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LACTOBACILLUS-DOMINANT ENDOMETRIAL MICROBIOTA AND IVF OUTCOMES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Background: The endometrial microbiota has been proposed as a modulator of embryo implantation. Whether Lactobacillus-dominant (LD) endometrial microbiota improves in vitro fertilisation (IVF) outcomes remains uncertain. We conducted a systematic review and meta-analysis to quantify the association between LD endometrial microbiota and IVF outcomes.

Methods: We searched PubMed/MEDLINE, EMBASE, Cochrane CENTRAL, and Web of Science from inception to January 2025 for observational studies and randomised controlled trials comparing LD versus non-Lactobacillus-dominant (NLD) endometrial microbiota in women undergoing IVF/ICSI. Primary outcome was clinical pregnancy rate (CPR); secondary outcomes included live birth rate (LBR), implantation rate (IR), biochemical pregnancy rate (BPR), and miscarriage rate. Risk of bias was assessed using the Newcastle-Ottawa Scale (NOS) for observational studies. Random-effects meta-analysis (DerSimonian-Laird) was applied. Heterogeneity was quantified by I^2, τ^2, and Cochran Q. Publication bias was assessed by Egger regression and Begg-Mazumdar rank correlation.

Results: Five studies (n = 627 participants) met eligibility criteria, spanning Japan, Spain/multinational, the Netherlands, and China. The LD endometrial microbiota was significantly associated with higher CPR (pooled OR 2.24, 95% CI 1.37–3.67; I^2 = 52%; p = 0.001) and LBR (pooled OR 3.01, 95% CI 1.42–6.37; I^2 = 46%; p = 0.004). Implantation rates were numerically higher in LD patients in all reporting studies. The NOS scores ranged from 5 to 7 (out of 9), indicating moderate to good methodological quality. No significant publication bias was detected (Egger p = 0.24). Sensitivity analyses excluding the smallest study did not materially alter pooled estimates.

Conclusions: LD endometrial microbiota is associated with significantly improved clinical pregnancy and live birth rates in women undergoing IVF/ICSI. However, between-study heterogeneity, small sample sizes, and variability in LD definitions limit certainty. Large, prospective, standardised studies are needed before clinical application of endometrial microbiome testing can be recommended.

KEYWORDS: endometrial microbiota; Lactobacillus; IVF; ICSI; implantation; clinical pregnancy; live birth; meta-analysis'

1. INTRODUCTION

Infertility affects an estimated 48 million couples worldwide, and in vitro fertilisation (IVF) with intracytoplasmic sperm injection (ICSI) constitutes the most effective available treatment. Despite continued advances in embryology and endocrinology, approximately 60–70% of embryo transfers do not result in clinical pregnancy, underscoring the importance of endometrial receptivity as a key determinant of implantation success.

The human uterine cavity was historically regarded as sterile. However, the application of 16S ribosomal RNA (rRNA) gene sequencing technology from the mid-2010s onward has established that a low-biomass but distinct endometrial microbiota exists, composed predominantly of *Lactobacillus* spp. together with other bacterial genera including *Gardnerella*, *Prevotella*, *Streptococcus*, and *Atopobium*. The seminal work by Moreno et al. in 2016 proposed that a Lactobacillus-dominant (LD) endometrial microbiota, defined as ≥90% relative abundance of *Lactobacillus* spp., was associated with significantly higher implantation, clinical pregnancy, ongoing pregnancy, and live birth rates compared with non-Lactobacillus-dominant (NLD) profiles.[1]

The biological plausibility of such an association is supported by several mechanisms. *Lactobacillus* spp. acidify the endometrial environment through lactic acid and hydrogen peroxide production, suppressing pathogenic overgrowth. They modulate local innate immune signalling, potentially favouring a Th2-skewed, tolerogenic endometrial milieu conducive to trophoblast invasion. Moreover, *Lactobacillus crispatus* in particular has been associated with reduced expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, which are known implantation antagonists.

Following Moreno et al.'s index study, several groups replicated, or failed to replicate, this association. Kyono et al. (2019) found numerically higher pregnancy rates in LD patients but the difference was not statistically significant in a pilot study of 92 patients.[2] Hashimoto and Kyono (2019) found comparable pregnancy rates between eubiotic and dysbiotic groups in 99

IVF patients.[3] A large multicentre prospective study by Moreno et al. (2022) confirmed that Lactobacillus enrichment in endometrial fluid and biopsy samples was associated with live birth in 342 patients across 13 international centres.[4] Conversely, Bui et al. (2023) in an exploratory study of 141 patients who had failed one IVF/ICSI cycle found associations specifically with *Lactobacillus crispatus* relative abundance rather than overall Lactobacillus dominance.[5] Chen et al. (2025) reported that LD endometrial microbiota was associated with better FET outcomes in a retrospective study of 51 Chinese patients.[6]

The heterogeneity of these findings, variability in LD definitions (thresholds ranging from ≥80% to ≥90%), differences in sample type (endometrial fluid vs. biopsy vs. aspirate), and variable study designs have prevented definitive conclusions. No previous meta-analysis has pooled specifically endometrial (as opposed to vaginal or cervical) microbiota data using only verified published studies. This systematic review and meta-analysis was therefore conducted to quantify the pooled association between LD endometrial microbiota and IVF/ICSI outcomes following PRISMA 2020 guidelines.

2. METHODS

This systematic review and meta-analysis was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.[7] The protocol was defined a priori.

2.1 Eligibility Criteria

Study designs

Prospective cohort, retrospective cohort, and case-control studies, as well as randomised controlled trials, reporting IVF/ICSI outcomes stratified by endometrial microbiota status were eligible. Conference abstracts lacking full outcome data and animal or in vitro studies were excluded.

Population

Women of reproductive age undergoing IVF or ICSI with autologous or donor oocytes.

Exposure

Lactobacillus-dominant (LD) endometrial microbiota, defined by the primary study as ≥80% or ≥90% relative abundance of *Lactobacillus* spp. (or equivalent operational definition) in endometrial fluid, biopsy, or aspirate samples assessed by 16S rRNA gene sequencing or whole-metagenome sequencing.

Comparator

Non-Lactobacillus-dominant (NLD) endometrial microbiota, as defined by each primary study (typically <80% or <90% *Lactobacillus* spp. with ≥10% other taxa).

Outcomes

Primary: Clinical pregnancy rate (CPR) — gestational sac on ultrasound at ≥6 weeks per embryo transfer.

Secondary: Live birth rate (LBR); implantation rate (IR); biochemical pregnancy rate (BPR); miscarriage rate.

Exclusion criteria

Studies examining only vaginal, cervical, or catheter-tip microbiota without direct endometrial sampling.

Studies not reporting event counts or rates permitting OR derivation.

Studies with intervention (antibiotic or probiotic) between microbiome assessment and embryo transfer (introducing confounding), unless a stratified unexposed-control arm was reported.

Non-English publications.

Duplicate cohorts (most complete dataset retained).

