Infertility and vaginal microbiome: review study

Infertilidade e microbioma vaginal: estudo de revisão

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ABSTRACT

Introduction: Infertility is an important public health problem and has many causal factors. Previous findings, based mainly on culture techniques, suggest an association between infertility and changes in the vaginal microbiome. The metagenomic approach allowed the discovery of new bacterial species, previously unidentified in the female genital tract, permitting a deeper knowledge of the role of vaginal microbiome in female reproductive health. **Objective:** To evaluate the association of changes in the vaginal microbiota with infertility, and its repercussions on the outcome of assisted reproduction techniques after a decade of the Human Microbiome Project. **Methods:** A systematic search was carried out in the MEDLINE database between September and November 2018, by selecting 14 studies, associating vaginal microbiome with infertility, or with results of assisted reproduction techniques. **Results:** The findings showed a higher prevalence of bacterial vaginosis, and increased microbial diversity in the vagina of infertile women. Regarding the success of assisted reproduction techniques, most studies did not show any significant association between bacterial vaginosis and reduction in pregnancy rates. **Conclusion:** Further studies are needed to better understand the influence of the balance of vaginal microorganism species on female reproductive health, addressing the microbiome composition in contexts beyond in vitro fertilization techniques.

Keywords: infertility; microbiota; vaginosis, bacterial; Gardnerella vaginalis; in vitro fertilization; reproductive techniques, assisted.

RESUMO

Introdução: A infertilidade é um importante problema de saúde pública e possui muitos fatores causais. Achados prévios, baseados principalmente em técnicas de cultura, sugerem uma associação entre infertilidade e alterações do microbioma vaginal. A abordagem metagenômica permitiu a descoberta de novas espécies bacterianas, anteriormente não identificadas, no trato genital feminino, possibilitando um conhecimento mais aprofundado do papel do microbioma vaginal na saúde reprodutiva feminina. **Objetivo:** Avaliar a associação das alterações da microbiota vaginal com a infertilidade e suas repercussões no resultado das técnicas de reprodução assistida após uma década do Projeto Microbioma Humano. **Métodos:** Foi realizada uma busca sistemática na base de dados MEDLINE, entre setembro e novembro de 2018, e selecionados 14 estudos que associavam o microbioma vaginal com a infertilidade ou com resultados de técnicas de reprodução assistida. **Resultados:** Os achados evidenciaram uma maior prevalência de vaginose bacteriana e um aumento da diversidade microbiana na vagina de mulheres inférteis. Com relação ao sucesso das técnicas de reprodução assistida, a maioria dos estudos não evidenciou uma associação significante entre a vaginose bacteriana e a redução nas taxas de gravidez. **Conclusão:** São necessários novos estudos para melhor compreender a influência do equilíbrio das espécies de microrganismos vaginais na saúde reprodutiva feminina, abordando a composição do microbioma em contextos além das técnicas de fertilização in vitro.

Palavras-chave: infertilidade; microbiota; vaginose bacteriana; Gardnerella vaginalis; fertilização in vitro; técnicas de reprodução assistida.

INTRODUCTION

The vaginal microenvironment and a brief history of taxonomy

Since the times of Aristotle, and later on, of Lineu, living organisms were classified into two kingdoms: the Kingdom Animalia, which included heterotrophic organisms that, in general, move in the environment, capture and ingest food, including the protozoan, considered unicellular animals, and metazoans, or multicellular animals; and the Kingdom Plantae (Vegetal Kingdom) which included the photosynthesizing autotrophs, prokaryotes or eukaryotes. In the latter, there were non-photosynthesizing bacteria and fungi, considered achlorophyllous plants[1]. Until then, the microorganisms that inhabited the vagina were in their set called vaginal flora.

With the advances in Biology, new proposals for classification emerged, and a system of four kingdoms was created. Bacteria were then included in the Moneran Kingdom, which represented all prokaryotes organisms[2]. In 1959, Whittaker presented a new proposal for classifying living organisms into five kingdoms, including the Fungi Kingdom[3].

