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REVIEW

The association between vaginal microbiota and female infertility: a systematic review and meta-analysis

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Abstract

Purpose This study aimed to explore the association between vaginal microbiota and infertility.

Methods We searched a range of electronic databases for appropriate articles, including PubMed, Web of Science, Embase, Chinese National Knowledge Infrastructure (CNKI), and Wanfang, from inception to 8th September 2019. Identified articles were then screened using strict inclusion and exclusion criteria. By referring to Tamarelle's method, we divided vaginal microbiota into two categories: low-*Lactobacillus* vaginal microbiota (LL-VMB) and high-*Lactobacillus* vaginal microbiota (HL-VMB). Patients were defined as HL-VMB if they had a Nugent score of 0–3, a negative Amesel/Spiegel's test, or if the vaginal community status was dominated by either *L. crispatus*, *L. iners*, *L. gasseri* and *L. jensenii* via 16S rRNA sequencing. Otherwise, cases were regarded as LL-VMB. Statistical analyses were performed with STATA 13.0 statistical software. Effect estimates are presented as odds ratios (ORs) with 95% confidence intervals (CIs).

Results Fifteen articles were included in our final analysis. The HL-VMB was negatively related to infertility; a fixed model showed that the pooled OR was 0.83 (95% CI 0.77–0.90). There was no significant publication bias, as determined by Begg's test ($P=0.488$) and Egger's test ($P=0.652$). Using a random effect model, the pooled OR for intermediate bacterial vaginitis (BV) and infertility was 1.39 (95% CI 1.10–1.76) and the pooled OR for positive BV was 1.72 (95% CI 1.10–2.69). Subgroup and sensitivity analyses further demonstrated that the associations identified were stable. However, the acquired evidence was not sufficient to make inferences with regards to the mechanisms underlying these relationships.

Conclusion This systematic review and meta-analysis identified a negative correlation between HL-VMB and female infertility. However, due to a variety of limitations, the evidence acquired does not allow us to identify the specific mechanisms underlying this association. Further high-quality studies are needed to verify the causal relationship and explore the molecular mechanisms involved.

Keywords Infertility · Vaginal microbiota · Vaginal microbiome · Meta-analysis

Abbreviations

BV	Bacterial vaginitis
CI	Confidence interval
CST	Community status type
HL-VMB	High-Lactobacillus vaginal microbiota
LL-VMB	Low-Lactobacillus vaginal microbiota
NGS	Next-generation sequencing
OR	Odds ratio
PCOS	Polycystic ovary syndrome
PID	Pelvic inflammatory disease
STI	Sexually transmitted infections

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Introduction

Although infertility is a non-life-threatening disease, it has serious adverse effects on society, economy, and the mental health of the coupled involved [1]. Infertility is now an

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important public health problem, with a global prevalence of 8–12% among couples of reproductive age [2]. The factors underlying infertility are complex and wide ranging. With the exception of age, which remains the key influential factor responsible for a decline in fecundity in females [3], a wide range of disease-related factors are known to play a crucial role in female infertility. For example, premature ovarian insufficiency, polycystic ovary syndrome (PCOS), and endometriosis are all recognized causes of female infertility [2]. However, approximately 40% of cases cannot be explained by anovulation or tubal pathology; these cases are defined as 'unexplained infertility' [4]. Consequently, there is an urgent need for further research in order to increase our understanding of female infertility.

Over the last few decades, a number of researchers have focused on infections of the genital tract caused by pathogenic bacteria, particularly *Chlamydia trachomatis* [5] and *Neisseria gonorrhea*; [6] these pathogens are widely regarded as major factors responsible for tubal infertility. With the development of high-throughput next-generation sequencing (NGS) technology, the function of many bacteria that are considered "normal" within the vagina have been redefined. As a result of this work, scientists have developed concern not only with regards to potentially 'harmful' bacteria, but also in terms of changes in the entire structure of the vaginal microbiota [7]. Females express the desire for their vaginal microbiota to remain normal; however, the methodology used to assess vaginal health has changed regularly in line with technological developments. In 1983, Spiegel [8] reported that a direct gram staining technique could be used to identify bacterial vaginosis. This staining technique was subsequently improved by Nugent in 1991. The Nugent score has since become the widely used method to quantify the number of *Lactobacillus*, *Gardnerella vaginalis*, and curved Gram-negative rods, within Gram-stained vaginal smear [9]. Using this classification system, a score of 0–3 corresponds to a normal vaginal microbiota (high *Lactobacillus* morphotypes).Meanwhile, Amsel's criteria, a method that can be used to diagnose bacterial vaginitis (BV); this system is now commonly used in the clinic [10]. In 2015, a meta-analysis [11] reported that the widespread use of Amsel's criteria showed that BV was significantly more prevalent in infertile women compared with antenatal women, especially in women with tubal infertility. However, only two articles were included in this previous meta-analysis; thus, publication bias cannot be ruled out.

