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The vaginal microbiome of trans men and women: a systematized narrative review

O microbioma vaginal de homens e mulheres trans: uma revisão narrativa sistematizada

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ABSTRACT

The vaginal microbiota is a complex ecosystem, influenced by genetic, environmental, and hormonal factors. It is plausible that changes in the vaginal/neovaginal microbiota of transgender people have implications for their health. This review aims to characterize the vaginal microbiota of trans men using testosterone and the neovagina of trans women. This systematic narrative review was conducted in January 2025, in accordance with the PRISMA protocol. The search was conducted in PubMed, the Biblioteca Virtual de Saúde, Scopus, Periódicos CAPES, and the Scientific Electronic Library Online, using Health Sciences Descriptors and the Medical Subject Headings vocabulary. Studies involving cisgender populations or other populations were considered when comparing or contextualizing the findings. Results showed that the neovagina with penile/scrotal grafts may contain *Actinobacteria*, *Bacteroidetes*, *Lactobacillus*, *Staphylococcus*, and *Streptococcus*. The sigmoid/rectal flap may contain *Bacteroidaceae* and *Enterobacteriaceae*. Trans men may harbor *Atopobium*, *Prevotella*, *Gardnerella*, and *Lactobacillus*. The use of estrogen, surgical technique, and graft origin may impact the neovaginal microbiota of trans women. The findings should be interpreted with caution due to the heterogeneity among populations, samples, and microbiological identification methods used in the included studies.

Keywords. Vagina, Transgender Persons, Microbiota, Microbiome, Human.

RESUMO

A microbiota vaginal é um ecossistema complexo, influenciado por fatores genéticos, ambientais e hormonais. É plausível que as alterações na microbiota vaginal/neovaginal de pessoas transgênero repercutam na saúde. Esta revisão tem como objetivo caracterizar a microbiota vaginal de homens trans que utilizam testosterona e a microbiota da neovagina de mulheres trans. Esta revisão narrativa sistematizada foi realizada em janeiro de 2025, de acordo com o protocolo PRISMA. A pesquisa foi realizada no PubMed, na Biblioteca Virtual de Saúde, no Scopus, nos Periódicos CAPES e na Scientific Electronic Library Online, utilizando Descritores de Ciências da Saúde e o vocabulário Medical Subject Headings. Foram considerados estudos envolvendo populações cisgênero ou outras populações, quando utilizados para comparação ou contextualização dos resultados. Os resultados revelaram que a neovagina com enxertos penianos/escrotais pode conter *Actinobacteria*, *Bacteroidetes*, *Lactobacillus*, *Staphylococcus* e *Streptococcus*. O retalho sigmoide/retal pode conter *Bacteroidaceae* e *Enterobacteriaceae*. Os homens trans podem albergar *Atopobium*, *Prevotella*, *Gardnerella* e *Lactobacillus*. A utilização de estrogênio, a técnica cirúrgica e a origem do enxerto podem impactar na microbiota neovaginal das mulheres trans. Os resultados devem ser interpretados com cautela devido à heterogeneidade das populações, das amostras e dos métodos de identificação microbiológica utilizados nos estudos incluídos.

Palavras-chave. Vagina, Transgênero, Microbiota, Microbioma Humano.

INTRODUCTION

Transgender people are defined as those whose gender identity is incongruent with their biological sex assigned at birth[1]. Trans men are those whose gender identity is congruent with the social roles assigned to men and are therefore men assigned female at birth. Trans women are the opposite: they are women born with a male genital phenotype. Since gender transition goes from one extreme to the other in these binary identities, use other terms to refer to such people: female-to-male (FTM) for trans men and male-to-female (MTF) for trans women.

Hormone therapy is an intervention that transgender people can use as a means of giving them physical characteristics consistent with the gender with which they identify. In the case of transgender men, hormone therapy is based on the administration of exogenous testosterone, which is intended to induce virilization[2]. For trans women, hormone therapy consists of the use of estrogens and anti-androgens. Another option for trans women is gender affirmation surgery, which involves multiple surgical approaches[3].