So far, all classifications characterized bacteria based on phenotypic markers, such as morphology, growth or pathogenic potential, as well as physiological and biochemical properties[4].

In 1990, a new classification was proposed based on the analysis of rRNA (Ribosomal Ribonucleic Acid), dividing living organisms into three domains (a taxonomic category superior to kingdom): Archea, Bacteria, and Eucaria, based on phenotypic data, chemotaxonomic, genotypic, and phylogenetic evolution[4,5].

Microbiota was then defined as the "set of microorganisms that exist within a given environment, and are revealed through molecular techniques"[6], and the consequent denomination of vaginal microbiota.

The Human Microbiome Project, initiated in 2007 by the National Institutes of Health (NHI), performed metagenomic studies through sequencing and analysis of high-performance DNA, which helped to characterize the bacterial population of various sites of the human body[7]. These molecular techniques take advantage of the 16S rRNA gene, which is unique to bacteria and contains several hypervariable regions that serve as identifiers for a genus or bacterial species[8]. Human microbiome was then defined as "the total of commensal,

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symbiotic and pathogenic microorganisms, and their genetic material existing in the human body"[9].

Thus, the vaginal microbiome was also characterized, and areas previously considered sterile, such as the uterine cavity and the placenta, were evidenced a microbiome themselves[10,11].

With the advances in molecular biology, microbial taxonomy and phylogenetic experienced rapid changes, making the microbial classification process quite complex[12], bringing us the following question: What will be the proper term to define the vaginal microenvironment in one or two decades when new genomic technologies will have surely emerged?

Vaginal microbiota and infertility

Historically, bacteria are identified using Gram staining or culture-based techniques. Only 20% of the bacteria in the human body, however, can be cultivated, and culture methods can therefore underestimate the diversity of such microbiome[13].

Information obtained from the combination of molecular biology methods with culture-based methods can clarify not only the role of bacteria in gynecological health, but also how the shift of vaginal microbiota affects the susceptibility to diseases.

Infertility, in turn, is defined as the inability to conceive after 12 months of regular sexual activity, without the use of contraceptive methods[14]. It is an important public health problem, globally affecting around 9% of women in the menacme, and approximately 1.5 million women in the United States[15]. It is estimated that one in every six couples will present problems with fertility during their reproductive life[16].

Several decades ago, studies showed that some microorganisms, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, have a well-established association with infertility[15-18].

The association between bacterial vaginosis (BV) and infertility has also been studied, with findings of BV prevalence up to three times higher among infertile women compared to fertile women[19].

Moreover, with the development of molecular biology techniques, new microorganisms associated with infertility were identified[20,21].

Disorders in the composition of bacterial communities seem to contribute to disease conditions, and there are growing evidences that vaginal microbiota, which is unique for each woman and presents variations associated with the menstrual cycle, sexual activity, stage of reproductive life, habits and external factors, plays an important role in determining the multiple facets of reproductive health[6,22].

OBJECTIVE

The objective of this review is to evaluate the association of vaginal microbiota alterations with female infertility, and its repercussions on the results of assisted reproduction techniques, addressing the findings of the last decade after the advent of the Human Microbiome Project.

METHODS

Research was carried out from September to November 2018. We sought to identify studies addressing associations between vaginal microbiome and women with infertility diagnosis. Studies in English, published over the last ten years (January 2008 to November 2018), such as meta-analyses, original cross-sectional, cohort and case-control studies were evaluated.

The levels of evidence of the mentioned studies were evaluated according to the Oxford Centre for evidence-based Medicine levels (March 2009 – www.cebm.net)[23].

A systematic search in the MEDLINE database was conducted using the following terms from the Medical Subject Headings (MeSH) Dictionary: ("Infertility") AND ("Microbiota" OR "Vaginosis, Bacterial" OR "Gardnerella vaginalis"). Complementary search was performed using the terms of the MeSH: ("Fertilization in Vitro" OR "Reproductive Techniques, Assisted") AND ("Microbiota" OR "Vaginosis, Bacterial" OR "Gardnerella vaginalis").

