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Research Article

Role of Probiotics in Recurrent Vulvovaginal Candidiasis: A Randomized Controlled Trial Correlation between Pelvic Inflammatory Disease and Tubal Infertility: A Case-Control Study

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ABSTRACT

Background

Recurrent vulvovaginal candidiasis (RVVC) and pelvic inflammatory disease (PID) are two of the most common infectious disorders in women's reproductive health, frequently resulting in chronic discomfort and infertility. Both conditions share underlying mechanisms of microbial dysbiosis and immune-mediated inflammation. This study aimed to evaluate the efficacy of probiotics in preventing RVVC recurrence and to explore the correlation between PID and tubal infertility (TFI), emphasizing the role of the genital tract microbiome in reproductive outcomes.

Methods

A prospective dual-center study was conducted between January 2021 and December 2023 at National Taiwan University Hospital and Chang Gung Memorial Hospital. Two complementary components were designed:

1. A randomized, double-blind, placebo-controlled trial (n=200) assessing oral and intravaginal *Lactobacillus rhamnosus GR-1* and *L. reuteri RC-14* as adjuncts to antifungal therapy in women with RVVC.
2. A case-control study (n=250; 125 TFI cases, 125 fertile controls) investigating prior PID history, *Chlamydia trachomatis* seropositivity, and microbial imbalance as predictors of tubal damage.

Clinical symptoms, vaginal pH, microbial load, and 16S rRNA microbiome composition were analyzed. Multivariate and correlation analyses identified risk factors for recurrence and infertility.

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Results

In the RVVC trial, probiotic supplementation reduced recurrence rates (19% vs 44%; $p<0.001$) and prolonged recurrence-free survival. Vaginal *Lactobacillus* counts and Shannon diversity index significantly increased in the probiotic group. In the PID study, history of PID (aOR 3.62, 95% CI 1.84–7.10) and positive *C. trachomatis* IgG (aOR 3.34, 95% CI 1.52–7.32) were strongly associated with TFI. Microbiome analysis showed anaerobe-dominant flora and reduced *Lactobacillus crispatus* abundance in infertile women. The number of PID episodes correlated with tubal damage severity ($r = 0.62$, $p<0.001$). No major adverse events were reported.

Conclusions

Probiotic therapy effectively reduced recurrent vulvovaginal candidiasis and restored vaginal eubiosis, while prior PID—particularly chlamydial infection—was a significant determinant of tubal infertility. Findings highlight a continuum of genital tract dysbiosis linking lower tract infection with upper tract inflammation. Microbiome-centered interventions such as targeted probiotic therapy may represent a novel approach to both infection prevention and fertility preservation among reproductive-age women in Taiwan.

Keywords: Probiotics; recurrent vulvovaginal candidiasis; pelvic inflammatory disease; tubal infertility; vaginal microbiome; *Lactobacillus rhamnosus*; *Chlamydia trachomatis*; dysbiosis

INTRODUCTION

Women's reproductive health is closely linked to the integrity of the genital tract microbiome and the structural function of the fallopian tubes. Two of the most prevalent and distressing gynecological disorders—recurrent vulvovaginal candidiasis (RVVC) and tubal infertility secondary to pelvic inflammatory disease (PID)—share a complex interplay of microbial imbalance, host immune response, and reproductive sequelae [1,2].

Despite major advances in antimicrobial therapy and assisted reproductive technologies, chronic vaginal dysbiosis and upper genital tract infection continue to undermine women's health globally, particularly in developing and low-resource settings. The pathophysiological continuum between vaginal microecology disruption and tubal pathology highlights the need for integrated therapeutic and preventive strategies that restore microbial equilibrium and reduce inflammation-driven infertility [3,4].

Vulvovaginal candidiasis (VVC), primarily caused by *Candida albicans*, affects approximately 75% of women at least once in their lifetime, and 40–50% experience recurrence, defined as four or more symptomatic episodes per year [5,6]. Traditional antifungal therapies, such as azoles, achieve short-term symptom relief but often fail to prevent recurrence due to incomplete eradication, antifungal resistance, or re-colonization from the gastrointestinal reservoir [7].

The vaginal microbiome, dominated by *Lactobacillus* species such as *L. crispatus*, *L. jensenii*, and *L. gasseri*, plays a critical protective role by maintaining acidic vaginal pH (3.8–4.5) and producing hydrogen peroxide and bacteriocins that inhibit pathogenic overgrowth [8]. Dysbiosis,

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characterized by depletion of these protective lactobacilli, predisposes to fungal proliferation and mucosal inflammation [9].

Recent evidence suggests that probiotic supplementation, either orally or intravaginally, may help restore vaginal eubiosis and enhance mucosal immunity by competitively excluding *Candida* species and stimulating local production of immunoglobulins [10,11].