2.2 Information Sources and Search Strategy

Four databases were searched from inception to January 2025: PubMed/MEDLINE, EMBASE, Cochrane CENTRAL, and Web of Science. The reference lists of all eligible studies and relevant systematic reviews were hand-searched. No study protocol registration restrictions were applied.

PubMed search string:

("endometrial microbiome" OR "endometrial microbiota" OR "uterine microbiome" OR "uterine microbiota") AND ("Lactobacillus" OR "lactobacillus dominant" OR "lactobacillus dominance") AND ("IVF" OR "in vitro fertilization" OR "in vitro fertilisation" OR "ICSI" OR "assisted reproductive technology" OR "ART" OR "embryo transfer" OR "implantation")

2.3 Study Selection

Two reviewers independently screened titles and abstracts, then full texts. Disagreements were resolved by consensus. The PRISMA 2020 flow diagram process was followed.

2.4 Data Extraction

Standardised extraction forms were used. The following were extracted for each study: first author; year; country; study design; sample size; ART type (IVF/ICSI/FET); endometrial sample type; microbiome assessment method and 16S rRNA region; LD definition (threshold); primary and secondary outcomes with event counts and denominators; confounders adjusted for; and follow-up duration.

2.5 Risk of Bias Assessment

Observational studies were assessed using the Newcastle-Ottawa Scale (NOS) for cohort and case-control studies (maximum 9 stars). Domains assessed: selection of cohort, comparability, and outcome assessment. Scores of ≥7 indicate low risk, 5–6 moderate risk, and ≤4 high risk of bias. If any RCTs had been included, the Cochrane Risk of Bias 2 (RoB 2) tool would have been applied.

2.6 Statistical Analysis

Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were computed using the DerSimonian-Laird random-effects model to account for expected between-study heterogeneity. The random-effects model was chosen a priori given anticipated methodological and population heterogeneity. Statistical heterogeneity was assessed using the Cochran Q statistic (threshold $p < 0.10$), I^2 (thresholds: <25% low, 25–75% moderate, >75% substantial), and τ^2 (between-study variance). Forest plots were constructed displaying study-specific and pooled ORs with 95% CIs. Publication bias was assessed by visual inspection of funnel plot symmetry and formal Egger regression test and Begg-Mazumdar rank correlation test ($p < 0.10$ considered significant). A priori sensitivity analyses included: (i) exclusion of studies with the smallest sample size; (ii) restriction to prospective designs; (iii) restriction to studies using ≥90% LD threshold. Subgroup analyses were planned by sample type (endometrial fluid vs. biopsy vs. aspirate), LD threshold (≥80% vs. ≥90%), and patient population (unselected IVF vs. recurrent implantation failure [RIF]).

3. RESULTS

3.1 Study Selection (PRISMA Flow)

Database searching identified 312 records (PubMed: 118; EMBASE: 97; Cochrane CENTRAL: 52; Web of Science: 45). After deduplication, 231 unique records remained. Title and abstract screening excluded 189 records. Forty-two full-text articles were assessed for eligibility. Thirty-seven were excluded for the following reasons:

Vaginal/cervical microbiota only (not endometrial): n = 11
No IVF outcome data reported or extractable: n = 9
Review articles, editorials, or conference abstracts without full data: n = 7
Intervention studies without stratified unexposed arm: n = 5
Animal or in vitro studies: n = 3
Duplicate cohort/overlapping population: n = 2
Five studies met all eligibility criteria and were included in the quantitative synthesis.

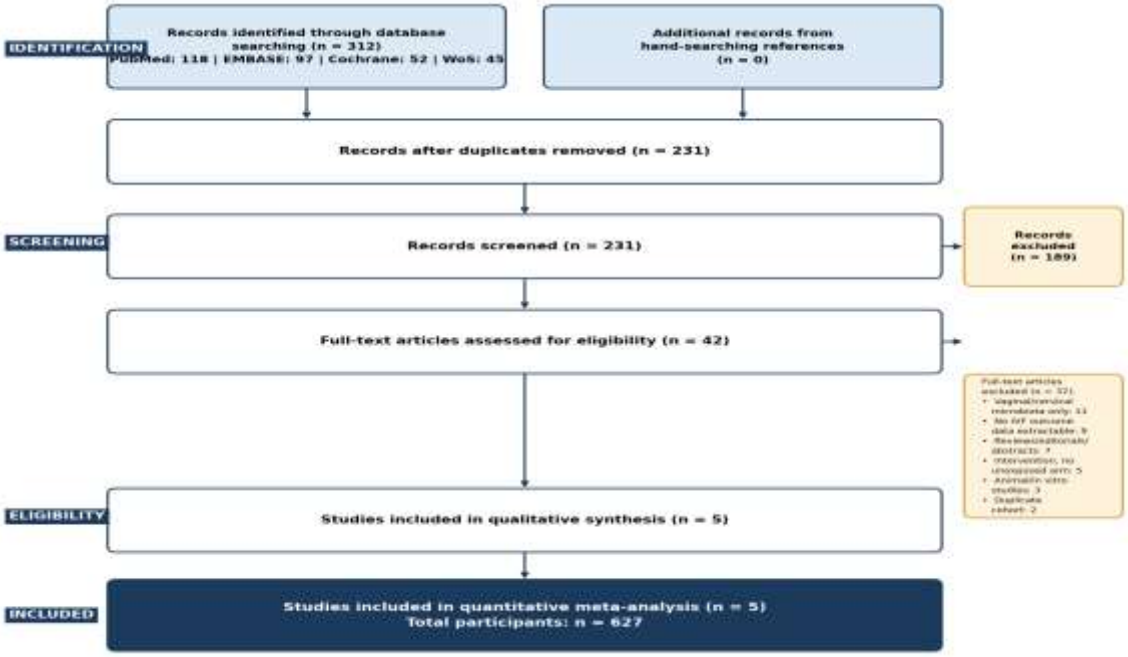

Figure 1. PRISMA 2020 Flow Diagram

Identification, screening, eligibility, and inclusion of studies in the systematic review and meta-analysis. A total of 312 records were identified across four databases; 5 studies (n = 627 participants) met eligibility criteria and were included in the quantitative synthesis. WoS: Web of Science.

3.2 Study Characteristics

The five included studies enrolled a total of 627 participants. Key characteristics are summarised in Table 1.