All available methods support the fact that under normal circumstances, the vaginal microflora is dominated by *Lactobacillus*. However, many studies have reported that not all types of *Lactobacillus* are beneficial bacteria. For example, *L. crispatus* appeared to have beneficial properties, while *L. iners* did not [12]. Using NGS, researchers were able to successfully classify the vaginal microbiota into five community status types (CST): CST I to V is dominated by *L. crispatus*, *L. gasseri*, *L. iners*, highly diverse, and *L. jensenii*, respectively [13]. Previous studies also showed that *L. iners*, *L. crispatus*, and *L. gasseri* can distinguish idiopathic infertile women from healthy women or those with vaginosis [14]. This work provided a new perspective in that the maintenance of a healthy/normal vaginal microbiota is more efficient than preventing BV if we are aiming to increase female fertility.

Thus far, 16S rRNA sequencing has rarely been used to explore the association between the normal vaginal microbiota and infertility. In this review, we still divided the vaginal microbiota into two categories: low-Lactobacillus vaginal microbiota (LL-VMB) and high-Lactobacillus vaginal microbiota (HL-VMB), in accordance with Tamarelle's methodology [15]. The objective of our study was to evaluate the statistical association between female infertility and vaginal microbiota. Furthermore, we propose that advanced technologies should be used to realign our understanding of how the vaginal microbiota is related to fertility and thus allow the development of methods to help improve fertility.

Materials and methods

This meta-analysis was conducted in accordance with the PRISMA statement [16].

Search strategy

We searched a range of electronic databases, including PubMed, Web of Science, Embase, Chinese National Knowledge Infrastructure (CNKI), and Wanfang, from inception to 8th September 2019. The search terms used were as follows: [(infertility OR sterility OR subfertility) AND (*genital OR vagin*) AND (microbiota OR microbiota OR vaginosis OR lactobacilli)]. The citation lists of any identified publications were also searched by hand to identify any additional references. Our searches did not involve any restrictions related to language or country of origin.

Inclusion and exclusion criteria

Titles and abstracts were first reviewed by two of the authors (HX and MJ) and only relevant publications were selected for full review. Studies in this meta-analysis were required to meet the following inclusion criteria: (1) observational studies that addressed infertile women and fertile controls; (2) the vaginal microbiota status was characterized by 16S rRNA gene amplicon sequencing, Nugent score, Amsel's criteria, or Spiegel's criteria; (3) human studies; and (4) original studies that provided clear data relating to vaginal microbiota status.

Articles were excluded if they were: (1) comments, reviews or conference abstracts; (2) repetitive studies; (3) animal studies; (4) devoid of a control group; (5) were linked to a control group that was not made up of fertile women; (6) the status of the vaginal microbiota was based on BV diagnosis records without any specific methodology; (7) not written in English or Chinese; (8) if they were studies related to clinical intervention.

Data extraction

Two investigators (HX and YJ) independently extracted a range of data, including date of publication, authors, study design, study population, sample size, methods used for microbiota characterization/diagnosis, and the specific type of infertility. Any disagreements were resolved through discussion with a third reviewer (WB). By referring to Tamarelle's method [15], we divided the vaginal microbiota into two categories: low-*Lactobacillus* vaginal microbiota (LL-VMB) and high-*Lactobacillus* vaginal microbiota (HL-VMB). Patients were defined as HL-VMB if they had a Nugent score of 0–3, a negative Amesel/Spiegel's test, or if the vaginal community status was dominated by either *L. crispatus*, *L. iners*, *L. gasseri* and *L. jensenii* via 16S rRNA sequencing. Otherwise, cases were regarded as LL-VMB.

Quality assessment

Two authors (HX and YJ) evaluated the quality of the studies included in our analyses based on the Newcastle–Ottawa Scale (NOS) (see Supplementary Table S1). The NOS considers three critical aspects: selection, comparability, and exposure. Two investigators scored the studies independently and any discrepancies between reviewers were resolved by reaching a consensus or by a third reviewer (WB).

