Seminovaginal microbiome: it takes two to tango

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ABSTRACT

Infertility, adverse pregnancy outcomes, and genital infections are conditions that can affect any couple in the global population. The reproductive tract microbiome appears to play a crucial role in the physiology of both the female and male reproductive tracts. Despite the presence of thousands of microbes in body fluids shared during unprotected sexual intercourse, they have traditionally been studied separately, with greater emphasis on the female (mostly vaginal) microbiome. Consequently, the concept of "seminovaginal microbiome" emerges to address both microbial niches as a whole that would provide more detailed understanding and potential solutions to the reproductive success. This systematic review discusses the state-of-the-art of the complementary microbiome, encompassing its diversity and composition, and how it is linked to the couples' health and disease, the success of assisted reproductive techniques and pregnancy, and the occurrence of microbe-associated diseases such as sexually transmitted diseases, prostatitis, bacterial vaginosis, and candidiasis. Additionally, the microbial interplay in homosexual couples and transsexual individuals is discussed.

Keywords: 16S rRNA gene; Infertility; Microbiota; Semen; Sexually Transmitted Diseases; Vagina

INTRODUCTION

The number of bacterial cells associated with the human body is estimated to be higher than the number of human own cells [1,2], having important functions in physiology and pathophysiology. The microorganisms that colonize our body are known as our microbiota which, in addition to bacteria, include viruses, fungi, yeasts, archaea, and protozoa [3,4]. Each individual has a unique mix of microbes, presumably as a result of genetic, epigenetic, and environmental factors that regulate bacterial colonization and its stability [5]. Our microbiota performs several beneficial roles for our body, including protective, structural, and metabolic functions [6].

At the same time, the fact that each human being is populated by a unique combination of microorganisms makes us more or less susceptible to various diseases, including those affecting the reproductive tract [7]. Impaired reproduction, adverse pregnancy outcomes, and genital infections are challenges that impact couples worldwide [8]. The microbiome, genetic content of the microbes, detected in the semen and vagina are shown to play a significant role in the functioning of the male and female reproductive systems [9–15]. Despite of the presence of thousands of microbes in body fluids shared during unprotected sexual activity, the male and female urogenital microbial niches have traditionally been studied separately, with a stronger emphasis on the vaginal microbiome [16,17]. Indeed, only a limited number of studies have focused on the analysis of interacting microbiomes of both partners [10,16,18–30], mainly due to the study question and the complexity involved in collecting simultaneous samples from couples. Thus, the concept of the "seminovaginal microbiome", which was proposed in 2015 [18], has not gained much attention, and its short- and long-term potential in human urogenital health and reproduction awaits full establishment and understanding.

The seminovaginal microbiome comprises all the microorganisms from seminal and vaginal ecosystems that are transferred and shared between the partners during unprotected sexual intercourse, influencing each other and impacting reproductive health and functions [18]. The broader concept of the shared microbes encompasses microorganisms residing in areas or bodily fluids that interact with the couple's gametes or reproductive organs during intercourse [31], including regions such as the oral or perianal areas [32–34]. Furthermore, this bidirectional exchange can influence the microbial makeup of either of the partner's or potentially both [26]. Indeed, studies have demonstrated that bacteria are shared among partners and that they influence the species composition of the reproductive tract of both partners [10,16,18–28]. Further, a hypothesis of the vaginal microbiome directly affecting male genital tract health, leading to chronic prostate infection, has been proposed [35]. Therefore, these microbial communities may have a far-reaching implication for individuals and the couples, which are currently understudied and poorly understood. Under the couples' microbial communication, we also address homosexual and transgender individuals. Through this systematic review, we aim to present the current knowledge regarding seminovaginal microbiome studies, to assess the shared microbes within couples, and to determine the potential impact of these shared microbiomes on couple's health.

RESULTS AND DISCUSSION

Identification and selection of articles

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart in **Figure 1** illustrates our search strategy and selection process. Initially 766 articles were identified and after removing duplicates (N=98), 663 articles were screened

by title and abstract. Big part of the records was excluded (N=635) as they assessed only one partner of the couple or other methods than high-throughput next-generation sequencing (NGS) methods were used. Thirty-three articles remained for the full-text evaluation, and 23 out of them were excluded based on our inclusion and exclusion criteria: no NGS methods used (N=8), not including male samples (N=10), and case report, review, or debate (N=5). Ten articles met the inclusion criteria and were selected via the systematic search. Additional records were included using snowballing method (N=4), resulting in total of 14 studies for the current systematic review (**Table 1**).

Quality assessment of the studies

The complete quality assessment is available in **Supplementary Table S1**. Overall, the quality of the studies identified in this systematic literature search was fair. Three out of 14 studies presented poor quality, nine studies were categorized with fair quality, and two studies with good quality. Information like inclusion criteria, study population characteristics, and whether the outcome measurement was clearly defined and reliable was accounted for most of the studies. However, items related to sample size justification, exposure and outcome measurements, and confounding variables (i.e., items #5, #6, #8, #10, #12, and #14) were the most frequently omitted, and thereby accounted for the lower quality score (**Supplementary Table S1**).

Seminovaginal microbiome in heterosexual couples

The seminal microbiome varied greatly among individuals, especially regarding the microbial composition and relative abundance [23,36,37]. The main genera found in semen included *Lactobacillus*, *Finegoldia*, *Prevotella*, *Corynebacterium*, *Staphylococcus*, and

Streptococcus, among others [14,38,39], however, the origin of these microbes in semen remains unclear [40–42]. The seminal microbiome is dynamic and can change due to the factors like individual health, lifestyle habits (such as hygiene and diet), age, ethnicity, and the use of antibiotics and probiotics [14]. Nonetheless, long-term studies that periodically sample the same individuals over time are needed for a comprehensive understanding of microbiome stability and fluctuations.

In healthy reproductive-aged women, the vaginal microbiome typically has limited diversity, mainly consisting of bacteria from *Lactobacillus* genus [43]. *Lactobacillus* spp. contribute to a low vaginal pH, suppressing harmful bacterial growth [44,45]. In fact, a healthy *Lactobacillus*-dominant vaginal microbiome is associated with better reproductive outcomes [46]. The vaginal microbial composition undergoes dynamic changes, varying daily and weekly [47], although it has been shown to maintain stable over several months [48]. These fluctuations appear to be influenced by factors including the menstrual cycle, sexual activity, hormonal contraceptive use, diet, exercise, and antibiotic/probiotic use [44,49,50].

The seminal microbiome is more diverse, albeit with a lower bacterial concentration than the vaginal microbiome [18,23,25–27,51]. This implies for a complex and enriched bacterial community in semen that could interact with the more concentrated but less diverse vaginal microbiota during sexual intercourse. Further, seminal neutral to slightly alkaline pH, around 7.5, can impact the acidic environment of the vagina during unprotected intercourse, potentially leading to shifts in microbial composition, including increased bacterial vaginosis (BV)-related bacteria [52]. A temporary replacement of vaginal *Lactobacillus* with *Gardnerella vaginalis* due to the neutralizing impact of the ejaculate has been shown [53]. Further, a considerable correlation between the presence of spermatozoa in vaginal samples and the Nugent score (i.e., a measure of BV) has been

demonstrated [54]. A pioneering NGS study examined the pre- and post-coital vaginal samples with semen samples from 23 infertile couples, revealing changes in the vaginal microbial composition after sexual intercourse and suggesting that a *Lactobacillus*-dominating microbiota protects against post-intercourse shifts while women with higher Nugent scores had larger shift of bacterial communities [18].

Likewise, sexual activity has been shown to influence seminal microenvironment. Sexually experienced men have been shown to display greater bacterial diversity and concentration than men at same age who have never had sex [55]. Further, sexually more active younger men are shown to exhibit a higher prevalence of typical vaginal microbial species in the semen such as *Lactobacillus crispatus*, *L. iners*, *G. vaginalis*, and *Atopobium vaginae* (recently renamed as *Fannyhessea vaginae*), while sexually less active older men harboured more environmental bacteria in the semen as *Pseudomonas*, *Gillisia*, *Flavobacterium*, and *Acidovorax* genera [56], which refers to the microbial differences due to sexual activity and/or age. Altogether, different factors such as the onset age of sexual activity, number of sex partners, condom use, and the time since the last sexual intercourse have been associated with the seminal microbial composition [55–57].

Genital (vaginal and penile) microbiome in heterosexual couples

The penile skin microbiome, like other skin microbiomes, is complex and diverse, consisting of various bacteria, fungi, and viruses [58]. A healthy penile skin microbiome is dominated by common skin bacteria from genera *Corynebacterium* and *Staphylococcus*, but also several anaerobes like *Prevotella*, *Finegoldia*, *Peptoniphilus*, *Porphyromonas*, and *Anaerococcus* [59,60] are present, harboring a richer but less abundant microbial

community compared to the vagina [27]. However, the penile microbiome may have fewer regulatory factors or may be less susceptible to perturbations than the vagina [24]. It has been shown that circumcision substantially modifies the penile skin microbiome, particularly by decreasing its α-diversity (i.e., variability of microbes within a sample) and reducing the presence of BV-associated genera and anaerobic bacteria [21,24,59,61–64]. Changes in this microenvironment can potentially influence the risk of urinary tract infections, sexually transmitted infections (STIs), and other conditions in the couple.