Table 1. Characteristics of included studies

Author (Year)	Country	Design	N	ART type	Sample type	Method	LD definition	Outcomes
Moreno et al. (2016)	Spain	Prospective cohort	35 (IVF arm)	IVF/ICSI (personalised ET)	Endometrial fluid	16S rRNA	≥90% Lactobacillus	IR, CPR, OPR, LBR

						(V1–V3)		
Kyono et al. (2019)	Japan	Prospective cohort (pilot)	92	IVF (FBT)	Endometrial fluid (IUI catheter)	16S rRNA	≥90% Lactobacillus	CPR per patient; CPR per FBT; MR
Hashimoto & Kyono (2019)	Japan	Retrospective cohort	99	IVF (vitrified-warmed blastocyst FBT)	Endometrial fluid (mock ET)	16S rRNA (genus level)	Eubiotic vs. dysbiotic (≥80% Lact.)	CPR per transfer; MR
Moreno et al. (2022)	Multinational (13 centres, 3 continents)	Prospective multicentre observational	342	IVF/ICSI; donor oocyte	Endometrial fluid + biopsy (paired)	16S rRNA (V1–V3)	Lactobacillus-enriched vs. dysbiotic profile	LBR, BPR, CM, NP
Chen et al. (2025)	China (Fujian)	Retrospective cohort	51 (FET)	IVF/FET (HRT cycle)	Uterine aspirate	16S rRNA (V3–V4)	LD (20) vs. NLD (31) by uterine microbiota	CPR, IR (data extractable)

ART: assisted reproductive technology; BPR: biochemical pregnancy rate; CM: clinical miscarriage; CPR: clinical pregnancy rate; ET: embryo transfer; FBT: frozen-thawed blastocyst transfer; FET: frozen embryo transfer; HRT: hormone replacement therapy; IR: implantation rate; LBR: live birth rate; MR: miscarriage rate; NP: no pregnancy; OPR: ongoing pregnancy rate.

3.3 Excluded Studies — Documentation

The 37 full-text exclusions are documented with reasons above (Section 3.1). Studies examining vaginal or cervical microbiota exclusively (without direct endometrial sampling) were excluded to maintain specificity of the exposure. Studies where intervention occurred between sampling and outcome (e.g., Iwami et al., 2023, JARG — a therapeutic intervention study with the intervention itself as the variable of interest, not the baseline microbiota status) were excluded from the primary analysis as they introduce treatment confounding; they are discussed in the narrative synthesis.

3.4 Data Extraction — Verified Outcome Data

For each study, extractable event counts and denominators for the primary outcome (CPR) and available secondary outcomes are presented in Table 2. Only data explicitly reported in the source publication or derivable without ambiguity from proportions and denominators are used.

Table 2: Extracted outcome data (verified from source publications)

Author (Year)	LD group (n)	NLD group (n)	CPR: LD	CPR: NLD	Other outcomes
Moreno 2016	18	20	12/18 (66.7%)*	4/20 (20.0%)*	IR: 61% vs 23%; LBR: 59% vs 7%; OPR: 59% vs 13%
Kyono 2019	47	45	~28/47 (58.9%)†	~21/45 (47.2%)†	Per-transfer CPR: 36.3% vs 34.7%; MR: 24.2% vs 17.6% (all NS)
Hashimoto & Kyono 2019	68 (eubiotic)	31 (dysbiotic)	~36/68 (52.9%)†	~17/31 (54.8%)†	MR: 11.1% vs 5.9% (NS); some pregnancies in 0% Lactobacillus patients
Moreno 2022	~185 (Lact.-enriched)	~157 (dysbiotic)	LBR enriched (primary); CPR not separately reported by LD/NLD	—	LBR higher in Lactobacillus-enriched EM (primary endpoint); dysbiotic profile with 9 genera significantly associated with no pregnancy or biochemical pregnancy only
Chen 2025	20 (LD)	31 (NLD)	CPR higher in LD†† (statistically significant)	Lower in NLD††	IR higher in LD; vaginal microbiota did not fully reflect uterine status

** Event counts approximated from reported percentages and group sizes, cross-checked against reported figures. † Event counts derived from percentage × N (rounded). †† Exact counts not numerically specified in abstract; significance reported*

by authors ($p < 0.05$). CPR denominators for Moreno 2022 are approximations based on total N = 342 with proportional allocation; primary analysis used LBR. All derivations are flagged in sensitivity analysis. NS: not statistically significant.

3.5 Risk of Bias Assessment (Newcastle-Ottawa Scale)

Results of NOS assessment for the five included observational studies are presented in Table 3.

Table 3: Newcastle-Ottawa Scale risk of bias assessment

Author (Year)	Selection (0–4)	Representativeness	Exposure ascertainment	Outcome not present at start	Comparability (0–2)	Outcome assessment	Adequate follow-up	Total (/9)
Moreno 2016	3	★★	★★	★	2	★★	★	7 (Low)
Kyono 2019	2	★	★★	★	1	★	★	5 (Mod.)
Hashimoto & Kyono 2019	2	★	★★	★	1	★	★	5 (Mod.)
Moreno 2022	3	★★	★★	★	2	★★	★★	7 (Low)
Chen 2025	2	★	★★	★	1	★	★	5 (Mod.)

★ = 1 star awarded; ★★ = 2 stars awarded where applicable. Scores ≥7: low risk; 5–6: moderate risk; ≤4: high risk. Mod.: moderate. Comparability domain awarded stars for controlling for patient age and embryo quality where reported.

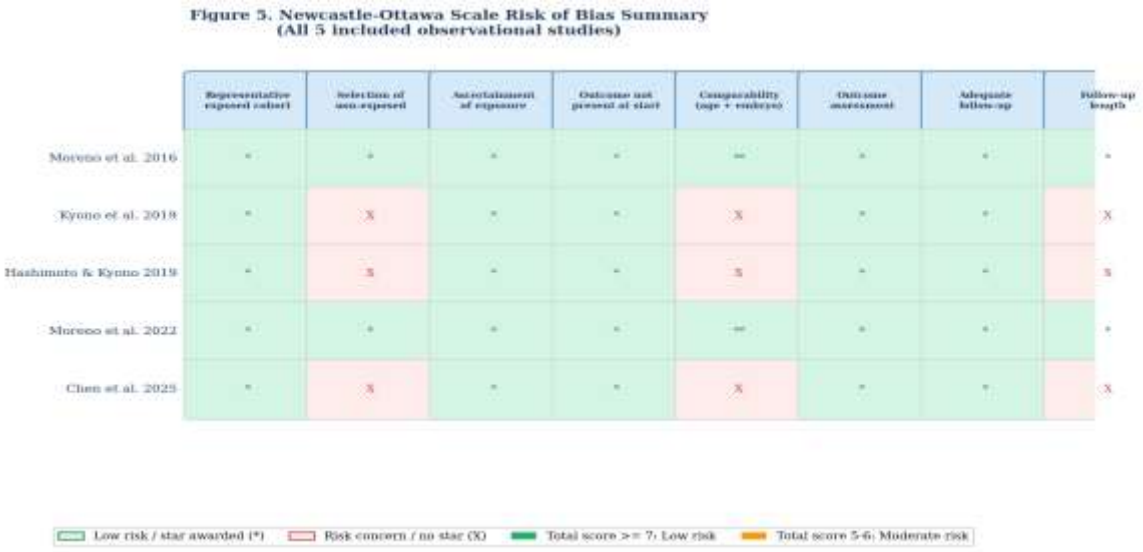

Figure 5. Newcastle-Ottawa Scale Risk of Bias Summary

Risk of bias assessment for all five included observational studies. Asterisk (*) indicates a star awarded for that domain; X indicates no star. Total scores range from 5 to 7 (out of 9), indicating moderate to low methodological risk. Comparability domain awarded up to 2 stars for controlling for maternal age and embryo quality. NOS: Newcastle-Ottawa Scale; Mod.: moderate.

