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Vaginal probiotics as therapeutic adjuncts for improving embryo transfer success rates: a systematic review and meta-analysis

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Abstract

Background Infertility treatments are continually evolving, with vaginal probiotic supplementation before embryo transfer (ET) being explored as a potential method to improve clinical outcomes. So, this systematic review evaluated the effect of vaginal probiotics on pregnancy rates following ET.

Methods Studies were identified through PubMed, Scopus, Web of Science, Cochrane, and clinical trial registries up to October 17, 2024. We included prospective interventional studies (RCTs or quasi-experimental) focusing on pregnancy outcomes post-ET. We excluded non-prospective studies, non-vaginal routes of probiotic administration, and studies with insufficient methodological or statistical details. The data was extracted from each qualifying study by two reviewers and recorded using an electronic form. Results were synthesized using a random-effects model, with Mantel–Haenszel (MH) risk ratio (RR) and 95% confidence intervals (CI) calculated for ET outcomes. Also, subgroup analyses were done to explore the history of recurrent implantation failure (RIF) as a probable source of heterogeneity.

Results We included six studies with 850 participants (419 in intervention and 431 in control groups). Vaginal probiotics showed a non-significant increase in clinical pregnancy rates compared to the control group (157 per 419 [37.47%] versus 136 per 431 [31.55%], respectively; RR: 1.19; $P=0.07$), with similar findings in women with and without a history of RIF. No significant differences were found in biochemical pregnancy (RR: 1.04; $P=0.74$) or ongoing pregnancy rates (RR: 1.09; $P=0.53$). A non-significant reduction in miscarriage risk was observed (RR: 0.67; $P=0.12$).

Conclusions Vaginal probiotics may offer a non-significant increase in clinical pregnancy rates and a slight non-significant reduction in miscarriage risk. However, considering the potential limitations of the included studies, findings should be interpreted with caution. Further research is needed to explore the potential of personalized probiotic therapy.

PROSPERO Registration Code CRD42024550798.

Keywords Probiotics, Synbiotics, In-vitro fertilization, Assisted reproductive techniques, Intracytoplasmic sperm injections, Embryo transfer

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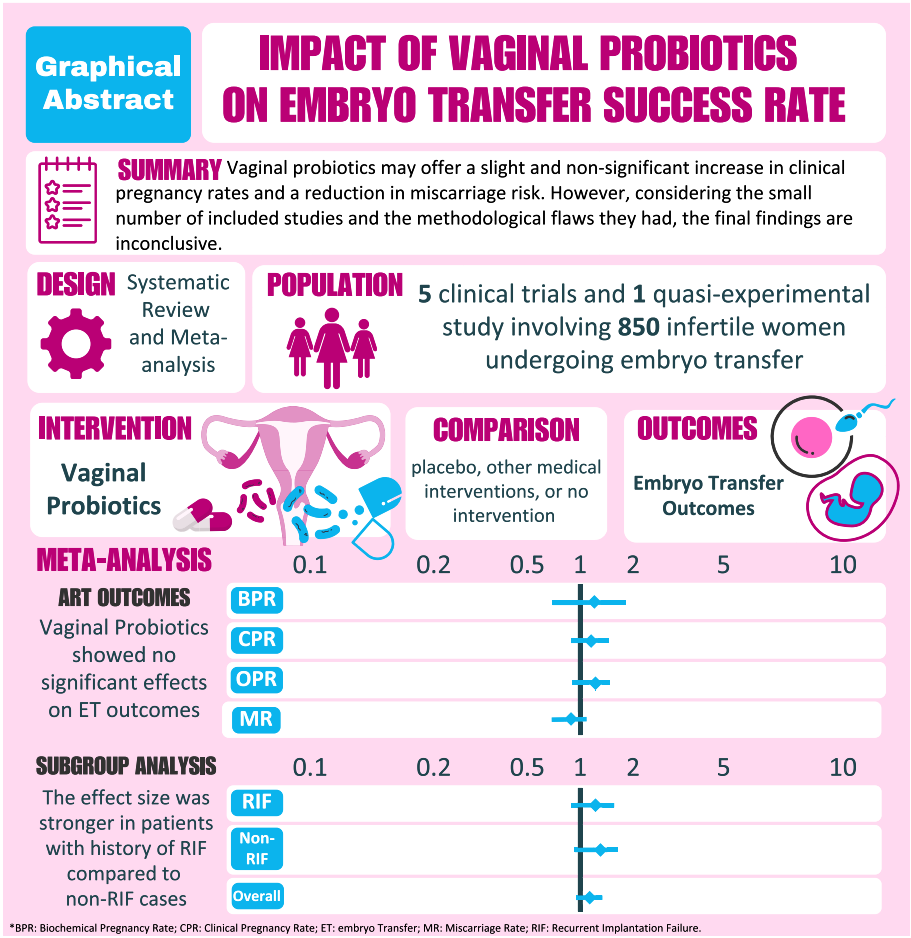
Graphical Abstract



Introduction

Infertility affects approximately 1 in 6 couples globally [1], presenting a complex challenge to reproductive health professionals. Significant advances in infertility treatments like assisted reproductive techniques (ART) have emerged as a beacon of hope for many aspiring parents. Embryo implantation is a pivotal stage in ART, requiring a delicate balance between the embryo and the endometrium. Various research over the years [2–4] shows that approximately 30% of conceptions do not progress past the implantation stage after embryo transfer (ET). Additionally, another 30% of conceptions are lost shortly after implantation, either at the biochemical stage or before any clinical becomes evident.

Recent research suggests the human microbiome, particularly the vaginal and endometrial microbiota, plays a critical role in reproductive success and embryo implantation [5]. Approximately 80% of reproductive-age women have a vaginal microbiome dominated by *Lactobacillus* species, which is indicative of a healthy vaginal ecosystem [6]. Recent research has revealed that, alongside vaginal microbiota, the microbiota of the endometrium is also linked to infertility [7, 8]. This challenges the traditional belief that the endometrium is a sterile environment. Accordingly, several studies suggest that dysbiosis at the time of ET may lead to reduced pregnancy rates while *Lactobacillus* abundance poses a positive impact on ART outcomes [9–11].

Therefore, the administration of probiotics has been suggested as a strategy to alter the endometrial microbiome, which may improve the chances of embryo implantation and a successful pregnancy. Numerous primary studies have examined the effects of probiotic supplementation before ET on ART outcomes and pregnancy rates, yielding inconclusive and mixed results [12, 13]. To date, this area has not been systematically reviewed.

Moreno et al.'s review [14] addressed the influence of vaginal probiotics on reproductive health and associated dysbiosis, demonstrating a moderate alteration of the vaginal microbiota. Blancafort et al. [15] assessed the potential of probiotics to improve fertility outcomes, emphasizing advantages for non-symptomatic women of reproductive age, yet they did not focus on infertile women undergoing ART. So, this systematic review and meta-analysis aimed at providing new perspectives on the efficacy of probiotics as an ART add-on.

Methods

This systematic review and meta-analysis aimed at evaluating the effectiveness of probiotic supplementation prior to ET in comparison to various controls (placebo, alternative treatments, or no intervention at all), in enhancing clinical outcomes for infertile women following ET. Our methodology adhered to the Cochrane Handbook for Systematic Reviews of Interventions and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Appendix file 2).

Study PICOs

The study population in this review included infertile women undergoing ET procedures as part of ART. The primary intervention examined was the administration of vaginal probiotics. This included any probiotic formulations designed for intravaginal use. The effects of vaginal probiotics were compared against several controls, including a placebo, other medical interventions such as antibiotics, or no intervention at all.

The outcomes of this review were the key ET success indicators. These included the implantation rate, chemical pregnancy rate, clinical pregnancy rate, miscarriage rate, ongoing pregnancy rate, and live birth rate. The implantation rate is defined as the number of gestational sacs visualized in ultrasound assessments divided by the number of transferred embryos. Chemical pregnancy refers to the number of chemical pregnancies diagnosed by the detection of beta human chorionic gonadotropin in serum or urine divided by the total number of ET cycles. Clinical pregnancy is defined as the number of clinical pregnancies, diagnosed by ultrasonographic observation of fetal heartbeats, per total ET cycle. Miscarriage is the loss of a pregnancy before viability (less than 20 weeks of gestation) per total ET cycle. Ongoing pregnancy includes each pregnancy showing a positive heartbeat at ultrasound after 12 weeks of gestation divided by the total number of ET cycles [16].

Inclusion and exclusion criteria

We included studies in our review based on the following criteria:

- The study was designed as a prospective interventional study (including randomized clinical trials [RCTs] or quasi-experimental studies).
- The intervention involved vaginal probiotic administration before or during the ET cycle.
- The control group received an alternative active intervention, no intervention, or a placebo.
- The study population consisted of infertile women undergoing ET.

We excluded observational (including cohort, case–control, case series, case report, or cross-sectional) and experimental (animal models and cell cultures) studies. Studies were excluded if they involved any route of probiotic administration other than vaginal. Additionally, studies lacking sufficient details regarding methodology or results were also omitted from our review.

