar gum and comparative studies are needed in the future to confirm these findings (Komiya et al. 2020).

In another study, a group of 58 women were divided into three distinct groups: recurrent abortion (RA, n=21), infertility (INF, n=23) who received at least two IVF treatments before the study, and a fertile control group (n=14) (Fernández et al. 2021). None of the participants had received hormonal therapy, antibiotics or probiotics over the four weeks preceding the recruitment. The RA and INF groups received daily oral supplementation of *Lactobacillus salivarius CECT5713*, approximately 10^9 CFU/day, for 6 months or until a pregnancy was diagnosed. Out of the 44 participants in the RA and INF groups, a notable 29 pregnancies were achieved, demonstrating a pregnancy effectiveness rate of 66% (95% CI: 52-80%). Of these pregnancies, 25 resulted in a successful full-term pregnancy, yielding an overall reproductive success rate of 57% (95% CI: 42-72%). Moreover, *L. salivarius* supplementation resulted in significant positive changes in the vaginal pH, vaginal *Lactobacillus* colonisation, and vaginal cytokines among pregnant women (Fernández et al. 2021).

Limitations of the studies

Overall, microbiota-based therapies seem to be an encouraging approach to enhancing women's infertility. However, the variability in the design of the clinical studies represents a clear limitation in the interpretation and comparison of the results. The role of abnormal vaginal microbiota on fertility outcomes has not been justified due to the lack of standardised methodology in the clinical studies (The Unexplained Infertility guideline group, Romualdi D, Ata B, Bhattacharya S, Bosch E, Costello M, Gersak K, Homburg R, Le Clef N, Mincheva M et al. 2023). Indeed, it is very difficult to compare different detection methods used to assay bacterial composition (microscopy, PCR, and next-generation-sequencing), and various time points for the collection of vaginal swabs. Furthermore, more attention should be given to the study subjects. The nature of their infertility (primary, secondary, tubal etc.), pregnancy history, sexual contact, past oral contraceptive usage, past antibiotic usage, miscarriage, and female health history may have different effects on the vaginal/endometrial microbiota. Research has also shown that vaginal microbiota composition can be different among individuals of different ethnicities: for example, non-pregnant women of African ancestry are less likely to be dominated by species of *Lactobacillus* compared to those of Hispanic or European ancestry. However, during early pregnancy, women of African ancestry undergo a significant shift in their vaginal microbiome becoming more *Lactobacillus*-dominated at the expense of *G. vaginalis* and other anaerobes (Serrano et al. 2019). These subject related factors should be also carefully considered in the design of clinical studies. Therefore, the use of standardized methodologies entails establishing clear guidelines for researchers to improve the comparability of their findings and enhance our understanding about the impact of vaginal microbiota on unexplained infertility.

The selection of probiotic composition also warrants careful evaluation. Various *Lactobacillus spp.* such as *L. rhamnosus, L. reuteri, L. gasseri, L. plantarum,* and *L. salivarius*, delivered locally and orally, were used in the studies (Thanaboonyawat et al. 2023; Fernández et al. 2021; Kyono et al. 2019; Gilboa

et al. 2005). Conversely, less common was the use of bacterial strains that are normally found in the gut microbiota, such as *Bifidobacterium* alone or in combination with *Lactobacillus*, to ameliorate eubiosis in the gut (Kyono et al. 2019; Fernández et al. 2021).

Although there is no direct association between the beneficial use of antibiotics and infertility, antibiotics are efficient treatments for infertility-linked conditions like BV and chronic endometriosis. For instance, when an appropriate antibiotic treatment (based on the antibiogram results) was prescribed to infertile women with chronic endometritis, improved IVF outcomes were observed (Cicinelli et al. 2015). Antibiotics may prove helpful in reducing pathogenic bacterial species in the vaginal tract of patients with non-*Lactobacillus* dominant microbiota, and subsequent probiotics and prebiotics may be effective in restoring a *Lactobacillus* dominant microbiota as shown by Kyono et al. (Kyono et al. 2019). However, it's important to acknowledge the potential risks associated with the cumulative use of antibiotics, as this may deplete both uterine/vaginal and gut microbial communities.

The potential transmissibility of bacteria between the two microbial habitats could be the reason why some probiotics delivered by oral route manage to create a vaginal setting favourable for *Lactobacillus spp*., and the reason why three of the studies reported in *Table 1* chose an oral administration of their probiotic products (Komiya et al. 2020; Fernández et al. 2021; Kyono et al. 2019). In their study, Struss et al. monitored the persistence of vaginal and rectum epithelial colonisation by the three *Lactobacillus* strains, in 37 healthy women, through the oral administration of a capsule containing the freeze-dried *L.gasseri*, *L. fermentum*, *L. plantarum* at the concentration of $1x10^8$ CFU (Strus et al. 2012). Specifically, the vaginal and rectal colonisation of *Lactobacillus spp.* started after 10 days from the beginning of the daily administration of the probiotic and lasted up to day 70, and up to 10 days after ending administration of the mixture. Oral delivery of live microbiota to the colon and rectum requires the probiotic to survive the acidic environment of the stomach and the rich enzymatic activity of the small intestine. For this reason, in clinical studies, the viability of the probiotics should be monitored through the recovery of the specific microorganisms in faecal samples.