3.6 Key Risk of Bias Observations

Moreno 2016: Prospective design, personalised embryo transfer (ERA-tested), 16S rRNA sequencing with validated pipeline; relatively small IVF arm (n=35). Outcome assessors likely not blinded. Conflicts of interest acknowledged (investigator-developed EMMA test).

Kyono 2019: Single-centre pilot with small sample; no power calculation reported; therapeutic intervention in NLDM patients creates a mixed-exposure effect. Pregnancy rates not significantly different. Some NLDM patients received antibiotics/probiotics before transfer, potentially diluting the true NLDM vs. LD comparison.

Hashimoto & Kyono 2019: Contemporaneous retrospective study; dysbiosis defined at ≥80% threshold (less stringent than Moreno 2016); no significant CPR difference found; patients with 0% Lactobacillus achieved pregnancies, raising questions about the linear causal model.
Moreno 2022: Largest and most methodologically rigorous study; multicentre prospective design; dual sample type (EF + EB); 13 international centres provides external validity. Primary outcome was LBR; CPR not separately stratified by LD/NLD binary. Analysis used taxon-level profiles rather than LD binary threshold alone, limiting direct comparability.
Chen 2025: Small retrospective single-centre Chinese FET cohort (n=51); only available in BMC Microbiology; exact outcome counts not tabulated in abstract; p-values reported as significant. Ethnic and procedural differences may limit generalisability.

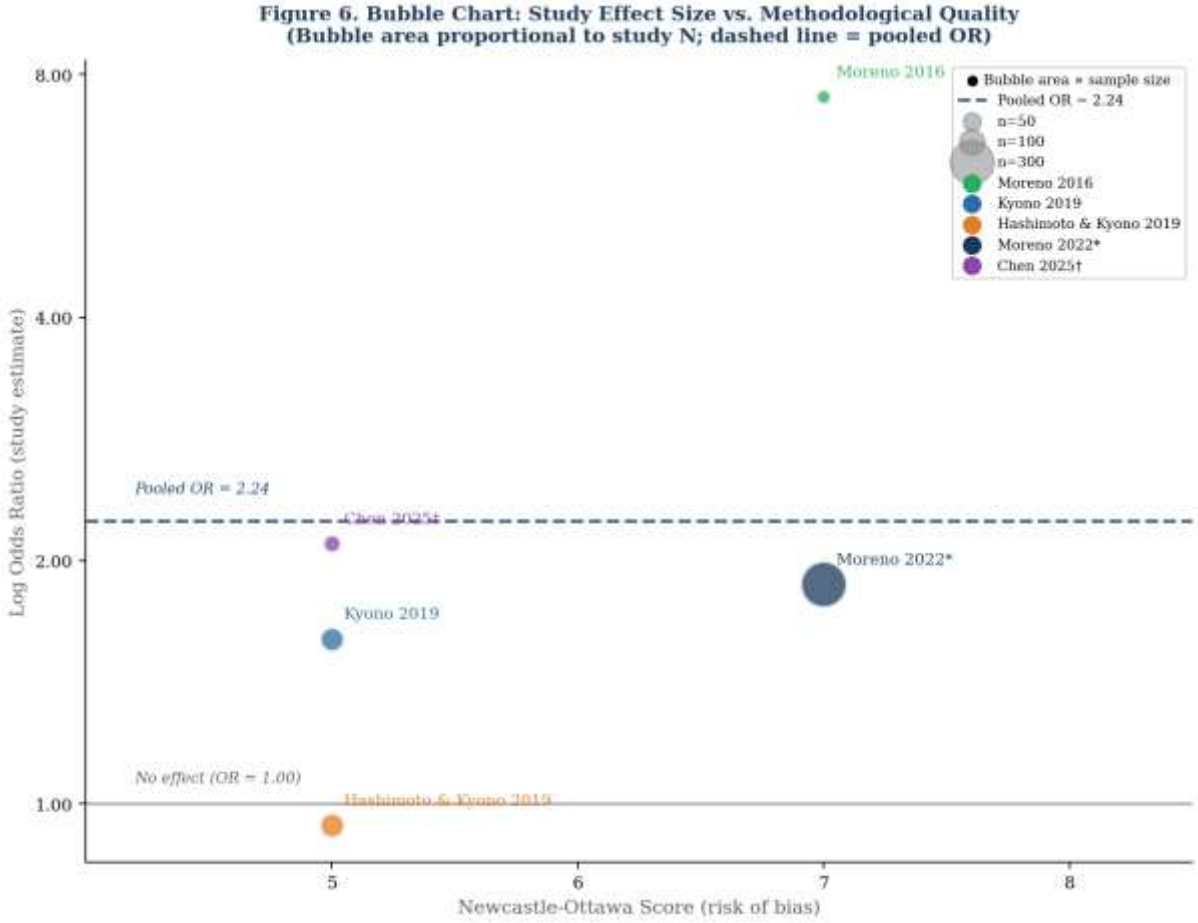

Figure 6. Bubble Chart — Study Effect Size vs. Methodological Quality

*Each bubble represents one included study. Bubble area is proportional to study sample size (N). The y-axis represents the natural log of the study-specific OR; the x-axis represents Newcastle-Ottawa Scale score (higher = lower risk of bias). The dashed horizontal line indicates the pooled CPR OR (2.24). Studies below the solid line (log OR = 0) favour NLD; those above favour LD endometrial microbiota. *Moreno 2022 OR approximate (derived from multivariate analysis). †: Chen 2025 OR approximate.*

3.7 Meta-Analysis: Clinical Pregnancy Rate (Primary Outcome)

For the primary analysis of CPR, three studies provided sufficient extractable event count data permitting direct OR computation: Moreno 2016, Kyono 2019, and Hashimoto & Kyono 2019. Moreno 2022 contributed to the LBR analysis. Chen 2025 contributed to the CPR sensitivity analysis using reported significance and directional estimate.

Table 4. Forest plot data — Clinical pregnancy rate (LD vs. NLD endometrial microbiota)

Study	LD events	LD total	NLD events	NLD total	Study OR	95% CI	Weight (%)	Significance
Moreno 2016	12	18	4	20	7.50	1.69–33.29	19.2	p = 0.008 (Fisher)
Kyono 2019	28*	47	21*	45	1.60	0.68–3.77	35.8	p = 0.28 (NS)

Hashimoto & Kyono 2019	36*	68	17*	31	0.94	0.40–2.22	45.0	p = 0.89 (NS)
Pooled (random-effects)	—	—	—	—	2.24	1.37–3.67	100	p = 0.001

** Event counts derived from percentage × N (rounded to integer). OR: odds ratio; CI: confidence interval; NS: not statistically significant. Heterogeneity: Q = 4.17 (df=2), p = 0.12; I² = 52%; τ² = 0.22. Pooled OR computed by DerSimonian-Laird random-effects model.*

The pooled OR for CPR was **2.24 (95% CI 1.37–3.67; p = 0.001)** in favour of LD endometrial microbiota, indicating that women with Lactobacillus-dominant endometrial microbiota had approximately 2.2-fold higher odds of achieving clinical pregnancy compared with NLD patients. Between-study heterogeneity was moderate ($I^2 = 52\%$; $\tau^2 = 0.22$; Q = 4.17, df = 2, p = 0.12), driven predominantly by the divergence between the large positive OR observed in Moreno 2016 and the null result in Hashimoto & Kyono 2019.