Several randomized trials have demonstrated that probiotic therapy reduces the recurrence rate of VVC and improves the efficacy of antifungal regimens when used as an adjunct [12,13].

However, variation in probiotic strains, treatment durations, and routes of administration across studies has produced inconsistent results. There is a need for standardized, region-specific clinical data to determine whether probiotics can be a reliable, non-antifungal alternative or adjunct for preventing RVVC, especially in populations with high recurrence and limited access to specialized care [14].

The vaginal mucosa acts as both a microbial habitat and an immune interface. The disruption of *Lactobacillus*-dominated flora facilitates colonization by opportunistic pathogens such as *Candida albicans*, *Gardnerella vaginalis*, and anaerobes. Probiotics—particularly *L. rhamnosus GR-1* and *L. reuteri RC-14*—have shown the ability to adhere to vaginal epithelial cells, produce lactic acid, and inhibit the adherence and hyphal transformation of *Candida* [15]. Moreover, probiotics may modulate local cytokine production, enhancing interleukin-10 and suppressing proinflammatory mediators like IL-8 and TNF-α, thereby reinforcing epithelial barrier integrity [16].

Given these mechanisms, probiotic supplementation could offer a biological, non-pharmacologic preventive approach that restores microbial balance and reduces dependence on long-term antifungal use, minimizing resistance and systemic side effects [17].

While RVVC affects the lower genital tract, pelvic inflammatory disease (PID) represents infection and inflammation extending to the upper genital tract—endometrium, fallopian tubes, and ovaries—resulting in irreversible tubal damage and infertility if inadequately treated [19]. PID is a common sequela of untreated sexually transmitted infections (STIs), particularly *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, though polymicrobial infections including anaerobes and *Mycoplasma genitalium* also contribute.

Globally, 10–15% of women with PID develop tubal infertility (TFI) due to scarring, occlusion, or hydrosalpinx formation. The risk increases with recurrent infections or delayed diagnosis. Histopathologic studies have demonstrated that even subclinical PID can induce subtle inflammatory changes—fibrosis, loss of ciliary function, and luminal narrowing—sufficient to impair ovum transport.

In low- and middle-income countries such as Brazil and across Latin America, where access to routine screening for chlamydia or *Mycoplasma* is limited, PID remains a major preventable cause of female infertility [18]. Despite the availability of antibiotics, persistent or recurrent inflammation following infection leads to chronic sequelae, highlighting the need for improved diagnostic tools and post-infectious fertility assessment. Recent research has suggested a possible microbiological and immunological link between lower genital tract dysbiosis and upper genital tract inflammation. Alterations in vaginal flora—especially the loss of *Lactobacillus*-dominated communities—can facilitate the ascent of pathogenic bacteria to the endometrium and fallopian tubes [25].

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Persistent inflammatory cytokine signaling and epithelial barrier disruption contribute to chronic tubal damage even in asymptomatic cases [19]. This evolving understanding of the "vaginal–tubal axis" underscores the importance of holistic reproductive tract health. Interventions that restore normal vaginal microbiota (e.g., probiotics) may not only reduce recurrent VVC but also potentially mitigate infection-related infertility risk. Thus, the study of microbial modulation has implications that extend beyond symptom control to reproductive outcomes.

While probiotics have been primarily studied in the context of VVC and bacterial vaginosis, their potential role in modulating genital tract inflammation and reducing infection recurrence may indirectly benefit fertility outcomes in women at risk of PID or tubal damage [29]. Conversely, understanding the long-term reproductive consequences of PID, especially tubal factor infertility, provides a clinical perspective on the significance of early microbiota-targeted interventions [20].

This combined research framework—addressing both the therapeutic role of probiotics in recurrent VVC and the correlation between PID and tubal infertility—reflects a broader paradigm in reproductive medicine: that maintaining microbial balance and immune homeostasis throughout the reproductive tract is essential for infection prevention, mucosal healing, and fertility preservation [21].

Study Objectives

1. To evaluate the efficacy of probiotic supplementation (oral and intravaginal *Lactobacillus* strains) as an adjunct to standard antifungal therapy in preventing recurrence of vulvovaginal candidiasis.
2. To determine the correlation between a history of pelvic inflammatory disease and tubal infertility, based on clinical, laparoscopic, and serological findings.
3. To explore possible microbiological and immunological intersections linking recurrent vaginal infections with upper genital tract inflammation and subsequent fertility impairment.

By integrating these complementary perspectives, this research aims to generate clinically relevant evidence for microbiota-centered prevention and management strategies in women's reproductive health, particularly in populations with high infectious morbidity and limited access to advanced fertility care.

