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Review

The relation of the vaginal microbiota to early pregnancy development during in vitro fertilization treatment—A meta-analysis

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ABSTRACT

An abnormal vaginal microbiota composition has been shown to lead to pre-term births, miscarriage, and problems with conceiving. Studies have suggested that dysbiosis reduces successful early pregnancy development during IVF. However, conflicting reports exist. This meta-analysis aims to answer the following question: what is the aggregated effect found by studies investigating the influence of the vaginal microbiota composition on early pregnancy rates after IVF treatment?

A systematic review was performed using the Medline and EMBASE databases, using search terms for healthy vaginal microbiota, abnormal vaginal microbiota, fertility and pregnancy. The search resulted in six included articles. Of these, all six were used for further meta-analysis. The main outcome measures were the clinical pregnancy rate, determined through ultrasound proven fetal heartbeat and/or hCG results before 10 weeks gestation, in relation to the vaginal microbiota composition.

We found a correlation between abnormal vaginal microbiota and lower rates of early pregnancy development after IVF treatment (OR = 0.70, 95% CI = 0.49 - 0.99). One study showed the reverse correlation. However, heterogeneity between study methodologies in various forms was found.

In conclusion, women with an abnormal vaginal microbiota are roughly 1.4 times less likely to have a successful early pregnancy development after IVF treatment when compared to women with normal microbiota.

Contents

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Introduction

Over the past decades, bacterial microbiota in humans has received increasing attention. Through technological advances in molecular typing it has become possible to more easily and accurately characterize an individual's microbiota. This has led to links between host microbiotas and inflammatory diseases such as in Crohn's disease, as well as links with susceptibility to infections [1–4]. The vaginal commensal microbiota controls pH levels and provide a physical barrier to opportunistic pathogens. These characteristics have been shown to influence the vaginal environment and possibly influence conception and development of the child during pregnancy.

A healthy vaginal microbiota is currently defined by a composition dominated by one of the multiple anaerobic *Lactobacilli* [5]. Through the production of lactic acid, *Lactobacilli* lower the pH level of the vaginal environment, which protects the vagina from invasion and infection by opportunistic pathogens. Many *Lactobacilli* also produce hydrogen peroxide (H_2O_2), bacteriocins, glycogen, and glycerol, which aid in the defense against pathogens and in return secure the dominant habitation of *Lactobacilli* [5–8]. The most commonly found phylotypes of *Lactobacillus* spp. are *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii* [9–12]. Another common phylotype is a diverse microbial profile, without dominance of *Lactobacilli*, and is often related to Bacterial Vaginosis (BV) [11]. BV is the most common vaginal disorder in women and occurs in up to 20% of pregnant women [5]. BV is a disruption of the ecological vaginal balance by overgrowth of a typically non-*Lactobacillus* anaerobic bacterium [13]. This results in an alteration of the milieu and composition of the vaginal microbiota. Notable BV related bacteria are *Gardnerella vaginalis*, *Mobiluncus* spp., and *Atopobium vaginae* [14–17]. Symptoms of BV include watery discharge with a fishy malodour. However, roughly 50% of women who have BV are asymptomatic or have less obvious symptoms [18]. Clinically, BV is determined based on the widely accepted Nugent criteria, which take into account the presence and abundance of various vaginal bacteria [13].

Recent evidence corroborates that BV increases risk of preterm delivery and pregnancy loss [18–23]. This risk is potentially twice as high when compared to women with a healthy vaginal microbiota [24]. Persistency and the relative amount of BV related bacteria in the vagina increase the potential for negative pregnancy outcomes [24–27]. Additionally, women without previous pregnancy are at higher risk of second trimester pregnancy loss when the vaginal microbiota contains low amounts of *Lactobacillus* spp. or no *Lactobacillus* spp. at all [28]. Although BV has received the most attention, abnormal vaginal microbiota is not always BV and other conditions have separate effects on pregnancy outcome [14]. For instance, loss of *Lactobacillus* without BV-related bacterial growth is more strongly associated with preterm birth than BV [28]. All of these studies indicate a significant role of the vaginal microbiota in the development of the child during pregnancy, but primarily focus on later stage negative outcomes of pregnancy, as these appear more clearly expressed.