Search strategy

Core databases

A systematic search was conducted using PubMed, Scopus, Web of Science, and Cochrane databases from inception to October 17, 2024. References and the citation list of published articles were also hand-searched to identify additional eligible studies. Full details of the search strategy, search terms, and database-specific indexing terminology are provided in Appendix file 1.

Grey literature

Following the Cochrane Collaboration guidelines, we conducted comprehensive searches in the following databases:

- Open Grey: We searched the Open Grey database for unpublished theses, conference papers, and technical reports relevant to our research topic.
- ClinicalTrials.gov: We examined the "ClinicalTrials.gov" registry for ongoing and completed clinical trials that may not have been published in peer-reviewed journals.
- WHO International Clinical Trials Registry Platform (ICTRP): We explored the ICTRP for registered trials from various international registries.
- Google Scholar: We used Google Scholar to identify grey literature, including dissertations, theses, and conference abstracts.
- ProQuest Dissertations & Theses Global: We searched for relevant dissertations and theses to ensure comprehensive coverage of unpublished academic work.

In addition to database searches, we reviewed the reference lists of included studies and relevant systematic

reviews to identify additional grey literature. We also contacted experts in the field to inquire about any unpublished data or ongoing studies that might be pertinent to our review.

Study selection

The process of study selection and data collection was meticulously carried out by two independent reviewers (S.A. and N.E.), who examined the titles and abstracts from electronic databases against our predetermined criteria for eligibility. Studies deemed potentially relevant had their full texts retrieved for a more comprehensive evaluation. In cases of uncertainty regarding a study's inclusion, it was further reviewed and deliberated upon with a third reviewer (R.K.).

Data extraction

The data extracted from each qualifying study was verified by two reviewers (R.K. and S.A.). This information was systematically recorded using a standardized electronic data capture form designed for this review.

Handling of missing data

We carefully reviewed each included study for completeness of the reported data. In cases where essential data were missing or unclear, such as participant numbers or outcome measures, we attempted to contact the corresponding authors via email to obtain the missing information. If no response was received within two weeks, we proceeded with the available data.

For studies where the data were insufficient for inclusion in the meta-analysis and additional data could not be obtained, we included a narrative summary in the systematic review. We did not perform any statistical imputation for missing data; thus, our analyses were based on the available data from the published studies.

Quality appraisal

To ensure the integrity and validity of our systematic review and meta-analysis, two robust tools were employed for quality appraisal. The Cochrane Collaboration's Risk of Bias 2 (ROB 2) was used for RCTs [17], and the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) was used for non-randomized interventional studies [18].

ROB 2 tool

It focuses on five domains including:

1. Randomization Process: Assessing whether the allocation sequence was adequately generated and concealed.
2. Deviations from Intended Interventions: Evaluating if participants and personnel were appropriately blinded and if any deviations from the intended interventions occurred.
3. Missing Outcome Data: Determining the extent and handling of incomplete outcome data.
4. Measurement of the Outcome: Assessing if the outcome assessors were blinded and if the outcome measurements were reliable.
5. Selection of the Reported Result: Evaluating the likelihood of selective reporting of outcomes.

Each domain was judged as 'low risk of bias,' 'some concerns,' or 'high risk of bias.' The overall risk of bias for each RCT was determined based on the judgments in the individual domains.

ROBINS-I tool

It assesses the risk of bias through seven domains:

1. Confounding: Assessing the presence of confounding variables and if they were accounted for in the analysis.
2. Selection of Participants: Evaluating the selection process of study participants and any potential biases introduced.
3. Classification of Interventions: Assessing if the classification of interventions was accurate and free from bias.
4. Deviations from Intended Interventions: Evaluating if participants were appropriately managed according to the intended interventions.
5. Missing Data: Determining the extent and handling of missing data.
6. Measurement of Outcomes: Assessing if the outcome measurements were reliable and if outcome assessors were blinded.
7. Selection of the Reported Result: Evaluating the likelihood of selective reporting of outcomes.

Similar to ROB 2, each domain was appraised, and an overall risk of bias judgment was made for each non-randomized study.

Both tools were applied by two independent reviewers (A.M-H. and R.K.) to minimize subjective bias, and any discrepancies were resolved through discussion or consultation with a third reviewer (F.A.).

Statistical analysis

We extracted ART outcomes from each of the included studies according to treatment strata and calculated Mantel–Haenszel (MH) risk ratio (RR) with the corresponding 95% confidence interval (CI) for each endpoint

in the probiotics versus control groups. All meta-analyses were conducted using a random effects model to account for potential heterogeneity among studies. Statistical analyses were performed using Comprehensive Meta-Analysis (CMA) software (Version 3.0, Biostat Inc., USA).

Assessment of heterogeneity

Heterogeneity refers to the variation in study outcomes between the included studies. We assessed heterogeneity using two statistical measures:

1. Q Statistic: The Q statistic is a measure of the total variance observed in the studies. It tests the null hypothesis that all studies share a common effect size. A high Q value relative to the degrees of freedom (df) indicates the presence of heterogeneity.
2. I-Squared (I^2) Statistic: The I^2 statistic quantifies the proportion of total variation in study estimates that is due to heterogeneity rather than chance. It ranges from 0 to 100%, with higher values indicating greater heterogeneity.
    - 0% to 25%: Low heterogeneity
    - 25% to 50%: Moderate heterogeneity
    - 50% to 75%: Substantial heterogeneity
    - 75% to 100%: Considerable heterogeneity

Sensitivity analysis

To address the potential impact of high-risk bias studies on our primary outcome (clinical pregnancy rate), we performed a sensitivity analysis. We excluded studies that were designed as non-randomized interventions and quasi-experimental.

Subgroup analyses

Subgroup analyses were used to identify the effect of probiotics on ART outcomes considering the history of recurrent implantation failure (RIF) as a possible source of heterogeneity. RIF refers to the failure to achieve a clinical pregnancy after the transfer of good-quality embryos in at least three consecutive ET cycles to a morphologically normal uterus [19].

Assessment of publication bias

To assess publication bias, we employed CMA software to generate a funnel plot for the primary outcome of clinical pregnancy rate. The funnel plot is a scatter plot where the effect sizes from individual studies are plotted against a measure of study precision. In the plot, observed studies were represented by blank dots. We then conducted a trim-and-fill analysis to identify and adjust effect sizes for any 'missing' studies due to publication bias. This method imputes hypothetical studies to symmetrize the plot, represented by black-filled dots, and adjusts the effect size estimates accordingly. We also assessed publication bias using Egger's regression test.

Results

Summary of the literature search

Our initial search across electronic databases and registries retrieved a total of 1013 studies. Additionally, 8 publications were identified through manual searches and citation tracking. Utilizing Endnote, a reference manager software, we identified and removed 183 duplicate entries. A preliminary review of the titles led to the exclusion of irrelevant studies, narrowing the field to 39 articles. After reviewing the remaining full-text articles, we included six studies in the meta-analysis. The study flow of our literature search and study selection is depicted in the PRISMA 2020 flow diagram [19] (Fig. 1). For transparency, we have also provided a complete list of studies that were excluded by abstract and full-text screening along with their details and reasons for exclusion at each screening phase, in a supplementary table (Appendix file 3).

Study characteristics

Table 1 summarizes the key characteristics of all included studies, including methodological approaches and ET cycle specifics. All studies, except one that was published in 2005 [20], were conducted within the last two years (2023–2024). The studies were conducted in Iran (2 studies) [21, 22], China (2 studies) [23–25], Thailand (one study) [26], and Israel (one study) [20]. All studies compared vaginal probiotics versus blank control (no intervention), except one that compared vaginal probiotics versus placebo [21].

Four studies were designed as clinical trials (CTs) [20–22, 26]. One of these [20] CTs was a pseudo-randomized CT without blinding. One study [25] utilized mixed-methods designs. Wei et al. [25] combined a prospective cohort study with an embedded RCT to assess the efficacy of transvaginal *Lactobacillus* supplementation. One study [23] was a prospective interventional study, though details on randomization and blinding were not mentioned. In the absence of specific details on randomization and blinding, this study was conservatively categorized as a quasi-experimental study.