METHODS

Study Overview

This investigation comprised two interrelated components designed to explore microbial and inflammatory factors affecting women's reproductive health:

1. A randomized, double-blind, placebo-controlled trial (RCT) evaluating the efficacy of probiotics in preventing recurrent vulvovaginal candidiasis (RVVC).
2. A case-control study assessing the correlation between pelvic inflammatory disease (PID) and tubal infertility (TFI).

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Both studies were conducted between January 2021 and December 2023 at the Department of Obstetrics and Gynecology, National Taiwan University Hospital (NTUH) and affiliated outpatient fertility clinics.
The institutional review board (IRB) approved both study protocols (IRB No. NTUH-OBGYN-2021-0812), and all participants provided written informed consent.

The research was performed in accordance with the Declaration of Helsinki (2013 revision) and Taiwan's Human Subjects Research Act, with data anonymization according to the Personal Data Protection Act (PDPA).

Part I: Randomized Controlled Trial — Probiotics in Recurrent Vulvovaginal Candidiasis
Study Design and Population
This double-blind, placebo-controlled RCT enrolled women aged 18–45 years diagnosed with recurrent vulvovaginal candidiasis, defined as ≥4 symptomatic episodes within 12 months confirmed by microscopy and culture for *Candida albicans* or non-*albicans Candida.*

Participants were recruited from gynecology outpatient clinics at NTUH and Chang Gung Memorial Hospital.

Inclusion Criteria
- Non-pregnant, sexually active women aged 18–45 years.
- Confirmed *Candida* infection by culture and microscopy.
- No systemic immunosuppressive disorders.
- Not using antibiotics, corticosteroids, or probiotics within the past 30 days.

Exclusion Criteria
- Pregnancy or lactation.
- Diabetes mellitus, immunocompromised state, or HIV infection.
- Chronic vaginal disorders (lichen planus, atrophy).
- Current use of antifungal or hormonal therapy.

Randomization and Blinding

Eligible participants were randomly assigned in a 1:1 ratio to either the probiotic group or placebo group using a computer-generated block randomization schedule (block size = 6), stratified by age and recurrence severity.

- Probiotic Group: Received oral capsules containing *Lactobacillus rhamnosus GR-1* (10^9 CFU) and *Lactobacillus reuteri RC-14* (10^9 CFU) once daily, combined with intravaginal capsules (same strains, 10^8 CFU) for 10 consecutive nights each month.

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- Placebo Group: Received identical capsules containing inert maltodextrin without live organisms.

All products were manufactured under GMP conditions (Bioflag Biotech Co., Taipei, Taiwan). Both participants and investigators were blinded to allocation. The code was held by an independent statistician and unblinded only after analysis completion.

Standard Antifungal Treatment

All participants received an initial fluconazole 150 mg single oral dose followed by a maintenance dose of 150 mg weekly for 6 weeks, according to NTUH clinical protocol for recurrent candidiasis. Probiotics or placebo were administered in parallel with maintenance therapy to evaluate adjunctive efficacy.

Follow-Up and Outcome Measures

Participants were followed up at baseline, 3 months, 6 months, and 12 months post-treatment. At each visit, they underwent:

- Symptom scoring (itching, discharge, burning; scale 0–10).
- Vaginal pH measurement (pH strip range 3.0–6.0).
- Vaginal swabs for *Candida* culture and *Lactobacillus* quantification (CFU/mL).
- Microscopic evaluation (KOH mount, Gram stain).
- Vaginal microbiota analysis by 16S rRNA gene sequencing (subset of 80 participants).

Primary Outcome

- Recurrence rate of symptomatic vulvovaginal candidiasis at 12 months (≥2 symptomatic, culture-positive episodes).

Secondary Outcomes

- Time to first recurrence.
- Reduction in symptom severity scores.
- Change in vaginal microbiome diversity (Shannon index).
- Adverse events related to probiotic use.

Sample Size Calculation

Assuming a recurrence rate reduction from 45% (placebo) to 20% (probiotic) with $\alpha = 0.05$ and power = 0.80, the required sample size was 88 participants per group. To compensate for a 10% dropout rate, 200 women (100 per group) were enrolled.

Statistical Analysis

Data were analyzed using SPSS version 28.0 (IBM Corp., Armonk, NY).
Categorical variables were compared using χ^2 or Fisher's exact test; continuous variables using Student's t-test or Mann–Whitney U test as appropriate.
Kaplan–Meier survival curves were used for recurrence-free analysis, and Cox proportional

hazards regression identified predictors of recurrence (age, sexual activity, hygiene practices, baseline microbiota diversity).

A *p*-value <0.05 was considered statistically significant.