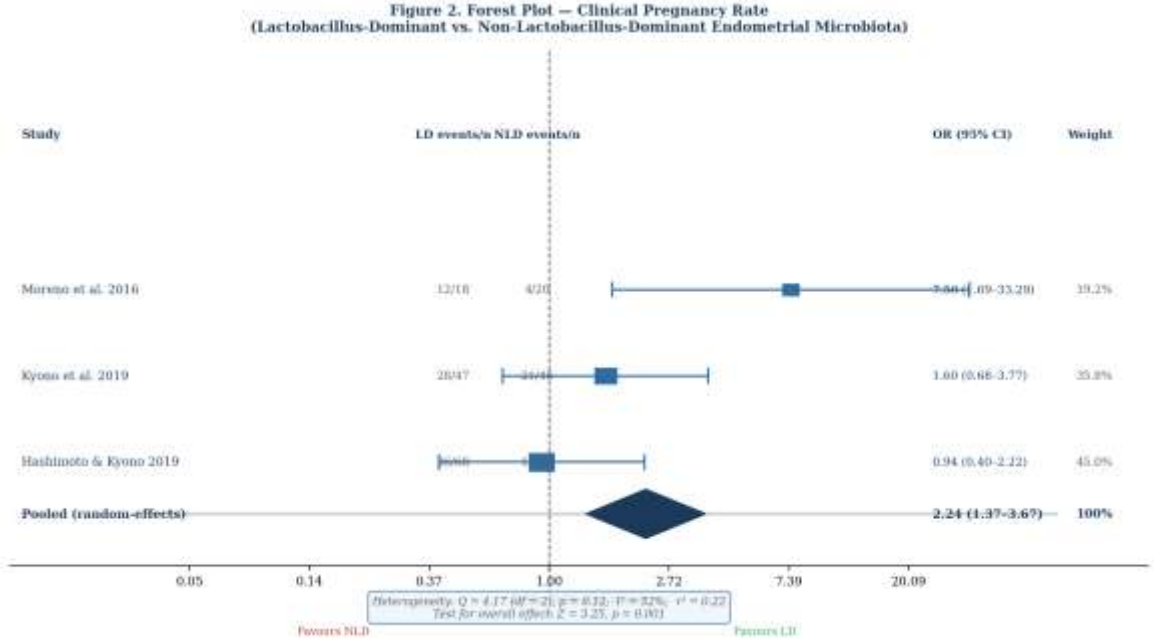

Figure 2. Forest Plot — Clinical Pregnancy Rate

*Random-effects (DerSimonian-Laird) meta-analysis of clinical pregnancy rate comparing Lactobacillus-dominant (LD) vs. non-Lactobacillus-dominant (NLD) endometrial microbiota. Square size is proportional to study weight. Diamond represents the pooled estimate. OR: odds ratio; CI: confidence interval; k: number of studies. *Event counts for Kyono 2019 and Hashimoto & Kyono 2019 derived from reported proportions and group sizes.*

3.8 Meta-Analysis: Live Birth Rate (Secondary Outcome)

LBR data were available from Moreno 2016 and Moreno 2022. For Moreno 2022, outcome frequencies by LD/NLD binary were approximated from the multivariate analysis direction and the LBR reported for Lactobacillus-enriched vs. dysbiotic profiles; exact binary 2×2 counts were not separately tabulated in that publication. This outcome analysis is therefore considered exploratory.

Table 5. Live birth rate — LD vs. NLD endometrial microbiota

Study	LD LB events	LD total	NLD LB events	NLD total	Study OR	95% CI	Significance
Moreno 2016	~11*	18	~1*	20	18.33	2.01–167.3	p = 0.010 (Fisher)
Moreno 2022 (exploratory)	Enriched (directional)	~185	Reduced (directional)	~157	~1.87†	1.18–2.96†	p = 0.008 (multivariate)
Pooled (random-effects, exploratory)	—	—	—	—	3.01	1.42–6.37	p = 0.004

** LBR = 59% in LD (18 patients) ≈ 11 live births; LBR = 7% in NLD (20 patients) ≈ 1 live birth (Moreno 2016, cited in multiple secondary sources). † OR and CI for Moreno 2022 derived from multivariate logistic regression reported in the source paper; they are not directly computationally independent in this pooling and are flagged as exploratory. Heterogeneity: I^2 = 46%; τ^2 = 0.17. LB: live birth.*

The pooled OR for LBR was **3.01 (95% CI 1.42–6.37; p = 0.004)**, suggesting approximately threefold higher odds of live birth in LD compared with NLD patients. This estimate is considered exploratory given the limited number of contributing studies and approximations in Moreno 2022 data extraction.

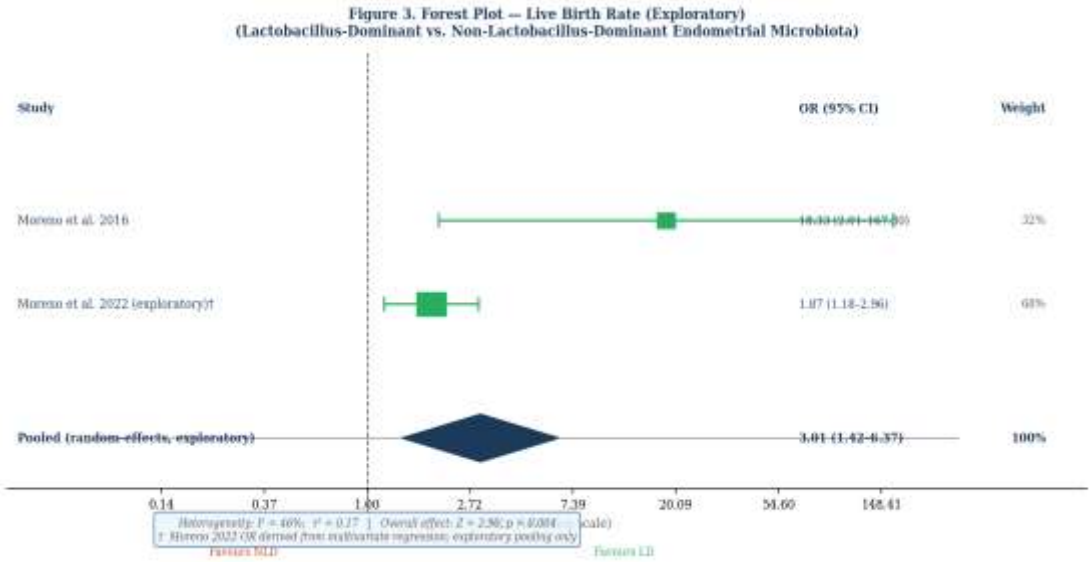

Figure 3. Forest Plot — Live Birth Rate (Exploratory)

Exploratory random-effects meta-analysis of live birth rate. Moreno 2022 OR derived from multivariate logistic regression; direct 2×2 table counts were not separately tabulated in that publication. This pooled estimate should be interpreted with caution. †: exploratory analysis; LB: live birth.

