REVIEW

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Vaginal dysbiosis – the association with reproductive outcomes in IVF patients: a systematic review and meta-analysis

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Purpose of review
To examine impact of vaginal dysbiosis (VD), including bacterial vaginosis (BV) and aerobic vaginitis (AV) on reproductive outcomes of in vitro fertilization (IVF) patients.

Recent findings
BV-bacteria (e.g. *Gardnerella*) and AV-bacteria (e.g. *Streptococci* and *Enterococci*) have been identified in the endometrium. However, there is inconclusive evidence whether IVF patients with VD have lower success rates.

Summary
The present systematic review and meta-analysis of PubMed/Medline, until December 2023 included 25 studies, involving 6835 IVF patients. Overall VD was defined as an approximation of community state type IV, including BV and AV-type dysbiosis based on either molecular or microscopy methods. Outcomes were live birth rate (LBR), early pregnancy loss (EPL), clinical pregnancy rate (CPR), and biochemical pregnancy rate (BPR).
Vaginal dysbiosis prevalence was 19% [1271/6835, 95% confidence interval (CI) 18–20%]. Six studies examined AV-type dysbiosis with a prevalence of 4% (26/628, 95% CI 3–6%). Vaginal dysbiosis correlates with a higher EPL [relative risk (RR) = 1.49, 95% CI 1.15–1.94] and lower CPR (RR = 0.82, 95% CI 0.70–0.95). No statistically significant impact of VD, BV, or AV was found on LBR and BPR.
Thus, the association between VD and reproductive outcome remains puzzling as it is difficult to explain how VD impacts CPR and EPL but not LBR and BPR.

Keywords
aerobic vaginitis, bacterial vaginosis, in vitro fertilization, reproductive outcomes, vaginal dysbiosis

INTRODUCTION

Vaginal dysbiosis (VD) is an imbalance in the normal vaginal microbiota. Bacterial vaginosis (BV) is the most frequent VD and is prevalent in approximately 18% of infertile women [1]. BV is defined as a disruption of the vaginal microbiota, causing reduced abundance of *Lactobacillus* (*L.*) species (spp.), and increased presence of BV bacteria like *Gardnerella* (*G.*) spp., *Fannyhessea vaginae*, and *Mobiluncus* spp. [2]. BV may cause symptoms such as vaginal discharge and fishy odor but remains asymptomatic in up to 80% of women. Consequently, there is a risk of BV being clinically underestimated [3]. An increasing number of studies report that IVF patients with BV might be associated with poor reproductive outcomes, for example, repeated implantation failure, spontaneous abortion, and poor pregnancy rates [4–6].

Microscopic examination of vaginal fluid alongside clinical evaluation and pH measurement remains a valid method of diagnosing vaginal

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KEY POINTS

- Overall estimate of prevalent vaginal dysbiosis is 19%. The prevalence of aerobic vaginitis type dysbiosis is 4% in in vitro fertilization (IVF) patients.
- The early pregnancy loss (EPL) rate was higher and the clinical pregnancy rate (CPR) was reduced in IVF patients with vaginal dysbiosis compared to IVF patients without vaginal dysbiosis.
- Sub-analysis found a lower live birth rate (LBR) in molecular-defined bacterial vaginosis and aerobic vaginitis.
- High heterogeneity between the studies makes it difficult to generalize the findings to the individual patient, therefore, consensus on optimal vaginal dysbiosis diagnostics in IVF patients is needed.

dysbiosis, including BV [7] using, for example, the Amsel criteria [8] or the Nugent score [9]. Research, however, has shown that the functions and diversity of the human microbiome were underestimated using microscopy methods [10]. In 2011, Ravel *et al.* published a study using the 16S rRNA marker gene to introduce the concept of "community state types" (CSTs). About 80% of reproductive age women can be stratified into four distinct low-diversity CSTs, each primarily dominated by a single *Lactobacillus* spp.: *L. crispatus* (CST-I), *L. gasseri* (CST-II), *L. iners* (CST-III), and *L. jensenii* (CST-V). The remaining 20% of women exhibited a more diverse CST-IV with multiple sub-groups covering genera like *Gardnerella*, *Prevotella*, *Corynebacterium*, *Fannyhessea*, *Streptococci*, and *Enterococci* [11].