In Table 2 we offered a detailed account of the probiotic supplementation strategies employed across the reviewed studies. It includes information on the types of probiotics used, dosages, frequencies, and durations of treatment. The duration of probiotic supplementation ranged from a single dose to 1 month. All studies used probiotics

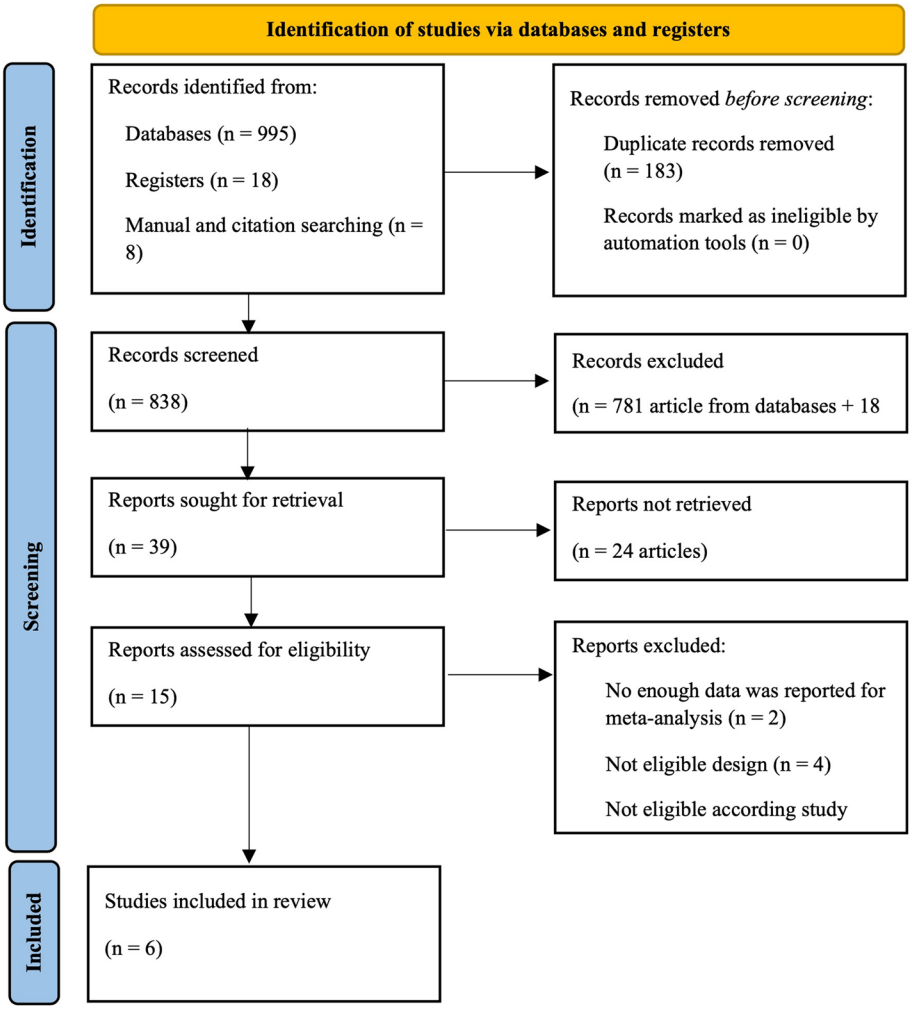


Fig. 1 PRISMA (preferred reporting items for systematic reviews and meta-analyses) 2020 flow diagram

containing different strains of *Lactobacillus* except one study that used *Enterococcus* species [23].

Quality of included studies and risk of bias

The summary of the risk of bias assessment for RCTs is shown in Fig. 2. Briefly, Jafarabadi [22] and Thanaboonyawat [26] exhibited a low risk of bias across most domains, with some concerns raised due to the absence of a placebo in the control group. Davari Tanha [21] study was assessed to have a high risk of bias, primarily due to the lack of concealment and absence of detailed reporting on randomization procedures. Wei [25] and Gilboa [20] encountered a high risk of bias, with no detailed information on concealment and randomization methods. Both non-randomized interventional studies were scrutinized for biases inherent to their design. Pengying [23] was the only study with quasi experimental design so were assessed by ROBINS-I tool. This study was impacted by various sources of bias, including not accounting for embryo quality and stage, unclear timing of probiotic therapy, and outcome assessors' awareness of interventions.

Results of meta-analysis

Clinical pregnancy rate

A total of six studies were included in the meta-analysis for clinical pregnancy rates. The pooled clinical pregnancy rate was relatively higher in probiotic group compared to control group (157 per 419 [37.47%] versus 136 per 431 [31.55%] respectively. The pooled MH risk ratio was 1.19 (95% CI: 0.99 to 1.44), indicating a non-significant increase in clinical pregnancy rates with the use of vaginal probiotics ($Z = 1.82$, $p = 0.07$). Heterogeneity was low ($Q = 5.10$, $df = 5$, $p = 0.40$, $I^2 = 1.89$), suggesting that the variation in

Table 1 Characteristics: methodological features and details of embryo transfer cycles

Study ID	Country	Study Design	Sample size and groups	Population	ET Method	Embryos Quality	Embryonic stage	Endometrial Preparation regimen in FET cycles	Outcome measures
Jafarabadi, 2024 [22]	Iran	RCT	Randomized: 166 (Intervention group = 83, Control group *n* = 83); Included in final Analyses: 163 (Interventional group = 82, Control Group = 81)	Infertile women aged < 40 with a Hx of RIF	FET	Only good quality embryos (Grad A)	Cleavage	HRT (Esteradiol followed by luteal phase support with IM Progesterone befor ET and vaginal Progesterone after ET)	BPR, CPR, IR
Wei, 2024 [25]	China	RCT	Randomized:60 (Intervention group = 30, Control group *n* = 30)	Infertile women aged 20–40, with a Hx of RIF	FET	NM	Cleavage	HRT (Esteradiol followed by luteal phase support with vaginal Progesterone)	CPR, MR
Davari Tanha, 2023 [21]	Iran	RCT	Randomized:103 (Interventional group = 54, Control group *n* = 49); Included in final Analyses: 94 (Interventional group = 49, Control Group = 45)	Infertile women aged < 40	FET	Only good quality embryos (Grad A and B)	Mixed (Cleavage and Blastocyst)	HRT (Esteradiol followed by luteal phase support with IM and vaginal Progesterone)	BPR, CPR
Thanaboonyawat, 2023 [26]	Thailand	RCT	Randomized:340 (Intervention group = 170, Control group *n* = 170); Included in final Analyses: 316 (Interventional group = 158, Control Group = 158)	Infertile women aged 18–39	FET	Only good quality embryos (grade 4BB or higher)	Mixed (Cleavage and Blastocyst)	HRT (Esteradiol followed by luteal phase support with vaginal Progesterone)	BPR, CPR, IR, MR, OPR, LBR
Pengying, 2023 [23]	China	CT (Randomization and Blinding was NM)	Total: 100 (Intervention:50, Control: 50)	Infertile women aged 25–40 with a Hx of RIF	FET	NM	NM	HRT (Esteradiol followed by luteal phase support with IM Progesterone)	CPR, IR, MR, OPR
Gilboa, 2005 [20]	Israel	pseudo-randomized CT	Total: 117 (Intervention: 50, control:67)	Infertile women aged < 38	Fresh ET	NM	NM	-	CPR

Studies are sorted according publication date

BPR Biochemical pregnancy rate, *CPR* Clinical pregnancy rate, *CT* Clinical trial, *EMT* Endometrial microbiome testing, *ET* Embryo transfer, *FET* Frozen embryo transfer, *HRT* Hormone replacement therapy, *HX* History, *IM* Intramuscular, *IR* Implantation rate, *LBR* Live birth rate, *MR* Miscarriage rate, *NM* Not mentioned, *OPR* Ongoing pregnancy rate, *RCT* Randomized clinical trial, *RIF* Recurrent implantation failure

Table 2 Details of probiotic supplementation in reviewed studies

Study ID	Brand	Active Ingredients	the number of alive and active microorganisms in one serving	Excipients/ Inactive Ingredients	Intervention Duration	Dosage	Time interval between probiotic therapy and ART	assessment of endometrial microbiota composition	Individualized treatment based on microbiota composition
Jafarabadi, 2024 [22]	Lactovag (Zist Takhmir, Iran)	Different strains of *Lactobacillus* including *Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus acidophilus,* and *Lactobacillus gasseri*	10^9 CFU	maltodextrin	2 weeks	1 Tablet per day	2 weeks started with FET cycle	No	No
Wei, 2024 [25]	Dingjunsheng (Inner Mongolia Shuangqi Pharmaceutical Co., Ltd., China)	live *Lactobacillus* (species is NM)	NM	NM	30 consecutive days	1 capsule per day	before ET cycle beginning	16S rRNA sequencing	No
Davari Tanha, 2023 [21]	Lactovag (Zist Takhmir, Iran)	*Lactobacillus rhamnosu*	10^9 CFU	Inulin	2 weeks	1 capsule per day	2 weeks befor FET cycle beginning	No	No
Thanaboonyawat, 2023 [26]	Gynoflor (Medinova AG, Switzerland)	*Lactobacillus acidophilus* + estriol	10^9 CFU	Lactose, Anhydrous disodium phosphate, Microcrystalline cellulose, Sodium starch glycolate, Magnesium stearate	6 Days	1 Tablet per day	At the same day of luteal phase support	No	No
Pengying, 2023 [23]	Lacidophilin (Xi'an Zhenghao Biopharmaceutical Co., Ltd., China)	*Enterococcus* (species is NM)	6×10^6 CFU	NM	7 Days	2 capsules per day	Was not clearly mentioned	No	No
Gilboa, 2005 [20]	Femina (Altman Ltd, Israel)	*Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Bifidobacterium longum*	3×10^9 CFU	NM	Single Dose	2 capsules in a single dose	Immediately after oocyte retrieval	No	No