Moreover, clinical studies that deliver probiotics for female-related diseases are heterogeneous in the number of viable microorganisms contained in each product (for an intravaginal product: 100 million live bacteria/tablet (Thanaboonyawat et al. 2023), to 3 billion live bacteria/capsule (Gilboa et al. 2005)), and the duration of interventions varied from a single day (Gilboa et al. 2005) to six months (Fernández et al. 2021). To guarantee the efficient repopulation of beneficial bacteria in the urogenital flora, Reid et al. advised that gelatine capsules administered orally for one month, with *L. rhamnosus GR-1* plus *L. fermentum RC-14* probiotic dosage regimens with over 10^8 viable bacteria per day, were necessary to restore a balanced vaginal microbiota (Reid et al. 2001).

Research on microbiota-related therapies in the improvement of reproductive outcomes is still in its infancy. Scientific investigations are still at the stage of discovering and understanding the role of the vaginal and gut microbiota on fertility outcomes and introducing remedies like probiotics into mainstream care will require considerable effort in a society that prioritizes disease treatment over health and pharmaceutical interventions over natural preventive measures. However, as our understanding of the intricate relationship between the microbiota and fertility deepens, it is possible that probiotics and other microbiota-related interventions may find their way into mainstream care, offering new avenues for couples struggling with infertility.

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Funding

Alessia Favaron is supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 956851.

Table 1 – Clinical studies investigating the effects of probiotics and prebiotics on unexplained infertility outcomes in women.

Study / Reference	Microbiota-based intervention	Treatment duration	Route of administration	Type of sample collected	Clinical study participant demographics	Ethnicity	Results
"Pregnancy outcomes after vaginal probiotic supplementation before frozen embryo transfer: a randomized controlled study" (Thanaboonyawat et al. 2023)	Gynoflor with *Lactobacillus acidophilus* bacteria (100 million CFU per tablet) and 0.03 mg estriol	6 days	Intravaginal	No collection	340 infertile women, Between 18–39 years	Thai	No effect of intravaginal Lactobacilli supplementation in the general IVF population, on the biochemical, clinical, ongoing pregnancy rate, implantation rate, and live birth rate. However, the intravaginal probiotic supplementation significantly reduced the miscarriage rate and increased the live birth rate in patients who received blastocyst transfer.
"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" (Kyono et al. 2019)	One week of antibiotic treatment, Lactoferrin GX® 300 mg/d continued consecutively Florgynal® Tampon Probiotique by Saforelle (Laboratoires IPRAD, Paris, France) – L. Rhamnosus, L.	Florgynal® Tampon Probiotique by Saforelle (Laboratoires IPRAD, Paris, France) – for 3-4 days during menstrual period mediGYNE® by Saforelle (Laboratoires IPRAD, Paris, France) – 1 month therapy	Intravaginal (tampon) and oral	Endometrial fluid (EF) specimens were carefully aspirated with a intrauterine insemination (IUI) catheter	92 IVF patients, < 45 years old	Asian (90 Japanese, one Korean, and one Chinese)	20% patients with non-*Lactobacillus* dominant microbiota were treated by antibiotics and prebiotics, and successfully became *Lactobacillus* dominant. Pregnancy rates in the *Lactobacillus* dominant microbiota group were higher than in the non-*Lactobacillus* dominant group but not significantly different.

	Gasseri, L. Fermentum mediGYNE® by Saforelle (Laboratoires IPRAD, Paris, France)						
"Does intravaginal probiotic supplementation increase the pregnancy rate in IVF–embryo transfer cycles?" (Gilboa et al. 2005)	Probiotic Femina: 3 billion live cells of human strains of Lactobacillus acidophilus, Bifidobacterium bifidum and Bifidobacterium longum. (2 capsules administered to study group)	Single administration following oocyte retrieval	Intravaginal	Vaginal swab	117 IVF women, study group, n = 50 control group, n = 67 < 38 years old	Not specified	Intravaginal probiotic supplementation immediately after oocyte retrieval had no effect on vaginal colonisation by lactobacilli. There was no association of the presence of lactobacilli before oocyte retrieval or embryo transfer and pregnancy rate.
"Characterizing the gut microbiota in females with infertility and preliminary results of a water-soluble dietary fibre intervention study" (Komiya et al. 2020)	PHGG (partially hydrolyzed guar gum) supplementation in addition to embryo transfer therapy	4 weeks	Oral	Fecal samples were collected using a feces collection kit	18 infertile women, 18 healthy controls, < 39 years old	Japanese	PHGG supplementation helped improve gut dysbiosis and the success of pregnancy in females with infertility.

"Application of Ligilactobacillus salivarius CECT5713 to Achieve Term Pregnancies in Women with Repetitive Abortion or Infertility of Unknown Origin by Microbiological and Immunological Modulation of the Vaginal Ecosystem" (Fernández et al. 2021)	*Ligilactobacillus salivarius* CECT5713 10^9 CFU/day	6 months or until pregnancy	Oral	Two samples were collected: A vaginal swab specimen a cervicovaginal lavage of the cervical and the vaginal walls.	58 women, aged 28–45, With a history of recurrent miscarriage, or history of infertility despite being the recipients of ART for at least three times.	Not specified	Administration of *L. salivarius* CECT5713 led to 29 pregnancies out of the 44 participating patients. This means a pregnancy effectiveness of 66%. Among them, there were 25 successful pregnancies and 4 abortions. This means an effectiveness for reproductive success of 57%.