A recent longitudinal study revealed that the composition of the penile microbiome is stable over a one-year period in 50-60% of men [24]. The penile skin microbiome has been correlated to the vaginal microbiome in a number of studies with inconclusive results [19–21,24,27,30,65]. The penile skin bacterial communities from couples with BV were significantly more similar to their female partner's vaginal communities than to the vaginal communities of a non-partner women [19], being in line with research where BV in women has been positively associated with the relative abundance of penile taxa [24,27]. However, these associations between the vaginal and penile microbes are often derived from studies following treatment for BV, limiting our understanding of the microbial cross-talk in healthy states.

A pairwise comparisons of microbial composition between the vagina-penis and the vagina-semen in couples with infertility have shown that the vaginal and penile samples were more similar than the vaginal and semen samples, and that the penile and semen samples displayed higher similarity when they were collected from the same individual compared to the same sample types from different men [27]. This study concludes that the male genital microbiome has a minimal influence on the bacterial colonization in females, although the authors acknowledge that the information of sexual activity was missing [27].

Another study collected daily vaginal and penile specimens from a female participant and the male partner through 3 weeks, where a dynamic interaction between the microbiomes of sexual partners were characterized [65]. The study revealed an increase in the abundance of *Streptococcus mitis* post-coitally in urethra, oral cavity, and urine in the female, suggesting sexual transmission of this microorganism. *S. mitis* is a bacterium usually associated with the oral cavity but has also been detected in the urogenital tract [65–67]. A case report of a woman with no previous vaginal and oral infections, however developed recurrent vaginal problems and gingivitis after starting a relationship and revealed lower *Lactobacillus* in the vagina and higher *Corynebacterium* levels in the penis [33]. Corynebacteria include many species that are mostly commensals, but they have also been associated with prostatitis syndrome [56,68]. Intriguingly, other studies indicate that a *Corynebacterium*-dominated and low-diversity penile microbiome might have beneficial health associations for men and their female partners [24,60]. Despite substantial progress in characterizing these microbial sites, the findings are changeable and controversial, and the dynamics of microbial interplay between the penile skin and vagina needs further investigation.

Microbiome in homosexual couples

Same-sex couples also experience an exchange of microbial communities during sexual intercourse, albeit with different implications due to the anatomical distinctions. Men who have sex with men have been found to harbor unique rectal microbiome compared to men who have no sex with men [69], which might influence the susceptibility to human immunodeficiency virus (HIV) infection and other STIs. Also, the seminal microbiome can vary substantially between the men of different sexual preferences, as rectal microbiome of homosexual men engaging in condomless receptive anal intercourse have

shown *Prevotella*-rich microbiome with decreased diversity [70,71], which could have different consequences for men's health.

Among same-sex female couples, shared vaginal microbiota is common and has been linked to BV, demonstrating a higher incidence of BV [72,73]. Additionally, female long-term partners seem to share *Lactobacillus* strains, which could be beneficial to the health of both partners [74]. In contrast, women who continually change partners have been more likely to have BV [75]. These are the first studies in the field and the understanding of the microbial interactions, colonization, and long-term effect on health among same-sex partners clearly warrants more research.

Microbiome in transgender and gender-diverse individuals

Hormones and tissue structure shape the genital microbiome, meaning both hormone treatments and gender reassignment surgeries may have effects on microbial communities in transgender individuals (i.e., transbiota) [76]. As in cisgender people, changes in genital microbiota likely have considerable impacts on the health of transgender people.

For some transfeminine people (i.e., person assigned male at birth who identifies as female), gender-affirming care may involve the use of hormone therapy, such as estrogen and/or progestin therapy, and may also include the surgical creation of a vulva and/or vaginal canal (neovagina) [77]. The neovaginal microbiome differs from that of the cisgender women in several key aspects, primarily due to the differences in the tissue used to create the neovagina [78]. Different tissues may lead to different colonization of microbes [79]. The local microenvironment, including the epithelium characteristics, plays a significant role in shaping the microbiome, which can have implications for the individual's health and quality of life as some individuals sometimes experience

bothersome neovaginal symptoms after vaginoplasty [76,77]. Commonly reported symptoms include discharge and malodor, which suggest microbial dysbiosis [76]. In cisgender women, the vaginal microbiome is typically dominated by *Lactobacillus* species, which help maintain a low pH and protect against pathogenic organisms. However, the neovaginal epithelium, particularly when created from penile skin, lacks glycogen and retains some degree of cornification (i.e., keratinization), which makes it less conducive to supporting a *Lactobacillus*-dominated microbiota [80]. Similarly, estrogen therapy can influence the local microenvironment of the neovagina as it affects the vaginal epithelium in cisgender women by increasing glycogen production. However, the neovaginal epithelium, especially when created from penile skin, may not respond to estrogen in the same way as the natal vaginal epithelium due to the differences in tissue characteristics [77]. Studies have shown that the neovaginal microbiome may instead be populated by a variety of bacterial genera, such as *Prevotella* and *Peptostreptococcus* which are also prevalent in conditions like BV in cisgender women [78]. However, the optimal composition of the neovaginal microbiome is not yet well understood. Additionally, the role of hormone therapy in shaping the neovaginal microbiome and its potential treatments for gynecological symptoms require further investigation.

Transmasculine individuals (i.e., person assigned female at birth who identifies as male), who may undergo testosterone therapy as part of the gender-affirming treatment, can experience changes in the vaginal microbiome due to the hormonal alterations [76]. Among this population, genital surgery is relatively uncommon; however, testosterone therapy can lead to atrophic changes in the vaginal mucosa, which can alter the local microenvironment and potentially affect the composition of the microbiome [77]. Individuals receiving testosterone therapy also frequently experience bothersome symptoms, including vaginal dryness, itching, and pain with sexual activity [76].

Testosterone therapy suppresses circulating estrogen, leading to changes in the vaginal epithelium that can be similar to the post-menopausal period in cisgender females which includes reduced glycogen deposition, reduced epithelial proliferation and turnover, and a marked reduction in the availability of free glycogen in the mucosa [81]. Thus, the use of testosterone in transmasculine individuals can decrease the prevalence of *Lactobacillus* and increase in vaginal pH, making the environment more hospitable to a diverse array of bacterial species [77]. As a result, the vaginal microbiome in transmasculine individuals shows higher abundance and prevalence of genera such as *Streptococcus*, *Corynebacterium*, *Finegoldia*, *Peptoniphilus*, *Anaerococcus*, and *Bifidobacterium* [82,83].

With increasing social acceptance and improving access to the gender-affirming medical treatment advances, the demand for gynecological services among transgender individuals is on the rise. However, more research is needed to elucidate the microbiome in the genitalia of transgender individuals and to see what implications it has on their own health and that of their partner.

Effect of seminovaginal microenvironment on seminal parameters

Recent studies are providing new knowledge into the field by demonstrating that couples having unprotected sexual intercourse share certain bacterial genera that can impact seminal parameters [14]. In the semen, relative abundance of *Lactobacillus* is significantly lower than in the vagina and their roles are not well-defined [26]. However, increased abundance of *Lactobacillus* in the seminal microbiome has been correlated with improved sperm motility and concentration and with normal morphology, probably because it prevents lipid peroxidation [37,84,85]. Accordingly, *Lactobacillus* spp. have garnered considerable attention due to their probiotic potential for semen quality maintenance and

how probiotic interventions with *Lactobacillus* strains have influenced the seminal microbiome [86]. Interestingly, an *in vitro* study investigated the potential impact of vaginal isolated microorganisms on sperm motility, where several vaginal bacteria, including different *Lactobacillus* spp., *G. vaginalis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*, effectively adhered to spermatozoa and significantly reduced sperm motility and penetration in a viscous medium, suggesting a potential detrimental impact on fertility [51]. While this work analyzed each species separately for the adhesion test, which is not mimicking the *in situ* environment, this work highlights the possible dual nature of *Lactobacillus*. This genus has been positively associated with anti-inflammatory cytokines, possibly reducing the generation of pro-inflammatory cytokines [87], while abundant adhesion of *Lactobacillus* spp. to sperm cells seems to reduce sperm functions, which could negatively impact reproductive health [51]. Contrary, *G. vaginalis* has been found to negatively affect sperm health and to associate with infertility in men (**Figure 2**) [86] which has also been linked with BV when it outnumbers *Lactobacillus* spp. in women [18]. Another study demonstrated that the adhesion of *E. coli* to spermatozoa correlated to diminished embryo quality by promoting spermatozoa agglutination via their plasma membranes and their subsequent destruction by inducing cell apoptosis [84,88,89]. While specific vaginal bacteria indeed compromise sperm motility, understanding and manipulating the vaginal microbiome might prove to be a novel strategy in fertility treatment.