Studies are sorted according publication date

CFU colony-forming units, *ET* embryo transfer, *FET* frozen embryo transfer, *NM* not mentioned

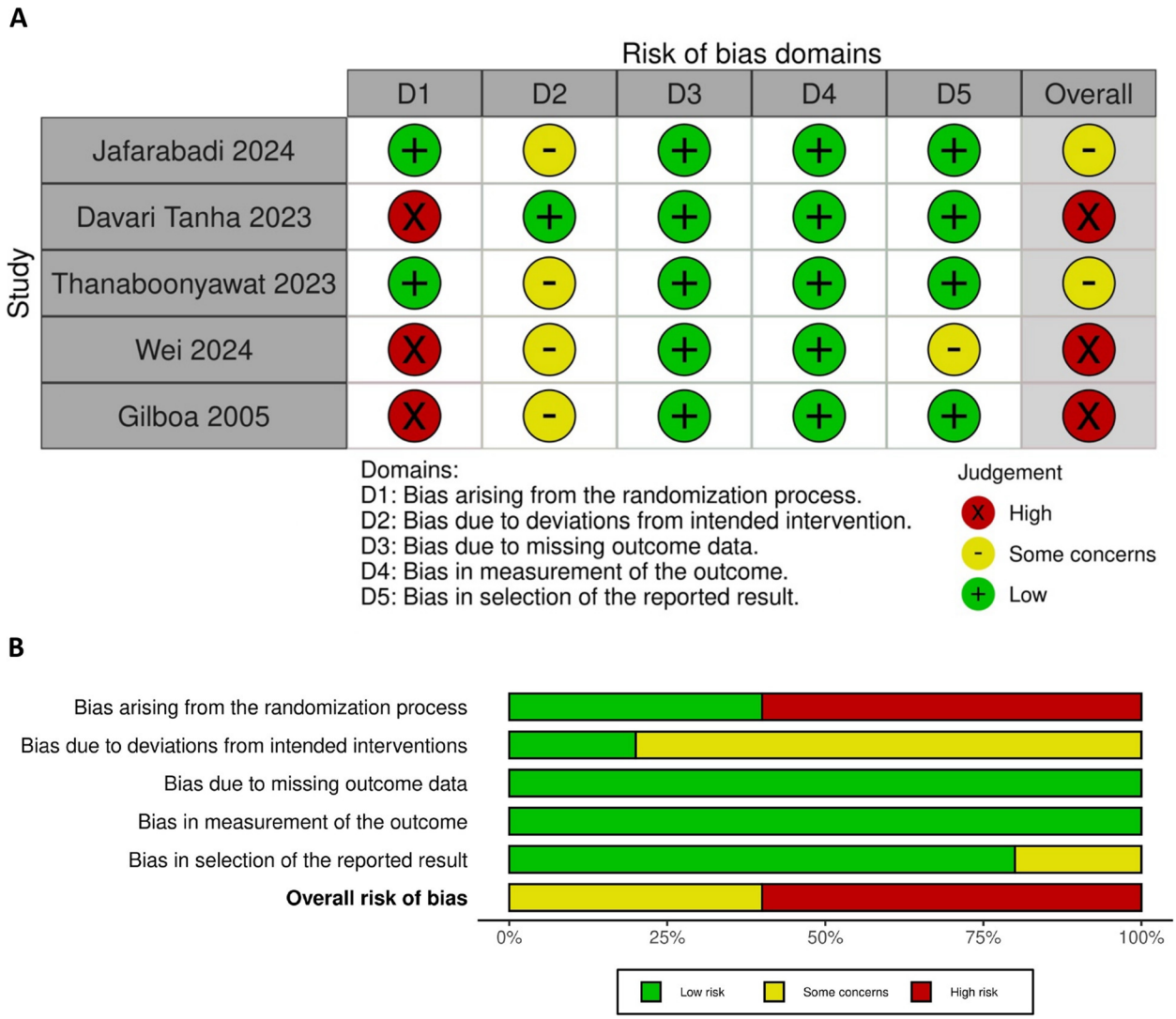


Fig. 2 Risk of bias in randomized clinical trials according to ROB 2 Tool. **a** Methodological risk of bias summary: review authors' judgments about each methodological bias item for each included study; (**b**) Methodological risk of bias graph: review authors' judgments about each methodological bias item presented as percentages across all included studies

effect estimates across studies was negligible and that the results are consistent across the included studies (Fig. 3).

For sensitivity analysis, we excluded three studies that were designed as non-randomized interventions Wei [25] and Gilboa [20] and quasi-experimental Pengying [23]. After excluding these studies with high risk of bias, no significant changes were observed in findings. The pooled clinical pregnancy rate was relatively higher in probiotic group compared to control group (99 per 289 [34.25%] versus 83 per 284 [29.22%] respectively. The pooled MH risk ratio was 1.27 (95% CI: 0.88 to 1.82), indicating a non-significant increase in clinical pregnancy rates with the use of vaginal probiotics (Z = 1.28, $p = 0.20$). Heterogeneity remained low (Q = 2.04, df = 2, $p = 0.36$, $I^2 = 2.15\%$), further supporting the robustness of the findings despite the exclusion of studies with high risk of bias (see Supplementary Figure [Appendix file 4]).

Funnel plot analysis suggested no publication bias for clinical pregnancy rates. The trim-and-fill method indicated that there were no missing studies that could be included to adjust the model, and Egger's regression test was not significant (two-tailed $p = 0.118$, one-tailed $p = 0.059$) (Fig. 4).

Subgroup analyses were conducted to explore the effect of vaginal probiotics on clinical pregnancy rates in women with and without history of RIF. In both

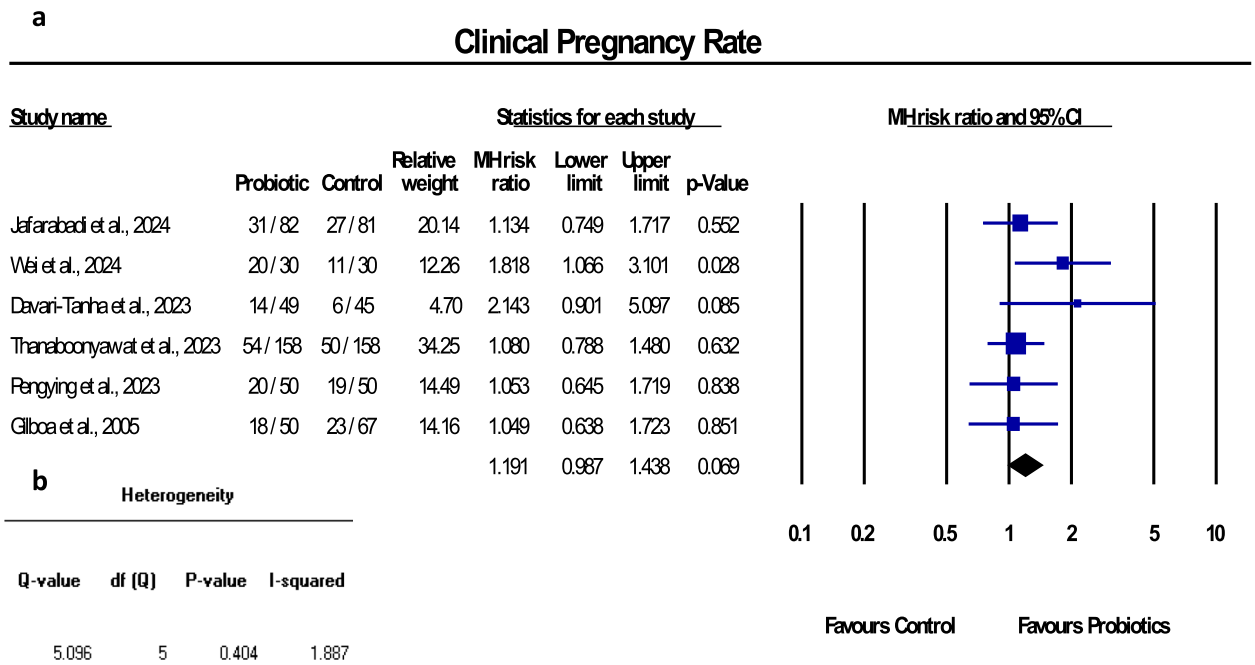


Fig. 3 Comparison of clinical pregnancy rate between probiotics and control groups. **a** Forest plot detailed Mantel–Haenszel risk ratio and 95% confidence intervals for clinical pregnancy rate. **b** Chi-square-based Q statistic and I^2 value for heterogeneity assessment