In eubiosis, the vaginal microbiota of cisgender women of reproductive age is composed predominantly of *Lactobacillus* spp., including *Lactobacillus crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*, which produce abundant local lactic acid to maintain an acidic pH (3.8-4.5)[4]. The literature indicates that vaginas with a predominance of *Lactobacillus* spp. are less prone to develop bacterial sexually transmitted infections (STIs), such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, and viral infections, such as human papillomavirus (HPV) and human immunodeficiency virus (HIV)[5-8].

Among trans women, despite estrogenization, vaginoplasty surgery uses tissues with different constitutions from the cisgender vagina, such as penile, scrotal, or intestinal tissue. The effects of these processes on the vagina of transmasculine individuals and the neovagina of trans women have not been fully characterized; however, the hormonal influence and tissue structure shape the genital microbiome, suggesting that both hormone therapy and gender affirmation surgeries lead to changes in the microbiota. Therefore, as occurs in cisgender people, alterations in the genital microbiota are likely to have a distinct impact on the health of transgender people[9]. In addition, studies involving cisgender women or other populations may serve as comparative or contextual references. Still, readers should interpret their findings with consideration of differences in population, sample type, and microbiological methods.

Given the relevance of the vaginal microbiota to sexual and reproductive health, further knowledge about its characteristics in transgender populations is important for gynecological care. This review aims to characterize the vaginal microbiota of trans men using testosterone and the neovagina of trans women constructed using several surgical methods, considering the methodological heterogeneity among the available studies.

MATERIAL AND METHODS

Study characterization

This study is a systematic narrative review of the literature, conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[10]

and registered in the International Prospective Register of Systematic Reviews (PROSPERO; CRD42024535446).

Search strategies and eligibility criteria

The search, reading, and tabulation of scientific output were conducted between January and March 2025.

The research question was defined using the PICO strategy[11], guiding the investigation of the components of the vaginal microbiota in trans men undergoing testosterone therapy and of the neovaginal microbiota in trans women. The PICO components were defined as follows: P (Population) = trans men undergoing testosterone therapy and trans women; I (Interest) = the composition and microbial components of the vaginal and neovaginal microbiota; and Co (Context) = the vaginal environment of trans men and the neovaginal environment of trans women. Studies involving cisgender women or other populations were considered only when they allowed comparison or contextualization of the findings related to transgender populations, without replacing the main population of interest of this review.

The study used the following inclusion criteria: a) original articles, b) available in full text, c) written in English, Spanish or Portuguese, d) with no time frame, and e) related to the subject of the study, indexed in the following data libraries: U.S. National Library of Medicine (PubMed), Biblioteca Virtual de Saúde (BVS), Scopus, Periódicos CAPES and Scientific Electronic Library Online (SciELO), which index the Medical Literature Analysis and Retrieval System Online (MedLine) and Latin American and Caribbean Literature in Health Sciences (LILACS) databases, among others. These libraries/databases were chosen owing to their national and international scope, as well as their role as references in the production of scientific knowledge in the area, enabling broad retrieval of the available literature. The exclusion criteria were as follows: a) theses, dissertations, monographs, event proceedings, conference abstracts, study protocols, experience reports, case reports and review articles; b) non-original studies; c) studies whose full text was not available; d) studies whose population did not include transgender people, except when used only as contextual support in the discussion; e) studies without original microbiological, microbiome, microflora or flora-related outcome data; and f) articles not related to the subject of the study.

To select the studies, controlled descriptors available in the Health Sciences Descriptors (DeCS) and PubMed's Medical Subject Headings (MeSH), in Portuguese, Spanish, and English, were used. The descriptors were divided into four groups: Group 1 (microbiota and its variations), Group 2 (trans men, trans women, and their variations), Group 3 (vagina and variations), and Group 4 (sex reassignment surgery and variants). The search was carried out using the descriptors in Group 1, along with language, plus the Boolean operator "AND" to cross-reference them with the descriptors in Groups 2, 3, and 4. In addition, for synonymous descriptors, the Boolean operator "OR" was used. The reference lists of the included studies were also manually screened to identify additional relevant articles.