RESULTS

Thirty studies were initially identified, fourteen of which were selected after reading the abstract. Those that evaluated the microbiome of the male reproductive tract and studies not performed in humans were excluded, according to the flowchart shown in **Figure 1**.

Studies were divided into two groups: those comparing the vaginal microbiota of fertile and infertile women; and those evaluating the association of vaginal microbiome with the results from assisted reproduction techniques.

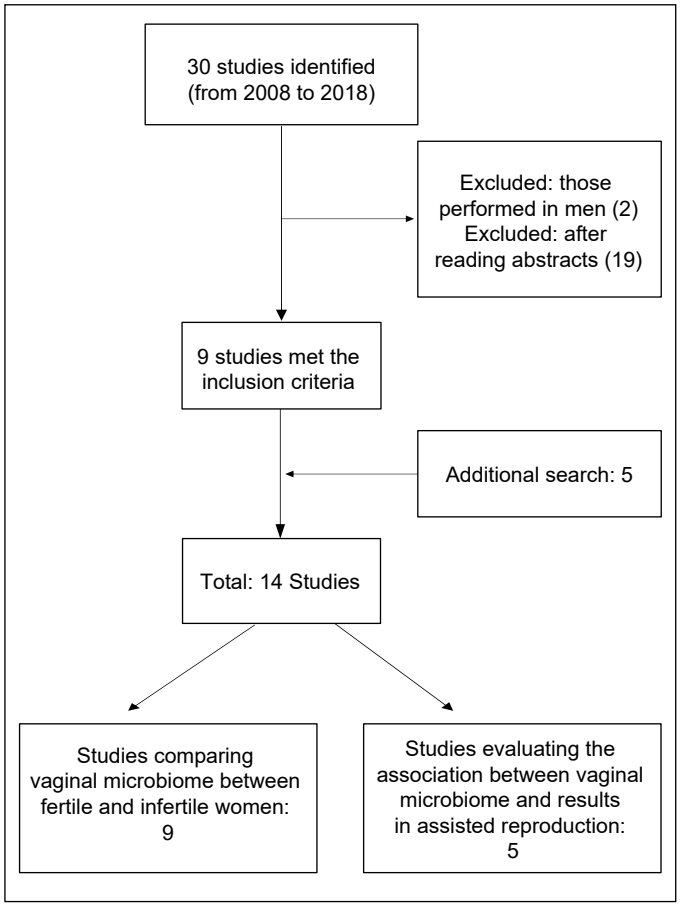

Figure 1 – Flowchart of the selected studies.

Vaginal microbiome and infertility

Eight studies[17-30] were selected by comparing the vaginal microbiome of fertile and infertile women, totaling 3,611 patients, in addition to a systematic review with meta-analysis[19], which evaluated twelve articles, and a total of 3,229 patients. The origin of each study and the diagnostic methods used to characterize the microbiota are described in **Table 1**.

Mania-Pramanik et al.[24] evaluated a group of 510 women: 112 (21.96%) had a diagnosis of infertility; 115 (22.5%) a history of repeated miscarriage; 100 (19.6%) women presented signs and symptoms of lower genital tract infections; 102 (20%) were healthy pregnant women (gestational age from 2 to 3 months); and 81 (15.9%) were asymptomatic women. Reproductive tract infections, such as bacterial vaginosis (BV), *Candida sp.*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Human papillomavirus* (HPV) were investigated with the Nugent score, fresh examination, and Polymerase Chain Reaction (PCR). The BV rate was higher (25.9%; 29/112) among infertile women when related to the other groups, evidencing a statistically significant association (p=0.0001).