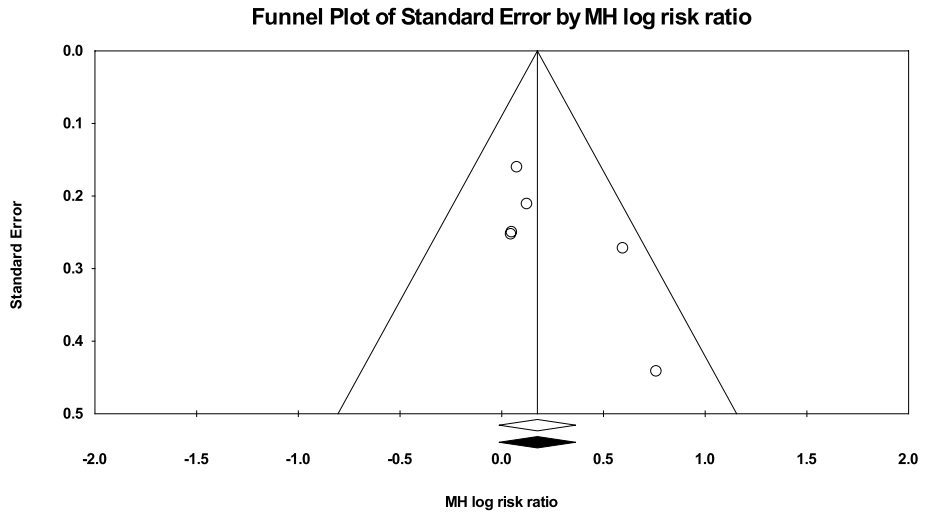


Fig. 4 Assessment of publication bias in clinical pregnancy rates. This funnel plot visualizes potential publication bias in studies measuring clinical pregnancy rates. The Y-axis indicates the precision of each study, while the X-axis shows the effect size. The plot's foundation lies in the principle that larger studies provide more precise estimates of the true treatment effect. Studies included in the analysis are marked with blank (white) dots. A black-filled dot represents a hypothetical 'missing' study inferred through trim-and-fill analysis. The asymmetry of the funnel plot hints at possible publication bias, suggesting that the reported effect size of vaginal probiotics on clinical pregnancy rates might be inflated. Without this bias, the observed effect (white diamond) would likely gravitate towards a diminished or nonexistent impact (black diamond)

subgroups, meta-analysis showed similar findings indicating a non-significant increase in clinical pregnancy rates with the use of vaginal probiotics (Fig. 5).

Biochemical pregnancy rate

Three studies were included in the meta-analysis for biochemical pregnancy (positive BhCG test) rates. The pooled MH risk ratio was 1.04 (95% CI: 0.84 to 1.28),

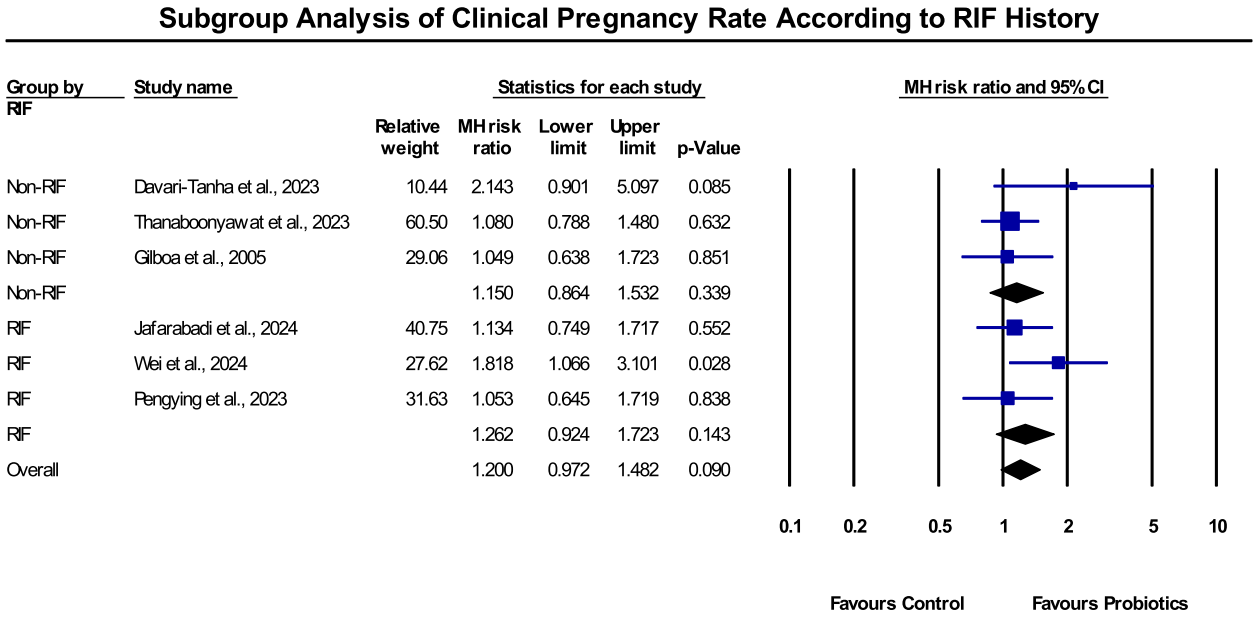


Fig. 5 Subgroup analysis of clinical pregnancy rate considering history of RIF.Forest plot detailed Mantel–Haenszel risk ratio and 95% confidence intervals for clinical pregnancy rate in the RIF and non-RIF subgroups for probiotic and control groups. RIF: Recurrent Implantation Failure

indicating no significant difference between groups (Z = 0.33, $p = 0.74$). No heterogeneity was observed (Q = 1.02, df = 2, $p = 0.60$, $I^2 = 0\%$), suggesting consistency in the effect estimates across the included studies (Fig. 6).

Ongoing pregnancy rate

Two studies were included in the meta-analysis for ongoing pregnancy rates. The pooled MH risk ratio was 1.09 (95% CI: 0.83 to 1.45), indicating no significant difference between groups (Z = 0.63, $p = 0.53$). No heterogeneity was observed (Q = 0.01, df = 1, $p = 0.91$, $I^2 = 0\%$), suggesting consistency in the effect estimates across the included studies (Fig. 7).

Miscarriage

Three studies were included in the meta-analysis for miscarriage rates. The pooled MH risk ratio was 0.67 (95% CI: 0.40 to 1.12), indicating a non-significant

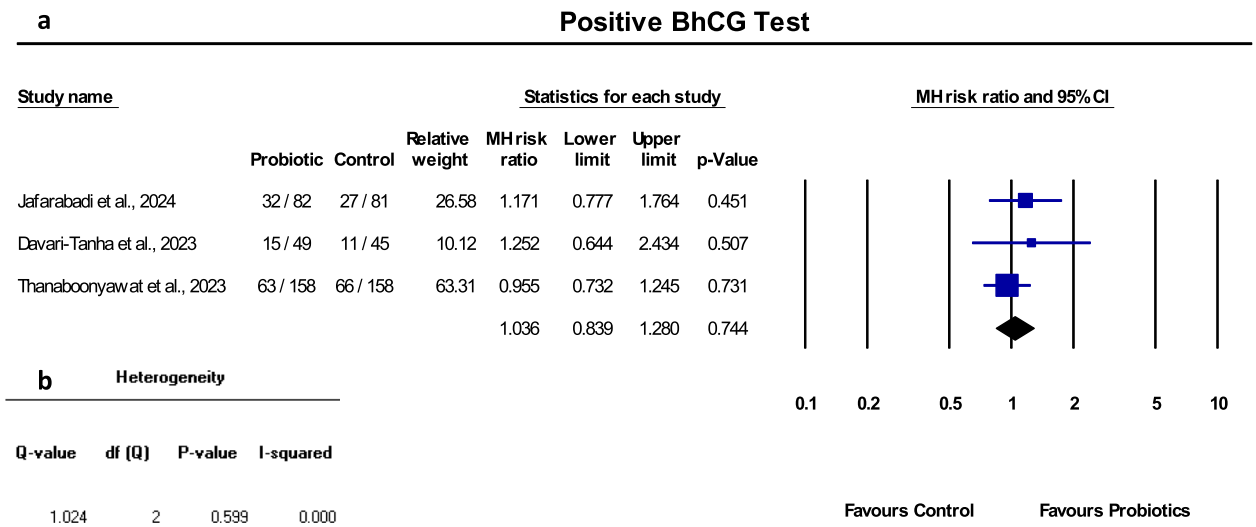


Fig. 6 Comparison of biochemical pregnancy rate between probiotics and control groups. **a** Forest plot detailed Mantel–Haenszel risk ratio and 95% confidence intervals for biochemical pregnancy rate (determined by BhCG detection in blood or urine). **b** Chi-square-based Q statistic and I^2 value for heterogeneity assessment