Also, the impact of *Klebsiella pneumoniae* and *S. agalactiae*, common opportunistic bacteria in the vagina, on sperm parameters has been studied *in vitro*, as well as their capacity to interact with and be transported by human spermatozoa [90]. The findings revealed that the presence of *K. pneumoniae* adversely impacted sperm motility, specifically the progressive motility that is crucial for successful fertilization (**Figure 2**).

Additionally, the bioactive substances released by this bacterial species negatively influenced sperm health, increasing the number of necrotic sperm cells. Similarly, the soluble factors of *S. agalactiae* led to an increase in lipid peroxidation in the sperm membrane, a process that can damage cell structures and potentially impair sperm function (**Figure 2**) [91]. These authors observed a robust interaction between the spermatozoa and *K. pneumoniae* and *S. agalactiae* and concluded that human spermatozoa might act as vehicles for these bacteria, facilitating their spread within the female reproductive tract [90].

Diseases related to sexual intercourse

A notable example of the interaction and mutual influence between the seminal and the vaginal microbes is observed in the development of various diseases. These microbial interactions within the host tissues and organs can have significant implications for reproductive health and fertility, and the chances of achieving a successful pregnancy. Therefore, these diseases are an important focus of study. The microbes-associated diseases in reproductive tract can be divided into two big groups – specific infections (sexually transmitted diseases, STDs) and the "ecological diseases" that are associated with microbial imbalance (the most well-known example being BV). It is important to highlight that causative agents of STDs never belong to normal microenvironment.

Sexually transmitted diseases, STDs. A STD is primarily transmitted through unprotected vaginal, anal, or oral sex from one partner to another. However, some infections can also be transmitted through blood and from mother to child during pregnancy, childbirth, or breastfeeding [92]. The STDs can be caused by viruses, bacteria, or parasites. The most common bacterial STDs include chlamydia (*Chlamydia*

trachomatis), mycoplasma (*Mycoplasma genitalium*), gonorrhea (*Neisseria gonorrhoeae*), and syphilis (*Treponema pallidum*). Viral infections include human papillomavirus (HPV), herpes (herpes simplex virus [HSV]), HIV, and hepatitis B (HBV). Some STDs like trichomoniasis are caused by parasites (*Trichomonas vaginalis*) [93–95]. In women, *C. trachomatis, M. genitalium*, and *N. gonorrhoeae* cause mostly cervicitis while *T. vaginalis* causes mostly vaginitis. In male partner, the main clinical expression of STDs is urethritis. In rare cases, urethritis can be caused also by non-STD-microorganisms, like *Haemophilus influenzae* and *H. parainfluenzae*, especially among homosexual men [96]. Knowing which microorganism is causing the infection is crucial for appropriate treatment strategies. Viral infections typically cannot be completely cured but can be managed and their symptoms alleviated. On the other hand, bacterial infections offer a broader range of treatment possibilities, as they can often be effectively treated with antibiotics. Therefore, accurately identifying the specific microorganism responsible for the infection is vital in determining the most appropriate and effective course of treatment [21,97]. In case of bacterial and parasitic STDs, both (or all) partners need to be treated simultaneously.

HIV is the causative agent of acquired immunodeficiency syndrome (AIDS), a STD with a high prevalence despite today's advancements [98]. It is understood that microabrasions during sexual intercourse in both male and female genital tracts serve as the primary route for HIV to access its target cells, as they degrade the protective barrier formed by the epithelia [22,99]. Predisposing factors such as inflammatory reactions and an altered state of the microbiota have also been identified [98,100]. Specifically, vaginal dysbiosis (i.e., BV) and the changes induced by semen on the vaginal microenvironment have been found not only to disrupt the microbiota barrier but also to recruit immune system cells, which are susceptible to HIV infection [101–103]. Furthermore, anaerobic microorganisms present

within the penile skin have been shown to increase the likelihood of infection in the male genital tract during sexual intercourse [104]. The role of the microorganisms in HIV transmission still requires further investigation, as there is a hypothesis that a favorable seminovaginal microbiota may potentially reduce viral entry to some extent [100].

Microbial dysbiosis. Microbial dysbiosis or the "ecological diseases" are common in both sexes. Individuals can develop microbial dysbiosis even without engaging in intercourse, however, participating in sexual intercourse can substantially heighten the probability of acquiring these diseases due to the factors such as the composition of their partner's microbiota, the characteristics of bodily fluids, or physical injury sustained during sexual activity. Among the sexually enhanced diseases, specifically related to women, the BV is a commonly occurring vaginal condition that is linked to various obstetric and gynecological complications and has substantial implications for healthcare costs [34]. The etiology of BV is not fully established; in the past, it has been suggested that it may be transmissible, and that *G. vaginalis* may be the main etiological agent [63]. However, the nature of BV is more complicated. In women with BV, the composition of the vaginal microbiome is characterized by a decrease in *Lactobacillus* spp. and an increase in specific microaerophilic and anaerobic bacteria collectively referred to as BV-associated bacteria. These bacteria included *G. vaginalis*, *A. vaginae*, *Mobiluncus mulieris, Prevotella* spp., *Sneathia* spp., and others [21]. Sexual activity is clearly linked to the development of BV but likely through a more complex mechanism than specific STIs. It has been hypothesized that the change in the vaginal pH resulting from semen is what drives the shift in microbiota that results in BV [105]. When BV is linked to sexual activity, it typically arises not only due to an alkalization of the vaginal microbiota caused by semen's pH but also the transmission of bacteria from the woman's perianal region [34]. Additionally, the transfer of *G. vaginalis* from the seminal to the vaginal microbiota may

contribute to this condition [57]. In either case, sexual intercourse diminishes the abundance of *L. crispatus* in the vagina, compromising the woman's defense and making it more susceptible to conditions like BV and different STDs [34,57]. It is interesting to observe that circumcision has been shown to reduce the abundance of anaerobic bacteria in the penile microbiota and has been associated with a reduced risk of BV in female partners [29]. Therefore, it is probable that circumcision impacts not only a woman's risk of BV recurrence, but also the effectiveness of male partner treatment strategies [21]. Although male circumcision reduces BV-associated bacteria on the penis and decreases BV in female partners, the link between the penile microbiota and female partner BV is not well understood [62]. Gynecological evaluation of BV is based mostly on the Amsel criteria assessment [106] while laboratory confirmation of BV is mostly based on Nugent scoring [107]. It involves assessing the presence of specific bacterial morphotypes [107]. Large Gram-positive rods (*Lactobacillus* morphotypes) are assessed for a decrease in quantity, with a score ranging from 0 to 4. Small Gram-variable rods (*G. vaginalis* morphotypes) are also evaluated and scored from 0 to 4. Additionally, curved Gram-variable rods (*Mobiluncus* morphotypes) are considered and scored from 0 to 2. A total score of 7 to 10 indicates the presence of BV.

Another "ecological disease" with special relevance in females is vulvovaginal candidiasis (VVC), a disease caused by the excessive proliferation of fungi of the genus *Candida* in the vaginal microenvironment [108]. *Candida albicans* is usually the main cause of the infection, although other species such as *C. krusei, C. tropicalis* and *C. parapsilosis* can also be the cause [109]. *Candida* spp. are present in the healthy vaginal microbiota in small amounts without causing harm, but as an opportunistic pathogen, it can take advantage of situations as physiological imbalance to proliferate [110]. Therefore, the development of VVC may be due to genetic and/or environmental factors, as well as the

use of antibiotics [110] and sexual intercourse [111]. It can be transmitted directly from the seminal microbiota or the composition of semen can favor the growth of *Candida* strains already present in the vaginal microbiota [111]. It has been demonstrated that the semen can promote VVC by presenting factors that stimulate the growth of the fungus, particularly favoring the development of hyphae [108]. It has been also observed that the proliferation of *C. albicans* was decreased, at least partially, by an increase in semen viscosity [108]. Interestingly, previous research has shown that semen presents antifungal factors [112], however these do not appear to be effective against *Candida* growth in the vagina [108].