During IVF transfer a transfer catheter is inserted through the vaginal cavity into the uterus. During this transfer it is not uncommon for the catheter tip to become contaminated with mucus originating in the vagina [29,30]. This mucus is commonly filled with large amounts of bacteria from the vaginal microbiota. We hypothesize that the effect that vaginal microbiota can have on uterine implantation and early development is more expressed during IVF due to its larger presence during transfer.

In this study we aim to systematically review and perform a meta-analysis on the effect of vaginal microbiota composition on the early pregnancy development rate of IVF treatments, defined as the absence of first trimester pregnancy. Through this we hope to shed light on the effect of abnormal vaginal microbiota on the earlier stages of pregnancy.

Methods

Here we have systematically reviewed the effect of human vaginal microbiota on early IVF outcomes. This review was written in compliance with the PRISMA-statement for reporting systematic reviews.

Information sources and search

The Medline, Central, and EMBASE scientific databases were used to conduct the searches. All included scientific articles were written in English between 1980 and the 6th of April 2018. Articles were evaluated by two of the authors, and discrepant articles were judged by a third author.

The full search strategy and terms can be found in Table 1. We applied the search methodology described in the PRISMA-statement: disease or disorder, outcome measure, methodological terms, patient characteristics, and prognostic factors. Additional sources for articles included references from already included articles.

Study selection

Articles were selected for further screening when at least one of the following terms was found in the title or abstract: vaginal microbiota composition, vaginal microbiota, bacterial vaginosis, or abnormal vaginal microbiota associated with IVF outcome. The primary outcome measures were the implantation and/or early pregnancy development rate. Inclusion and exclusion criteria can

Table 1
Search strategy.

Categories	[MeSH] term	Free PubMed terms
Disease	Infertility, Female	Infertility Subfertility
Outcome measure	Fertilization in Vitro Pregnancy	Conception IVF success IVF outcome fertilization
Methodological terms		Prospective studies Prognosis Prediction model prognostic factor
Patient characteristics		Female Fertile Human Infertile Reproductive age Subfertile
Prognostic factors	Microbiota	Vaginal microbiome Vaginal microbiota vaginal microflora Bacterial dysbiosis Bacterial vaginosis

be found in Table 2. The Nugent criteria for scoring of vaginal microbiota to determine BV was used in our study to determine a normal or abnormal microbiota in the cases. Vaginal samples with Nugent scores ranging from 0 to 6 were considered indicative of normal microbiota. Vaginal samples with a Nugent score of 7 or higher were considered indicative of abnormal microbiota.

The data extraction included the following study characteristics: author and year of publication, source of data, sample size, methodology, outcomes to be predicted, and results of the study. The methodology, outcomes, and results were further examined for data on the subjects of age, indication of IVF, pituitary down-regulation, use of antibiotics, timing of sampling, country/ethnicity, smoking status, the IVF cycle of the sampling, and the transfer stage.

Risk of bias in individual studies

We assessed the risk of bias based on the Quality in Prognostic Studies (QUIPS) tool [31]. The different criteria were: (1) Study Participation, (2) Study Attrition, (3) Prognostic Factor Measurement, (4) Outcome Measurement, (5) Study Confounding, and (6) Statistical Analysis and Reporting. For each criterium a 'risk of bias' judgement was made in which potential bias variables have been assessed. Each domain has been rated with a high, moderate or low risk of bias [31,32]. The section of the results of the individual studies reveals outcomes of the single studies.

Statistical meta-analysis

Analyses of the overall effect of the vaginal microbiota on IVF outcome were carried out by integrating the quantitative findings in a random or fixed effect model.

With the aid of the statistical MedCalc Software (Ostend, Belgium), weights were assigned to the different studies for more insight of the pooled effect. When studies shared a common true effect the fixed effects model had been chosen. When the true effect of the studies was assumed to vary extensively, the random effects model was used for estimation of the weighted average of the effect reported in the studies [33]. A p-value < 0.10 in the test for heterogeneity indicated the random effects model due to significant heterogeneity. Principal summary measure were the odds ratio, together with a 95% confidence interval. The inconsistency (I^2) represented the percentage of observed variation across studies. Values larger than 0% indicate increasing heterogeneity and are presented in a forest plot.

