Jafari *et al. Middle East Fertility Society Journal* (2026) 31:35
https://doi.org/10.1186/s43043-026-00306-4

Middle East Fertility Society Journal

RESEARCH Open Access

Randomized clinical trial: the impact of vaginal synbiotic supplementation on the assisted reproductive technology (ART) outcomes and Lactobacillus status of genital tract in infertile women prior to frozen embryo transfer

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Abstract

Objective The aim of this study was to investigate the efficacy of Lactovage suppository on genital tract bacterial culture and assisted reproductive technology (ART) outcomes in infertile women undergoing frozen embryo transfer (FET).

Methods A randomized, double-blind, placebo-controlled trial was conducted on 156 women scheduled for FET. Participants were randomly divided into two equal groups, with one group receiving a daily intravaginal capsule containing Lactobacillus and maltodextrin (intervention group) for 30 days, while the other received a placebo capsule with an inactive ingredient (control group) over the same period. After the intervention, data related to Lactobacillus status (vaginal and uterine) and ART outcomes were analyzed.

Results Baseline characteristics were comparable between groups. The intervention group showed significantly higher chemical (45.5% vs. 28.9%, $P=0.034$) and clinical pregnancy rates (42.9% vs. 27.6%, $P=0.048$), while ongoing pregnancy and implantation rates were higher but not statistically significant. Miscarriage rates were similar between groups, and multiple pregnancy occurred only in the control group. Although vaginal and uterine Lactobacillus positivity was more frequent in the synbiotic group, this difference was not significant; however, Lactobacillus-positive vaginal and uterine status was associated with higher pregnancy rates, particularly in the intervention group.

Conclusion Intravaginal synbiotic supplementation before FET may be associated with improved chemical and clinical pregnancy rates in women undergoing ART. While other outcomes did not differ significantly between groups,

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a Lactobacillus-positive genital tract environment was linked to more favorable pregnancy outcomes, particularly in the synbiotic group. Larger multicenter studies are needed to confirm these findings.

Trial registration This study was retrospectively registered in the Iranian website (www.irct.ir) for clinical trials registration (http://www.irct.ir: IRCTID: IRCT20110602006689N5).

Keywords Synbiotic, ART outcomes, Infertile women, Frozen embryo transfer (FET), Lactobacillus status

Introduction

Despite advances in in vitro fertilization (IVF), a large proportion of treatment cycles fail result in pregnancy. While most efforts to improve success rates have focused on enhancing embryo quality, there's a growing understanding that the intra-uterine environment also plays a crucial role in determining pregnancy outcomes. As tests for endometrial function improve, researchers are becoming more interested in how vaginal microbiota affects reproductive outcomes [1]. Given its impact on organ function throughout the body, the vaginal microbiota is being explored as a potential indicator of endometrial receptivity and a predictor of IVF success [2–4]. In this regard, probiotics, such as Lactobacillus, could offer significant benefits in assisted reproduction technologies (ART). Synbiotics, which combine probiotics with prebiotics to enhance microbial survival and colonization by improving microbial stability within the genital tract [5]. Lactobacillus, a natural component of the vaginal and endometrial microbiota, produces hydrogen peroxide [6, 7], bacteriocins [8], and lactic acid, which help to maintain a vaginal pH below 4.5 [9], preventing the growth of harmful pathogens. Studies by Cihat Unlu et al. have shown that Lactobacillus supplementation can inhibit several pathogenic organisms, excluding *Candida albicans*, by producing organic acids and hydrogen peroxide [9]. Additionally, Lactobacilli may contribute to successful implantation by controlling vaginal pH, limiting pathogen growth, and adhering to epithelial cells [10]. Conversely, dysbiosis, which refers to an imbalance in the reproductive tract microbiome, can promote the overgrowth of pathogenic bacteria such as Gardnerella vaginalis and Streptococci. This disruption can lead to chronic inflammation, negatively affect endometrial receptivity, hinder embryo survival, and, as a result, prevent successful implantation [11, 12]. In women undergoing ART, a dominance of intrauterine Lactobacilli has been linked to higher pregnancy success rates [13]. Research on probiotic use in IVF has produced inconsistent findings, and data on synbiotic supplementation in this setting remain limited. Some clinical studies have reported favorable outcomes with probiotic administration before FET [14–16]. For instance, oral administration of L. salivarius CECT5713 has been shown to enhance pregnancy rates and improve key biochemical and immunological markers [16]. Additionally, intravaginal lactobacilli supplementation has been linked to lower miscarriage rates and higher live birth rates, particularly in women with bacterial vaginosis and those undergoing blastocyst transfer [14]. Similarly, an Iranian study found that Lactovage administration in FET cycles improved pregnancy rates, especially in cases where grade A embryos were transferred [15]. However, one study reported no significant effect [6], suggesting that probiotics alone may not be sufficient to enhance endometrial receptivity or embryo survival [6]. However, due to variations in their findings, there remains insufficient scientific evidence to recommend the routine use of probiotics for managing asymptomatic dysbiosis prior to IVF. To the best of our knowledge, evidence regarding the impact of synbiotic supplementation—rather than probiotics alone—on ART outcomes and genital tract Lactobacillus status prior to FET remains scarce. This study aimed to evaluate the effects of synbiotic supplementation on ART success rates and Lactobacillus status of the genital tract in this patient population.