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is an "ecological disease" in the male genital tract, being fairly frequent among middle-aged men. In addition to the subjective discomfort and lowered quality of life [113], CP is associated with reduced semen quality, including negative effect on sperm concentration, motility, vitality, and morphology [114]. In only a tenth of CP/CPPS patients the causative agent can be revealed, mostly as well-known urinary tract pathogens like *E. coli*, *Enterobacteriaceae*, or *Enterococcus*. In vast majority of CP/CPPS patients, the single causative agent cannot be detected, instead, the role of the microbial imbalance, ascending of imbalanced communities into prostate gland and polymicrobial infection is suggested [115]. The semen of CP patients contains fewer health-supporting *Lactobacillus* and has higher species diversity than that of healthy men resembling BV in women [56]. Bacteria that individually might have low virulence and are unable to cause disease can do so when in association with others. Their virulence factors may be different, too – prostatitis-related male genital tract bacteria have shown higher biofilm production and anticomplement activity than the bacteria isolated from healthy men [68,116]. In low number of cases, prostatitis may be developed as a consequence of a STD [117]. Also, *G. vaginalis*-caused prostatitis has been described [118]. This is consistent with the finding that *G. vaginalis* has been found to be

the predominant microbe in half of the women whose partners had inflammatory prostatitis but only in 13% of women whose couple was without the disease [18]. Moreover, it has been hypothesized that the source for the chronic inflammatory stimulus in the prostate is the dramatically altered lower female genital microbiota in women with menopause [35]. Altogether various microorganisms that include bacteria, viruses, and fungi can participate in the infections/diseases related to sexual intercourse, however the detailed mechanisms of their role need to be established.

Assisted reproductive technology (ART) outcomes

Due to the direct clinical interest, studies have started to elucidate the link between the seminovaginal microbiome and ART outcomes. Correlations between the specific bacterial communities and positive *in vitro* fertilization (IVF) outcomes (the most used ART method) have been described (**Figure 2**). Higher microbial concentrations of the classes *Alphaproteobacteria*, *Gammaproteobacteria*, and genus *Corynebacterium* in semen have been associated with lower embryo quality, while a higher abundance of fam. *Enterobacteriaceae* and genus *Lactobacillus* was correlated with better embryo quality [119]. Further, in semen samples, the increased mean proportions of *L. jensenii* and *L. iners* and decreased proportions of *Proteobacteria* and Gram-negative anaerobes have been associated with IVF success [10].

Concurrently, in the vaginal samples, increased proportions of *L. gasseri* and decreased proportions of *Bacteroides* and other *Lactobacillus* were associated with IVF success [10]. Similarly, a positive outcome of intrauterine insemination was linked with an increased proportion of *L. crispatus* in the vagina, whereas no differences were detected in the semen [16]. In another study, women with BV or a vaginal microbiome dominated by *L.*

iners or *L. gasseri* demonstrated reduced ART success rates compared to women with a *L. crispatus*-dominant or other lactic-acid-bacteria-predominant microbiome [26]. This finding corroborates previous research highlighting the protective role of *L. crispatus* in reproductive health [15,16,120]. In men, those with a seminal microbiome dominated by *Acinetobacter* in combination with other bacteria had the highest ART clinical pregnancy rates, while the seminal microbiome dominated by Gram-negative anaerobic and/or microaerophilic bacteria such as *Prevotella*, *Porphyromonas*, *Dialister*, *Campylobacter* associated with poorer ART outcomes [26]. On the couple level, those who had beneficial microbiome types had superior ART success rate of 53% compared to the rest of the couples (25%) [26]. Interestingly, healthy couples seem to have lower microbial diversity than the couples undergoing ART [26], meaning that an increased diversity in the reproductive microbiome may not necessarily be beneficial for fertility. Indeed, healthy vaginal microbiome is typically characterized by low diversity and dominance by one or few *Lactobacillus* spp., while there are conflicting results of the seminal microbial diversity and male health [43,44,67]. Specifically, conditions like HIV infection and azoospermia are associated with lower microbial diversity [121–123], while prostatitis tends to correlate with increased diversity [56]. This is somewhat counterintuitive when compared to the general perception of the gut microbiome, where high diversity is considered as indication of good health [124,125], underscoring the complexity of the reproductive microbiome's role in fertility.

Understanding the influence of the microbiome on reproductive functions becomes more complex due to the variability of the microbial communities, which can be influenced by numerous factors including sexual activity, hormonal shifts, microbial treatments, and various other causes. One possible mechanism by which genital tract microorganisms can affect fertility is by inducing infection- or dysbiosis-related oxidative stress (OxS) in both

partners (**Figure 3**) [91,126,127]. Interestingly, a strong correlation between the partners' OxS levels has been revealed in the couples undergoing infertility treatment [126]. OxS can damage sperm DNA, decrease sperm motility, and interfere with the normal function of the female reproductive tract, all of which are detrimental to the fertility [128]. Clearly more research is required to understand better the complementary microbial interplay in infertility treatment protocols in order to be able to favor beneficial microenvironment.

Microbiome modulation strategies

In the clinical setting, there is a high interest and demand to improve seminovaginal microbial composition in order to treat dysbiosis in the reproductive tract, to prevent harmful microenvironment for gametes, and to improve fertility outcomes. New therapies, such as pro- and prebiotic administration, together with microbiota transplants are gaining popularity for modulating microbial composition [13]. Several studies highlight the potential of microbiome modulation as a strategy for improving reproductive health in both men and women [129]. The first intervention study of probiotic treatment performed in couples with infertility assessed the effect of a 6-month treatment with oral probiotic *Ligilactobacillus salivarius* PS11610 on the genital dysbiosis [25]. Oral intake of the probiotic resulted in the clearance of dysbiosis in 67% of the couples. Along the treatment, the vaginal microbiome mainly increased the abundance of *Lactobacillus* in relation to the total bacterial counts, while seminal microbiome displayed slightly lower levels of pathogens and staphylococci and changes in the microbial composition [25]. Further, the systemic immunological status in both partners was assessed, and a switch from pro-inflammatory to anti-inflammatory profile post-treatment was found. Although being preliminary, altogether the intake of *L. salivarius* PS11610 slightly enhanced the rates of pregnancy and childbirth among 17 couples with unexplained infertility

undergoing ARTs [25]. The microbial modulation is a highly promising way to improve reproductive health and outcomes in the couple, nevertheless thorough research and extensive testing in randomized, placebo-controlled trials is warranted.

Microbial modification of penile skin via BV treatment

Several studies have focused on the treatment of male counterparts when their female partners are experiencing recurrent BV. The rationale for this approach is based on the fact that sexual transmission may play a role in recurrent BV, since BV-associated bacteria have been detected in different parts of the male genitourinary tract (i.e., penis, urethra, urine, and semen) [21]. Despite the logical rationale of treating both partners in cases of recurrent BV [20], previous randomized controlled trials (RCTs) that targeted male partners have not successfully decreased the recurrence of BV [130–135]. Nonetheless, a recent review presented that the reliability of the evidence from these RCTs ranged from low to very low [136]. Notably, none of the past trials evaluated the use of topical antibiotics for men. However, other authors hypothesized that while oral antibiotics may effectively target bacteria from internal areas of the male reproductive tract, cutaneous bacteria colonizing the penis may be more effectively eradicated with topical antibiotics [20]. Therefore, it was proposed that a combination of oral and topical antimicrobial treatments could be necessary to eliminate BV-associated bacteria.

In a previous study, the female participants diagnosed with BV received oral or intravaginal antibiotic (i.e., standard BV therapy) while their male partners received combined topical and oral antimicrobial treatment with both treatments lasting for 7 days [20]. The obtained results showed that while the immediate outcome was promising, with reductions in BV-associated bacteria and increased *Lactobacillus* colonization, BV-

associated bacteria re-emerged in the penile microbiome after 3 weeks and the beneficial effects did not sustain in a long term [20]. As the next step, the same antibiotic intervention in women with BV and their male partners was carried out and followed up in a 12-weeks period of time [21]. Again, the combined oral and topical treatment in men aimed to address multisite carriage of BV-associated bacteria. At 12 weeks post-treatment, the majority of women experienced suppression of BV-associated bacteria and an increase in *Lactobacillus* spp., suggesting that a male's combined therapy could be more effective than oral treatment alone. However, the male genital microbiome did not significantly differ from the baseline and after 12 weeks, with BV-associated bacteria re-emerging at male sites, cutaneous penile and urethra [21]. These works bring to light the challenges in managing recurrent BV and underscore the importance of considering both partners in the treatment strategies. Despite the re-emergence of BV-associated bacteria in men over time, the beneficial effects seen in women suggest that treating men may still play a role in managing recurrent BV [20,21]. However, these studies also highlight gaps in our understanding of the male genital microbiome and the role it plays in BV recurrence. While it seems logical to treat both partners in cases of recurrent BV, the appropriate treatment strategy and the factors that influence treatment success remain unclear. In addition, it should not be forgotten that small amounts of BV-bacteria are members of normal microbiota, and eradication of any member of normal microbiota tends to be highly challenging [67].

CONCLUSIONS

This systematic review provides the state-of-the-art knowledge of the complementary microbiome between couples, and how it can influence reproductive parameters and health. Considering there are thousands of works performed on the vaginal and seminal

microbiome analyses separately, it is surprising how few studies have assessed the complementary seminovaginal microbiome in the couples and how little we know of the bidirectional microbial communication.