Results

Our database searches initially yielded twenty articles, with an additional 5 articles included from other sources. None of these were duplicate findings. Nineteen articles were then removed based on the title and abstract, leaving six articles for further screening. Full-text assessment of these six articles led to no additional exclusions. All six articles were deemed fit for inclusion in the meta-analysis on the basis of the study outputs. A schematic overview of this procedure is shown in Fig. 1.

Synthesis of results

The search produced six studies addressing the relationship between vaginal microbiota composition and outcome of IVF treatment (Fig. 1). The selected six studies provided a total cohort for meta-analysis of 1095 participants wherein 893 women were classified with a normal vaginal microbiota and 202 women with abnormal vaginal microbiota [34–39]. The total OR was significant for the distribution of normal vaginal microbiota composition versus abnormal vaginal microbiota of women with or without early pregnancy development after IVF treatment (CI 95% 0.49 - 0.99). The OR of 0.70 represents a negative correlation between abnormal vaginal microbiota and early pregnancy development (Table 3, Fig. 2).

For the total OR we chose the random effect model due to the heterogeneity of the different studies included. However, when tested, we found no significant heterogeneity across the studies ($p = 0.15$).

Results of individual studies

The recent study of Haahr et al. investigated the diagnostic performance of qPCR assays and conventional Nugent scoring in predicting IVF outcome for an infertile population from Denmark [34]. Haahr et al. conducted this study to distinguish between normal vaginal microbiota and abnormal vaginal microbiota, and to elucidate the difference of the predictive capacity of these methods on IVF outcome. The prevalence of BV assessed by the Nugent score was 21%, compared to the prevalence of 28% of abnormal vaginal microbiota assessed by qPCR. Abnormal vaginal microbiota was defined by high levels of *Gardnerella vaginalis* and/or *Atopobium vaginae*. There was no significant difference in abnormal vaginal microbiota between qPCR- and Nugent-determined BV in predicting the IVF failure. However, the pregnancy rate was significantly lower in the abnormal vaginal microbiota group, with an OR of 0.13 (95% CI 0.03-0.60).

Mangot-Betrand et al. revealed a 9.45% BV prevalence in a population of 307 infertile patients [35]. Consequently, they assessed the impact of BV on the pregnancy rate after women underwent IVF. Participants with BV- showed higher embryo implantation rates compared to BV+. Nonetheless, the difference was not significant (36.3% vs. 27.6%, p = 0.418).

Liversedge et al. found no significant difference in conception rate in patients with abnormal vaginal microbiota compared to patient with normal microbiota (OR 1.15, CI 95% 0.66–2.03) [38]. In this study population, BV was much more prevalent in the group of patients with tubal disease ($p = 0.02$), while a normal microbiota was much more frequent in patients without tubal disease ($p = 0.004$). Selim et al. found significant differences in pregnancy rates to be correlated with single isolated bacteria [36]. Specifically, decreased pregnancy rates were found in patients who tested positive for *Staphylococcus aureus* and *Streptococcus viridians* compared with patients tested negative. However, they found no significant differences in pregnancy rates between participants with abnormal vaginal microbiota and those with normal microbiota (OR 0.61, CI 95% 0.22–1.64). Eckert et al. investigated the impact of the vaginal microbiota and vaginal inflammation on

Table 2
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Women of reproductive age	Diagnosis of abnormal microbiota without Nugent criteria or qPCR
Subfertility or infertility (otherwise healthy)	Reviews
In-vitro fertilization	
Ultrasound proven fetal heartbeat and/or hCG results before 10 weeks gestation	