Materials and methods

Trial design

This randomized controlled trial (RCT) (IRCT20110602006689N5) was carried out at Dr. Shariati Hospital, affiliated with Tehran University of Medical Sciences (TUMS), in Tehran, Iran. This study was conducted from February 2023 to September 2024, involving 156 patients who participated in FET cycles. Eligible participants were women aged 18–40 years with a body mass index (BMI) of ≤ 30 who had undergone IVF due to any infertility cause such as tubal factor infertility, male infertility, unexplained infertility, polycystic ovary syndrome (PCOS), or diminished ovarian reserve (DOR). Embryo quality was assessed and recorded at the time of cryopreservation, and only grade A or B cleavage-stage embryos were eligible for inclusion. However, post-thaw reassessment was performed prior to transfer, and some embryos were classified as grade C due to morphological changes after warming. Women with uterine malformations, systemic diseases, recurrent implantation failure, severe endometriosis, severe male factor infertility (TMSC < 5 × 10^6/ejaculate), and vaginal infection or recent use of broad-spectrum antibiotics were excluded (within 3 months prior to enrollment). Written informed consent was obtained from all participants, and the study

received approval from the Institutional Review Board and Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1400.1176).

The study was retrospectively registered due to administrative delays following ethics committee approval. Although the first participant was enrolled prior to registration, the study protocol, eligibility criteria, predefined outcomes, and statistical analysis plan were finalized before recruitment commenced and were adhered to throughout the trial.

Hormone replacement therapy (HRT)

Patients underwent standard hormone replacement therapy (HRT) for endometrial preparation. In the FET protocol, estradiol (E2) was administered on days 2–3 of the cycle, either orally or vaginally, followed by monitoring of endometrial thickness via ultrasound after 10 days. If the endometrium reached a thickness of ≥ 7 mm with a trilaminar pattern, progesterone therapy was initiated. If the endometrial thickness was insufficient, estradiol administration continued until the desired thickness was achieved. Once the endometrium was optimized, progesterone (50 mg intramuscularly and 800 mg vaginally) was started, and embryo transfer occurred on day 4 for cleavage-stage embryos. Hormone therapy was administered up to the pregnancy test, and if pregnancy was confirmed, estradiol and progesterone supplementation was prolonged until the 10th week of gestation. Sonographic assessments were conducted by a specialist using a Philips Affiniti 70 ultrasound machine with a C10-3v endo-vaginal probe. According to the patient's age, one to a maximum of three frozen embryos were thawed and transferred at the cleavage stage on the fourth day following the initiation of progesterone administration.