Statistical analysis

Associations between the vaginal microbiota and infertility were estimated by pooled odds ratios (ORs) and 95% confidence intervals (Cis) using the Mantel–Haenszel method. Heterogeneity between studies was tested using the Cochran's Q two-sided homogeneity test [17]; the I^2 statistic was also used as a critical factor to determine which model should be used to pool the effect size (if $I^2 < 50\%$, then a fixed model was used; otherwise, a random model was used). Publication bias was then evaluated by Begg's funnel plots and Egger's regression test, which measures the degree of funnel plot asymmetry. Subgroup analyses were performed based on the language used to write the original papers, the different methods used to diagnose vaginal microbiota, different types of infertility, and fertile control groups, to reduce heterogeneity. Several sensitivity analyses were performed to evaluate the robustness of our results by excluding some articles that were considered to be low quality. All analyses were performed using STATA (version 13.1, StataCorp, College Station, TX) and the '*metan*' command was used to estimate the ORs. A two-sided $P \leq 0.05$ was considered to be statistically significant.

Results

Study selection and characteristics

In total, we screened 1290 records for eligibility; 38 of these articles were selected for full-text review. However, 4 of these articles were not written in English or Chinese, 13 studies only focused on infertile women and did not feature controls, and 6 articles did not provide specific data, such as the frequencies of women with/without normal vaginal microbiota, or defined diagnostic methods for vaginal disorders. Thus, 15 studies met our inclusion criteria for meta-analysis [18–32] (Fig. 1).

Of the 15 articles that met our inclusion criteria; 10 were written in English and 5 were written in Chinese. Collectively, these articles reported 3277 cases and 4524 controls. With the exception of Adamson et.al. [20], all other studies involved study designs that were based on two parallel groups (infertile and fertile women). Because infertility status and vaginal microbiota data were collected at the same time, it was hard to infer a causal correlation between these factors. Hence, we considered these articles as cross-sectional studies, rather than case–control studies, despite of their original definition. Most of the studies ($n = 9$) focused on women who were diagnosed with infertility and seeking clinical treatment; in these studies, the specific type of infertility was ignored. Two studies related specifically to primary infertility [20, 27], while four studies referred specifically to tubal infertility [22, 24, 30, 31]. We also found that the control (fertile) groups were quite diverse. For example, some papers used women with a history of pregnancy ($n = 6$), antenatal women ($n = 2$), and even healthy women but without a detailed history of pregnancy ($n = 7$). Most researchers used the Nugent method ($n = 10$) to diagnose the vaginal microbiota; only two studies were based on 16S sequencing data. The others were based on Amsel or Spiegel's criteria. Further details of the included studies are shown in Table 1.

The overall association between high-*Lactobacillus* vaginal microbiota and infertility

Measures of association between HL-VMB and infertility in the selected studies ranged from 0.64 (95% CI 0.54–0.78) to 1.01 (95% CI 0.70–1.47). The pooled OR was 0.83 (95%