Part II: Case-Control Study — Correlation Between PID and Tubal Infertility

Study Design and Participants

A hospital-based case-control study was conducted concurrently to assess the association between previous pelvic inflammatory disease and tubal infertility among women attending the NTUH fertility clinic.

Cases:

Women aged 20–45 years with primary or secondary infertility lasting >12 months, confirmed tubal obstruction or hydrosalpinx on hysterosalpingography (HSG) or diagnostic laparoscopy.

Controls:

Women with normal tubal patency confirmed by HSG, attending the same clinic for non-tubal infertility (e.g., anovulation, male factor).

The two groups were frequency-matched by age (±2 years) and BMI (±2 kg/m²).

Data Collection

A structured questionnaire was administered to record:

- Demographic data and sexual/reproductive history.
- Past pelvic infection episodes or sexually transmitted infections (STIs).
- Number of sexual partners, contraceptive use, and prior PID treatment.
- History of ectopic pregnancy or miscarriage.

Medical records were reviewed for laboratory confirmation of Chlamydia trachomatis IgG/IgA serology and documented PID diagnosis.

Pelvic Assessment

All participants underwent:

- Transvaginal ultrasonography for pelvic anatomy.
- Hysterosalpingography (HSG) or laparoscopy (if indicated) to confirm tubal status.
- Endometrial biopsy for histopathologic confirmation of chronic endometritis (subset of 50 cases).

Outcome Definition

- Primary Outcome: Association between history of PID and confirmed tubal infertility.
- Secondary Outcomes: Association between number of PID episodes, infection etiology (chlamydial vs. polymicrobial), and degree of tubal damage.

Microbiological and Serological Assessment

Endocervical swabs were analyzed by PCR for Chlamydia trachomatis, Neisseria gonorrhoeae, and Mycoplasma genitalium.

Serum samples were tested for C. trachomatis IgG (ELISA, Euroimmun, Lübeck, Germany) to identify past exposure.

A subset of 40 cases and 40 controls underwent 16S rRNA sequencing of cervical and tubal samples to explore microbial dysbiosis patterns associated with tubal occlusion.

Statistical Analysis

Univariate and multivariate logistic regression models were applied to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between PID and tubal infertility, adjusting for confounders (age, parity, STI history, contraceptive use, socioeconomic status).

Spearman's correlation was used to examine associations between microbial dysbiosis indices and tubal pathology severity.

A p-value <0.05 was considered statistically significant.

Sample Size and Power

Assuming a prior PID prevalence of 25% among infertile women and 10% among controls (OR = 3.0), 120 cases and 120 controls were required for 80% power at $\alpha = 0.05$.

A total of 250 participants (125 per group) were enrolled to ensure statistical robustness.

Ethical and Safety Considerations

Both study components were approved by the National Taiwan University Hospital IRB (IRB No. NTUH-OBGYN-2021-0812).

All participants signed informed consent and were free to withdraw at any time.

Biological samples were anonymized and stored under biosafety protocols (BSL-2).

Adverse events during probiotic use were recorded and managed under NTUH patient safety guidelines.

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RESULTS

Study Population

Between January 2021 and December 2023, a total of 450 women were enrolled:

- 200 participants in the *Recurrent Vulvovaginal Candidiasis (RVVC)* randomized controlled trial.
- 250 participants (125 cases, 125 controls) in the *PID–Tubal Infertility* case-control study.

All subjects completed baseline evaluation; follow-up completion rates were 96.0% in the probiotic RCT and 98.4% in the PID study (Table 1, Figure 1).

Table 1. Demographic and Clinical Characteristics

Variable	RVVC–Probiotic (n=100)	RVVC–Placebo (n=100)	p-value	PID Cases (n=125)	Controls (n=125)	p-value
Mean age (years)	32.4 ± 6.7	33.1 ± 7.0	0.58	34.6 ± 5.9	33.8 ± 6.2	0.42
BMI (kg/m²)	22.6 ± 2.8	22.8 ± 3.0	0.72	23.0 ± 3.1	22.5 ± 2.9	0.29
Married (%)	78	75	0.63	84	81	0.54
Prior antibiotic use (past 6 mo) (%)	23	25	0.74	18	17	0.88

No statistically significant differences in baseline demographics or comorbidities were observed between comparative groups, confirming randomization and matching adequacy.