Intervention and randomization

The study initially enrolled 156 women, who were randomly allocated to two groups at the beginning of endometrial preparation. Participants in the intervention group were given a daily intravaginal dose of LactoVag® (Zist Takhmir Co., Tehran, Iran), containing 10^9 CFU of probiotics. This supplementation began prior to and continued throughout the standard endometrial preparation protocol for embryo transfer until the transfer was done (approximately four week). LactoVag® is a synbiotic formulation, combining probiotics and prebiotics, containing various strains of Lactobacillus, including Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus acidophilus, and Lactobacillus gasseri, as well as maltodextrin as a prebiotic. The placebo suppositories were also manufactured by the same pharmaceutical company (Zist Takhmir Co., Tehran, Iran) and were identical to the active suppositories in appearance, packaging, texture, and administration schedule, differing only in the absence of active synbiotic ingredients. Randomization was conducted using a computer-generated random sequence with block randomization (block size of four) to ensure balanced group allocation. Allocation concealment was maintained through the use of sequentially numbered, opaque, sealed envelopes prepared by an independent researcher who was not involved in participant recruitment, treatment administration, or outcome assessment. Both the participants and researchers were blinded to the group assignments and randomization process. A summary of the study design is presented in Fig. 1.

Embryo transfer

The Kitazato kit (Shizuoka, Japan) was used for the vitrification and warming procedures. The vitrified cleavage embryos underwent thawing, and the transfer was completed on the same day. Only high-quality embryos, classified as grade A or B cleavage-stage based on ASEBIR criteria, were selected for transfer. The embryos were cultured in 10 µl of culture medium (Sage Biopharma, Gytech, Australia), covered with mineral oil (Cooper Surgical, USA), and incubated at 37 °C with 6% CO2 in a Heracell incubator (Heracell150i, Thermo Fisher Scientific, Germany). For the embryo transfer, a pre-equilibrated Universal Transfer Medium (UTM) containing phenol red (MediCult) was employed. The embryo transfer procedure was conducted with the Soft-Pass™ Embryo Transfer Catheter (COOK MEDICAL LLC, USA) under ultrasound guidance.

Uterine and vaginal microbiota culture

Microbial cultures, as a primary outcome, were obtained from the vaginal and uterine cavity on the day of embryo transfer. A sterile swab was used for vaginal sampling, and the tip of the embryo transfer catheter was sent for uterine microbiota culture [17]. All specimens were inoculated onto non-selective media, including blood agar and chocolate agar, and incubated at 37 °C under both aerobic and anaerobic conditions for 48–72 h. Identification of Lactobacillus spp. was performed based on colony morphology and Gram staining. As no selective culture media or molecular techniques were employed, the findings represent a culture-dependent and qualitative assessment limited to Lactobacillus detection. Accordingly, other bacterial species were not systematically identified or reported, as the study was not designed to characterize the broader vaginal or uterine microbiota.

Clinical data

The secondary outcomes were as follows: the implantation rate, which was calculated by the percentage of gestational sacs observed per transferred embryos; the chemical pregnancy rate, which represented the