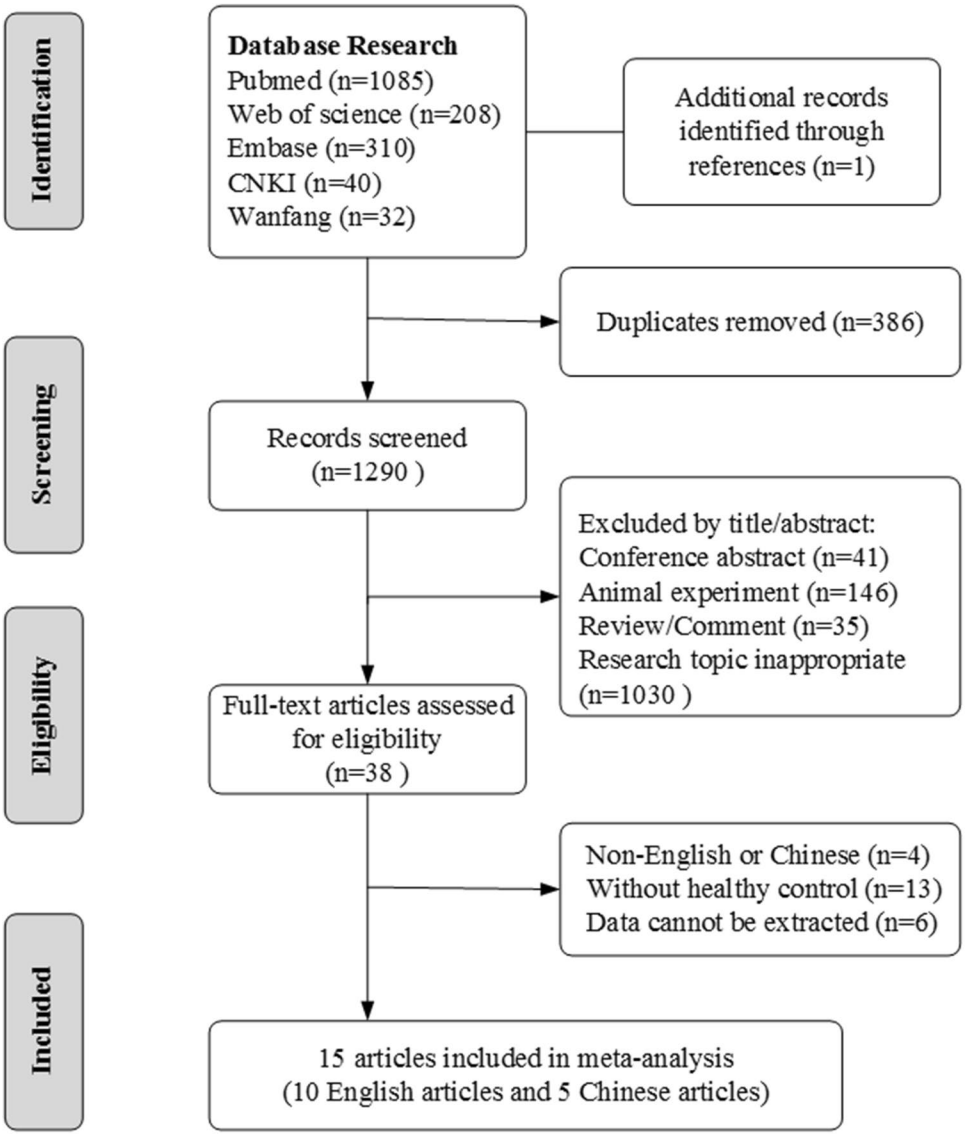


Fig. 1 A summary of the strategies used for literature searches and selection

CI 0.77–0.90) when determined by a fixed model ($I^2 = 0.0\%$) (Fig. 2), thus implying that HL-VMB was inversely related to infertility. Publication bias was not statistically significant, as determined by Begg's test ($P = 0.488$) and Egger's test ($P = 0.652$) (Fig. 3).

The association between the extent of BV and infertility

Six articles [20, 21, 25, 26, 30, 31] provided specific raw data relating to intermediate BV (a Nugent score of 4–6) and a positive BV (a Nugent score of 7–10). Using a random effect models, we found that the pooled OR for intermediate BV was 1.39 (95% CI 1.10–1.76; $I^2 = 0.0\%$) and that the pooled OR for positive BV was 1.72 (95% CI 1.10–2.69; $I^2 = 64.6\%$). Our data showed that the BV was positively associated with female infertility and that the effect was greater for those with positive BV than those with intermediate BV (Fig. 4).

Sensitivity analysis

Data arising from the subgroup analyses are shown in Table 2 and Figures S1–S5. All these analyses were performed using fixed models. The pooled OR for the five articles that focused on Chinese women was 0.90 (95% CI 0.80–1.00; that for the three articles that focused on Indian women was 0.87 (95% CI 0.70–1.08). HL-VMB, as diagnosed by Nugent score, was negatively associated with infertility (OR 0.89, 95% CI 0.81–0.97); however, when diagnosed by 16S rRNA sequencing, there was no statistical significance (OR 0.74, 95% CI 0.33–1.65). Although different types of infertility all showed negative relationships with HL-VMB, none of these were

Table 1 Characteristic of the included studies

First author	Year	Country	Study design	Type of infertility (A/B/C/D)[a]	Type of control group (E/F/G/H)[b]	Diagnosis method	Sample size (case/control)	NOS
Morgan DJ	1997	UK	Cross-section	A	E	Nugent	199/1379	5
Mania-Pramanik J	2009	India	Cross-section	A	G(E)	Nugent	112/81	7
Adamson PC	2011	India	Nest case–control	B	F	Nugent	113/784	9
Xu Y	2011	China	Cross-section	A	G	Nugent	300/389	4
Salah RM	2013	Egypt	Cross-section	A (D)	G	Spiegel	874/382	5
Tomusiak A	2013	Poland	Cross-section	A	F	Nugent	101/60	7
Durugbo	2015	Nigeria	Cross-section	D	F	Amsel	178/178	8
Qin XM	2016	China	Cross-section	A	G	Nugent	150/100	7
Zheng Y	2016	China	Cross-section	A	G	Nugent	560/560	6
Babu G	2017	India	Cross-section	A (B + C)	G	Nugent	116/84	7
Yang LL	2018	China	Cross-section	D	H	Nugent	126/120	5
Kyono K	2018	Japan	Cross-section	A	G	Sequencing	102/7	6
Wee BA	2018	Australia	Cross-section	A	G	Sequencing	15/16	7
Liu Y	2019	China	Cross-section	A (D)	F	Nugent	220/280	7
Moragianni D	2019	Greece	Cross-section	A	F	Nugent + Amsel	111/104	8