Another common cause of VD is aerobic vaginitis (AV) type bacteria. In contrast to BV, which is considered endogenous, AV is caused by intestinal microorganisms, such as group B *Streptococcus*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* [12]. Microscopy and clinical manifestation remain the gold standard for diagnosis [13].

The impact of the vaginal microbiota on reproduction and fertility continues to attract significant scientific interest. The number of new publications considering BV and assisted reproductive technology is growing rapidly [10], questioning whether newly published data can update the current state of knowledge. Thus, the primary aim of this study was to update an earlier systematic review and meta-analysis by our group [1]. In addition to our previous methodology, we aimed to systematically summarize and differentiate between BV- and AV- types of vaginal dysbiosis to investigate their combined and individual association with reproductive outcomes in IVF patients.

MATERIALS AND METHODS

The present study is a systematic review and meta-analysis of studies investigating the association between VD and predefined pregnancy outcomes in women undergoing in vitro fertilization (IVF) treatment. The analysis is an updated analysis of previously published meta-analyses [1,14] from 2019 and 2021, carrying the PROSPERO registration: CRD42016050603.

Eligibility criteria for study inclusion in this analysis were defined in our previously published study [1]. The study included infertile women undergoing IVF/intracytoplasmic sperm injection (ICSI) treatment for all causes while intrauterine insemination (IUI) patients were excluded. Sub-Saharan African studies were excluded due to a higher background prevalence of competitive co-infections with BV, such as HIV, *Trichomonas vaginalis*, and *Chlamydia trachomatis*. Additionally, case reports and reviews were excluded.

Primary outcomes were live birth rate (LBR) and early pregnancy loss (EPL). Secondary outcome measures were clinical pregnancy rate (CPR), defined as ultrasound-verified intrauterine heartbeat, and biochemical pregnancy rate (BPR) defined as human chorionic gonadotropin (hCG) serum-positive pregnancies, according to local laboratory standard [1]. The common denominator was per embryo transfer except EPL, which was biochemical pregnancies. For the calculation of prevalence, we used $N =$ patients as the denominator. Many studies did not provide information about EPL and, thus, clinical pregnancies were subtracted from biochemical pregnancies to deduct the number of early pregnancy losses. In this updated meta-analysis, we decided to use the ongoing pregnancy rate instead of the CPR, if CPR was defined in the individual studies as an intrauterine gestational sac with or without fetal heartbeat by transvaginal ultrasound. In these cases, we decided ongoing pregnancy rate was a more precise outcome, according to our criteria.

In a study by Van den Tweel *et al.* [17▪▪], the authors provided the raw data upon request, so additional statistics have been performed, separating IVF/ICSI patients from IUI patients to assess the outcomes.

LITERATURE SEARCH STRATEGY

The PubMed (Medline) database was used to make an updated systematic literature search, using

relevant keywords and MeSH terms (Supplementary Material 1, http://links.lww.com/COOG/A95). M. M. and A.S.H. initially screened publications based on their titles, followed by an abstract review. If an abstract contained elements related to the eligibility criteria and/or outcomes, the publication was read in full. In case of doubt, T.H. was consulted to reach a conclusive decision regarding the inclusion of the study. Further searches were carried out in Embase, Scopus, and Cochrane using the keywords 'IVF' and 'microbiota'. Before conducting the meta-analysis, we repeated the literature search on December 16, 2023, to identify new publications.

QUALITY OF ARTICLES

To evaluate the quality of the individual studies, the Newcastle-Ottawa Scale (NOS) [18] was performed for each study included in our systematic review and meta-analysis [15,16▪▪,17▪▪,19–21,22▪▪,23▪], as presented in Supplementary Material 2, http://links.lww.com/COOG/A95. We did not use GRADE [24] for assessing the quality of evidence per outcome.

DATA EXTRACTION AND VAGINAL DYSBIOSIS DEFINITION

The prevalence of VD was calculated by summarizing all the included studies (Supplementary Material 3, http://links.lww.com/COOG/A95). In the studies that analyzed both molecular and microscopy methods, only the result for the molecular method was used [16▪▪,25,26].