A study[25] evaluated the vaginal microbiota, with Gram scoring, culture and molecular biology (BD Affirm™ VPIII [Becton, Dickinson and Company, Franklin Lakes, NJ, USA], and COBAS Amplicor™ [Roche, Milan, Italy]), of 952 women divided into two groups: fertile women with vaginal discharge (N=556); and asymptomatic infertile women who would undergo assisted reproduction procedures (N=396). Statistical analysis showed a significant association (p≤0.001) between the presence of *Ureaplasma urealyticum*, *Streptococcus agalactiae*, *Gardnerella vaginalis*, and *Enterobacteriaceae,* or *Enterococci* in the vaginal microbiota with the decrease in *Lactobacilli* species. The study stated that the reduction of *Lactobacilli*, and the presence of a high number of polymorphonuclear in the vaginal content are important parameters to be considered when analyzing the health status of the female urogenital tract.

In a prospective cohort study[26], evaluating 874 infertile women, and 382 fertile controls, the prevalence of BV evaluated with the modified Spiegel method was also significantly higher among infertile women. The prevalence of BV was of 45.5% (398/874) in infertile women, compared to 15.4% (59/382) in fertile women (p<0.001). The highest prevalence of BV in infertile patients was found in women with Polycystic Ovary Syndrome (PCOS) (60.1%), and Infertility Without Apparent Cause (IWAC) (37.4%).

Van Oostrum et al.[19], in a systematic review with meta-analysis, evaluated 12 Nugent scores studies, and demonstrated that BV is significantly more prevalent in infertile women (OR=3.32; 95%CI 1.53-7.20). On the other hand, in this study, infertile women for tubal factor had a significantly higher prevalence of BV (OR=2.77; 95%CI 1.62-4.75), compared to women with other infertility causes.

The prevalence of BV diagnosed with the Amsel criteria among patients with infertility for tubal factor was evaluated in a study with 356 women (178 fertile; 178 with tubal infertility)[27]. Bacterial vaginosis was observed in 50 women (28.1%) with tubal infertility, compared to 14 (7.9%) fertile women (p<0.001). Infertile women showed a higher risk of bacterial vaginosis when belonging to a lower socioeconomic level (OR=11.89; 95%CI 5.20-27.69), using vaginal showers (OR=19.15; 95%CI 7.2-47.75), using agents that dry out the vagina (OR=17.04; 95%CI 6.91-43.24), initiating their sexual activity early (OR=32.08; 95%CI 12.02-88.89), or having a history of sexually transmitted infections (OR=12.42; 95%CI 5.36-29.35).

The prevalence of asymptomatic BV diagnosed with the Nugent score in fertile women (N=84), and interfile women (N=116) was also evaluated in another study[28]. It was observed that the vaginal microbiota of healthy women was dominated by *Lactobacillus* (40, 27.8%), whereas the percentage of microbiota with predominance of *Lactobacillus* in the group of infertile women was relatively low (4, 3.5%). Asymptomatic bacterial vaginosis was present in 27.6% (32/116) of infertile women, while in the fertile women group only 7.1% (6/84) had asymptomatic BV (p≤0.05).

Wee et al.[29] examined the vaginal, cervical and endometrial microbiota through 16S rRNA sequencing of 15 women with a history of infertility, compared with 16 fertile women (controls), and observed that infertile women were more likely to present more often two most prevalent microorganisms: *Ureaplasma* in the vagina (p=0.042), and *Gardnerella* in the cervix (p=0.044). Four out of five women with infertility colonized by *Ureaplasma* also had vaginal microbiota dominated by *L. inners* (p=0.015). There was no statistically significant difference in the expression of genes selected in the endometrium and microbiome composition between cases and controls.

Table 1 – Comparison studies of reproductive tract microbiome among fertile and infertile women.