NOS Newcastle–Ottawa Scale for article quality assessment

[a]A: Infertility for clinical treatment. B: Primary infertility. C: Secondary infertility. D: Tubal infertility. The type in the brackets means the data of this subtype can be extracted

[b]E: Antenatal women; F: The women with pregnancy history; G: Healthy women without infertility history and they were not pregnant when enrolled. But the pregnancy history is unknown; H: Other patients without tubal infertility

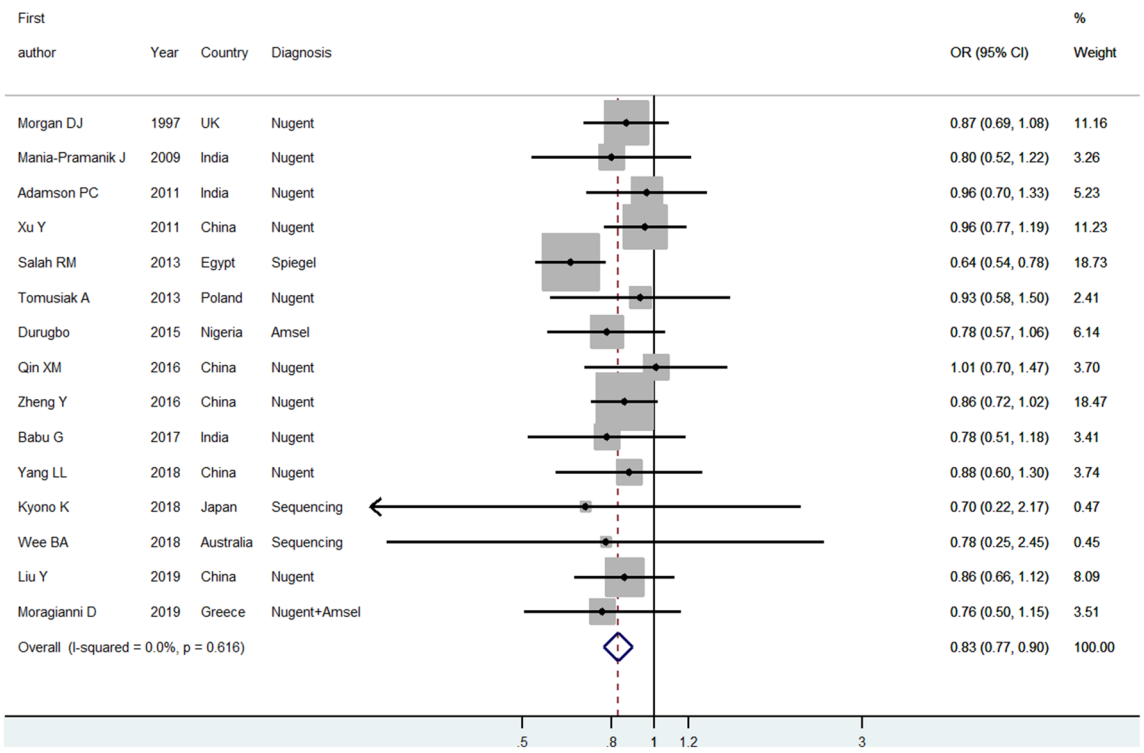
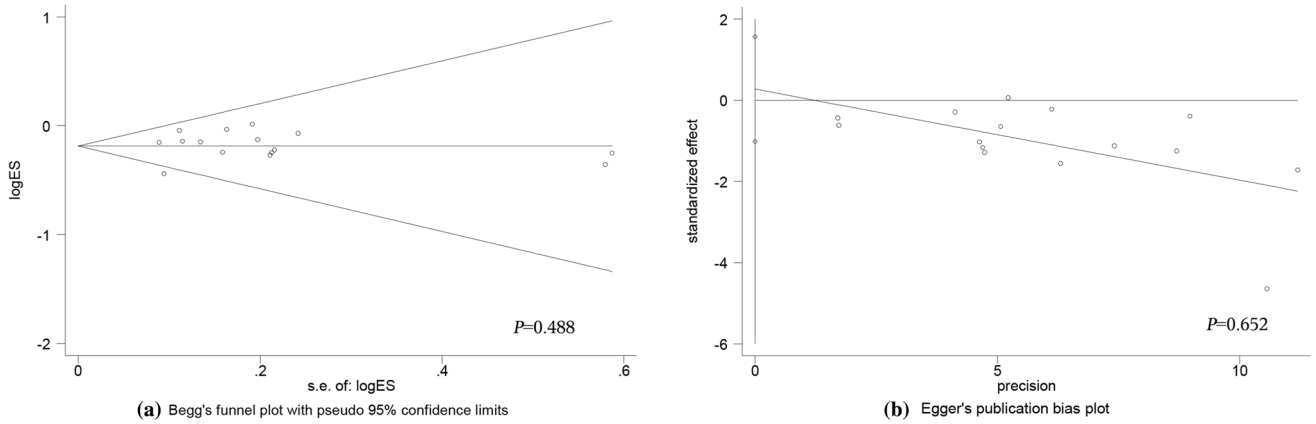
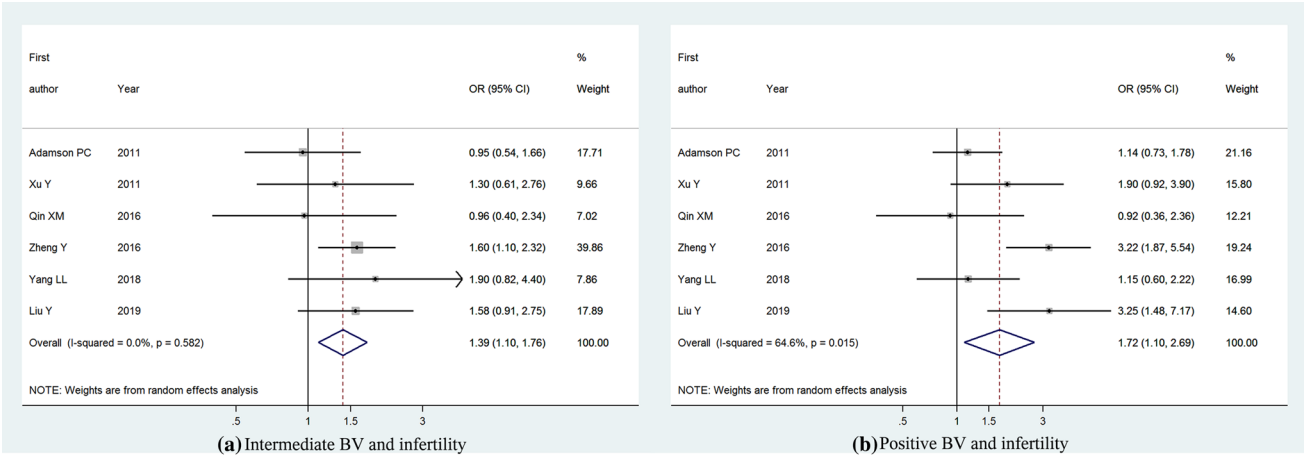