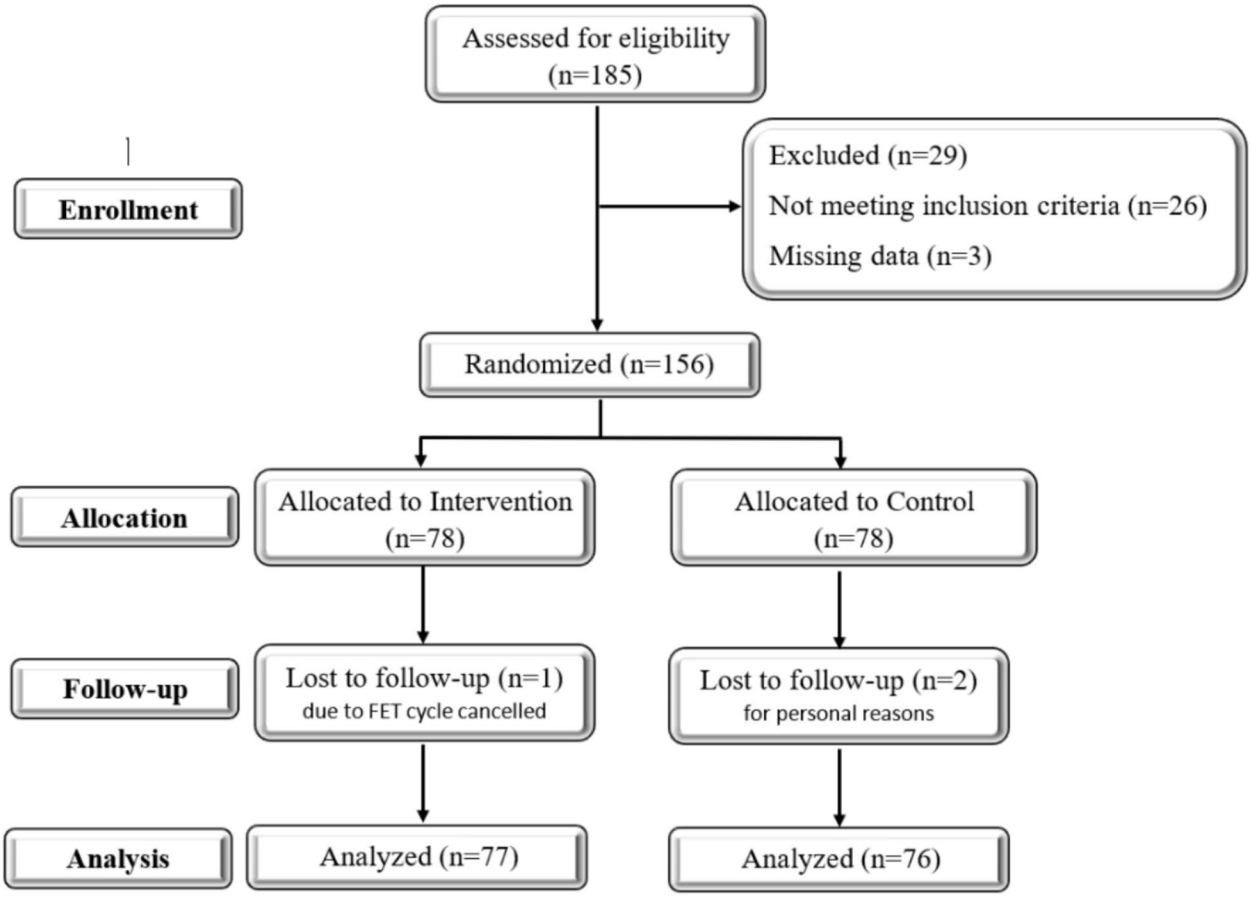


Fig. 1 Summary of patient flow through the study

percentage of embryo transfer (ET) cycles with a positive serum β-hCG test 14 days post-ET [18]; the clinical pregnancy rate, determined by the percentage of ET cycles resulting in pregnancies confirmed by ultrasound findings, such as a gestational sac or heart rate, 5–6 weeks post-transfer [19]; the ongoing pregnancy rate, referring to the percentage of ET cycles leading to pregnancies that reached or surpassed 20 weeks of gestation; the miscarriage rate, calculated by the ratio of miscarriages to the total number of clinical pregnancies [20] and the multiple pregnancy rate, which was the ratio of cycles with multiple pregnancies to the total number of clinical pregnancies [21].

Sample size and statistical analysis

Based on the findings of Wang Ya et al. [22], the sample size was calculated to detect a significant difference in genital tract microbiota culture between groups with 80% statistical power at a two-sided significance level of 0.05. After accounting for an anticipated 15% attrition rate, a total of 156 participants (78 per group) were required. Data analysis was performed using IBM SPSS Statistics version 24. The Kolmogorov–Smirnov test was employed to assess the normality of the data distribution. Qualitative data were expressed as percentages, while quantitative data with a normal distribution were presented as means with standard deviations. Differences between the two groups (placebo and treatment) were evaluated using the chi-square test and independent sample t-test, with a *P*-value of less than 0.05 considered statistically significant.