The studies were selected in two stages. In the first stage, titles and abstracts were reviewed against the eligibility criteria. In the second stage, the potentially eligible studies were read in full. Articles that did not meet the established criteria or were not directly related to the objective of the review were excluded. When studies presented mixed populations, data related to transgender people were prioritized. When data

from transgender participants were not clearly separable, but the study addressed neovaginal tissue, graft characteristics, or microbiological findings relevant to the objective of the review, the study was retained only as contextual or methodologically limited evidence, without being interpreted as an isolated estimate of the transgender microbiome. When it was not possible to separate the transgender population from other groups, and the study did not provide microbiological evidence directly relevant to the vaginal or neovaginal environment, it was not considered part of the main corpus of the review. It could be used only as contextual support when relevant.

Data extraction and synthesis

To simplify data extraction, the researchers designed a collection tool incorporating information on the library/database, author and year, study title, origin, language, method, objective, level of evidence, and author highlights. Whenever available, additional data were extracted for the study population, type of biological sample analyzed, microbiological identification method, and the main microorganisms reported in each study. The extraction of microbiological findings was based on the microorganisms described in the original studies, taking into account the methodological particularities reported by each article, such as culture, cytology, polymerase chain reaction, sequencing, or other microbiological approaches, when available. These methodological differences were considered during the narrative synthesis, since they may influence the identification and comparability of the reported microorganisms. Therefore, direct comparisons between studies using different microbiological methods were avoided or interpreted with caution, especially when studies employed techniques with varying levels of sensitivity and specificity.

The studies were classified according to the level of evidence proposed by Melnyk et al[12]: Level I, systematic reviews or meta-analyses of randomized controlled trials or evidence-based clinical guidelines based on such reviews; Level II, at least one well-designed randomized controlled trial; Level III, well-designed clinical trials without randomization; Level IV, well-designed cross-sectional, ecological, cohort, or case-control studies; Level V, systematic reviews of descriptive and qualitative studies; Level VI, a single descriptive or qualitative study; and Level VII, expert opinions, experience reports, consensus documents, regulations, and legislation. However, the authors did not perform a formal risk-of-bias assessment.

RESULTS

Study selection and characteristics

A total of 26 studies were initially identified in the databases: eight in PubMed, five in BVS, five in Periódicos CAPES, and eight in Scopus. In addition, we retrieved eight articles from additional sources. From the total number of articles identified, 17 were duplicates. We analyzed the remaining 17 articles based on their titles and abstracts and excluded three: two were unrelated to the theme, and one did not meet the inclusion criteria. The remaining 14 studies were evaluated in full. Four were excluded after full-text assessment: one because it was a conference abstract, one because

it corresponded to a study protocol, one because it involved a population outside the scope of the review, and one because it presented a mixed population without sufficient separation of data related to transgender people. Therefore, 10 studies were included in the main corpus of this review. The flowchart for the selection of studies, according to the PRISMA guidelines[10], is shown in **Figure** below.