The previous studies have mainly assessed either female part or male part separately, where vaginal microbiome studies have been published a magnitude more than the seminal studies (~2000 *vs.* ~200 studies), meaning that the vaginal microenvironment is better characterized. Nevertheless, both partners play an important role in the couples' host protection and participate in reproductive functions. We believe that it is time to adopt the holistic view of the couples' microbial community, to discontinue focusing only on one side of the communication in order to unravel the true potential and interplay of the seminovaginal microbiome in reproductive physiology and pathophysiology.

The current studies of the seminovaginal microbiome are highlighting the weak points in these study designs, such as lack of power calculation, repetitive measures, and control for confounding factors, together with limited positive and negative controls and relatively small sample sizes (see **Supplementary Table S1**). Considering all these shortcomings, which are important in NGS-based studies, the different studies are barely comparable and it is hard to draw solid conclusions and the field yarns for well-designed studies. Different guidelines for designing and conducting microbiome studies have been proposed [11], and three minimal standard requirements that researchers should follow for human microbiome studies have been presented [137].

Further, the currently applied NGS-based microbiome analysis methods are analyzing DNA sequences, which does not necessarily equate with the presence of live bacteria [138]. DNA molecules are able to persist decades, even when being breakdown products (e.g., DNA from dead bacteria) or be present as background DNA contamination [139]. Thereby, DNA-based analysis can be used to characterize a microbiome but not to conclude that

the detected sequences are functionally active microbes. RNA-based and [12] culture-based techniques [140], such as culturomics could be a new tool to investigate the complementary seminovaginal microbiota for detecting alive microbes in eubiosis and dysbiosis and to be able to develop the microbiota modulation strategies. Culturomics, the "renaissance" of the culture-based techniques in microbiology, is a method combining a vast array of enriched broths, agar mediums, and incubation settings to culture all viable microbes, which has been successfully applied to female reproductive tract [140]. Altogether, we are facing a big gap from the analysis of DNA sequences to the functionally active microbes, which hopefully will be unraveled in the coming years.

The potential of the modulation of seminovaginal microbiome carries high clinical relevance in maintaining and restoring eubiosis and in preventing and treating dysbiosis. Nevertheless, our knowledge of the detailed composition and dynamics of the complementary microbiome is lacking. It is clear that when investigating an individual, considering also the partner is essential, as it truly takes two to tango. In short, overcoming the limitations of the current microbiome analysis methods and by adopting the concept of couple's microbiome as holistic concept would provide a better understanding of the seminovaginal microbiota and its implications. This, in turn, can lead to improved strategies for addressing challenges related to reproductive health and success, ultimately promoting optimal reproductive outcomes.

MATERIAL & METHODS

The search strategy was performed following the PRISMA (**Supplementary Table S2**) [141]. The review protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD42022323201).

Data source and search strategy

A systematic search of the literature in PubMed, Web of Science, and Scopus was independently conducted up to 24th November 2023 by two researchers (N.M.M. and A.C-G.). The strategy performed for literature search combined keywords and medical subject heading (MeSH). The search was focused on male and female reproductive niches, microbiota/microbiome, and human reproduction related words. Detailed search query is reported in **Supplementary Table S3**.

Study selection

The study population consisted of couples at their reproductive age. All types of studies describing the microbial composition of genital tract in female (i.e., vagina) and male (i.e., semen, penile skin) genital tracts of couples via the NGS were included. The exclusion criteria were conference abstracts, letters to editors, study protocols, editorials/opinions, case reports, review articles, or studies assessing the microbial composition in only one of the partners and studies written in any language other than English or Spanish. Time (from 2007 to the present) and human-species filters were applied.

Study selection was carried out independently by two investigators (N.M.M. and A.C-G.), and discrepancies were resolved through consultation with a third independent researcher (S.A.). Initially, the articles resulting from the systematic search underwent screening based on their titles and abstracts, following a full-text screening of the remaining articles. After the systematic search and study selection, we employed the

snowballing method to hand-search additional records, ensuring comprehensive coverage by retrieving extra studies from reference lists of review articles and prior selections.

Data extraction and synthesis

The primary outcome of this review was to identify the shared microbiome profiles within the couple. Data from selected articles were manually extracted by two investigators (N.M.M. and A.C-G.) and tabulated their characteristics. For every eligible study during full-text screening the following information was gathered: 1) reference information; 2) study aim; 3) study design; 4) study population (number of participants, condition, age, country/ethnicity, possible treatment); 5) sampling (body niche, collection procedure, follow-up); 6) top identified taxa in each of the individuals of the couple and the shared ones; and 7) main study conclusions.

Quality assessment and risk of bias

To evaluate the quality and possible bias in the study design of the selected articles, two researchers (N.M.M and I.L-B.) independently scored each included work using the Quality Assessment Tool from the National Heart, Lung, and Blood Institute-National Institute of Health [142]. Possible inconsistencies were resolved through common agreement. Studies were scored on 14 criteria using the "Quality assessment tool for observational cohort and cross-sectional studies" and quality was categorized as poor quality (<5 points), fair quality (between 5-8 points), or good quality (>8 points).

ACKNOWLEDGEMENTS

The authors thank Dr. Merli Saare from the University of Tartu for the stimulating discussion regarding the microbiome in transgender individuals. This study is part of a PhD Thesis conducted within the Official Doctoral Program in Biomedicine of the University of Granada, Spain.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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TABLE

Table 1. Systematic search results and comprehensive overview of the data extracted from microbiome studies of 16S rRNA gene sequencing in different-sex reproductive-age couples.

Reference information	Study aim	Study design	Population (N, diagnosis, ethnicity, age [y], treatment)	Sampling	Top identified taxa	Main conclusions
(Mändar *et al.*, 2015)	To compare seminal and vaginal microbiomes in couples and to assess the influence of sexual intercourse on vaginal microbiome	Longitudinal	23 couples with infertility (Estonia) Men 32.2 (24-43) y Women 29.9 (21-39) y Tx No	Semen aliquot Vaginal swab F/U <24 hours	Men *Varibaculum*, *Flavobacterium*, *Prevotella*, *Porphyromonas*, *Dysgonomonas*, *Atopobium*, *Corynebacterium* Women *Lactobacillus*, *Gardnerella*, *Streptococcus*, *Veillonella*, *Pseudomonas*, *Atopobium* Shared 85% of all detected phylotypes *Lactobacillus*, *Veillonella*, *Streptococcus*,	Seminal communities were significantly more diverse, but with lower total bacterial concentrations than those of the vagina Significant decrease in the relative abundance of *L. crispatus* after intercourse and high concordance between semen and vaginal samples

					Porphyromonas, *Atopobium*	
(Zozaya *et al.*, 2016)	To examine the diversity, community composition, prevalence, and relative abundance of genital bacteria in monogamous couples	Cross-sectional	65 couples where the female has BV (Africa-American) Men 30 ± 8.4 y Women 27.3 ± 6.6 y Tx No 31 couples without BV (African-American) Men 32.2 ± 12 y Women 28.4 ± 7.7 y	Penile swab Vaginal swab F/U No	Men *Corynebacterium, Peptoniphilus*, *Anaerococcus*, *Staphylococcus*, *Finegoldia*, *L. iners*, *Streptococcus*, *Gardnerella* Women *L. iners*, *Gardnerella*, *L. crispatus*, *Megasphaera*, *Enterobacter* Shared most OTUs in vaginal and penile samples of BV couples showed a strong positive correlation, while correlations among non-BV couples were strikingly lower *Peptoniphilus*, *Gardnerella*, *Lactobacillus*, *Barnesiella*, *Prevotella*, *Megasphaera*, *Dialister*	Diversity of BV couples was higher in penis and vagina compared to non-BV couples The penile skin communities of BV-males were significantly more similar to the vaginal communities of their sexual partner
(Plummer *et al.*, 2018)	To investigate the impact of dual-partner BV treatment on the	Longitudinal (pilot trial)	21 couples where the female has BV (mainly Australia)	Penile swab Vaginal swab F/U 4 weeks	Men *Corynebacterium*, *Finegoldia*, *Peptoniphilus*,	Correlations between prevalent taxa in vagina and penile microbiome

	vaginal and penile microbiome		Men 33.1 ± 9.1 y Tx oral metronidazole (400mg twice daily for 7 days) and clindamycin applied topically to penile skin (2% cream twice daily for 7 days) Women 28.6 ± 6.4 y Tx oral metronidazole (400mg twice daily for 7 days) or intravaginal clindamycin (2% applicator vaginally for 7 nights)		*Prevotella*, *Staphylococcus*, Women *Gardnerella, L. iners*, *Prevotella*, *L. crispatus*, *Staphylococcus*, *Escherichia*/*Shigella* Shared *Dialister*, *Prevotella*, *Staphylococcus*	The vaginal microbiome was not more similar to the cutaneous penile microbiota of their sexual partner, when compared to non-partner males Re-emergence of BV-associated bacteria in the penile microbiome post-treatment was common
(Campisciano *et al.*, 2020)	To focus on the possible differences in the genital microbiome and HPV presence in couples diagnosed with unexplained infertility compared with couples diagnosed with explained infertility	Observational prospective	47 couples attending an infertility clinic (Caucasian) Men 38 (28–44) y Woman 38 (28–44) y Tx No	Semen aliquot Cervicovaginal lavage F/U No	Men *Prevotella*, *Staphylococcus*, *Lactobacillus* Women *Lactobacillus*, *Streptococcu* Shared *Lactobacillus*	Comparing HPV-positive samples with HPV-negative samples, *Prevotella* significantly differed *Porphyromonas bennonis* and *Prevotella bivia/disiens* were identified as seminal biomarkers Different microbial composition between the genital tracts of couples with unexplained infertility