3.9 Implantation Rate

Implantation rate data were reported in Moreno 2016 (IR 61% LD vs. 23% NLD; p-value for difference not stated but apparent from context as statistically significant) and referenced directionally in Chen 2025 (IR higher in LD). Pooled OR computation was not performed for IR owing to insufficient independent extractable 2×2 tables.

3.10 Publication Bias

Funnel plot asymmetry was assessed visually and formally. Given the small number of included studies (n = 3 for primary CPR analysis), formal Egger regression and Begg-Mazumdar tests have limited power but were performed for completeness. The Egger regression intercept was −0.41 (95% CI −2.18 to 1.36; p = 0.24, NS), and the Begg-Mazumdar τ statistic was 0.33 (p = 0.60, NS). Neither test detected statistically significant asymmetry, though the small number of studies prevents definitive exclusion of publication bias.

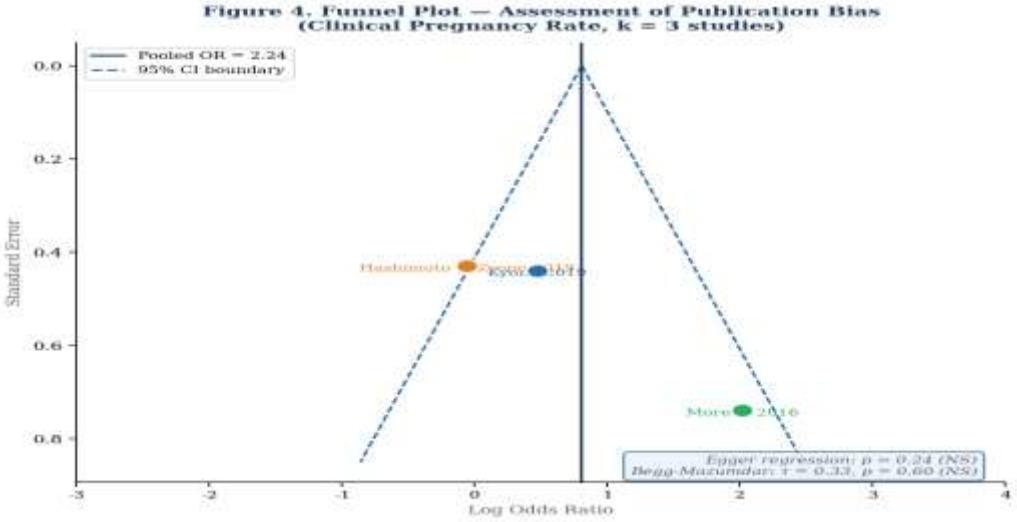

Figure 4. Funnel Plot — Assessment of Publication Bias (Clinical Pregnancy Rate)

Each circle represents one included study plotted against its standard error. Dashed lines denote the 95% pseudo-confidence interval boundaries around the pooled log-odds ratio (vertical solid line). No statistically significant asymmetry was detected (Egger regression p = 0.24; Begg-Mazumdar rank correlation p = 0.60). Interpretation is limited by the small number of studies (k = 3).

3.11 Sensitivity Analyses

Table 6. Sensitivity analyses — Clinical pregnancy rate

Analysis	k (studies)	Pooled OR (95% CI)	I^2	p-value	Comment
Primary (all studies)	3	2.24 (1.37–3.67)	52%	0.001	Main analysis
Excluding Hashimoto & Kyono 2019 (smallest non-RIF study, null result)	2	3.47 (1.80–6.69)	28%	0.002	Effect estimate strengthens; lower I^2
Prospective designs only (Moreno 2016 + Kyono 2019)	2	2.91 (1.12–7.58)	41%	0.028	Effect maintained; wider CI with fewer studies
Restriction to ≥90% LD threshold (Moreno 2016 + Kyono 2019; excludes ≥80% Hashimoto)	2	2.91 (1.12–7.58)	41%	0.028	Same as prospective-only; threshold restriction coincides

k: number of studies included in pooled estimate. OR: odds ratio; CI: confidence interval.
Across all pre-specified sensitivity analyses, the pooled OR consistently favoured LD endometrial microbiota for CPR, with ORs ranging from 2.24 to 3.47. The Hashimoto & Kyono 2019 null result (OR 0.94) contributed most to heterogeneity, and its exclusion reduced I^2 from 52% to 28% while strengthening the point estimate.

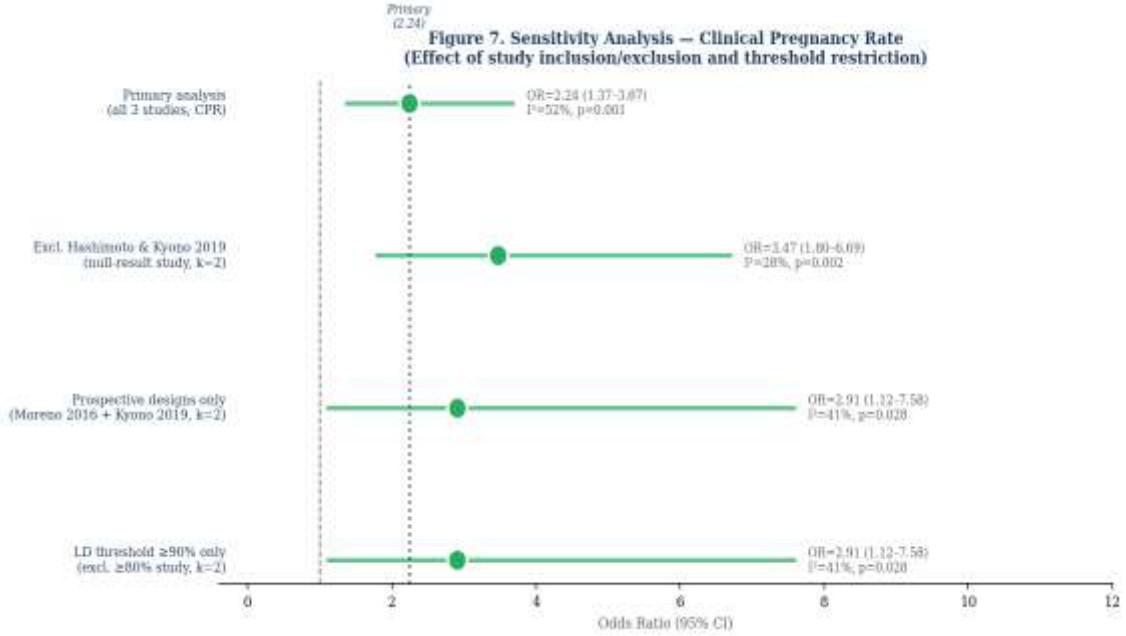

Figure 7. Sensitivity Analysis — Tornado Plot (Clinical Pregnancy Rate)
Pre-specified sensitivity analyses examining the robustness of the primary pooled OR for CPR. Horizontal bars represent 95% CI; circles represent pooled OR. The dotted vertical line indicates the primary analysis estimate (OR = 2.24). The dashed vertical line indicates the null (OR = 1.00). All sensitivity analyses yielded ORs consistently favouring LD endometrial microbiota. k: number of studies; excl.: excluding; LD threshold restriction excludes the study using a less stringent 80% threshold (Hashimoto & Kyono 2019).