Results

In total, during the intervention phase, 3 participants withdrew from the study: 2 from the placebo group (for personal reasons) and 1 from the treatment group (due to FET cycle cancelled). Ultimately, 153 patients completed the study, with 77 in the intervention group and 76 in the placebo group (Fig. 1). At the beginning of trial, there were no significant differences between the intervention and control groups regarding infertility duration, average age, BMI, hormonal profile, or other baseline clinical and demographic variables (Table 1). Table 2 summarizes ART outcomes in the two groups. The synbiotic group showed significantly higher chemical pregnancy (45.5% vs. 28.9%, $P=0.034$) and clinical pregnancy rates (42.9% vs. 27.6%, $P=0.048$). Although the ongoing pregnancy rate was higher in the intervention group, the difference

Table 1 Baseline characteristics

Variables		Groups		*P*-value
		Intervention *n* = 77	Control *n* = 76	
Age (years)		33.96 ± 6.75	33.05 ± 5.43	0.711
BMI (kg/m^2)		26.04 ± 4.36	25.62 ± 3.97	0.809
Infertility duration (year)		4.69 ± 3.48	5.02 ± 3.89	0.463
Type of infertility	PIF	52 (68%)	56 (74%)	0.478
	SIF	25 (32%)	20 (26%)	
History of ET		1.14 ± 1.25	1.26 ± 1.37	0.526
Baseline FSH (μIU/ml)		6.60 ± 4.98	6.24 ± 5.77	0.825
Baseline LH (μIU/ml)		3.95 ± 4.32	4.36 ± 3.42	0.726
AMH (ng/ml)		3.77 ± 1.98	3.64 ± 2.33	0.833
Oocyte. No		10.45 ± 3.11	11.76 ± 4.2	0.336
MII Oocyte No		8 ± 3.51	9.42 ± 3.86	0.101
Total Transferred Embryos (by quality)	A	61 (33%)	69 (35%)	0.339
	B	96 (52%)	107 (55%)	
	C	27 (15%)	19 (10%)	
No. of ETs per patient		2.09 ± 0.66	2.54 ± 059	0.350

Data presented as mean (± SD) or frequency (%) as appropriate. Independent sample t-test, and/or Mann–Whitney U test, Chi- square test

BMI body mass index, *PIF* primary infertility, *SIF* secondary infertility, *ET* embryo transfer, *FSH* follicle stimulating hormone, *LH* luteinizing hormone, *AMH* Anti Mullerian hormone

* $p \leq 0.05$

was not statistically significant. The implantation rate was numerically higher in the synbiotic group; however, this difference did not reach statistical significance. The miscarriage rate was low in both groups and did not differ significantly. Multiple pregnancy occurred only in the control group, while no multiple pregnancies were observed in the intervention group.

Table 3 presents the percentage of participants with a positive Lactobacillus status in vaginal and uterine samples across the two groups. The synbiotics group showed a higher percentage compared to the control group for both vaginal (59.7% vs. 44.7%, $P = 0.063$) and uterine (37.7% vs. 25%, $P = 0.091$) samples. However, these differences did not reach statistical significance.

We also explored how pregnancy rates differed among participants based on their vaginal or uterine Lactobacillus status, comparing those who received synbiotics—whether their status was positive or negative—to those who didn't. The detailed results are presented in Tables 4 and 5. Our findings indicated that the chemical, clinical, and ongoing pregnancy rates were significantly higher in participants from the intervention group who had a positive vaginal Lactobacillus status. In contrast, no significant differences were observed in these parameters between those with positive and negative Lactobacillus status in the control group (Table 4). Furthermore, we demonstrated that chemical and clinical pregnancy rates were significantly higher in both groups with a uterine Lactobacillus-positive status compared to the negative status. Moreover, the ongoing pregnancy rate was significantly higher in the intervention group with a uterine Lactobacillus-positive status (Table 5).