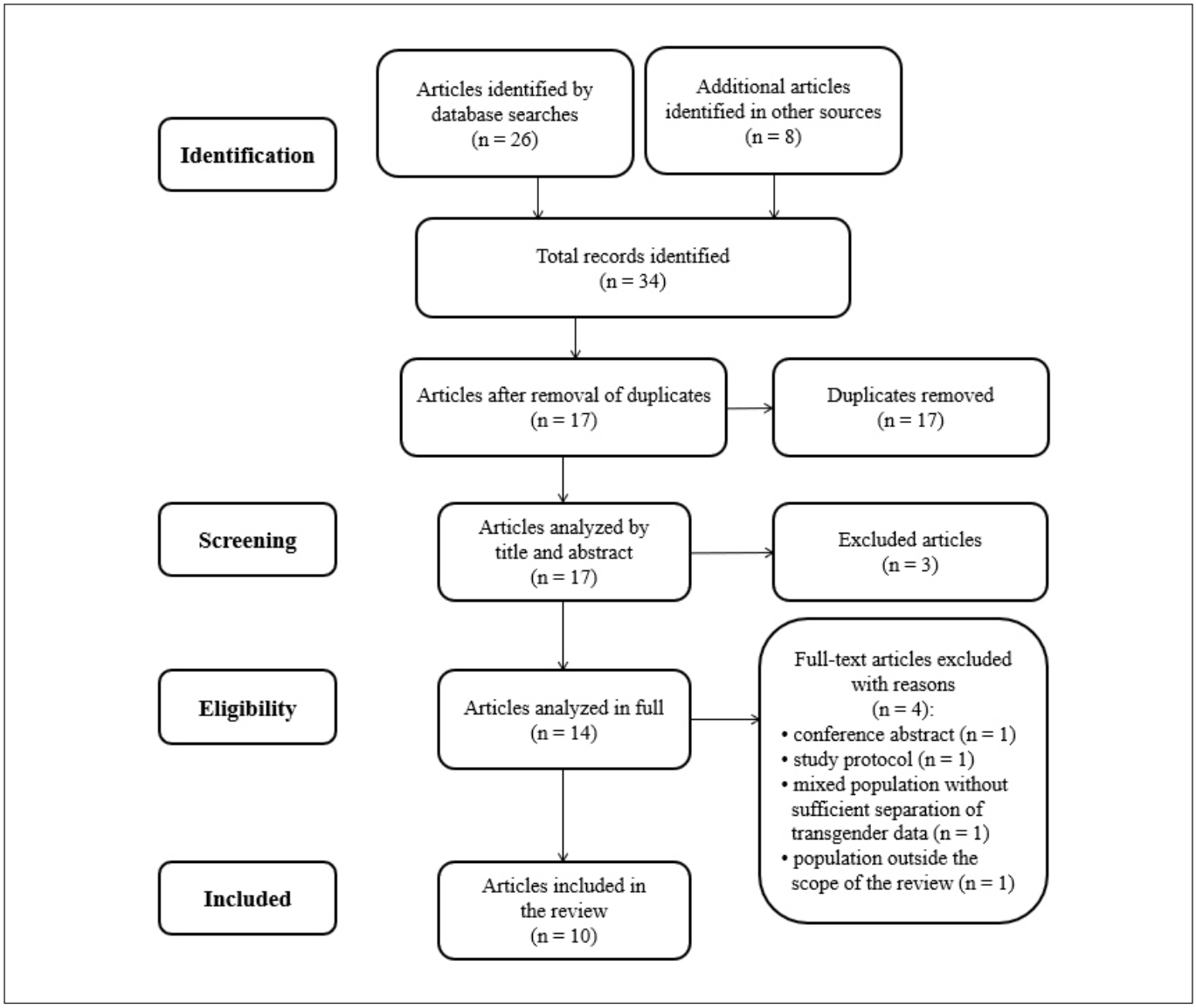

Figure. Flowchart of the study selection process for inclusion in the systematized narrative review. Adapted from PRISMA[10]

All 10 included studies were written in English[13-22]. Among the countries, one article was from Brazil[13], two from the United States[14,15], three from Austria[16-18], one from South Korea[19], one from the Netherlands[20], one from Belgium[21], and one from Switzerland[22]. A summary of the characteristics of the selected articles is shown in **Table 1**. Because the studies used different designs, populations, biological samples, and microbiological identification methods, their findings were interpreted narratively and with caution.