Study/Year	Country	Population	Diagnosis Method	Microbiome Site
Graspeuntner et al. (2018)(17)	Germany	N=210	Culture, PCR, 16S rRNA	Cervical
Van Oostrum et al. (2013)(19)	Belgium	-------	Nugent score	Vaginal
Mania-Pramanik et al. (2009)(24)	India	N=510	Nugent score, PCR and fresh examination	Vaginal
Casari et al. (2010)(25)	Italy	N=952	Gram, culture, molecular biology	Vaginal
Salah et al. (2013)(26)	Egypt	N=1,256	Spiegel modified	Vaginal
Durugbo et al. (2015)(27)	Nigeria	N=356	Amsel	Vaginal
Babu et al. (2017)(28)	India	N=200	Fresh examination, Gram and Nugent score	Vaginal
Wee et al. (2017)(29)	Australia	N=31	16S rRNA	Vaginal, cervical, and endometrial
Campisciano et al. (2017)(30)	Italy	N=96	rDNA V3-16S	Vaginal and cervical

PCR: polymerase chain reaction.

The vaginal and cervical microbiota were characterized by sequencing the rDNA V3-16S gene in a study[30] that evaluated 14 women with idiopathic infertility, 13 women with non-idiopathic infertility, 39 fertile women with BV and 30 healthy women (controls). The affected groups (idiopathic, non-idiopathic infertility, and BV) had α-diversity (diversity of microorganisms within the same sample) greater than that of the control group. The controls were significantly different from the group with idiopathic infertility ($p<0.05$), non-idiopathic infertility ($p<0.01$), and fertility with vaginosis ($p<0.01$). An unequal distribution of *Lactobacilli* was observed among the studied groups. *L. inners* acted as a marker of microbiome health based on its prevalence in each group: controls (51%), compared to idiopathic infertility (29%), non-idiopathic infertility (18%), and vaginosis (15%). There was a decrease in the prevalence of *L. crispatus* in infertile women, and with BV (25 and 6%, respectively) in relation to women with idiopathic infertility (31%), which, in turn, was lower than in controls (36%). There was higher prevalence of *L. gasseri* in the group of women with idiopathic infertility.

In order to evaluate the bacterial composition and other microorganisms of the reproductive tract of infertile women with infectious cause, a study[17] recruited a group of 210 women, as follows: 26 women with non-infectious infertility; 21 women with infectious infertility, 89 fertile women; and 54 sex workers. Three cervical samples were collected: the first, for conventional culture of commensal and pathogenic bacteria of the urogenital tract; the second, for PCR test for *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis* and *U. urealyticum*; and, the third, for 16S rRNA gene sequencing. Women with infectious infertility differed significantly in the frequency of *C. trachomatis* infections compared to fertile controls ($p<0.01$) and women with non-infectious infertility ($p<0.05$). No differences were observed between groups with HPV, HSV, *Treponema pallidum*, HIV, or hepatitis B and C infections. Despite the increase in the rate of positive tests for *U. urealyticum/parvum* (41.30%), *N. gonorrhoeae* (7.90%), *M. genitalium* (9.50%), and *M. hominis* (34.90%) among sex workers, no significant differences were observed between the other groups.

The analysis of the microbiota using amplification of the 16S sequence of cervical smears revealed significant differences between the group of women with infectious infertility and the fertile controls' as to the prevalence of *Gardnerella* (10.08% *vs*. 5.43%). The α-diversity varied between groups: among fertile women, communities dominated by *Lactobacillus* prevailed; among women with infectious infertility, communities dominated by *Gardnerella* occurred more often; and a diversity of communities in other groups was observed.

Results in assisted reproduction

Five studies[19-33] evaluated the association between vaginal microbiota and the results of assisted reproduction techniques, as shown in **Table 2**.

Selim et al.[31] investigated with culture and Nugent score the impact of vaginal bacterial microbiota on the rates of live births during Intracytoplasmatic Sperm Injection (ICSI). In women with bacterial vaginosis, intermediate microbiota, and normal microbiota, the conception rates were of 35 (9/26), 42 (14/33), and 58% (7/12), respectively, with no statistically significant difference between groups (p=0.06). The conception rate was of 29% (2/7) in those with *S. viridians*, and 22% (2/9) with *Staphylococcus aureus* isolated from the embryonic transfer catheter tip, 39% (18/46) when no bacteria was isolated from the catheter tip, and 80% (8/10) when the *Lactobacilli* H2O2 producers were recovered ($p<0.001$).