Fig. 2 Forest plots showing the association between high-*Lactobacillus* vaginal microbiota and infertility. *OR* odds ratio



(a) Begg's funnel plot with pseudo 95% confidence limits **(b)** Egger's publication bias plot

Fig. 3 Publication bias plot based on **a** Begg's test and **b** Egger's test



(a) Intermediate BV and infertility **(b)** Positive BV and infertility

Fig. 4 Forest plots showing the association between infertility and **a** intermediate bacterial vaginitis and **b** positive vaginitis

statistically significant. When compared to females with a specific history of pregnancy, or healthy fertile women, the proportion of those with HL-VMB was lower among infertile women (OR 0.85, 95% CI 0.74–0.99 and OR 0.81, 95% CI 0.73–0.89).

To confirm that our results were robust, several other sensitivity analyses were conducted. First, we analyzed the entire dataset using a random model; the results remained the same (OR 0.83, 95% CI 0.77–0.89). We noted that the original data in one article [22] showed a mistake in logic, in which the total number of patients with bacterial vaginosis was not consistent with the total numbers of patients in different subclasses of infertility (including PCOS, unexplained infertility, tubal infertility, and infertility with endometriosis). Consequently, we removed this article from our analysis and carried out sensitivity analysis again. The pooled OR was 0.873 (95% CI 0.803–0.948; $I^2 = 0.0\%$). We also tried excluding 4 articles with a NOS score < 6 [18, 21, 22, 30]; this analysis showed that the pooled OR still showed a stable association (OR 0.857, 95% CI 0.774–0.948; $I^2 = 0.0\%$).