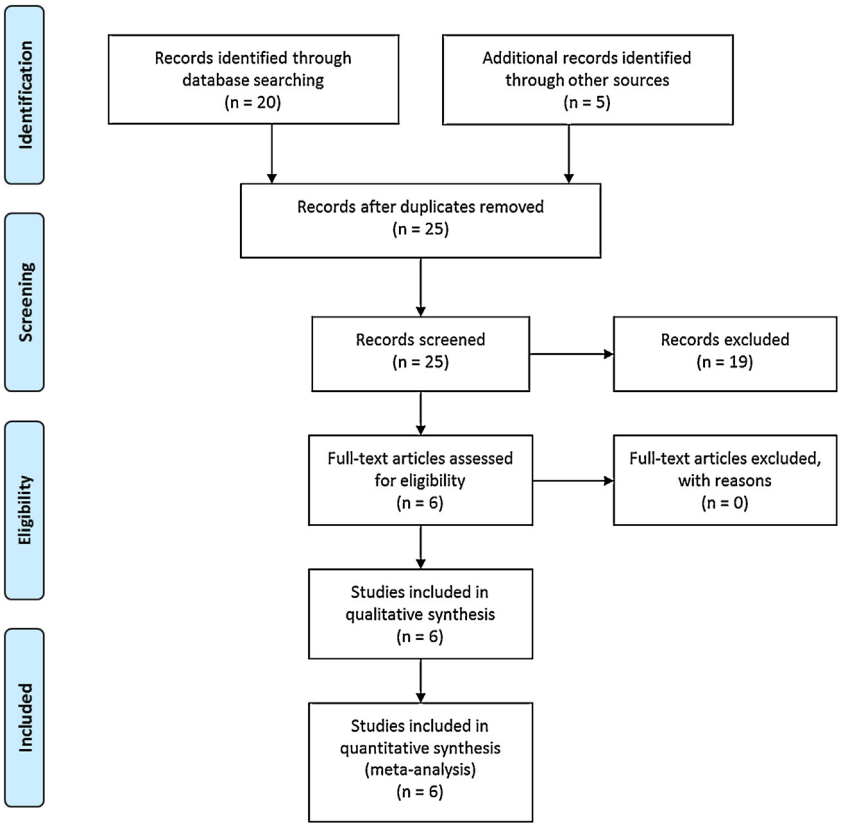

Fig. 1. Flowchart of the article selection process.

Table 3
Meta-analysis data of the included IVF articles.

Studies	Analysis method	Clinical pregnancy rate?		Total OR (95% CI)
		Abnormal VF	Normal VF	
Haahr et al. [34]	qPCR	2 (9)	27 (44)	0.13 (0.03 - 0.60)
Mangit-Bertrand et al. [35]	qPCR + Nugent score	8 (28)	92 (33)	0.77 (0.33 - 1.81)
Selim et al. [36]	Nugent score	9 (35)	21 (47)	0.61 (0.22 - 1.64)
Eckert et al. [37]	Nugent score	3 (30)	38 (47)	0.49 (0.12 - 2.01)
Liversedge et al. [38]	Nugent score	24 (32)	64 (30)	1.15 (0.66 - 2.03)
Gaudoin et al. [39]	Nugent score	7 (18)	53 (26)	0.61 (0.26 - 1.47)
Total (fixed effects)		53 (26)	295 (33)	0.70 (0.49 - 0.99)
Total (random effects)		53 (26)	295 (33)	0.66 (0.40 - 1.08)

Data are represented as n (percent of patients per group). Heterogeneity testing resulted in a Cochran's Q score of 8.09 with 5 degrees of freedom and a P value of 0.15. I^2 for inconsistency was 38.23% with a 95% confidence interval of 0.00–75.44. VF = Vaginal flora.

conception within IVF trajectories and found no significant differences of conception rates between participants with abnormal vaginal microbiota and women with normal vaginal microbiota composition. These findings were confirmed by the study of Selim et al. (57). Besides the quantifications of the vaginal culture they also cultured the embryo transfer catheter-tip. The rate of conception in participants with *Streptococcus viridans*-positive catheter tips compared with catheter tips with no bacteria was 18% and 39% respectively ($p < 0.001$).

In Table 4 we describe a number of characteristics that may have affected the IVF outcome of patients in the included studies, and therefore affected the outcome of the meta-analysis.