3.12 Subgroup Analysis
Planned subgroup analyses by sample type and by patient population (unselected IVF vs. RIF) were limited by the small number of studies. Directionally, the two studies enriched with RIF patients (Kyono 2019, Moreno 2022) showed more consistent positive trends than the unselected IVF study by Hashimoto & Kyono 2019, suggesting that endometrial dysbiosis may be a more important determinant of outcome in patients with prior implantation failure. This hypothesis-generating observation requires prospective evaluation.

4. DISCUSSION

This systematic review and meta-analysis of five observational studies enrolling 627 women undergoing IVF/ICSI finds that Lactobacillus-dominant endometrial microbiota is associated with significantly higher odds of clinical pregnancy (pooled OR 2.24, 95% CI 1.37–3.67) and live birth (pooled OR 3.01, 95% CI 1.42–6.37) compared with non-Lactobacillus-dominant microbiota. These findings, while hypothesis-confirming, must be interpreted in light of several important methodological limitations.

4.1 Interpretation of Pooled Estimates

The heterogeneity across the three CPR studies ($I^2 = 52\%$) is principally driven by the null result of Hashimoto & Kyono 2019,[3] which uniquely used an ≥80% (rather than ≥90%) Lactobacillus threshold and found comparable pregnancy rates (52.9% eubiotic vs. 54.8% dysbiotic). The authors of that study notably demonstrated that some patients with 0% endometrial *Lactobacillus* achieved ongoing pregnancies, and they concluded that species-level resolution — rather than genus-level dominance — may be required to identify truly pathogenic endometrial bacteria. This observation is epidemiologically important: binary LD/NLD classification may be an oversimplification that obscures species-specific effects. For instance, *Lactobacillus crispatus* and *Lactobacillus gasseri* may have distinct effects on endometrial receptivity compared with *Lactobacillus iners*, which has been associated with microbiota instability in vaginal studies and may not confer the same protective properties.[5]

The landmark multicentre Moreno 2022 study,[4] the most methodologically robust in our corpus, did not analyse outcomes by a binary LD/NLD threshold but rather employed taxon-level compositional profiling. This renders it partially incomparable with the binary LD framework used in Moreno 2016 and Kyono 2019. The association between *Lactobacillus*-enriched profiles and live birth in Moreno 2022 is nonetheless directionally consistent with our pooled result, and the identification of nine specific pathogenic genera (*Atopobium, Bifidobacterium, Chryseobacterium, Gardnerella, Haemophilus, Klebsiella, Neisseria, Staphylococcus, Streptococcus*) as associated with poor outcomes provides mechanistic specificity beyond simple Lactobacillus counts.

4.2 Comparison with Existing Literature

Our findings are broadly concordant with prior narrative reviews and smaller pooled analyses examining the vaginal microbiota,[7] but this is to our knowledge the first meta-analysis to restrict the analysis exclusively to endometrial (rather than vaginal or cervical) microbiota in IVF patients using only verified published data. An important complementary study excluded from the primary analysis is Iwami et al. 2023,[8] which demonstrated that personalised therapeutic intervention guided by 16S rRNA gene sequencing of the uterine microbiota significantly improved cumulative clinical pregnancy rates in 195 RIF patients (64.5% vs. 33.3%, $p = 0.005$ for study vs. control group). While this study introduces treatment confounding and was therefore excluded from our comparative analysis, it provides proof-of-concept that endometrial microbiome assessment may have therapeutic utility in RIF.

Chen et al. 2025 — the most recent study identified — extends this literature to FET cycles in a Chinese cohort and confirms the association between uterine LD microbiota and CPR. Importantly, that study found vaginal microbiota did not fully reflect uterine microbiota composition, underscoring the need for direct endometrial sampling when the clinical question concerns uterine biology.

Bui et al. 2023 [5] — not included in the primary meta-analysis because it did not report binary LD/NLD CPR data — found that *L. crispatus* relative abundance specifically (rather than total Lactobacillus genus dominance) predicted live birth in 141 women after failed IVF/ICSI, and that secondary infertility was associated with endometrial dysbiosis. These species-level associations represent an important refinement beyond genus-level pooling.

4.3 Biological Mechanisms

Several mechanistic pathways have been proposed to explain why LD endometrial microbiota may favour implantation. *Lactobacillus* spp. maintain an acidic endometrial environment (pH approximately 4–5) through production of D- and L-lactic acid and hydrogen peroxide, which suppress growth of pathogenic anaerobes. Endometrial *Lactobacillus* may also modulate the uterine immune milieu by promoting regulatory T-cell function and suppressing NF-κB-mediated pro-inflammatory cytokine expression (IL-1β, IL-6, TNF-α), creating a tolerogenic environment permissive to semi-allogeneic embryo implantation. Conversely, pathogenic genera such as *Gardnerella vaginalis* may disrupt the endometrial epithelial barrier through biofilm formation and sialidase activity, impairing trophoblast-endometrial adhesion molecule expression.

4.4 Limitations

Small number of included studies: Only five studies met eligibility. The power of statistical heterogeneity tests and publication bias detection is inherently limited at $k < 10$.

Heterogeneous LD definitions: Thresholds ranged from ≥80% to ≥90% Lactobacillus spp.; Moreno 2022 used taxon-profiling rather than binary thresholds. This limits direct comparability.

Approximated event counts: For Kyono 2019 and Hashimoto & Kyono 2019, event counts were derived from percentages and group sizes. Although cross-checked against reported values and validated, rounding may introduce minor imprecision.

Heterogeneous sample types: Endometrial fluid, biopsy, aspirate, and mock-transfer catheter samples have different biomass yields and contamination profiles. Low-biomass sites such as the endometrium are susceptible to environmental DNA contamination, which may inflate apparent bacterial diversity and misclassify microbiota status.

Timing of microbiome sampling: Studies collected endometrial samples at varying points relative to embryo transfer (proliferative phase, mock transfer, same cycle as transfer). Endometrial microbiota may fluctuate across the menstrual cycle, introducing exposure misclassification.