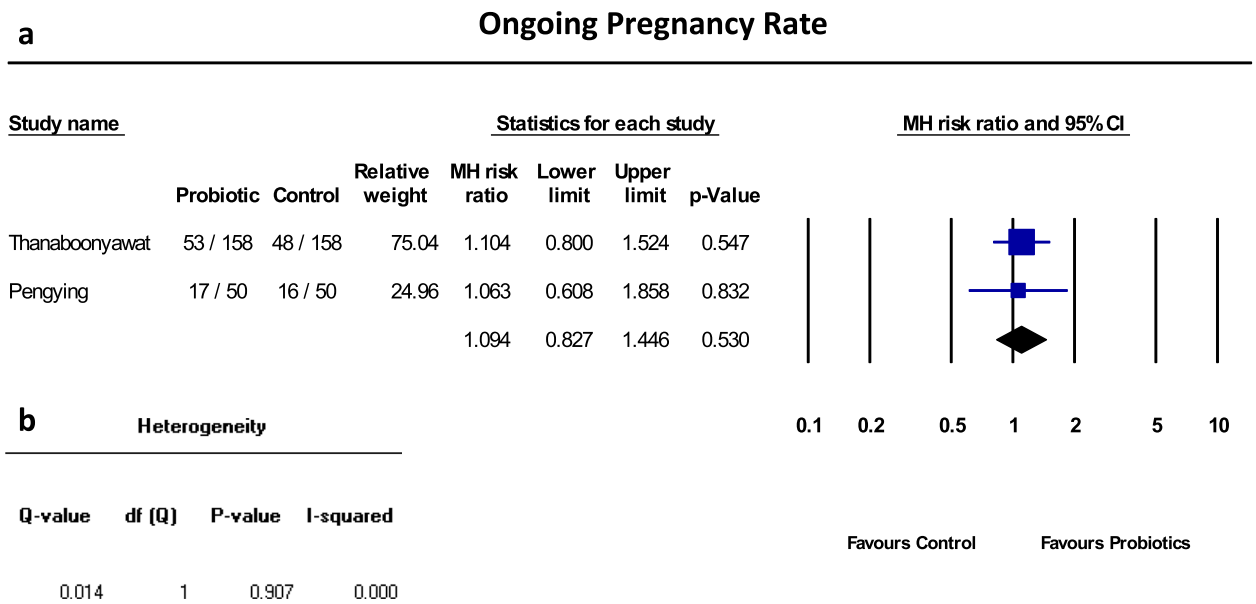


Fig. 7 Comparison of ongoing pregnancy rate between probiotics and control groups. **a** Forest plot detailed Mantel–Haenszel risk ratio and 95% confidence intervals for ongoing pregnancy rates. **b** Chi-square-based Q statistic and I^2 value for heterogeneity assessment

reduction in miscarriage rates with the use of vaginal probiotics ($Z = -1.54$, $p = 0.12$). Heterogeneity was low ($Q = 2.29$, $df = 2$, $p = 0.32$, $I^2 = 12.61\%$), suggesting that the results were relatively consistent across the included studies with some minor variation (Fig. 8).

Other outcomes

Reported data for implantation and live birth rates was insufficient so meta-analysis was not possible for these outcomes.

Discussion

Summary of study findings

In this systematic review and meta-analysis, we evaluated the efficacy of vaginal probiotics as therapeutic adjuncts on ET success rates. Our findings indicated a non-significant increase in clinical pregnancy rates in the probiotic group compared to controls, with negligible heterogeneity observed among the studies. Subgroup analysis revealed similar non-significant increases in clinical pregnancy rates in both women with and

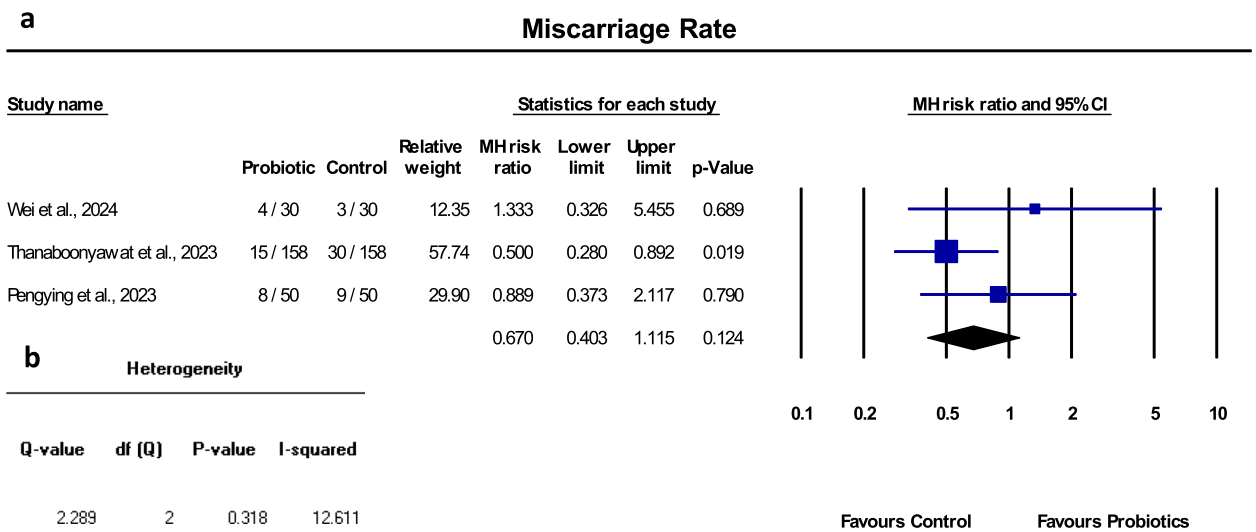


Fig. 8 Comparison of miscarriage rate between probiotics and control groups. **a** Forest plot detailed Mantel–Haenszel risk ratio and 95% confidence intervals for miscarriage rate. **b** Chi-square-based Q statistic and I^2 value for heterogeneity assessment

without a history of RIF. The analysis of biochemical pregnancy rates did not demonstrate a significant difference between the probiotic and control groups. Similarly, the ongoing pregnancy rates showed no significant improvement with probiotic use. A noteworthy finding was the non-significant reduction in miscarriage rates in the probiotic group, suggesting a potential role of vaginal probiotics in sustaining early pregnancy. Due to limited data, we could not conduct a meta-analysis for implantation and live birth rates. Overall, the very low to zero heterogeneity observed across all outcomes supports the robustness of our findings and suggests a high level of consistency in the effect estimates.

Comparison of study findings with previous reviews

To date, our study is the only systematic review and meta-analysis focusing on effect of vaginal probiotics as therapeutic adjuncts on ET outcomes. There is only one systematic review with relatively similar objective by Santana et al. [27]. This review assessed the role of probiotics on reproductive outcomes in infertile women undergoing ART. It only included a qualitative presentation of each study's findings without performing a quality assessment or meta-analysis. Another systematic review and meta-analysis by Moreno et al.'s [14] addressed the influence of vaginal probiotics on reproductive health and associated dysbiosis, demonstrating a moderate alteration of the vaginal microbiota. However, it did not focus specifically on infertile women undergoing ART. Blancafort et al. [15] assessed the potential of probiotics to improve fertility outcomes, emphasizing benefits for non-symptomatic women of reproductive age, but again, did not focus on infertile women undergoing ART.

Hypothesis and potential mechanisms of action

The administration of probiotics as adjuvant treatments before ET, is based on a hypothesis that altering the endometrial microbiota composition can provide a favorable environment for embryo implantation. A shift in the population of *Lactobacillus* species, for instance, has been implicated in diminished endometrial receptivity [8, 9, 28, 29]. An eubiotic endometrial microbiome is postulated to mildly activate the local immune response, thereby facilitating normal endometrial cells remodeling essential for implantation [30]. Beneficial metabolites produced by these microbes are thought to boost the endometrial support for the embryo implantation [31]. Additionally, probiotics containing *Lactobacillus* strains can decrease the expression of pro-inflammatory cytokines (IFN-γ, IL-17, and IL-23) and increase the expression of anti-inflammatory cytokines (IL-4 and IL-10) in different tissues [32–34]. On the other hand, an optimal balance of cytokines is crucial for successful implantation, as excessive inflammation can disrupt this delicate process. So, by reducing the levels of pro-inflammatory cytokines and enhancing anti-inflammatory cytokines, probiotics may help create a more conducive environment for embryo implantation. However, this hypothesis requires further studies to elucidate the precise mechanisms and confirm the beneficial effects of probiotics on embryo implantation. Moreover, these microbes are theorized to prevent pathogen colonization through spatial antagonism, thereby protecting the endometrial niche [35]. Conversely, in a dysbiotic endometrial environment, ET outcomes may be adversely affected by alterations in bacterial communities, as such changes can initiate immune reactions and alter the metabolic profile [31].

Limitations of included primary studies

One of the limitations of the included studies is related to variability in probiotic interventions. Different doses of probiotics can lead to varying levels of vaginal and endometrial colonization and activity of these microorganisms. Duration of probiotics administration also can affect the establishment and maintenance of beneficial vaginal and endometrial flora.