Risk of bias within the studies

The risk of bias has been assessed using the QUIPS tool [31]. The results of the evaluation of the risk of bias are shown in supplementary table S1. Haahr et al. gave an accurate description of the source population of 130 patients undergoing IVF treatment, in two fertility clinics in Denmark [34]. A baseline study sample description provided the characteristics of the participants with exception of the sampling frame and the recruitment. Furthermore, no method for sampling frame and recruitment, or in- and exclusion criteria were described, suggesting that the association between the prognostic factor and the outcome is different for participants and eligible nonparticipants. Gaudoin et al. mentions an underrepresentation of certain IVF indicators in the included patient groups, but does not correct for that in their study [39]. In the study of Liversedge et al. patients having an IVF treatment in the clinic were asked for their participation with no further eligibility criteria mentioned [38]. Within their study population the prevalence of BV was twice that found in patients attending obstetrics and gynecology clinics in the UK (using the same method and criteria to define the grade of the vaginal microbiome).

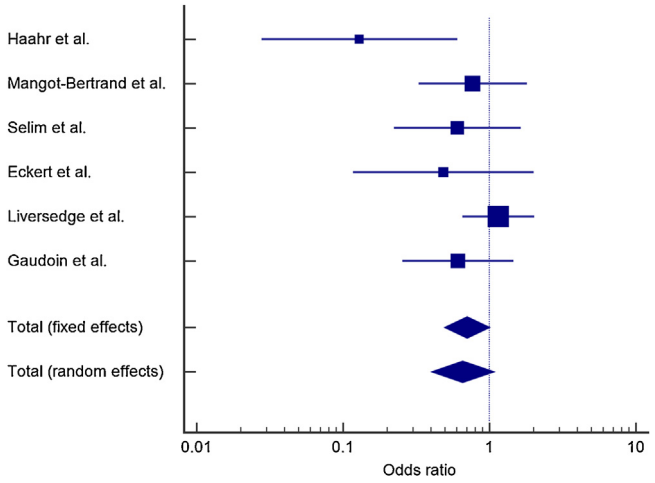

Fig. 2. Forest plot of clinical pregnancy rates following IVF procedure in patients with abnormal vaginal microbiota (BV) compared with patients with normal vaginal microbiota The figure represents the individual raw OR's with 95% Confidence intervals and the combined OR of the fixed effect model and the random effect model. The data of these models can be seen in Table 3. The size of the squares for the individual studies was proportional to the weight of the study.

It is not clear how this difference can be explained. Attrition bias was likely when missing data was inadequately reported. Haahr et al. described that only 65% of the 130 patient were eligible for analysis due to exclusion of vaginal swabs older than 2 months from embryo transfer [34]. Patient characteristics were provided from the whole cohort of 130 participants but no separate description of the loss to follow-up participants were provided. The same applies to the studies of Liversedge et al., Haahr et al., Selim et al., Eckert et al, and Gaudoin et al. [34,36–39]. High risk of bias was found within the domain "study confounding". Important confounders such as duration of infertility were not provided in the key characteristics table in the study of Haahr et al. [34]. Haahr et al. described blinded measurements to prevent misclassification bias. Herewith they prevented inter-rater variability. Liversedge et al. also described two microscopists, who were blinded to each other's results and the clinical information of the patient [38]. The weighted kappa (0.88) was measured for concordance of the assessors. The other studies provided no information of their blinding which makes the risk of bias very likely. Lastly, the statistical analysis contributed to high risk of bias within the studies. Selim et al. for instance showed a table categorizing the vaginal gram stain and the proportions of biochemical pregnancy and early pregnancy loss [36]. This data was used to summarize the potential predictors as 'influenced by vaginal, embryo transfer catheter microbiology and pH value' without statistically discriminating them. Often the presentation of the data was insufficient to assess the adequacy of the analytic strategy.

Discussion

In this review we have used meta-analysis to show the importance of the microbiota composition during the conception and initial gestation through IVF. The positive effect that healthy vaginal microbiota was found to have on the outcome of IVF treatment underlines the importance of microbiota, and microbiota-focused studies investigating the early pregnancy development during IVF. However, further analysis of the included studies showed widespread heterogeneity in the methodologies of the studies. This means that more studies using more similar methodologies are needed to draw a strong conclusion through meta-analysis.