With 16S rRNA sequencing techniques, a study[21] evaluated the composition of the vaginal microbiota on the day of embryo transfer in women undergoing *in vitro* fertilization (IVF). Through a sophisticated calculation of the diversity index (Shannon Diversity Index), comparing vaginal fluid swabs of women who had a live newborn to those who did not succeed, it was demonstrated that a lower rate of diversity of vaginal microbiota correlated with the highest rate of live births (p=0.01).

With the Nugent score and PCR, a study[32] evaluated the prevalence of BV in 307 infertile women submitted to *in vitro* fertilization, and the impact of BV on the pregnancy rate after IVF. The embryo implantation rate did not decrease significantly, when comparing the normal vaginal microbiota women group to the BV women group (36.3 *vs.* 27.6%, respectively; p=0418), nor the rate of clinical pregnancy (33.1 *vs.* 27.6%, respectively; p=0.68). Obstetric results (frequency of early miscarriage, premature rupture of membranes, gestational age at delivery and delivery or birth weight) also showed no statistically significant differences.

In the systematic review by Van Oostrum et al.[19], it was demonstrated that BV was not associated with decreased conception rates (OR=1.03; 95%CI 0.79-1.33), nor with increased risk of abortion in the first trimester (OR=1.20; 95%CI 0.53-2.75), but associated with a significantly elevated risk for pre-clinical gestational loss (OR=2.36; 95%CI 1.24-4.51). None of the studies evaluated in the review found an association between abnormal microbiota and conception rates after IVF.

Another study[33] evaluated the diagnostic performance of PCR tests, compared to the Nugent score for abnormal vaginal microbiota, and to predict the success rate of the treatment of women submitted to IVF. The prevalence of BV by the score was of 21% (27/130), whereas the prevalence of abnormal vaginal microbiota defined by

Table 2 – Studies evaluating the association between the reproductive tract microbiota and the results of assisted reproductive techniques.

Study/Year	Country	Population	Diagnosis Method	Microbiome Site
Van Oostrum et al. (2013)(19)	Belgium	-------	Nugent score	Vaginal
Hyman et al. (2012)(21)	USA	N=30	16S rRNA	Vaginal
Selim et al. (2011)(31)	Egypt	N=71	Culture and Nugent score	Vaginal
Mangot-Bertrand et al. (2013)(32)	India	N= 307	PCR and Nugent score	Vaginal
Haahr et al. (2016)(33)	Denmark	N= 130	PCR and Nugent score	Vaginal

PCR: polymerase chain reaction.

PCR was of 28% (36/130), with high concentrations of *Gardnerella vaginalis,* and/or *Atopobium vaginae*. The PCR approach showed sensitivity and specificity, respectively, of 93 and 93% for BV defined with the Nugent score. In addition, PCR allowed the stratification of Nugent's intermediate microbiota. A total of 84 patients completed IVF treatment. The overall rate of clinical pregnancies was of 35% (29/84). Curiously, only 9% (2/22) with abnormal microbiota defined by PCR obtained a clinical gestation (p=0.004).

Table 3 summarizes selected studies with their respective levels of evidence and results.

CONCLUSION

Studies suggest that bacterial vaginosis and abnormal vaginal microbiota are significantly more prevalent in infertile women, compared to fertile patients, and that the healthy vaginal environment has lower microbial diversity.

The role of vaginal microbiome in the success or failure of assisted reproduction techniques (ART) is still unclear, and most of the selected studies did not reveal a significant association between bacterial vaginosis and reduction in pregnancy rates. Hence, further studies addressing the microbiome composition in contexts beyond the *in vitro* fertilization techniques are needed.

Participation of each author

Dr. Muse Santiago de Oliveira did the conception of research, conducted research of articles in MEDLINE, and wrote this article. Dr. Francisco das Chagas Medeiros conducted research of articles and helped to write this article. Dr. José Eleutério Jr. helped in the conception of the idea and reviewed this article.