Discussion

Disturbances in the composition of human bacterial communities have been shown to contribute to a range of disease states, and there is a growing body of evidence to suggest that the vaginal microbiota, which is unique to each female, plays an important role in determining many aspects of reproductive health [33]. Many studies have suggested that infections of the reproductive tract, or BV, represent the most serious risk factors for infertility, particularly tubal infertility [34]. Our meta-analysis also provides some intermediate evidence to suggest that HL-VMB might be negatively associated with female infertility by integrating data arising from 15 related articles and 7801 individuals. To our knowledge,

Table 2 Subgroup analysis for the association between high-Lactobacillus vaginal microbiota and infertility

Subgroup	Articles	Pooled OR	95% CI	I^2 (%)
Country				
India	3	0.87	0.70–1.08	0.0
China	5	0.90	0.80–1.00	0.0
Others	7	0.75	0.67–0.84	0.0
Diagnosis method				
Nugent	10	0.89	0.81–0.97	0.0
Sequencing	2	0.74	0.33–1.65	0.0
Others[a]	3	0.69	0.59–0.80	0.0
Type of infertility				
Primary infertility	2	0.88	0.68–1.14	0.0
Secondary infertility	1	0.97	0.48–1.97	–
Tube infertility	4	0.84	0.71–1.00	0.0
Type of control women				
The women with pregnancy history	6	0.85	0.74–0.99	0.0
Antenatal women	2	0.85	0.70–1.03	0.0
Healthy women[b]	7	0.81	0.73–0.89	39.7

[a]Including the methods of Spiegel, Amsel or Nugent + Amsel

[b]Healthy women without infertility history and they were not pregnant when enrolled. But the pregnancy history is unknown

this is the first study to systematically evaluate the statistical association between vaginal microbiota and infertility.

It is relatively clear that some sexually transmitted infections (STIs) can cause infertility. *C. trachomatis* may synthesize a large amount of heat shock protein (hsp60) and thus induce a pro-inflammatory immune response in the epithelia of the human fallopian tube, thus resulting in scarring and tubal occlusion [35]. *N. gonorrhoeae* may attack the epithelial cells of the fallopian tubes, thus exerting impact on ovum transportation and fertilization [6]. However, we still do not know exactly how LL-VMB or BV exerts influence on the process of fertilization. By reviewing the literature, we have identified three possible pathways. The first is the chronic inflammation hypothesis. While there is a close association between pelvic inflammatory disease (PID) and BV, the causal mechanisms underlying this association remain unclear [36]. A proportion of female infertility is known to be attributable to subclinical PID [37]. Furthermore, BV is often accompanied by a rise in pH, mucosal cell damage, and a local inflammatory response. Although vaginal inflammation would not affect the ovum directly, it is still possible that the microbiota might play a role. A recent study also suggested that there is a microbiota continuum that exists along the female reproductive tract, including the cervical canal, uterus, fallopian tubes, and peritoneal fluid [38]. Because some PIDs are chronic, and without clinical symptoms, many females only realize these issues following a diagnosis of infertility. The second hypothesis refers to susceptibility to sexually transmitted infections (STIs). A recent meta-analysis [15] provided evidence for a protective role for HL-VMB against HPV and *C. trachomatis*. Furthermore, many studies have shown that BV is a risk factor for the acquisition of STI/HIV[39] The third possibility refers to non-causal association. PCOS is a very common cause of female infertility and represents a complex of endocrine diseases characterized by hyperandrogenism, oligo-/anovulation, and ovarian cysts [40]. Changes in estrogen or the hypothalamic–pituitary–ovarian axis are known to be related to PCOS [40] and the vaginal microbiota [41], although the mechanisms involved remain unclear. There is some evidence to suggest that the gut microbiota is associated with PCOS [42], although whether the vaginal microbiota exhibits a similar association remains unknown. In addition, hyperglycemia has been proven to be associated with a reduction in female fecundity [43]. Vulvovaginitis is also known to be more prevalent among patients with diabetes [44]. Further studies are now needed to systematically explore the causal associations between vaginal microbiota, infertility, and other confounding/mediating factors.

It is a pity that only one article we included in our current analysis was a cohort study; most studies were cross-sectional. It was evident that vaginal samples and information relating to fertility status were collected during the same period of time; as a consequence, it is difficult to identify any specific causal mechanisms. The greatest difficulty related to the confirmation of the specific time of onset for infertility. Because infertility is diagnosed by clinical pregnancy outcome (the inability to achieve a clinically recognized pregnancy after regular unprotected sexual intercourse for more than a year [45]), when a female is diagnosed as being infertile, it is difficult to identify what happened previously in the vaginal microbiota, because no previous samples were collected. If these females were not attempting to get pregnant, there would be no way of knowing whether they were fertile or infertile. The best way to overcome this difficulty is by creating a pre-pregnancy cohort, as described previously by Adamson [20] and Hong [46]. In such cohorts, every couple is attempting to get pregnant; consequently, some very useful information can be collected. After 1 year, couples who have not achieved pregnancy would be diagnosed with clinical infertility. Then, it is possible to carry out a nested case–control study; such analysis can provide strong evidence to support specific causal associations [47].