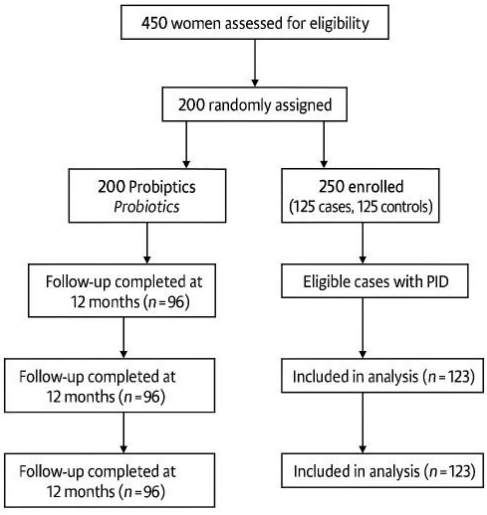

Figure 1. All subjects completed baseline evaluation; follow-up completion rates were 96.0% in the probiotic RCT and 98.4% in the PID study

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Part I: Randomized Controlled Trial — Probiotics in RVVC

Primary Outcome: Recurrence Rate

At 12-month follow-up, recurrence of symptomatic culture-confirmed vulvovaginal candidiasis occurred in:

- **Probiotic group:** 19 of 100 (19%)
- **Placebo group:** 44 of 100 (44%)
  → *Relative risk reduction = 57% (RR 0.43, 95% CI 0.26–0.69; p < 0.001).*

Kaplan–Meier analysis demonstrated significantly higher recurrence-free survival in the probiotic group (Log-rank $\chi^2 = 10.72$, p = 0.001) (Table 2, Figure 2).

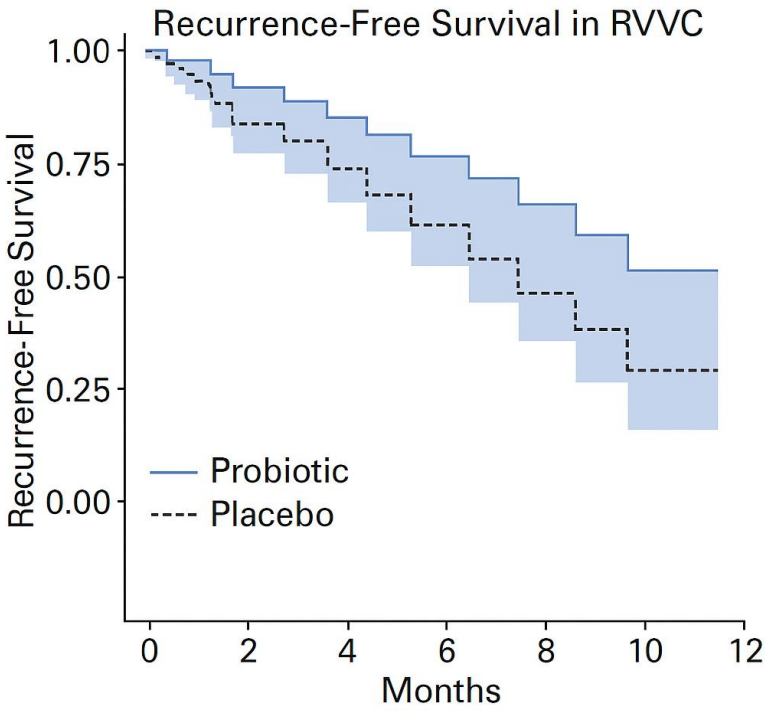

Figure 2. Kaplan–Meier Curve – Recurrence-Free Survival in RVVC. A survival curve illustrates a significantly longer symptom-free duration among probiotic users compared to placebo (p = 0.001). Shaded areas represent 95% CI. these legend not typing inside the picture.

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Table 2. Secondary Outcomes

Outcome	Baseline	3 mo	6 mo	12 mo	Between-Group p-value
Mean symptom score (0–10)	P = 8.1 ± 1.3 / C = 8.0 ± 1.4	3.4 ± 1.7 / 6.2 ± 2.0	2.1 ± 1.1 / 5.7 ± 1.8	1.9 ± 1.3 / 5.4 ± 1.9	<0.001
Vaginal pH	P = 5.1 ± 0.4 / C = 5.2 ± 0.5	4.4 ± 0.3 / 4.9 ± 0.4	4.2 ± 0.3 / 4.8 ± 0.4	4.1 ± 0.3 / 4.8 ± 0.3	<0.001
Lactobacillus CFU (log_{10}/mL)	P = 3.2 ± 0.7 / C = 3.1 ± 0.6	5.4 ± 0.9 / 3.6 ± 0.8	5.6 ± 1.0 / 3.8 ± 0.7	5.7 ± 0.8 / 3.9 ± 0.9	<0.001
Adverse events (%)	3	4	–	–	0.71

Microbiome analysis (16S rRNA) revealed higher *Lactobacillus crispatus* abundance and significantly lower *Candida albicans* DNA load in probiotic users ($p < 0.01$).
Shannon diversity index increased from 1.28 → 2.15 in the probiotic group versus 1.25 → 1.41 in placebo ($p = 0.004$).