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Conflict of interests

There is no conflict of interest to be reported.

Table 3 – Summary of selected studies results.

Reference	Type of study	No. Patients	Results (P value)
Graspeuntner et al. (2018) (B3B)(17)	Case-control study	N=210	Frequency of *C. trachomatis* greater in women with infectious infertility compared to fertile controls (p<0.01), and women with non-infectious infertility (p<0.05).
Van Oostrum et al. (2013) (B2A)(19)	Systematic review with meta-analysis	N=3,229	BV more prevalent in infertile women, compared to women in prenatal monitoring (OR=3.32; 95%CI 1.53-7.20), and associated with an increased risk of early gestational loss (OR=2.36; 95%CI 1.24-4.51). BV was not associated with decreased rates of conception (OR=1.03; 95%CI 0.79-1.33) nor with increased risk of abortion in the first semester (OR=1.20; 95%CI 0.53-2.75).
Hyman et al. (2012) (B2B)(21)	Cohort study	N=30	The diversity of species varied in different hormonal environments, and on the day of embryo transfer correlated with the outcome (live births/no live births) (p=0.01).
Mania-Pramanik et al. (2009) (B3B)(24)	Cross-sectional study	N=510	Statistical analysis between negative and positive women for BV revealed a statistically significant association (p=0.0001) with infertility.
Casari et al. 2010 (B3B)(25)	Case-control study	N=952	Significant association (p≤0.001) between the decrease in *Lactobacilli* and the increased prevalence in *Ureaplasma urealyticum*, *Streptococcus agalactiae*, *Gardnerella vaginalis*. *Enterobacteriaceae,* or *Enterococci* in the vaginal flora.
Salah et al. (2013) (B2B)(26)	Cohort study	N=1,256	BV higher prevalence in infertile women than in fertile women (45.5% *vs.* 15.4%). The highest prevalence was observed in PCOS (60.1%), and IWAC (37.4%) patients. Cumulative pregnancy rates among patients with PCOS and IWAC were significantly higher among patients who were treated for BV.
Durugbo et al. (2015) (B3B)(27)	Cross-sectional study	N=356	Prevalence of higher BV among women with tubal infertility compared to fertile women (p<0.001).
Babu et al. (2017) (B3B)(28)	Case-control study	N=200	Asymptomatic BV present in 27.6% of infertile women, and in 7.1% of fertile women (p≤0.05).
Wee et al. (2017) (B3B)(29)	Case-control study	N=31	Infertile women showed two predominant microorganisms: *Ureaplasma* in vagina (p=0.042), and *Gardnerella* in cervix (p=0.044); not adjusted.
Campisciano et al. (2017) (B3B)(30)	Case-control study	N=96	The α-greater diversity in patients with idiopathic infertility (p<0.05), non-idiopathic (p<0.01), and BV (p<0.01) compared to the control group.
Selim et al. (2011) (B2B)(31)	Cohort study	N=71	In women with bacterial vaginosis, intermediate flora and normal flora, the conception rates were of 35 (9/26), 42 (14/33), and 58% (7/12), respectively (p=0.06).
Mangot-Bertrand et al. (2013) (B2B)(32)	Cohort study	N=307	There was no significant decrease in the rates of embryo implantation by comparing the groups with normal vaginal flora and BV (p=0.418), nor in the clinical pregnancy rates between the two groups (p=0.68).
Haahr et al. (2016) (B2B)(33)	Case study	N=130	BV prevalence with Nugent and PCR were highly correlated; women with abnormal microbiota defined by PCR were significantly less likely to obtain a clinical pregnancy (9%), compared to the overall rate of 35% (p=0.004).

p≤0.05 (statistically significant); BV: bacterial vaginoses; OD: odds ratio; 95%CI: confidence interval of 95%; PCOS: polycystic ovary syndrome; IWAC: infertility without apparent cause; PCR: polymerase chain reaction.

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