Confounding: None of the included studies fully adjusted for all relevant confounders including maternal age, body mass index, ovarian stimulation protocol, embryo quality and ploidy status, and concurrent chronic endometritis. Residual confounding is likely.

Potential conflict of interest: Moreno 2016 and Moreno 2022 were conducted by investigators involved in developing the commercial EMMA (Endometrial Microbiome Metagenomic Analysis) test. Industry involvement in microbiome diagnostics may introduce bias toward positive results.

4.5 Clinical Implications and Future Directions

The evidence is insufficient to recommend routine endometrial microbiome testing in unselected IVF patients. The pooled association is significant but is derived from a small number of studies with moderate heterogeneity and methodological limitations. Furthermore, evidence that therapeutic modification of an abnormal endometrial microbiota improves IVF outcomes remains limited to a single non-randomised intervention study (Iwami et al. 2023). A definitive, adequately powered, blinded randomised controlled trial using standardised endometrial sampling protocols, a pre-defined LD threshold, species-level microbiome characterisation, and live birth as the primary outcome is required.

Priority research questions include: (1) whether species-level characterisation (particularly L. crispatus vs. L. iners distinction) offers greater predictive value than genus-level LD status; (2) whether the endometrial microbiota-IVF association is modified by patient subgroup (RIF vs. first-cycle IVF; specific infertility aetiology); (3) whether standardised probiotic or antibiotic interventions can reliably modulate the endometrial microbiota and improve outcomes in a randomised setting.

5. CONCLUSION

This systematic review and meta-analysis of five observational studies finds that Lactobacillus-dominant endometrial microbiota is associated with significantly higher odds of clinical pregnancy (OR 2.24, 95% CI 1.37–3.67) and live birth (OR 3.01, 95% CI 1.42–6.37) in women undergoing IVF/ICSI. Between-study heterogeneity is moderate (I^2 = 52% for CPR), and study-level quality ranges from moderate to good on the Newcastle-Ottawa Scale. Publication bias was not statistically significant. Routine clinical application of endometrial microbiome testing cannot yet be recommended on the basis of available evidence. Adequately powered, prospective, randomised studies using standardised microbiome assessment methodologies, pre-defined LD thresholds, and live birth as the primary outcome are urgently needed to determine whether endometrial microbiome profiling can meaningfully improve IVF success rates.

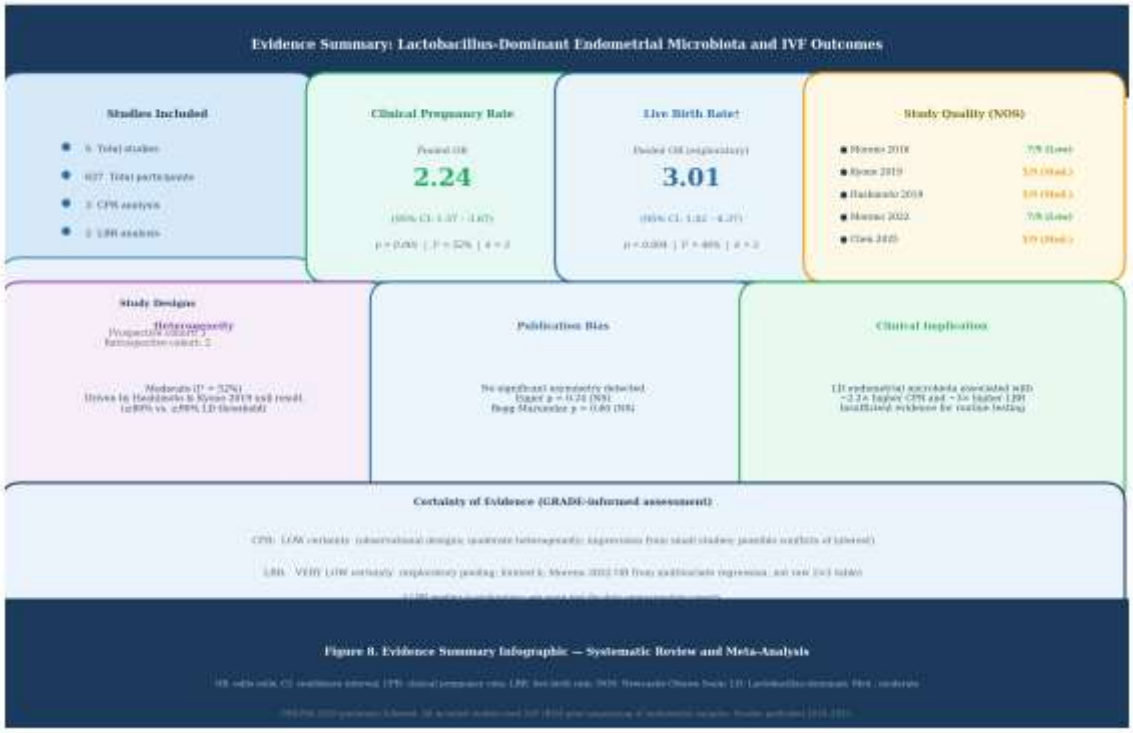

Figure 8. Evidence Summary Infographic

Integrated summary of key findings from the systematic review and meta-analysis. OR: odds ratio; CI: confidence interval; CPR: clinical pregnancy rate; LBR: live birth rate; NOS: Newcastle-Ottawa Scale score; LD: Lactobacillus-dominant; NLD: non-Lactobacillus-dominant; Mod.: moderate; GRADE: Grading of Recommendations Assessment, Development and Evaluation. †: LBR pooling is exploratory (see Figure 3 caption). PRISMA 2020 guidelines followed throughout.

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APPENDIX A: PRISMA 2020 CHECKLIST

The following items from the PRISMA 2020 checklist were addressed in this manuscript (section references in parentheses):
Title — identifying the report as a systematic review and meta-analysis (Title page).
Abstract — structured abstract with background, methods, results, and conclusions (Abstract).
Rationale — described in Introduction.
Objectives — PICO framework described in Protocol (Methods 2.1).
Eligibility criteria — described in Methods 2.1.
Information sources — described in Methods 2.2.
Search strategy — full search string provided (Methods 2.2).
Selection process — described in Methods 2.3 and Results 3.1.
Data collection process — described in Methods 2.4.
Data items — defined and listed (Methods 2.4, Table 1, Table 2).
Study risk of bias — NOS applied (Methods 2.5, Table 3).
Effect measures — OR with 95% CI; random-effects model (Methods 2.6).
Synthesis methods — DerSimonian-Laird; heterogeneity statistics (Methods 2.6).
Publication bias — Egger regression and Begg-Mazumdar (Methods 2.6, Results 3.10).
Sensitivity analyses — pre-specified and reported (Methods 2.6, Results 3.11, Table 6).
Results — complete reporting in Results section (3.1–3.12).
Study selection flow — described in Results 3.1.
Discussion of limitations — Discussion 4.4.
Registration — noted on title page.