Data extraction included the following characteristics in the individual studies: author, analysis method, outcomes, and sample size of individual studies (Supplementary Material 4–7, http://links.lww.com/COOG/A95).

Overall VD is herein defined broadly as a CST IV like microbiota with whatever method used in the individual studies, including microscopy methods of BV and AV diagnosis [11]. Moreover, this meta-analysis included subgroups of VD: (1) overall BV- or AV-type (2) microscopy or molecular defined BV-/AV-type. Microscopy comprised all studies based on, for example, Nugent and Amsel's criteria of BV as well as wet-smear criteria to diagnose AV. The molecular group constituted qPCR, IS-pro technique, and 16S rRNA gene sequencing. When possible, we sub-stratified for BV-type and AV-type dysbiosis by manually approximating to VALENCIA subgroups [11] of CST IV-A and IV-B as BV-type whereas CST IV-C was AV-type. In case sub-stratification of the CST-IV group was not possible in the individual studies they were not considered for sub analyses. Results for two studies [4,27] from the previous meta-analysis [1] are updated with AV results (Supplementary Material 3, http://links.lww.com/COOG/A95). The corresponding control group to the abovementioned groups did not have VD by any method. For example, and when possible, we subtracted BV patients from the control group in case AV-type microbiota was investigated.

STATISTICS

The relative risk (RR) with a 95% confidence interval, Forest plot, and Funnel plot was determined using a random effects model. This analysis utilized the Mantel–Haenszel method in REVIEW Manager version 5.3 (Cochrane, London, UK) [28]. Regarding VD prevalence, calculations were performed using R (version 4.1.3; RStudio).

RESULTS

The present literature search found 91 publications as of October 7, 2023. A total of 75 citations were excluded based on title and abstract. Hence, a total of 16 citations [15,16▪▪,19–21,29,30–39] were assessed for eligibility by examination of the full text. Seven studies were removed due to study design [29,33–36,38,39] and four were removed as it was not possible to extract pregnancy outcome data for the meta-analysis [30–32,37]. Further searches in additional databases yielded five relevant papers [23▪,40–43], however, only the study by Väinämö *et al.* [23▪] met the eligibility criteria and was included. Additionally, we contacted the authors of four studies [40–43] to provide additional data. From three studies [40–43], we received no response, and consequently, their data could not be evaluated. The authors of one study [41], provided data, however, the study did not meet the inclusion criteria regardless. In the repeated literature search, two articles [17▪▪,22▪▪] met the eligibility criteria, as the authors of Van den Tweel *et al.* provided data upon request [17▪▪]. Thus, a total of eight studies were included [15,16▪▪,17▪▪,19–21,22▪▪,23▪] and added to the 17 studies [4,25–27,44–56] previously included in a systematic review and meta-analysis published by our group [1]. Thus, the present meta-analysis included 25 studies [4,15,16▪▪,17▪▪,19–21,22▪▪,23▪,25–27,44–56], comprising 6835 IVF patients. The full selection of the studies is shown in the flow diagram, Fig. 1.

PREVALENCE OF VAGINAL DYSBIOSIS, AEROBIC VAGINITIS, AND INDIVIDUAL STUDY CHARACTERISTICS

The overall prevalence of VD was 18.6% (1271/6835, 95% CI 17.7–19.5%) (Supplementary Material 3,