In another systematic review by van Oostrum et al. [40] the primary focus was on the relation between the later stages of IVF-induced pregnancy and microbiota [40]. However, part of their study was also focused on the effect of BV on conception rates. At the time, van Oostrum et al. found no impingement of BV on clinical conception rates. By including more up-to-date literature and specifically focusing on the early pregnancy development

Table 4
Clinical and participant characteristics of study populations.

Study	Haahr et al. [34]	Mangot-Bertrand et al. [35]	Selim et al. [36]	Eckert et al. [37]	Liversedge et al. [38]	Gaudoin et al. [39]
Age	31 (median)	33.5 (mean)	21-44 (range)	21-45 (range)	33 (median)	No info
Indication for IVF	Male factor / Tubal factor / Endometriosis / Unknown / Ovarian factor / Single or lesbian	Male factor / Tubal factor / Endometriosis / Mixed / Unexplained	No info	No info	Tubal factor (with and without hydrosalpinx) / Sperm dysfunction (with and without sperm antibodies) / Endometriosis / Ovulatory disorder / Unexplained	Tubal factor / Endometriosis / Ovulatory problem / Male factor (donor semen) / Unexplained
Pituitary Down-regulation	No info	No info	No info	No info	Long agonist protocol	Long agonist protocol
Antibiotics	No	No	Yes, beginning at ovum pick up (Metronidazol, Once daily for 5 days)	Yes, before ovum pick up (Doxycycline 100 mg, twice daily for 5 days)	Yes, after postive *C. trachomatis* immunofluoresence test (Ofloxacin), Timing unknown	No info
Timing of sampling	95% of samples between 2-4 weeks before start of IVF. Maximum 2 months before transfer	During ovum pick up	During ovum pick up	During embryo transfer	During ovum pick up	During ovum pick up
Country / Ethnicity	Denmark / 90% Caucasian	France / no info	Egypt / no info	USA (Washington) / no info	United kingdom / no info	United kingdom / no info
Smoking (%)	8/130 (6.2)	72/307 (235)	No info	No info	No info	No info
IVF cycle of sampling	No info	Ranked cycles (1,2,3, > = 4)	First cycle	First cycle	One cycle, no info on the cycle number	No info on the cycle number, multiple cycles were possible
Transfer stage	No info	No info	4-8 cell stage	4-8 cell stage	No info	No info

phase, we have indicated that there is an important effect. This can primarily be attributed to the increased size of the aggregated cases and control groups in the meta-analysis of this review. More recently, Haahr et al. reviewed the relation between dysbiotic vaginal microbiota and IVF outcome, with clinical and biochemical pregnancies as secondary outcomes [41]. Due to less stringent criteria, such as using the life birth rate instead of clinical pregnancy, they were able to analyse more studies. Notably, Amsel or Nugent criteria were not specifically required for definition of BV. Additionally, definitions of clinical and biochemical pregnancies were not specifically defined, even though our review noted these as subjects of high variability between studies. In line with our findings, the quality of the extracted evidence was scored as very low using the GRADE tool. Once more, our data highlight the need for an increase in study sizes and repetition of studies relating to urogenital microbiota and reproductive health, and importantly give an overview of the research characteristics most likely to affect the outcome of the studies.

The results from the meta-analysis shows a relatively large variation in the effects found in individual studies. Our findings show that the methodology of the studies is likely the reason for this variation, as it differed on multiple points. Firstly, all included studies had slightly different definitions of conception or early pregnancy. Our definition of an ultrasound proven fetal heartbeat and/or hCG results before 10 weeks gestation captured all included articles. However, this still left room for definitions of clinical pregnancy, including only hCG results in Selim et al. and fetal heartbeat proven by ultrasound at 4 weeks of gestation by Liversedge et al. [36,38]. We find these definitions unsatisfactory, as they skirt the edges of proper diagnosis of clinical pregnancies.