Female infertility can be classified into different types according to different criteria, in which the associations with vaginal microbiota might vary. From etiological point-of-view, tubal infertility is the most common disease associated with vaginal microbiota [24, 34]. It is also possible that a chronic inflammatory response caused by BV might account for tubal adhesion, at least in part [34]. Our subgroup analysis also identified a negative association between HL-VMB

and tubal infertility. However, there was only limited information relating for associations with ovulation failure and unexplained infertility. In addition, pregnancy histories allow us to classify infertility as either primary or secondary infertility. The etiology for these two types of infertility might vary [48]. It is also possible that the vaginal microbiota might play different roles in these two different forms of infertility. Thus, it is not appropriate that most studies only included infertile patients who were seeking medical treatment, but without dividing these cases into separate subgroups; this represented the main source of heterogeneity in our present study.

Another factor responsible for heterogeneity might relate to the different standards used to define the control groups. It is very challenging to fully define a fertile women. Two of the included studies [18, 19] used antenatal women as a control group, although it was clear that there was a dynamic change of the vaginal microbiota in pregnant women. MacIntyre [49] pointed out that the composition of the vaginal microbiota changed dramatically postpartum to become less dominated by *Lactobacillus* spp. dominant with increased levels of alpha-diversity. Some studies included a control group that featured women with a history of pregnancy. However, this practice cannot avoid the impact of secondary infertility. In a narrow sense, the "healthy women" who took part in the physical examinations could not be regarded as fertile women, because they would not know if they were fertile or not without attempting to get pregnant. It is recommended that future studies should clearly define both the infertile and fertile groupings and thus improve comparability between the two groups.

NGS has been used extensively to investigate the gut microbiota. While NGS has yet to be deployed extensively to study the vaginal flora, this technology could be very useful. Although we have developed many antibiotics to fight vaginal infection, we still know very little with regards to maintain the vaginal microbiota in an optimum condition. In a previous paper, Campisciano [14] explored the vaginal microbiota of infertile women using NGS, and found that there was an alteration in cases of idiopathic infertility that was caused by a reduction in *L. crispatus* and *L. iners*, and an increase in *L. gasseri*. However, Verstraelen [50] reported that the relative roles of *L. crispatus* and *L. iners* in the vaginal microbiota were different; *L. crispatus* might promote stability of the normal vaginal microflora while *L. gasseri* and/or *L. iners* might predispose women to the occurrence of abnormal vaginal microflora, at least to some extent. The majority of the studies we included in our present analysis used microscopy to detect *Lactobacilli* and could not, therefore, distinguish between different species. However, the sample size used in the sequencing-based study was relatively small. If we used our standard system for the classification of vaginal microbiota (HL/LL-VMB), then specific data could not be extracted easily and the statistical power would be low [28, 29]. The division of vaginal flora into two broad groups might not be appropriate for the vaginal flora, given its diversity. Some researchers even suggested that the stability of the vaginal microbiota is usually not expressed in terms of changes in the composition of taxa. In terms of CST consistency [51], it might be possible for us to make dynamic observations of at least one complete cycle in infertile women in future. Although the Nugent-Score is still the widely accepted standard with which to diagnose BV and assess the vaginal environment, this system can be significantly influenced by the subjective variability of the observer [7]. We, therefore, recommend that advanced genetic techniques should be deployed in future studies of the microbiota.

It is evident that our meta-analysis may be limited by the cross-sectional study design adopted by the original articles, some degree of heterogeneity from the use of differing definitions of cases/controls, and the different methods used to investigate the microbiota. Furthermore, none of the included studies used strict matching methods, or adjusted for key confounding factors, when comparing the composition of the vaginal microbiota. Consequently, it is likely that these factors affected the comparability of these groups. In addition, the results derived from microbiota testing are known to be significantly influenced by ethnicity, [51], although most of the patients involved in the studies included in our meta-analysis were of Asian origin; these factors may affect our ability to interpret our results. Furthermore, some original articles were written in Chinese and are, therefore, not globally accessible.

Overall, our results suggested that a healthy vaginal microbiota might be associated with a lower risk of infertility and that the development of molecular biological techniques could help us to understand the vaginal microbiota better. The current evidence is clearly inadequate due to the existence of a wide range of limitations. There is a clear need for more high-quality studies to be carried out so that we can identify the mechanisms underlying the relationships described in this paper.

Author contributions Literature search and screening: XH, JM, JY and BW; data extraction: XH, JY, JG and BW; data analysis: XH, HZ, MZ and XZ; results visualization: XH, MY and YX; manuscript draft and modification: XH, S.F, JM and BW; funding acquirement: BW. All authors reviewed the final version of the manuscript and approve it for publication.

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Compliance with ethical standards

Conflict of interest We declare that we have no conflict of interest.

Data sharing statement More original data can be obtained from Prof. Wang: wangbeilxb@163.com.

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