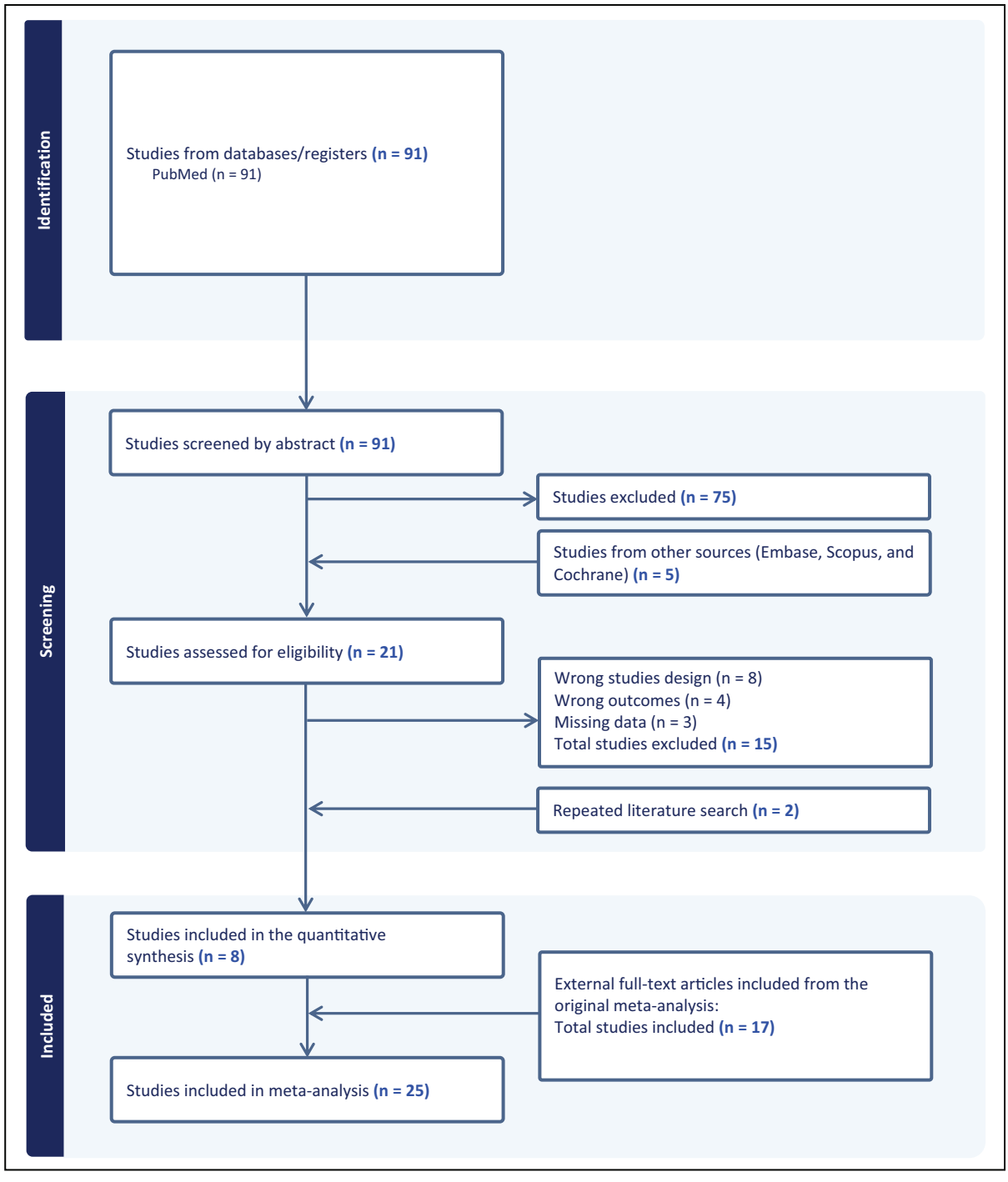

FIGURE 1. Vaginal dysbiosis – the association with reproductive outcomes in IVF-patients: a systematic PRISMA review and meta-analysis. IVF, in vitro fertilization. Created with www.covidence.org.

http://links.lww.com/COOG/A95). The prevalence of AV was 4.1% (26/628, 95% CI 2.7–6.0%) (Supplementary Material 3, http://links.lww.com/COOG/A95). In general, only six studies [4,16▪▪,19,21,22▪▪,27] were included in the present analysis considering AV.

The heterogeneity of VD prevalence was large, ranging from 4% (53) to 45% [20].