Secondly, the included studies had a number of variations in the IVF protocols. Table 4 shows the varying IVF characteristics as they were used in the respective studies. Especially notable is the varying time points of sampling for the studies. Sampling during the follicular puncture means that the increased estrogen levels are likely to affect the microbiota composition [42,43]. Sampling should ideally be done 2–4 weeks before the start of the IVF procedure, as hormonal levels will be lowest at that point. Additionally, some studies where antibiotics were given had sampling take place shortly after or during the antibiotic therapy. These antibiotics likely affected the compositions of the microbiota in the patients. For ideal sampling, antibiotic use prior to the sampling should be taken as an exclusion criterion for possible patients willing to participate in these studies. Other varying characteristics in Table 4 show the need for uniformity in sampling during IVF studies to produce a study that can be properly compared to previously performed studies. Furthermore, it shows the need for care on the side of the reader of these articles, as these varying characteristics mean study results are not always immediately translatable to other settings.

Another factor that can influence the outcomes of this study is microbiota on the endometrium, which was previously thought of as sterile. A number of studies found that an abnormal endometrial microbiota is associated with implantation failure in reproductive trajectories of subfertile women [44,45]. A study conducted by Moreno et al. [45] compared the vaginal microbiota with the endometrial microbiota in a cohort of healthy and fertile women [45]. All of the endometrial samples revealed bacterial colonization consisting of *Lactobacillus* and less abundant anaerobic bacteria such as *Gardnerella*, *Prevotella* and *Atopobium*. About 20% of the women showed bacterial colonization in their endometrial samples that differed significantly from their vaginal samples, which suggests that the endometrial microbiota may be an independent effector of conception. Even then, a link between the vaginal microbiota composition and the endometrial microbiota could further increase the value of knowing the vaginal microbiota composition before IVF treatment begins. In that sense, knowledge of the interactions between the vaginal microbiota, the endometrial microbiota, and the reproductive tract is crucial to improve the fertility trajectory of subfertile women [46]. Notable, a recent study by Benner et al. corroborates this by linking the uterine microbiota to the receptivity and fertility of the endometrium [47]. One aspect of special interest is chronic endometritis, which occurs in roughly 45% of all subfertile women [48]. The chronic inflammation is suggested to prevent embryo implantation. Antibiotic intervention of chronic endometritis after bacterial culture revealed improved reproductive results in women with recurrent implantation failure, showing a clear interaction between the host and colonizing bacteria [49]. Whether the vaginal microbiota can be linked to the development or severity of chronic endometritis still needs to be studied. A clear link would make diagnosis and treatment of chronic endometritis easier, and potentially lead to improvement of early pregnancy development rates in subfertile women.

There are limitations to consider in this study. The size of the effect found during the meta-analysis is notable. An odds ratio of 0.7 in favor of a healthy vaginal microbiota on the outcome of IVF treatment is much less than that suggested by most of the included studies. Although this result is still significant, it suggests the contribution to IVF failure of other factors that may act together with the resident microbiota.

Additionally, this study has some inherent limitations, including the length of time between included studies. This may mean that the methods applied in earlier studies do not represent their findings as accurately as more recent studies. We believe that the size of the meta-analyses partially corrected for this.

Conclusions

In this review and related meta-analysis we show in a large aggregated cohort that abnormal vaginal microbiota has a strong correlation with the failure of IVF through the absence of first trimester pregnancy. In addition to this, we conclude that there is currently too much heterogeneity in the methodology of studies into the vaginal microbiota during IVF, leading to poor comparability. We suggest researchers and readers of the literature to pay special attention to the possible confounding factors (e.g. hormone levels) that can effect study outcomes, and to make a concerted effort to have a uniform methodology with the current literature, unless deviation is strictly necessary.

Author's roles

MB and MS performed the meta-analysis and wrote Introduction, Materials and Methods, Results, and Discussion sections. SO and SAMé contributed by evaluating the review and ensuring the quality of the manuscript and aided in the evaluation of the data. All authors have approved the final article.

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Conflict of interest

All authors declare they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jogoh.2019.01.007.

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