						and those of couples with explained infertility
(Amato *et al.*, 2020)	To characterize the vaginal and seminal microbiome in couples with idiopathic infertility undergoing their first IUI and to correlate it with the clinical pregnancy rate after IUI	Observational prospective	23 couples with idiopathic infertility (Italy) Men 34 ± 4 y Women 33 ± 3 y Tx No	Semen aliquot Vaginal swab F/U No	Men *Tissierellaceae*, *Lactobacillaceae*, *Streptococcaceae*, *Prevotellaceae*, *Corynebacteriaceae* Woman *Lactobacillaceae*, *Pseudomonadaceae*, *Moraxellaceae*, *Pasteurellaceae*, *Enterobacteriaceae*, *Campylobacteraceae*, *Tissierellaceae*, *Veillonellaceae*, *Ruminococcaceae*, *Lachnospiraceae*, *Streptococcaceae* Shared *Tissierellaceae*, *Lactobacillaceae*, *Streptococcaceae*, *Prevotellaceae*, *Corynebacteriaceae*	Changes in the composition of the vaginal microbiome (but not the seminal microbiome) were associated with successful outcomes of IUI Potential role of *L. crispatus* in promoting a favourable environment for pregnancy
(Mehta *et al.*, 2020)	To identify factors associated with clinical BV among women with nonoptimal vaginal microbiome/molecular BV	Longitudinal	252 heterosexual couples (Kenya) Men median 27 y Women median 22 y Tx No	Penile swab Cervicovaginal lavage F/U 12 months	Men *Corynebacterium*, *Anaerococcus*, *Finegoldia*, *Sneathia sanguinegens*, *Streptococcus* Women *Prevotella*, *L. iners*, *Atopobium*	Penile bacteria were associated with increased odds of Nugent BV among women with nonoptimal CST Partner circumcision associated with a reduced

					vaginae, *Dialister*, *Megasphaera*, *Prevotella* Shared *Finegoldia*, *Streptococcus*, *S. sanguinegens*. *Dialister*, *Prevotella*	risk of BV in female partners
(Plummer *et al.*, 2021)	To assess the impact of concurrent BV partner treatment on genitourinary sites over a 12-week period	Prospective open-label pilot	34 couples where the female has BV (mainly Australia) Men 31 (27–37) y Tx oral metronidazole (400mg twice daily for 7 days) and clindamycin applied topically to penile skin (2% cream twice daily for 7 days) Women 30 (27–34) y Tx oral metronidazole (400mg twice daily for 7 days) or intravaginal clindamycin (2% applicator vaginally for 7 nights)	Penile swab Vaginal lavage F/U 12 weeks	Men *Staphylococcus*, *Finegoldia*, *Prevotella*, *Corynebacterium*, *L. iners*, *Peptoniphilus* Woman *L. iners*, *Gardnerella*, *Prevotella*, *Sneathia*, *Peptoniphilus* Shared *Candidatus Lachnocurva vaginae* (BVAB-1), *L. crispatus, L. gasseri*, *Corynebacterium*, *Finegoldia*, *Aerococcus*, *Prevotella*	Immediately post-treatment, concurrent partner treatment significantly reduced the abundance of BV-associated bacteria and altered the overall vaginal and seminal microbiome composition A significant positive correlation of taxa between sexual partners longitudinally, being less sustained in male microbiome

(Manzoor *et al.*, 2021)	To characterise the microbiome associated with fertile and infertile couples	Cross-sectional	23 couples with infertility (Pakistan) Men 33.97 ± 6.14 y Women 28.25 ± 5.47 y Tx No 22 fertile couples (Pakistan) Men 33.97 ± 6.14 y Women 28.25 ± 5.47 y	Genital swabs F/U No	Men *Corynebacterium*, *Staphylococcus*, *Lactobacillus*, *Anaerococcus*, *Finegoldia*, *Prevotella* Woman *Lactobacillus*, *Atopobium*, *Prevotella*, *Corynebacterium*, *Gardnerella* Shared *Corynebacterium*, *Staphylococcus*, *Anaerococcus*, *Lactobacillus*, *Prevotella*	Male genital microbiome was more diverse compared to female Genital samples indicated big variability from an individual to another Several fluctuations in the diversity and composition of the genital microbiome associated with fertility/infertility
(Okwelogu *et al.*, 2021)	To determine the microbiome compositions of the semen and vagina from couples seeking assisted reproductive health care, to investigate whether seminal microbiota differs substantially from the vaginal microbiome, and to identify bacterial taxa associated with positive IVF clinical outcomes	Cross-sectional	36 couples with infertility (Nigeria) Male 26-60 y Women 26-45 y Tx No	Semen aliquot Vaginal swab F/U No	Men *Lactobacillus*, *Gardnerella*, *Veillonella*, *Corynebacterium*, *Escherichia*, *Prevotella*, *Enterococcus, Megasphaera* Women *Lactobacillus*, *Prevotella*, *Gardnerella*, *Megasphaera*, *Olsenella*, *Sneathia* Shared many of the predominant genera (56%)	Seminal microbiome composition was more diverse but lower in bacterial concentrations compared to the vagina Significant association between the microbiome of semen and vaginal samples in couples with infertility Semen samples with positive IVF outcome were less diverse and significantly colonised by *L. jensenii* and *Faecalibacterium*, and significantly less colonised

					G. vaginalis, *L. iners*, *L. japonicus*, *L. jensenii*	by *Proteobacteria* and *Bacteroidetes* phyla, and *Prevotella* Vaginal samples with positive IVF outcome were significantly colonised by *L. gasseri* and presented higher *Firmicutes*/*Baceroidetes* ratio
(Mehta *et al.*, 2022a)	To determine how the vaginal microbiome and penile microbiome contribute to women's and men's HSV-2 serostatus	Prospective	231 heterosexual couples (Kenya) Men median 26 Women median 22 Tx No	Penile swab Cervicovaginal lavage F/U No	Men *Corynebacterium*, *Anaerococcus*, *Streptococcus*, *Finelgoldia*, *L. iners* Women *L. iners*, *G. vaginalis*, *L. crispatus*, *Sneathia amnii* Shared *Staphylococcus*, *Corynebacterium*, *Streptococcus*	Vaginal α-diversity measures were greater for women whose male sex partners were HSV-2 positive Penile richness was elevated for men whose female partners were HSV-2 positive Penile microbial composition was influenced by circumcision status
(Mehta *et al.*, 2022b)	To characterise penile microbiome composition over a 1-year period and to identify factors associated with penile microbiome composition over time	Prospective	218 heterosexual couples (Kenya) Men 26 (24-30) y Women 22 (20-25) y Tx No	Penile swab Cervicovaginal lavage F/U 12 months	Men *Corynebacterium*, *Streptococcus*, *S. sanguinegens*, *Finegoldia*, *Anaerococcus*, *L. iners*, *Prevotella*, *G. vaginalis*, *Veillonella*	Penile microbiome composition was stable over a 1-year period and was influenced by circumcision status, sexual practices, female partner's vaginal CST and BV status, and men's HSV-2 status