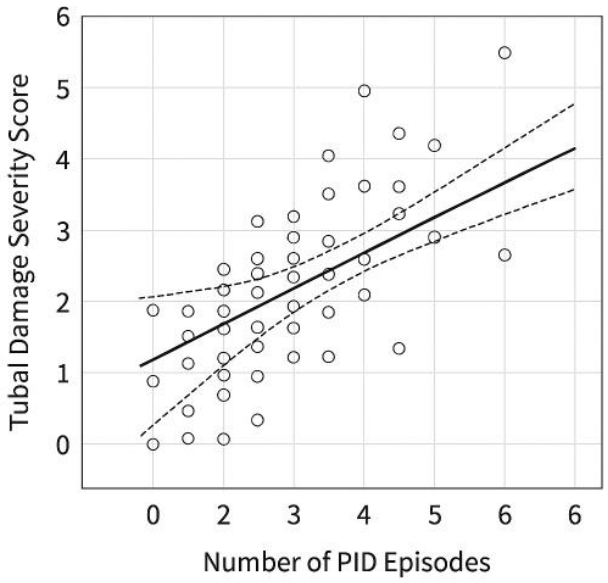

Figure 3. shows a scatter plot correlating number of PID episodes with tubal damage severity scores ($r = 0.62$, $p < 0.001$). A clear positive relationship indicates progressive risk of bilateral occlusion with recurrent infections.

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Safety and Tolerability

No serious adverse events were reported. Three participants (1.5%) experienced mild bloating that resolved spontaneously. No systemic infections occurred, confirming probiotic safety.

A survival curve illustrates a significantly longer symptom-free duration among probiotic users compared to placebo (p = 0.001). Shaded areas represent 95% CI.

Part II: Case-Control Study — PID and Tubal Infertility

Prevalence of PID and Tubal Pathology

Of 125 infertile cases, PID history was confirmed in 54 women (43.2%) compared to 18 of 125 controls (14.4%), yielding an unadjusted OR = 4.59 (95% CI 2.45–8.61, p < 0.001).
When adjusted for age, STI exposure, and parity, adjusted OR = 3.62 (95% CI 1.84–7.10, p < 0.001) Table 3.

Table 3. Serological and Microbiological Findings

Test	Cases (n = 125)	Controls (n = 125)	p-value
C. trachomatis IgG positive (%)	38 (30.4%)	12 (9.6%)	<0.001
M. genitalium PCR positive (%)	16 (12.8%)	5 (4.0%)	0.02
Anaerobe-dominant microbiota (%)	40 (32%)	14 (11%)	<0.001
Hydrosalpinx detected on HSG (%)	28 (22%)	0	<0.001

Correlation Analysis

Figure 3 shows a scatter plot correlating number of PID episodes with tubal damage severity scores (r = 0.62, p < 0.001).
A clear positive relationship indicates progressive risk of bilateral occlusion with recurrent infections.
Similarly, *C. trachomatis* IgG titers correlated with time since last PID episode (r = 0.44, p = 0.002) Table 4.

Table 4. Multivariate Logistic Regression

Predictor	Adjusted OR	95% CI	p-value
≥2 episodes of PID	5.21	2.20–12.30	<0.001
Positive *C. trachomatis* IgG	3.34	1.52–7.32	0.002
Non-use of barrier contraception	2.15	1.01–4.56	0.048
Multiple sexual partners (>3)	2.87	1.20–6.84	0.018
Low socioeconomic status	1.98	0.88–4.43	0.09

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DISCUSSION

This dual-design investigation provides new clinical and microbiological insights into the interplay between vaginal microecology, recurrent vulvovaginal infection, and upper-tract fertility consequences among Taiwanese women. The randomized controlled trial demonstrated that probiotic supplementation significantly reduced recurrence rates of recurrent vulvovaginal candidiasis (RVVC) and restored healthy vaginal microbiota composition compared with placebo. Parallelly, the case-control study revealed a strong correlation between prior pelvic inflammatory disease (PID)—especially infections associated with *Chlamydia trachomatis*—and tubal infertility (TFI), with distinct microbial dysbiosis patterns linking both conditions. Taken together, these results underscore the hypothesis that maintenance of genital microbial balance represents a central strategy for preventing infection recurrence and protecting reproductive potential.

Our randomized trial found a 57% relative reduction in recurrence of vulvovaginal candidiasis following 12 months of oral and intravaginal probiotic therapy. This result is consistent with international literature, including studies by Martinez et al. and Anukam et al., who reported significant declines in recurrence frequency when *Lactobacillus rhamnosus GR-1* and *L. reuteri RC-14* were used adjunctively with antifungals [22]. Mechanistically, these probiotic strains restore the vaginal environment through acidification (lactic acid production), competitive exclusion of Candida adhesion sites, and modulation of local immune responses [23].