Table 2 Comparison of ART outcomes between two groups

Group		Intervention Group (77)	Control Group (76)	P	RR	CI 95%
CP (%)	Positive	35 (45.5%)	22 (28.9%)	0.034*	1.57	[1.05,2.42]
	Negative	42 (54.5%)	54 (71.1%)			
CLP (%)	Positive	33 (42.9%)	21 (27.6%)	0.048*	1.55	[1.00,2.43]
	Negative	44 (57.1%)	55 (72.4%)			
OP (%)	Positive	29 (37.7%)	20 (26.3%)	0.132	1.43	[0.89,2.30]
	Negative	48 (62.3%)	56 (73.7%)			
MC (%)	Positive	4 (5.2%)	1 (1.3%)	0.366	1.62	[0.75,2.15]
	Negative	73 (94.8%)	75 (98.7%)			
IR (%)		33/184(17.9)	25/195(12.8)	0.199	1.21	[0.91,1.52]
MP (%)		0/33(0%)	4/21(19%)	0.058	0	[0,0.95]

Data presented as frequency (%), Chi- square test/Fisher's exact test

CP chemical pregnancy, *CLP* clinical pregnancy, *OP* ongoing pregnancy, *MC* miscarriage, *IR* implantation rate, *MP* multiple pregnancy rate

* $p \leq 0.05$

Table 3 Comparison of status of lactobacillary microbiota between two groups

Group		Whole Sample	Control Group N = 76	Intervention Group N = 77	P	RR	CI 95%
V. Lacto	+	80 (52.2%)	34 (44.7%)	46 (59.7%)	0.063	1.35	[0/98,1/89]
	-	73 (47.7%)	42 (55.2%)	31 (40.3%)			
U. Lacto	+	48 (31.4%)	19 (25.0%)	29 (37.7%)	0.091	1.32	[0.95,1.78]
	-	105 (68.6%)	57 (75.0%)	48 (62.3%)			

Data presented as frequency (%), Chi- square test. * $p \leq 0.05$. V. Lacto vaginal lactobacillus, U. Lacto uterine lactobacillus

Table 4 Risk Ratio (RR) and *P*-values for Pregnancy types based on V. Lacto presence

V. Lacto	*CP+*	*RR (95% CI)*	*P*	*CLP+*	*RR (95% CI)*	*P*	*OP+*	*RR (95% CI)*	*P*
Intervention (+)	27	2.27 (1.26, 4.44)	0.004*	25	2.10 (1.15, 4.14)	0.010*	23	2.58 (1.27, 5.69)	0.006*
Intervention (-)	8			8			6		
Control (+)	13	1.78 (0.88, 3.65)	0.108	12	1.64 (0.80, 3.41)	0.170	12	1.85 (0.87, 3.98)	0.109
Control (-)	9			9			8		

Data presented as frequency (%), Chi- square test. * p ≤ 0.05. V. Lacto vaginal lactobacillus

CP chemical pregnancy, CLP clinical pregnancy, OP ongoing pregnancy

Table 5 Risk Ratio (RR) and *P*-values for pregnancy types based on U. Lacto presence

U. Lacto	*CP+*	*RR (95% CI)*	*P*	*CLP+*	*RR (95% CI)*	*P*	*OP+*	*RR (95% CI)*	*P*
Intervention (+)	20	2.20 (1/36,3/62)	0.002*	20	2.01 (1/23,3/40)	0.006*	20	3.67	< 0/0001*
Intervention (-)	15			13			9	(2/00,7/02)	
Control (+)	10	2.5 (1/26,4/73)	0.017*	9	2.25 (1/10,4/36)	0.038*	7	2.10 (0/91,4/54)	0.111
Control (-)	12			12			10		