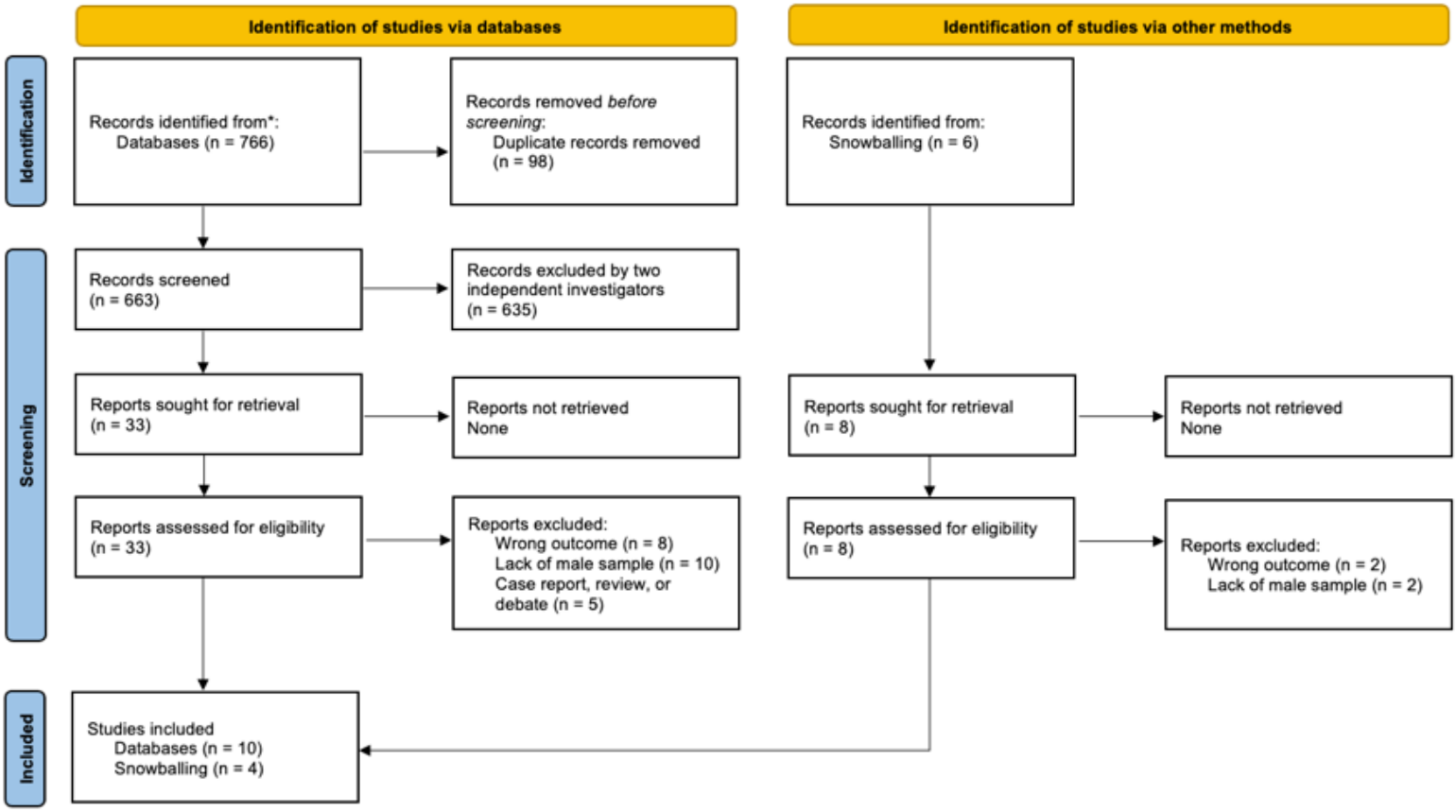
					Women *L. iners*, *G. vaginalis*, *L. crispatus* Shared *Lactobacillus* spp., *G. vaginalis*	BV was positively associated with the relative abundance of numerous individual penile taxa
(Iniesta *et al.*, 2022)	To determine the effect of a probiotic strain on the genitourinary dysbiosis	Interventional	17 couples with infertility and genitourinary dysbiosis (Caucasian) Men median 36 y Tx oral *Ligilactobacillus salivarius* PS11610 (10^9 CFU once daily for 6 months) Women median 35 y Tx oral *Ligilactobacillus salivarius* PS11610 (10^9 CFU twice daily for 6 months)	Penile swab, semen aliquot Vaginal swab F/U 6 months	Men *Peptoniphilus*, *Finegoldia*, *Lactobacillus*, *Streptococcus*, *Corynebacterium*, *Staphylococcus*, *Campylobacter*, *Prevotella*, *Anaerococcus* Women *Lactobacillus*, *Gardnerella*, *Streptococcus*, *Staphylococcus*, *Prevotella* Shared *Lactobacillus*, *Garnerella*, *Prevotella*	Male samples showed higher bacterial diversity than vaginal samples Post-treatment, the percentage of *Lactobacillus* in relation to the total bacterial counts increased in the vaginal microbiome At post-treatment, male urogenital microbiome showed slightly decreased pathogens and *Staphylococcus* spp. Post-treatment, shift from a proinflammatory to an anti-inflammatory profile of the couples at systemic level
(Koort *et al.*, 2023)	To determine the potential impact of female and male partners' reproductive tract microbiome composition on ART outcome	Cross-sectional	97 couples with infertility (Estonia) Men 37.4 (25–58) y Women 34.1 (25–46) y Tx No	Semen aliquot Vaginal swab F/U No	Men *Lactobacillus*, *Acinetobacter*, *Prevotella*, *Corynebacterium*, *Campylobacter*, *Flavobacterium*, *Finegoldia*, *Porphyromonas*	Semen microbiome diversity is higher compared to vagina Reproductive microbial communities of couples with infertility were significantly more diverse and with different predominance patterns in

			12 fertile couples (Estonia) Men 34.1 (22–42) y Women 32.3 (25–42) y		Women *L. crispatus*, *L. iners*, *Gardnerella*, *Atopobium*, *Bifidobacterium*, *Clostridium*, *Prevotella* Shared 96% of all detected OTUs (couples with infertility) and 65% (fertile couples) *Lactobacillus*, *Prevotellaceae*, *Bifidobacteriaceae*	comparison to fertile couples Couples with beneficial microbiome types had a significantly higher ART success rates compared to other couples Gram-negative anaerobes and microaerophiles associated negatively with ART success in both men and women
(Baud *et al.*, 2023)	To investigate the composition of genital microbiome in infertile couples and its potential impact on infertility, to explore the potential interaction between male and female microbiome, to investigate whether the microbiome of one partner could influence the composition of the other partner's microbiome	Cross-sectional	65 couples with infertility (Switzerland) Men reproductive age Women 25-4 y Tx No	Penile and semen swabs Vaginal swab F/U No	Men *Lactobacillus*, *Prevotella*, *Gardnerella*, *Corynebacterium*, *Staphylococcus*, *Porphyromonas*, *Peptoniphilus*, *Finegoldia*, *Campylobacter*, *Mobiluncus* Women *Lactobacillus*, *G.vaginalis*, *P. bivia*, *Atopobium* Shared *Lactobacillus*, *Prevotella*, *Staphylococcus*, *Ezakiella*	Vaginal samples had the highest bacterial load and male samples showed the highest diversity The paired vaginal and penis samples showed the lowest dissimilarity values compared to the vagina-semen A slight impact of male microbiome on the female bacterial colonisation

ART: assisted reproductive technology; BV: bacterial vaginosis; CFU: colony forming unit; CST: community state type; F/U: follow-up; IVF: *in vitro* fertilisation; HPV: human papilloma virus; HSV: herpes simple virus; IUI: intrauterine insemination; OTU: operational taxonomic unit; Tx: treatment; y: years old

FIGURES

Figure 1



*Records identified from PubMed, Web of Science, and Scopus databases.