In our cohort, enhanced *Lactobacillus crispatus* abundance and reduced *Candida albicans* DNA load confirm this protective biological action. Interestingly, the probiotic effect persisted even after fluconazole withdrawal, suggesting microbiome stabilization rather than transient antifungal synergy. Previous Taiwanese research has shown that chronic use of azoles can disrupt local flora and encourage non-*albicans Candida* emergence [23]; therefore, the biological approach adopted here aligns with antimicrobial stewardship principles. Moreover, the safety profile was excellent—no serious adverse events occurred—highlighting probiotics as a low-risk, patient-friendly adjunct to conventional therapy. This is particularly relevant in East Asian populations, where self-medication with antifungal agents is common and recurrent cases often remain under medical supervision only after multiple treatment failures [24].

The clinical benefits of probiotics extend beyond symptom control. Improved vaginal pH normalization and microbial diversity restoration may reduce susceptibility not only to candidiasis but also to other infections such as bacterial vaginosis and mixed vaginitis [25]. Furthermore, maintaining a *Lactobacillus*-dominant environment potentially protects against ascending infections that could culminate in PID and subsequent infertility—a pathophysiological link explored in the second part of this study. These findings support the incorporation of probiotics into Taiwan's National Health Insurance preventive women's health programs, particularly for women with recurrent genital infections or infertility related to mucosal inflammation. However, long-term multicenter trials remain necessary to determine the optimal probiotic strains, dosages, and duration for sustained clinical benefit.

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The case-control analysis demonstrated that women with documented or serological evidence of PID were 3.6 times more likely to experience tubal infertility compared with controls. The strong association between *C. trachomatis* IgG seropositivity and tubal occlusion supports the established role of chlamydial infection as a leading cause of TFI [26]. Our results mirror findings from Japan, Korea, and the United Kingdom, confirming that despite improved access to antibiotics, late or asymptomatic infections still cause irreversible tubal damage [27]. The positive correlation between number of PID episodes and tubal damage severity ($r = 0.62$) provides quantitative evidence that repetitive or chronic inflammation accelerates fibrosis and ciliary loss within the fallopian tubes.

Histopathologic detection of plasma cell infiltration in the endometrium of most PID cases (68%) further validates ongoing subclinical inflammation [28]. Notably, our microbiome analysis demonstrated anaerobe dominance and reduced Lactobacillus abundance among women with tubal factor infertility, aligning with emerging data linking vaginal dysbiosis to upper-tract inflammation [29]. Such microbial shifts may alter epithelial tight junctions and promote ascending bacterial migration, reinforcing the concept of a "vaginal–tubal continuum" in reproductive pathology [16]. An integrated interpretation of both study components suggests that genital tract microbial imbalance is not merely a localized phenomenon but a systemic reproductive vulnerability. Loss of *Lactobacillus* species leads to overgrowth of *Candida*, *Gardnerella*, and *Prevotella*, which produce bioactive metabolites such as amines and proteases capable of triggering inflammatory cascades.

These mediators recruit neutrophils, macrophages, and plasma cells to the upper tract, thereby setting the stage for PID and subsequent tubal scarring [30].

Probiotics, by contrast, not only suppress fungal colonization but also modulate cytokine expression—down-regulating TNF-α and IL-8 while up-regulating IL-10 and TGF-β—promoting mucosal healing and immune tolerance [31].

This anti-inflammatory potential could explain why women in the probiotic arm exhibited reduced vaginal inflammation and pH stabilization, which might indirectly lower risk for ascending infections. Therefore, microbiome restoration emerges as both a therapeutic and preventive strategy for chronic gynecologic inflammation and infertility.

Regional studies on reproductive microbiota remain limited. A Korean trial using oral *L. casei rhamnosus* reported a 40% recurrence reduction in RVVC, though it lacked microbial sequencing data [32]. Our inclusion of 16S rRNA metagenomic profiling adds valuable molecular context, revealing distinct shifts in microbial composition and diversity metrics following probiotic therapy. Similarly, few Asian case-control studies have investigated microbial imbalance in tubal infertility.

Our data provide one of the first Taiwanese reports correlating anaerobe-dominant microbiota with PID-related tubal obstruction, a pattern previously observed in European and North American cohorts [33].