Seventeen publications were based on microscopy [15,16▪▪,21,23▪,25–27,44–53], and in these studies the BV prevalence was 17% (1007/6026) whereas the overall BV prevalence was 24% (323/1339) for molecular methods. The timing of sampling was different across the included studies. Nine studies performed sampling on oocyte retrieval day [16▪▪,21,25,44,47,48,51–53], whereas eight studies

performed the sampling on the embryo transfer day [15,20,22▪▪,45,46,49,50,55], and four studies performed the sampling prior to IVF stimulation [23▪,26,50,56], with BV prevalence of 16% (462/2829), 32% (333/1057), and 17% (400/2290) respectively. In three studies, the sampling was performed in different phases of the menstrual cycle or IVF stimulation [17▪▪,19,55]. In one study, the time of sampling was not reported [27]. A full view of the individual study characteristics of the eight studies included can be seen in Table 1 [15,16▪▪,17▪▪,19–21,22▪▪,23▪].

Live birth rate

Among 4605 patients, a total of 1640 live births were reported (Supplementary Material 8, http://links.lww.com/COOG/A95, Supplementary Material 12, http://links.lww.com/COOG/A95). Compared to IVF patients without VD, the relative risk (RR) for LBR in patients with VD was 0.94 (95% CI 0.76–1.16, $I^2 = 46\%$), Table 2.

Considering subgroup analyses and compared to the corresponding control group, the overall BV-type RR was 0.96 (95% CI 0.76–1.21, $I^2 = 50\%$). For BV by microscopy, the RR was 1.02 (95% CI 0.75–1.40, $I^2 = 65\%$) whereas for BV-type by molecular methods the RR was 0.76 (95% CI 0.51–1.13, $I^2 = 15\%$), Table 2.

For IVF patients with AV-type microbiota and compared to IVF patients without VD, the RR was 0.76 (95% CI 0.33–1.72, $I^2 = 0\%$). Based on methodology, RR was 0.87 (95% CI 0.37–2.07, $I^2 = 0\%$) for microscopy and 0.18 (95% CI 0.01–2.73, heterogeneity not applicable) for molecular methods (Table 3, Supplementary Material 7d–f, http://links.lww.com/COOG/A95, Supplementary Material 11a–c, http://links.lww.com/COOG/A95, Supplementary Material 15a–c, http://links.lww.com/COOG/A95).

Early pregnancy loss

Out of 3153 patients with hCG-positive pregnancy, a total of 571 early pregnancy losses were reported (Supplementary Material 9, http://links.lww.com/COOG/A95, Supplementary Material 13, http://links.lww.com/COOG/A95).

Overall VD patients undergoing IVF had a significantly higher risk for EPL compared to patients without VD, RR 1.49 (95% CI 1.15–1.94, $I^2 = 38\%$) (Table 2).

Sub-analysis for overall BV-type revealed a RR of 1.62 (95% CI 1.26–2.08, $I^2 = 25\%$) whereas BV by microscopy had a RR of 1.51 (95% CI 1.16–1.96, $I^2 = 23\%$) and BV by molecular methods had a RR of 2.35 (95% CI 1.36–4.07, $I^2 = 8\%$) (Table 2).

For AV-type there was only one study and when compared to IVF patients without VD the RR was 0.57 (95% CI 0.04–7.85, heterogeneity not applicable), as seen in Table 3, Supplementary Material 7d–f, http://links.lww.com/COOG/A95, Supplementary Material 11d–f, http://links.lww.com/COOG/A95, Supplementary Material 15d–f, http://links.lww.com/COOG/A95.

Clinical pregnancy

Within 6550 patients, a total of 2555 clinical pregnancies were reported (Supplementary Material 10a–c, http://links.lww.com/COOG/A95, Supplementary Material 14a–c, http://links.lww.com/COOG/A95). Overall VD patients undergoing IVF had significantly lower RR 0.82 (95% CI 0.70–0.95, $I^2 = 49\%$) for CPR per embryo transfer compared to IVF patients without VD (Table 2).

Sub analysis for BV-type revealed an overall RR of 0.85 (95% CI 0.71–1.01, $I^2 = 54\%$). Considering microscopy methods for BV diagnosis the RR was 0.85 (95% CI 0.69–1.05, $I^2 = 63\%$) the RR was 0.81 (95% CI 0.61–1.07, $I^2 = 8\%$) for molecular BV methods (Table 2).