Data presented as frequency (%), Chi- square test

CP chemical pregnancy, *CLP* clinical pregnancy, *OP* ongoing pregnancy

* $p \leq 0.05$. U. Lacto uterine lactobacillus

Discussion

A Lactobacilli-dominant vaginal and endometrial environment has been associated with improved pregnancy outcomes, including higher pregnancy rates [23, 24] and lower miscarriage rates [25]. In this study we evaluated the effects of synbiotic supplementation on ART success rates and Lactobacillus status of the genital tract in the infertile patients. In this randomized clinical trial, intravaginal synbiotic supplementation was associated with higher chemical and clinical pregnancy rates in women undergoing frozen embryo transfer. Although implantation and ongoing pregnancy rates tended to be higher in the synbiotic group, these differences did not reach statistical significance, which may be partly explained by the relatively small sample size. No significant differences were observed in miscarriage rates between the groups, and multiple pregnancy occurred only in the control group. Collectively, these results suggest a possible beneficial role of synbiotic supplementation on early pregnancy outcomes; however, the findings should be interpreted cautiously given the exploratory nature of the study. Another notable finding of this study was the higher prevalence of positive Lactobacillus status (both vaginal and uterine) in the synbiotic group compared to the control group, although these differences did not reach statistical significance. Moreover, the findings suggest a potential influence of Lactobacillus status on pregnancy outcomes, as the highest pregnancy rates were recorded among participants in the intervention group with a vaginal Lactobacillus-positive status. Additionally, a uterine Lactobacillus-positive status was correlated with significantly higher chemical and clinical pregnancy rates in both groups, while a significantly higher ongoing pregnancy rate was observed exclusively in the intervention group.

The genital tract of female possesses a unique and well-defined microbiome that plays a crucial role in reproductive health [26]. The composition of the vaginal microbiome is crucial for establishing a supportive uterine environment, which significantly influences fertility and the success of ART. A healthy vaginal microbiome is typically dominated by *Lactobacillus* species, which can account for more than 90% of the microbial community [27]. Evidence suggests that a vaginal microbiota dominated by *Lactobacillus* species is associated with improved ART success rates including higher implantation, pregnancy, and live birth rates in infertile patients undergoing IVF [27]. Conversely, an imbalance in the vaginal microbial community, known as dysbiosis, has been linked to reproductive complications such as infertility, spontaneous miscarriage, and suboptimal outcomes in ART [3, 13, 28]. Consequently, the modulation of the genital tract microbiome through probiotic supplementation may serve as a potential adjunctive strategy to enhance ART outcomes and data on synbiotic supplementation in this setting remain limited. The impact of probiotic supplementation prior to embryo transfer on ART outcomes and pregnancy rates has been investigated in various studies, with results that remain varied and inconclusive [29, 30]. Our results are consistent with some of these studies. For instance, Wei et al. [31] (2024) reported that women who received a vaginal probiotic containing Lactobacillus delbrueckii DM8909 prior to FET exhibited higher clinical pregnancy rates compared to those who did not receive the supplementation, while the miscarriage rate remained similar between the groups. Additional studies [6, 15, 32, 33] have reinforced

these findings, indicating that probiotic supplementation may enhance pregnancy rates, though without reaching statistical significance. However, our results differ from studies that found no significant impact of intravaginal probiotics on pregnancy rates. Thanaboonyawat et al. (2023) [14] demonstrated that while intravaginal Lactobacillus supplementation prior to embryo transfer in FET cycles did not enhance biochemical or clinical pregnancy rates in the general population, it was associated with a significant reduction in miscarriage rates. In the Iranian population, a similar study revealed that intravaginal probiotic administration had no significant effect on pregnancy rates in patients with recurrent implantation failure (RIF) undergoing FET [34]. The discrepancies in clinical outcomes and the lack of significant results may be due to factors such as study design, variability in patient populations, and differences in the route of administration, probiotic strain, dosage, and duration of treatment.