The timing of probiotic administration in relation to the menstrual cycle and the scheduled day of ET was also variable among studies. Administering probiotics when mucus properties support optimal bacterial adhesion might enhance their efficacy. Bacterial colonization in the female genital tract is a complex process involving interactions among bacteria, host epithelial cells, and mucins, macromolecular glycoproteins that play a significant role in bacterial adhesion and colonization. Transmembrane mucins (MUC1, MUC4, MUC16) expressed on mucosal epithelial cells provide adhesive sites for vaginal bacteria, facilitating their colonization, while mucus-forming mucins (MUC5AC, MUC5B, MUC6) secreted by goblet cells can both support and inhibit bacterial attachment depending on their levels [36, 37]. Notably, the secretion of MUC5B, the major mucin component of vaginal mucus, fluctuates during the menstrual cycle, altering mucus properties and bacterial adhesion at different phases; during the ovulatory phase, neutral oligosaccharides on mucins are more abundant, enhancing bacterial binding affinity, whereas acidic oligosaccharides dominate during the follicular phase [36, 37]. These variations suggest that initiating probiotic therapy when mucin expression and composition are most favorable for bacterial adhesion may enhance the colonization efficiency of probiotic strains, thereby improving their beneficial effects on the endometrial microbiota.

Also, diverse probiotic strains were used across the included studies. This variability in strains could impact

the outcomes, as different probiotic strains have unique properties and health effects. Among the several available probiotic products, *Lactobacillus* is the most prominent genus in vaginal probiotics. Several *Lactobacillus* species have been identified as dominant or common members of the healthy vaginal tract, including *L. rhamnosus*, *L. fermentum*, *L. plantarum*, *L. gasseri*, *L. acidophilus*, *L. reuteri*, *L. casei*, and *L. paracasei* [38]. The primary and well-known mechanism of action of these strains relates to their ability to maintain a healthy vaginal microbiota and prevent bacterial vaginosis. Regulation of vaginal pH through the production of lactic acid is the most fundamental mechanism in these strains [37]; however, there may be additional mechanisms specific to different strains. For instance, some strains, like *L.s jensenii*, produce hydrogen peroxide, which has antimicrobial properties [39]. Certain strains like *L. crispatus* and *L. gasseri* produce bacteriocins, which are antimicrobial peptides that inhibit the growth of pathogenic bacteria [40, 41]. *L. gasseri* can also exert immunomodulatory effects via the Toll-like receptor 2 and NOD2-mediated phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway [38]. Additionally, *L.crispatus* has been shown to reduce the adhesion of both commensal and pathogenic *Gardnerella vaginalis* to HeLa cells [39], indicating that competitive exclusion of this bacterial vaginosis-associated species could play a key role in the health-promoting effects of *L. crispatus*.

In addition to the variability in probiotic interventions, individual host factors and the target population can also influence the efficacy of probiotics. Interindividual variability in vaginal and endometrial microbiota and host response to probiotics is a known confounder. Among the eligible included studies, none assessed the endometrial microbiota composition pre-intervention. However, there is a retrospective study by Chen et al. that considered a personalized treatment approach according to each patient's endometrial microbiota composition. They administered probiotics alone or in combination with antibiotics in patients with RIF and found significant improvements in pregnancy rate [24]. This raises a crucial question: Are probiotics universally effective, or do they specifically benefit patients with dysbiotic endometria? With advancements in microbiota assessment techniques like 16S rRNA sequencing, endometrial microbiota assessment before ET is feasible, allowing for the targeted prescription of probiotics to indicated patients [42]. While routine assessment of endometrial microbiota before ET may not be practical, it could be a valuable approach in RIF cases.

In addition to pre-existing microbiota composition, another notable limitation of the included studies is the broad spectrum of infertility causes among participants, contributing to significant heterogeneity. Since not all infertility causes are linked to altered endometrial receptivity, the indiscriminate application of probiotics across all infertile women may not be justified.

Another important issue is the failure of included studies in reporting the potential adverse effects of probiotics. While probiotics are generally considered safe and are widely used in various clinical settings, it is essential to acknowledge and discuss the potential adverse effects associated with their use in the context of ART. Adverse events related to probiotic use are typically rare and mild but can occur [43]. Probiotic strains of *Lactobacillus* can exert early immunostimulatory effects that may be directly linked to the initial inflammation of the response of human macrophages [44]. Consequently, vaginal administration of probiotics could potentially cause local discomfort, irritation, or allergic reactions. On the other hand, during the implantation process, a balanced production of inflammatory and anti-inflammatory cytokines is critical for success. The impact of probiotics on this delicate balance is not fully understood, and there is a potential risk that they may cause overactivated inflammatory reactions that could compromise embryo implantation. Additionally, exogenous probiotic strains might inadvertently disrupt the natural balance of the vaginal and endometrial microbiota. While the goal is to promote a favorable microbial environment, there is a risk of over-colonization by certain strains or suppression of beneficial native microbes, potentially leading to dysbiosis. In immunocompromised individuals, there have been rare reports of systemic infections caused by probiotic bacteria, such as bacteremia or sepsis involving *Lactobacillus* species [45]. Although such events are exceptionally uncommon, they underscore the need for cautious use in patients with compromised immune systems. The long-term safety of altering the endometrial microbiota on pregnancy outcomes is also not fully understood. Potential unintended consequences might include effects on fetal development or pregnancy complications, although current evidence is insufficient to establish such associations.

Another important limitation of some of the included studies was the lack of proper reporting and methodological standards. This includes detailed documentation of probiotic formulations, delivery methods, and adherence to intervention protocols. Also, it should be noted that with the exception of one study [21], none of the included studies used a placebo in their control groups. This absence of a placebo could potentially confound the effects of the intervention. Additionally, factors such as the semen microbiota of partners and the sexual habits of participants were not evaluated, yet these could significantly influence the findings. The potential impact of

these uncontrolled variables should be acknowledged and considered when interpreting the results of the studies.

Limitations of the present review

This meta-analysis has several limitations that should be considered when interpreting the results:

1. Limited Number of Studies and Outcomes: The number of included studies was relatively small, particularly for outcomes beyond clinical pregnancy rates. While we included all six available studies for the meta-analysis of clinical pregnancy, fewer studies reported on other important outcomes such as biochemical pregnancy, ongoing pregnancy, and miscarriage rates. Notably, critical outcomes like live birth rate and adverse pregnancy events were not reported in the included studies. The small number of studies and lack of data on these outcomes limit the statistical power and reliability of the analyses, potentially affecting the generalizability of the findings.
2. Limited Subgroup Analyses: We were only able to perform subgroup analysis based on RIF status due to the small number of studies and inadequate reporting of data. The inability to conduct more extensive subgroup analyses restricts our understanding of how different patient characteristics may affect treatment outcomes.
3. Diversity of Interventions: There was considerable variability in the probiotic interventions across studies. Although we focused exclusively on the vaginal route of administration, variations existed in probiotic strains, dosages, and treatment durations. This heterogeneity can significantly affect the consistency of the results and makes it challenging to draw definitive conclusions about the efficacy of specific probiotic regimens.
4. Methodological Quality of Included Studies: The methodological quality of the included studies varied. Some studies lacked proper randomization, blinding, and placebo controls, which could introduce bias and affect the validity of the results. The absence of placebo-controlled designs in most studies could confound the observed effects of the interventions.
5. Diversity of Included Population: The studies included participants with a broad spectrum of infertility causes. Since not all infertility etiologies are linked to altered endometrial receptivity, this diversity may contribute to heterogeneity and affect the applicability of the results to specific subgroups.
6. Lack of Pre-Intervention Microbiota Assessment: None of the included studies assessed the endometrial microbiota composition prior to intervention. Without this information, it is unclear whether the patients had dysbiotic endometrial environments that could potentially benefit from probiotic therapy. This gap limits the ability to tailor treatments and assess their true efficacy in different microbiota contexts.
7. Individual Host Factors Not Accounted For: Individual host factors such as genetics, diet, lifestyle, and pre-existing microbiota composition were not considered in the included studies. These factors can influence the efficacy of probiotic treatments and may lead to interindividual variability in outcomes.
8. Inadequate Reporting and Uncontrolled Variables: Some studies lacked detailed documentation of probiotic formulations, delivery methods, and adherence to intervention protocols. Additionally, factors such as the semen microbiota of partners and the sexual habits of participants were not evaluated, yet these could significantly influence the findings. The potential impact of these uncontrolled variables should be acknowledged when interpreting the results.

Clinical implications of study findings

The findings of this meta-analysis suggest that while vaginal probiotics may not significantly increase clinical pregnancy rates, they may have a possible role in sustaining early pregnancy. Although the reduction in miscarriage rates did not reach statistical significance, the observed trend is clinically noteworthy and warrants further investigation. This suggests that vaginal probiotics might enhance the endometrial environment or modulate immune responses favorably during the early stages of pregnancy.