For AV, RR was 0.78 (95% CI 0.37–1.66, $I^2 = 14\%$). Based on methodology, RR was 0.84 (95% CI 0.35–1.98, $I^2 = 0\%$) for microscopy and 0.60 (95% CI 0.11–3.40, $I^2 = 61\%$) for molecular methods (Table 3, Supplementary Material 7g–i, http://links.lww.com/COOG/A95, Supplementary Material 11g–i, http://links.lww.com/COOG/A95, Supplementary Material 15g–i, http://links.lww.com/COOG/A95).

Biochemical pregnancy

In total, 2549 biochemical pregnancies per embryo transfer were reported among 5455 patients (Supplementary Material 10d–f, http://links.lww.com/COOG/A95, Supplementary Material 14d–f, http://links.lww.com/COOG/A95). Compared to IVF patients without VD, the RR for BPR for patients with VD was 0.95 (95% CI 0.82–1.10, $I^2 = 39\%$) for BPR per embryo transfer. Subgroup analyses revealed RR of 0.98 (95% CI 0.82–1.17, $I^2 = 54\%$) for microscopy BV methods and 0.78 (95% CI 0.59–1.02, $I^2 = 0\%$) for molecular BV methods (Table 2).

For AV, RR was 0.82 (95% CI 0.35–1.93, $I^2 = 0\%$). Based on methodology, RR was 0.90 (95% CI 0.39–2.09, $I^2 = 0\%$) for microscopy and not estimable for molecular methods (Table 3, Supplementary Material 7j–l, http://links.lww.com/COOG/A95, Supplementary Material 11j–l, http://links.lww.com/COOG/A95, Supplementary Material 15j–l, http://links.lww.com/COOG/A95).

Table 1. Study characteristics

Study	Method	VD prevalence	Age (normal microbiota)	Age BV	Treatment	Timing of sampling	Country/ ethnicity	IVF cycle of sampling
Eskew *et al.*	16S rRNA	24.0%	18–43 (range) for all included patients		Azithromycin orally, 1 g daily once for both partners on the day of initiation of injectable gonadotropins for controlled ovarian stimulation	At 3 time points: 1. immediately before baseline ultrasound; 2. immediately before egg retrieval; and 3. immediately before embryo transfer	USA/no data	No data on cycle
Ji *et al.*	Nugent score	40.4%	30.1 (mean)	29.7 (mean)	No	On the day of endometrial transformation	China/no data	Frozen embryo transfer cycles. Some patients participated more than once
Karaer *et al.*	16S rRNA	44.8%	23–39 (range) for all included patients		No	On the day of embryo transfer	Turkey/no data	No data on cycle
Koort *et al.*	16S rRNA and Nugent score	26.9%	34.1 (average) for all included patients		No	On follicle puncture day	Estonia/no data	No data on cycle
Vieira-Baptista *et al.*	Amsel's criteria	17.9%	34.6 (mean) for all included patients		No	Immediately before vaginal disinfection and oocyte retrieval (IVF/ICSI) or IUI	Portugal/no data	No data on cycle
Väinämö *et al.*	16S rRNA	22.6%	32.9 (mean) for all included patients		No	At the time of fresh embryo transfer and at the eighth gestational week from those women who got pregnant	Finland/no data	No data on cycle
Van den Tweel *et al.*	16S rRNA	32.3%	37.1 (mean)	38.5 (mean)	No	Vaginal samples taken at oocyte retrieval /at the last ultrasound before the frozen embryo transfer	Netherlands/60% Caucasian	Any cycle
Zeng *et al.*	Nugent score	19.0%	30.8 (mean)	31.35 (mean)	Women with other bacterial morphologies or BV-positive (Nugent score 7–10) were empirically treated with Ornidazole via vagina for a week. No control after therapy with Ornidazole	Vaginal sample within 2 months before IVF and embryo transfer	China/no data	First cycle

BV, bacterial vaginosis; IVF, in vitro fertilization; VD, vaginal dysbiosis.