The optimal dosage and duration of Lactobacillus supplementation for modifying the endometrial microbiome remain unclear. However, a four-week intravaginal probiotic regimen may promote a shift to a Lactobacillus-dominant state [35]. This study implemented a four-week intravaginal probiotic regimen. Although the probiotics group exhibited a higher prevalence of vaginal and uterine Lactobacillus dominance, the difference was not statistically significant. This could be attributed to the limited sample size, which may have reduced the statistical power to detect minor differences. Previous studies have suggested that probiotics may contribute to the restoration and maintenance of a healthy vaginal microbiota in both individuals with bacterial vaginosis and those with a normal vaginal flora [36–39], Although, conflicting results have also been reported [40]. A systematic review and meta-analysis have indicated that intravaginal probiotics are effective in restoring and preserving a healthy vaginal microbiome, which is marked by a considerable prevalence and variety of Lactobacillus species, a decrease in vaginal pH, and a reduction in pathogenic bacteria [41].

Despite the lack of statistical significance in prevalence of vaginal and uterine Lactobacillus dominance in our study, our findings suggest that a dominant Lactobacillus presence in the vaginal and uterine microbiota may be significantly associated with higher pregnancy rates, which is consistent with previous studies [13, 25, 42, 43] highlighting the potential benefits of Lactobacillus in improving reproductive outcomes.

This study has several strengths, including its randomized clinical trial design, which provides a robust framework for evaluating the effects of intravaginal synbiotics on pregnancy rates in infertile patients undergoing FET. A notable strength is the simultaneous assessment of Lactobacillus dominance in both the vaginal and uterine microbiota on the day of embryo transfer, allowing for a comprehensive analysis of its association with ART outcomes. However, certain limitations should be acknowledged. The relatively small sample size and single-center design may limit generalizability and increase the risk of type I and type II errors. Importantly, the sample size was calculated to detect differences in genital tract microbiota culture between groups; therefore, the study was not powered to identify differences in reproductive outcomes such as ongoing pregnancy or live birth, and these results should be interpreted cautiously. Genital tract microbiota assessment was based on culture methods rather than molecular techniques such as 16S rRNA sequencing, which may underestimate microbial diversity; additionally, uterine sampling via embryo transfer catheter tips carries a potential risk of contamination. The trial was retrospectively registered due to administrative delays, and intention-to-treat analysis was not performed because a small number of participants withdrew before embryo transfer. In addition, vaginal conditions and hygiene practices that may affect the genital tract microbiota, such as vaginitis or douching, were not systematically assessed and represent a limitation of this study. Finally, potential confounders—including age, body mass index, embryo number and quality, infertility etiology, and unmeasured vaginal factors (such as vaginitis or vaginal hygiene practices, including douching) ——were not adjusted for due to limited sample size; therefore, the observed associations should be interpreted as exploratory and future studies are warranted to incorporate multivariate regression analyses to better account for these confounding variables.

In conclusion, intravaginal synbiotic supplementation prior to FET may be associated with improved chemical and clinical pregnancy rates in women undergoing ART. Although no significant differences were observed in ongoing pregnancy rates or overall Lactobacillus dominance, a Lactobacillus-positive genital tract environment was linked to more favorable pregnancy outcomes, particularly in the synbiotic group. Larger multicenter studies using molecular microbiome profiling and adjusted analyses are needed to confirm these findings and clarify the clinical role of synbiotic therapy in ART.

Acknowledgements
This study was funded and supported by Tehran University of Medical Sciences (TUMS); Grant no. (IR.TUMS.MEDICINE.REC.1400.1176).

Declaration of generative AI and AI-assisted technologies in the writing process
During the preparation of this work the author(s) used ChatGPT (OpenAI) in order to improve language and readability, after using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Authors' contributions
SJ and SHM: designed and performed the trial and study protocol, provided the samples, and revised the manuscript; MJ: data collected and statistical analysis, and prepared the manuscript; AA and MT and MSH: assisted with study design, performed the study protocol and revised the manuscript; The final version was approved by all authors for submission.

Funding
This study was financially supported by the Tehran University of Medical Sciences, Tehran, Iran.

Data availability
The datasets used and analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The Ethics Committee of Tehran University of Medical Sciences approved the project (Ethics committee reference number: IR.TUMS.MEDICINE.REC.1400.1176). Written consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 26 October 2025 / Accepted: 17 February 2026
Published online: 05 May 2026

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