Figure 2

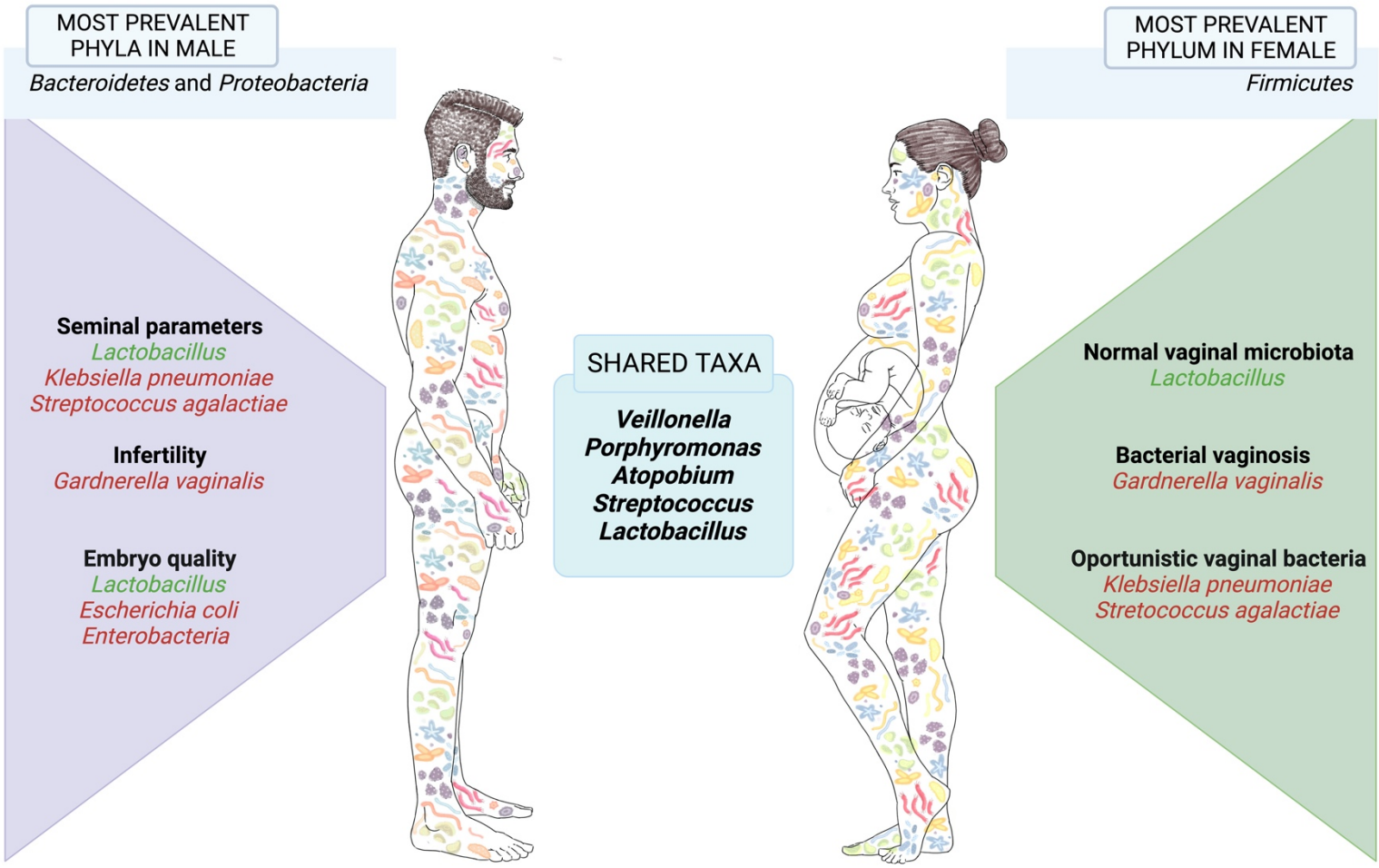

MOST PREVALENT PHYLA IN MALE
Bacteroidetes and Proteobacteria
Seminal parameters
Lactobacillus
Klebsiella pneumoniae
Streptococcus agalactiae
Infertility
Gardnerella vaginalis
Embryo quality
Lactobacillus
Escherichia coli
Enterobacteria
SHARED TAXA
Veillonella
Porphyromonas
Atopobium
Streptococcus
Lactobacillus
MOST PREVALENT PHYLUM IN FEMALE
Firmicutes
Normal vaginal microbiota
Lactobacillus
Bacterial vaginosis
Gardnerella vaginalis
Oportunistic vaginal bacteria
Klebsiella pneumoniae
Stretococcus agalactiae

Figure 3

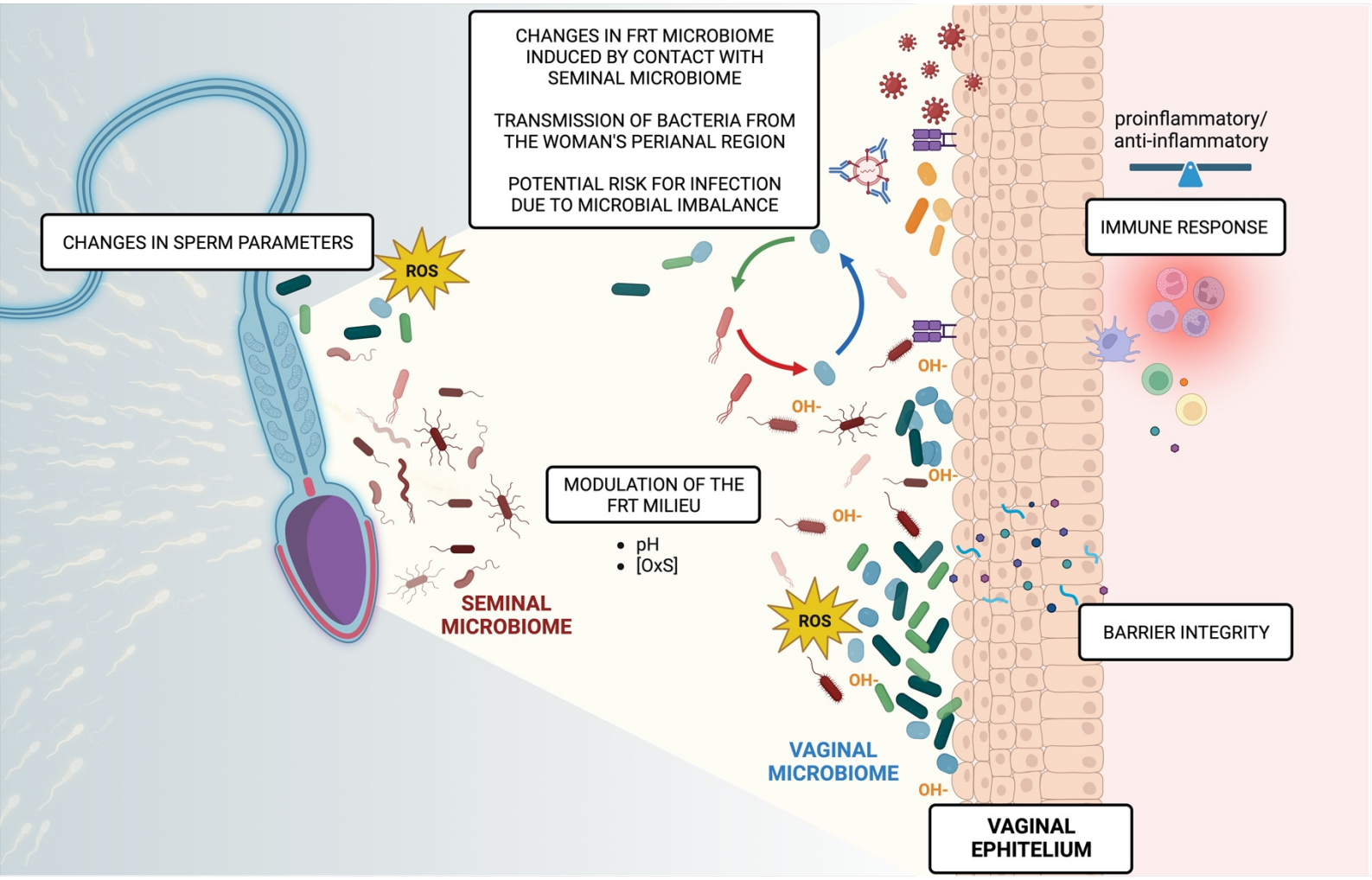

CHANGES IN FRT MICROBIOME INDUCED BY CONTACT WITH SEMINAL MICROBIOME
TRANSMISSION OF BACTERIA FROM THE WOMAN'S PERIANAL REGION
POTENTIAL RISK FOR INFECTION DUE TO MICROBIAL IMBALANCE
CHANGES IN SPERM PARAMETERS
ROS
proinflammatory/ anti-inflammatory
IMMUNE RESPONSE
OH-
MODULATION OF THE FRT MILIEU
pH
[OxS]
SEMINAL MICROBIOME
ROS
BARRIER INTEGRITY
VAGINAL MICROBIOME
VAGINAL EPHITELIUM

FIGURE CAPTIONS

Figure 1. PRISMA 2020 flow diagram for systematic review. Study identification, screening, and eligibility.

Figure 2. Overview representing the most prevalent phyla in seminovaginal interplay, taxa related to reproductive health, and shared taxa. Data compiled from 16S rRNA gene sequencing-based studies analyzing microbes in reproductive niches within couples. Green and red colors in the bacterial taxa indicate positive and negative effects on the specified outcomes, respectively (created with BioRender.com).

Figure 3. Potential mechanisms of the seminovaginal microbiome interplay (created with BioRender.com). FRT: female reproductive tract; OxS: oxidative stress; ROS: reactive oxygen species.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Quality assessment tool for observational cohort and cross-sectional studies. The first sheet shows the results of the quality assessment, the second sheet presents the questions corresponding to each item.

Supplementary Table 2. PRISMA 2020 checklist.

Supplementary Table 3. Search equations used in PubMed, Web of Science, and Scopus.

AUTHORS' CONTRIBUTION

N.M.M., A.C-G., and S.A. conceived and designed the study; N.M.M. and A.C-G. drafted the search strategy, performed literature search, and evaluated eligibility criteria; N.M.M.

and I.L-B. evaluated quality assessment; N.M.M and A.C-G. extracted data and drafted the manuscript; N.M.M., A.C-G., I.L-B., A.S-L., E.V., S.R-D., C.M.T., A.C-G., R.M., and S.A. interpreted the results; A.S-L. designed the figures. All authors revised and agreed with the final version of the manuscript.

FUNDING

This work was supported by MCIN/AEI/10.13039/501100011033 and ERFD A way of making Europe under grants Endo-Map PID2021-12728OB-100, ROSY CNS2022-135999, and PRE2018-085440; MCIN/AEI/10.13039/501100011033 and ESF Investing in your future under grant FPU19/01638; Plan de Recuperación, Transformación y resiliencia, Ayudas para la recualificación del sistema universitario español under grant Ayudas Margarita Salas UJAR01MS.