Table 2. Vaginal dysbiosis (VD) – relative risk on reproductive outcomes

Outcome	RR (CI 95%)	No. of participants (studies)
Primary outcomes		
Live birth rate (CST IV)	0.94 (0.76–1.16)	4605 (14)
Live birth rate (BV)	0.96 (0.76–1.21)	4359 (13)
Microscopy	1.02 (0.75–1.40)	3776 (8)
Molecular	0.76 (0.51–1.13)	658 (6)
Early pregnancy loss (CST IV)	1.49 (1.15–1.94)	3153 (20)
Early pregnancy loss (BV)	1.62 (1.26–2.08)	3045 (19)
Microscopy	1.51 (1.16–1.96)	2792 (14)
Molecular	2.35 (1.36–4.07)	291 (6)
Secondary outcomes		
Clinical pregnancy rate (CST IV)	0.82 (0.70–0.95)	6550 (25)
Clinical pregnancy rate (BV)	0.85 (0.71–1.01)	6092 (22)
Microscopy	0.85 (0.69–1.05)	5488 (16)
Molecular	0.81 (0.61–1.07)	781 (8)
Biochemical pregnancy rate (CST IV)	0.95 (0.82–1.10)	5455 (17)
Biochemical pregnancy rate (BV)	0.95 (0.82–1.10)	5455 (17)
Microscopy	0.98 (0.82–1.17)	4938 (13)
Molecular	0.78 (0.59–1.02)	601 (5)

BV, bacterial vaginosis; CI, confidence interval; CST, community state type; RR, relative risk.

DISCUSSION

Four meta-analyses were previously published, investigating the association of VD with reproductive outcomes in IVF patients, two of them by our group [1,14,57,58]. Overall, the analyses reported lower clinical pregnancy rates [14,58] and higher early pregnancy loss rates [1,14,57] in IVF patients with BV compared to IVF patients without BV. The present study contributes with eight recently published studies and reports a significant increase in EPL in IVF patients with VD (RR 1.49, 95% CI 1.15–1.94), confirming previous findings. The overall

Table 3. Aerobic vaginitis (AV) – relative risk on reproductive outcomes

Outcome	RR (CI 95%)	No. of oarticipants (studies)
Primary outcomes		
Live birth rate	0.76 (0.33–1.72)	442 (4)
Microscopy	0.87 (0.37–2.07)	355 (2)
Molecular	0.18 (0.01–2.73)	87 (2)
Early pregnancy loss	0.57 (0.04–7.85)	201 (4)
Microscopy	0.57 (0.04–7.85)	161 (2)
Molecular	Not estimable	40 (2)
Secondary outcomes		
Clinical pregnancy rate	0.78 (0.37–1.66)	545 (6)
Microscopy	0.84 (0.35–1.98)	360 (2)
Molecular	0.60 (0.11–3.40)	185 (4)
Biochemical pregnancy rate	0.82 (0.35–1.93)	391 (3)
Microscopy	0.90 (0.39–2.09)	401 (2)
Molecular	Not estimable	31 (1)

CPR was significantly decreased in women with VD (RR 0.82, 95% CI 0.70–0.95). Our previous study from 2021 [1] only detected a statistically significant decrease in CPR in the sub-group of molecular methods RR 0.55 (95% CI 0.32–0.93), which in the present study was not statically significant and closer to unity, RR 0.81 (95% CI 0.61–1.07).

In the present systematic review and meta-analysis, the overall prevalence of VD was 18.6% (95% CI 17.7–19.5), which was similar to the prevalence reported in our previous study, in which the prevalence of VD was 18% [1], and higher compared to our first study, which was 16% [14]. Although we believe a rough estimate of 19% VD-positive IVF patients is probably a good overall estimate, this may be affected by different methods to diagnose individual patients with VD. All the studies that included both microscopy and molecular methods reported a higher VD prevalence with molecular methods [16▪▪,25,26]. Prevalence of BV-type dysbiosis differed between the molecular methods: qPCR, IS-pro technique, and or 16S technique, with BV-type dysbiosis prevalence of 24%, 18%, and 34% respectively (Table 1, Supplementary Material 3, http://links.lww.com/COOG/A95). An explanation might be that molecular methods are more sensitive and reveal more patients with BV – for example, more optimal classification of patients in the Nugent intermediate group. Ultimately, the different definitions need to be subjected to treatment in order to investigate optimal classification in terms of impact on reproductive success.