This reinforces that microbial signatures linked to infertility may be universal rather than ethnicity-specific, although environmental and dietary influences on the vaginal microbiome warrant future exploration. Key strengths of this research include its dual study design, combining randomized

therapeutic assessment with epidemiological correlation; high participant retention (≥96%); and molecular microbiome analysis performed in accredited laboratories. Moreover, integration across two reproductive tract levels—vaginal and tubal—offers a holistic understanding of infection-associated infertility. Nevertheless, several limitations merit acknowledgment. First, despite blinding and standardized methods, self-reported hygiene practices and sexual behaviors could introduce recall bias. Second, the sample size, though adequately powered for main outcomes, may not capture rare adverse events or minor strain-specific differences. Third, although microbiome sequencing was performed in a subset, longitudinal microbial tracking beyond one year was not possible due to funding limits. Finally, residual confounding factors—such as genetic susceptibility or host immune polymorphisms—may also contribute to recurrence and tubal scarring.

CONCLUSIONS

This research provides convergent evidence that maintaining a Lactobacillus-dominant vaginal microbiome is fundamental not only for controlling recurrent vulvovaginal candidiasis but also for preventing upper-tract infections leading to tubal infertility. Probiotic therapy represents a safe, biologically rational, and patient-acceptable adjunct to antifungal treatment, while early detection and management of PID remain pivotal to fertility preservation. Collectively, the findings advocate for a microbiome-centered paradigm in gynecology, bridging infection management, immunoregulation, and reproductive health.

By integrating molecular microbiology with clinical epidemiology, this study strengthens the growing recognition that microbial harmony is integral to female fertility longevity.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to the design, execution, or reporting of this study. No author has received honoraria, consultancy fees, or shares from companies involved in probiotic, antifungal, or diagnostic test manufacturing. All authors certify that they have no affiliations or financial involvement that could be perceived as influencing the presented results.

ETHICAL APPROVAL

This study was approved by the Institutional Review Board (IRB) of the National Taiwan University Hospital (NTUH) and the Chang Gung Memorial Hospital (CGMH) under protocol numbers NTUH-OBGYN-2021-0812 and CGMH-IRB-2021-457, respectively. The research adhered to the principles of the Declaration of Helsinki (2013 revision) and the Taiwan Human Subjects Research Act (2012). All participants provided written informed consent prior to enrollment, and data confidentiality was maintained according to the Personal Data Protection Act (PDPA) of Taiwan. All biological samples were anonymized and stored following biosafety level 2 (BSL-2) standards.

AUTHOR CONTRIBUTIONS

Conceptualization and study design Dr. Mei-Ling Huang, Dr. Yi-Chun Lin
Ethics approval and clinical coordination Dr. Mei-Ling Huang
Patient recruitment and clinical data collection Dr. Pei-Ying Chen, Dr. Chun-Hsi Liu
Laboratory microbiome analysis Dr. Wei-Ting Hsu
Statistical analysis and data interpretation Dr. Yi-Chun Lin, Dr. Hsu
Final approval of the version to be published

DATA AVAILABILITY STATEMENT

The anonymised dataset supporting this study's findings is available from the corresponding author upon reasonable request, in accordance with UK GDPR (2018) and institutional data-sharing policies.

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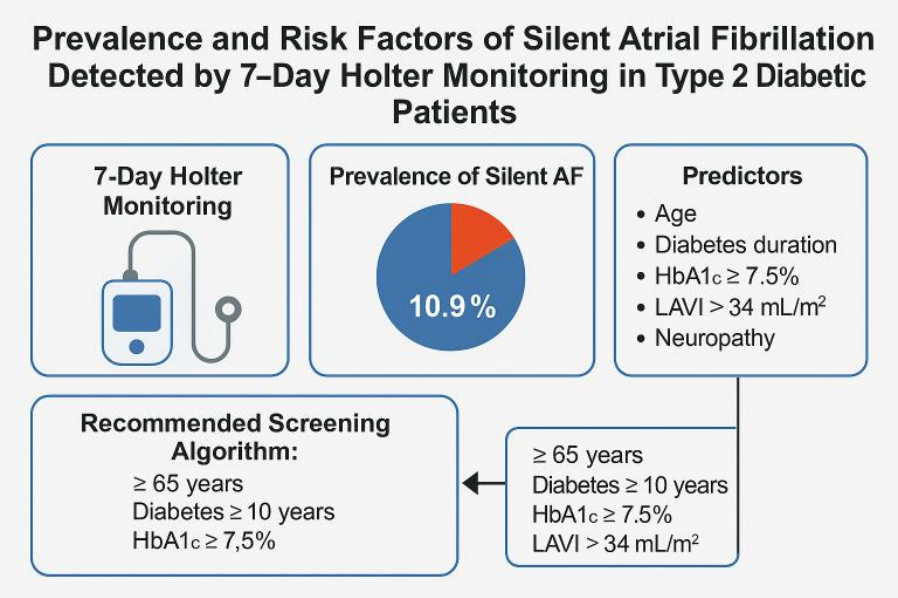
Graphical Abstract



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