For clinicians, these results highlight the potential benefit of incorporating vaginal probiotics as an adjunctive therapy, especially in infertile patients with a history of RIF or miscarriages. Given the critical role of the endometrial microbiota in implantation and pregnancy outcomes, assessing the endometrial microbiota composition before recommending probiotics could enable a more personalized treatment approach. Incorporating assessments of vaginal and endometrial microbiota into the standard evaluation of patients undergoing ART could help identify those who might benefit most from probiotic interventions. Although routine microbiota profiling may not be feasible in all settings due to cost and accessibility constraints, prioritizing it for patients with unexplained infertility or repeated ART failures could be valuable. This personalized approach aligns with the trend toward precision medicine in reproductive health care.

The variability in probiotic strains, dosages, and treatment durations observed across studies underscores the need for standardization in clinical practice. Clinicians

should be aware that different probiotic strains possess unique properties and mechanisms of action. Establishing standardized protocols for probiotic selection, optimal dosages, and appropriate treatment durations is not well established yet. Selecting probiotic strains with well-established safety profiles is crucial. Clinicians should opt for strains that have been extensively studied and are known to be safe for vaginal use, minimizing the risk of adverse reactions. Until consensus guidelines are developed, practitioners should consider using probiotics with well-characterized strains known for their beneficial effects on vaginal and endometrial health. Also, clinicians should vigilantly monitor patients receiving probiotic therapy for any adverse effects, particularly during the sensitive period surrounding ET and early pregnancy. Any adverse events should be thoroughly documented and reported to contribute to the overall understanding of probiotic safety in ART.

The timing of probiotic administration may also significantly influence their efficacy. Initiating probiotic therapy at a phase in the menstrual cycle when mucin expression and composition are most conducive to bacterial adhesion could enhance the colonization efficiency of beneficial strains. Clinicians should consider the menstrual cycle dynamics when prescribing probiotics to maximize their therapeutic potential.

While probiotics are generally considered safe, it is essential for clinicians to monitor patients for any adverse effects, especially since the patient population undergoing ART may have heightened sensitivities. Counseling patients about the possible benefits and limitations of probiotic therapy is important for setting realistic expectations and ensuring informed consent. Also, probiotic use may not be appropriate for all patients, including those with severe immunodeficiency, known allergies to probiotic components, and RIF resulting from confirmed immunological factors. It is essential to assess patient history and contraindications before starting therapy.

Finally, the integration of vaginal probiotics into clinical practice holds promise as a supportive strategy for improving reproductive outcomes in infertile women undergoing ART, particularly for patients with RIF or a history of miscarriage. However, the current evidence indicates a need for high-quality, well-designed RCTs to validate the efficacy of vaginal probiotics in improving ET outcomes and supporting their safety in ART context. By embracing standardized protocols and supporting ongoing research, the medical community can advance the understanding of probiotics in reproductive health.

Recommendations for future studies

To advance the understanding of vaginal probiotics as therapeutic adjuncts in ET outcomes, future research should consider the following recommendations:

1. Standardization of Probiotic Interventions:

    - Probiotic Strains: There are several recognized mechanisms for different *Lactobacillus* strains, such as the production of lactic acid, hydrogen peroxide, and bacteriocin, immune modulation, and competitive exclusion, providing some insight into how different strains may impact reproductive health. However, it should be noted that our understanding of the specific effects of individual probiotic strains on the reproductive system, particularly the endometrial microbiota, is limited. Most existing literature focuses on the role of probiotics in the gastrointestinal tract or the vaginal environment, with comparatively little research dedicated to their impact on the endometrium. Consequently, the differences that each probiotic strain may pose in the reproductive system and among infertile women remain largely unknown. Future studies should identify and standardize specific probiotic strains with the most therapeutic potential. Given that different strains, such as *L. crispatus*, *L. gasseri*, and *L. jensenii*, have unique mechanisms of action, pinpointing the most effective strains is essential.
    - Dosage and Duration: Determining the optimal dosage and duration of probiotic administration is crucial. Consistent dosing regimens will help establish the levels of colonization needed for beneficial effects on the vaginal and endometrial microbiota.
    - Timing of Probiotic Administration: Researchers should investigate the optimal timing of probiotic administration in relation to the menstrual cycle and ET. Since mucin composition and secretion fluctuate throughout the cycle, affecting bacterial adhesion, initiating therapy when mucin properties favor colonization may enhance efficacy. Also, finding the most effective and safe interval between probiotic therapy and the scheduled day of ET could maximize the positive impact on endometrial receptivity and implantation success

2. Personalized Treatment Approaches:

    - Microbiota Assessment: Implementing assessments of the endometrial and vaginal microbiota prior to intervention can help tailor probiotic therapy to individual needs, especially in patients with RIF.
    - Targeted Populations: Studies should consider stratifying participants based on specific infertility causes linked to altered endometrial receptivity to determine if probiotics are particularly beneficial in these subgroups.

3. Enhanced Study Designs:

    - Methodological Rigor: Future research should prioritize robust methodological designs, including proper randomization, blinding, and the use of placebo controls to minimize bias.
    - Comprehensive Reporting: Detailed documentation of probiotic formulations, delivery methods, adherence to protocols, and participant compliance should be standard to improve reproducibility and interpretation. Also, improved and standardized reporting of study data, including detailed participant characteristics and outcome measures, will facilitate more robust subgroup analyses.

4. Exploration of Mechanisms:

    - Immunological Investigations: Further studies are needed to elucidate the precise mechanisms by which probiotics influence cytokine balance and modulate the immune response during implantation.
    - Molecular Pathways: Research into specific signaling pathways affected by different probiotic strains will enhance understanding of how these interventions support embryo implantation.

5. Consideration of Host Factors:

    - Genetic and Lifestyle Factors: Accounting for individual host factors such as genetics, diet, and lifestyle can help in interpreting variations in probiotic efficacy and tailoring treatments accordingly.
    - Partner Factors: Evaluating the semen microbiota of partners and sexual habits may provide insights into additional variables that influence the vaginal and endometrial microbiota.

6. Larger, Multicenter Trials:

    - Increased Sample Sizes: Conducting studies with larger participant numbers will improve the statistical power and generalizability of findings.
    - Diverse Populations: Including a broader range of demographic and geographic populations can help determine the universal applicability of probiotic interventions. Future research should aim to explore the efficacy of probiotics across different infertility etiologies, age groups, and other relevant patient subgroups. This approach will help identify populations that may derive the most benefit from probiotic therapies.

7. Focus on Specific Outcomes:

    - Implantation and Live Birth Rates: Future studies should prioritize the reporting of essential reproductive outcomes, such as live birth rates, miscarriage rates, and adverse pregnancy events. Including these outcomes will provide a more complete understanding of the effectiveness and safety of probiotic interventions in the context of ART.
    - Long-term Effects: Investigating the long-term reproductive outcomes and potential impacts on offspring health will provide a more complete picture of probiotic benefits.
    - Potential Adverse Effects of Probiotic Use: Future studies should systematically assess and report any adverse events associated with probiotic administration to build a comprehensive safety profile. This will aid in identifying rare but significant risks, guiding safe clinical practices, and informing patient counseling.

By addressing these recommendations, future studies can overcome current limitations and contribute valuable knowledge to the field of reproductive medicine. This will aid in determining the true efficacy of vaginal probiotics in improving ET outcomes and potentially lead to personalized, effective interventions for infertile women undergoing ART.

Conclusion

In conclusion, our systematic review and meta-analysis suggest that vaginal probiotics may offer a non-significant increase in clinical pregnancy rates and a potential reduction in miscarriage rates when used as an adjunctive treatment in ET cycles. However, the evidence is not robust enough to draw definitive conclusions due to the small number of included studies, variability in study designs, probiotic interventions, and participant characteristics. Future well-designed randomized controlled trials with standardized probiotic regimens and comprehensive reporting are needed to better understand the role of vaginal probiotics in improving ET outcomes. Personalized approaches considering individual microbiota compositions and specific infertility causes may enhance the efficacy of probiotic treatments in ART.

Abbreviations

ART	Assisted reproductive techniques
ET	Embryo transfer
RCTs	Randomized clinical trials
ICTRP	International Clinical Trials Registry Platform
ROB-2	Risk of Bias 2
ROBINS-I	Risk of Bias in Non-randomized Studies of Interventions
CMA	Comprehensive Meta-Analysis
RIF	Implantation failure

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

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Authors' contributions
All authors contributed to the study conception and design and commented on previous versions of the manuscript. All authors read and approved the final manuscript. A detailed contribution statement is provided below: AM-H: Data collection (systematic search, and risk of bias assessment), manuscript original draft preparation, statical analyses, and study graphs and graphical abstract designing; RK: Data collection (study selection, data extraction, and risk of bias assessment) and manuscript editing; SA: Data collection (study selection and data extraction); NE: Data collection (study selection and data extraction); FA: Study conceptualization; supervision, interpretation of results; manuscript critical review and editing.

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Data availability
The data collection forms, data extracted from included studies, data used for all analyses, and the full list of screened titles in this review are not publicly available. However, they will be made available for reasonable requests. To obtain access to the raw data analyzed in this study, please contact [Arezoo Maleki-Hajiagha] at [Arezoomaleki.h@yahoo.com].

Declarations

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Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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