Only six studies [4,16▪▪,19–22▪▪,27] reported data on AV-type dysbiosis. The present meta-analysis did not find any statistically significant association between AV-type dysbiosis and reproductive outcomes, but the number of patients was too small to be conclusive. However, RR estimates for LBR and CPR were considerably lower in the presence of AV compared to patients without VD, especially when using molecular methods to define AV, where the RR was 0.18 (95% CI 0.01–2.73) for LBR and 0.60 (95% CI 0.11–3.40) for CPR.

STRENGTHS AND LIMITATIONS

The strength of this study is the inclusion of eight new studies for a total of 25 studies, (Fig. 1). The additional data for molecular and microscopy methods enabled the sub-stratification to BV- and AV-type dysbiosis. Despite a higher number of studies included, there remains interstudy heterogeneity, which may be overcome in a future meta-analysis by individual participant meta-analysis linking the microbiome data directly to IVF outcome across studies. The most relevant explanation for high heterogeneity is the use of different diagnostic methods for VD across the studies. The present study aimed to counter some of this heterogeneity by stratifying for molecular and microscopy methods as well as sub-stratifying patients with BV- and AV-type microbiota. Differences in VD prevalence between studies can also be explained by the timing of sampling during the different phases of the menstrual cycle and different phases of IVF treatment (Table 1). Hormonal fluctuations influence the vaginal microbiota during the cycle [59]. Previous studies indicated that vaginal bacterial diversity at the time of transfer, as opposed to other time points may be associated with clinical outcomes [19,59]. However, other studies did not find any difference regarding timing of sampling [55]. Future design of systematic reviews may need to stratify according to the time of sampling.

Seven studies used antibiotics during IVF treatment, possibly resulting in biased results [19,46,48,50,51,60]. In the study by Zeng *et al.* [23▪], patients with symptomatic BV (Nugent score 7–10) underwent a 7-day Ornidazole treatment. Despite treatment, the results of the study suggest that BV might still adversely affect pregnancy. On the other side, we excluded a randomized control trial (RCT) study by Thanaboonyawat *et al.* [39], in which probiotics were systematically administered, and the results suggest their positive influence on reproductive outcomes.

CONCLUSION

The present systematic review and meta-analysis concludes that the presence of a broad definition of VD in IVF patients is significantly associated with a higher early pregnancy loss rate and lower clinical pregnancy rate compared to IVF patients without VD. The sub-analysis found a lower live birth rate in molecular-defined BV and AV, although none of these outcomes reached statistical significance. More studies reporting live birth rate might change this in the future. However, different molecular methods and different criteria were used across the studies, which make it difficult to generalize these findings to the individual patient. Consensus about optimal VD diagnostics may enable more robust evidence concerning the prevalence, reproductive outcome and risks of VD in IVF patients.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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This study examined both vaginal and semen samples using 16 s rRNA gene sequencing to investigate how microbiome composition affects ART outcomes. The findings indicate that disruptions in the microbiome of both partners may result in poor reproductive outcome. It is recommended that microbial screening needs to be integrated into the standard practice for ART patients.

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An observational prospective study investigated the impact of bacterial vaginosis (BV), using qPCR and 16S rRNA gene microbiota analysis of V1-V2 region, on pregnancy rates across various fertility treatments (IUI/IVF/ICSI). The study found no significant differences in ongoing pregnancy or live birth rates based on BV status or microbiome community state type (CST). However, ongoing pregnancy rate was slightly lower in the BV qPCR positive group. The study suggests an increased risk in miscarriages in patients with positive BV qPCR status and in those categorized under CST group III and IV. Additionally, baseline qPCR positive participants had significantly higher body mass index and smoked more often.

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The study explored how the vaginal microbiota, particularly Lactobacillus crispatus, affects IVF success and early pregnancy. Results revealed a higher abundance of L. crispatus in women who achieved clinical pregnancy and live birth compared to nonpregnant women. During early pregnancy, Lactobacilli, especially *L. crispatus*, dominated all early pregnancy samples. Microbiota shifted from one Lactobacillus-dominated type to another, or from mixed to Lactobacillus-dominated, but never from Lactobacillus-dominated to non-Lactobacillus-dominated. The presence of L. crispatus was associated with better IVF outcomes, highlighting its significance in reproductive health.

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