Table 1. Characteristics of the selected articles

Reference	Population	Sample	Microbiology analysis method	Limitations
13	Transgender women	Neovaginal and rectal	Mass spectrometry-based metaproteomics, with additional 16S rRNA gene analysis	Small sample size; no comparability
14	Transgender men and nonbinary individuals *vs* cisgender women	Self-collected vaginal swabs	Broad-range PCR targeting the V3-V4 region of the 16S bacterial rRNA gene; next-generation sequencing and phylogenetic placement	Small sample size; low comparability
15	Transmasculine individuals *vs* cisgender women	Pap tests; compared with institutional cytology data	Cervicovaginal cytology specimens, HR-HPV testing and vaginal flora	Small sample; absence of molecular studies; low comparability
16	Transsexual women	Neovaginal smears	PCR-DGGE	Small sample; focus on specific species of bacteria
17	Transsexual women	Neovaginal and rectal smears	PCR-DGGE	Small sample; focus on *Lactobacillus* species between sites
18	Transsexual women	Neovaginal smears	Culture and real-time PCR	Small sample; limited evidence of a causal relationship
19	Trans women *vs* cisgender women	Vaginal wall biopsy	pH measurement, culture of normal flora and microscopy	Small sample; mixed population; microbiological findings were reported for the overall cohort and are not trans-specific. Use only with caution for rectosigmoid graft context
20	Trans women *vs* cisgender women	Rectal, neovaginal and skin swabs	Interspacer profiling (IS-pro), a PCR-based bacterial profiling technique	Small sample; mixed population

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Reference	Population	Sample	Microbiology analysis method	Limitations
21	Transsexual women	Swab samples	Gram stain, culture, tDNA-PCR and species-specific PCR for *A. vaginae*, *G. vaginalis* and *M. curtisii*. Direct microbiological evidence for penile skin-lined neovagina	Small sample; focus on specific species of bacteria; methodology that is not sufficiently detailed
22	Transgender *vs* cisgender women	Swab samples	Cytology and HPV PCR	Small sample; mixed population; absence of molecular methods

Note: Direct comparisons between studies were avoided or interpreted with caution because the studies differed in population, sample type, surgical technique, and microbiological method. Study protocols were excluded from the main corpus of the review
Source: Prepared by the authors, 2025

The included studies characterized the vaginal/neovaginal microbiota of trans men and trans women. In the following section, these results are summarized into four groups: 1) Vaginal microbiota and vaginal flora of trans men using testosterone and comparison with cisgender women, 2) Evaluation of the neovaginal and rectal microbiota or flora of trans women, 3) Evaluation of microbial changes after rectal/sigmoid graft vaginoplasty, and 4) Microbiome or microflora of the neovagina constructed with penile/scrotal flap. **Table 2** summarizes the main microorganisms observed in the neovagina constructed via penile inversion/scrotal graft, intestinal graft, and in the vaginas of transgender men using testosterone, considering the methodological limitations of each study.

Table 2. Summary of the main microorganisms observed in the neovagina constructed via scrotal graft/penile inversion, intestinal graft, and in the vaginas of transgender men using testosterone, according to the microbiological method used

Context	Studies	Main microorganisms/flora findings	Interpretative caution
Trans men using testosterone	14, 15	McPherson et al reported that transgender men were less likely to have *Lactobacillus* as the primary genus and had higher microbial diversity Taxa with relative abundance included *Atopobium*, *Prevotella*, *Peptostreptococcus*, *Sneathia* and *Finegoldia*, with *Gardnerella* also detected among transgender men. Lin et al found lactobacilli substantially decreased in most reviewed cytology slides	Do not merge these studies as equivalent evidence: study 14 used 16S rRNA sequencing, whereas study 15 used cytology/histology. Both suggest a reduction in *Lactobacillus*, but with different methodological strength

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Context	Studies	Main microorganisms/flora findings	Interpretative caution
Penile inversion/ scrotal graft neovagina broad microbiological profile	13, 21	Birse et al identified *Porphyromonas*, *Peptostreptococcus*, *Prevotella*, *Mobiluncus* and *Jonquetella* as abundant neovaginal taxa, with additional findings including *Jonquetella anthropi* and *Synergistaceae*. Weyers et al found mixed aerobic and anaerobic microflora; frequent species included *Staphylococcus epidermidis*, *Streptococcus anginosus* group spp., *Enterococcus faecalis*, *Corynebacterium* sp., *Mobiluncus curtisii*, and *Bacteroides ureolyticus*	Both studies support a mixed/anaerobic profile, but they used different methods and should not be compared as prevalence estimates. The presence of BV-associated taxa does not equal diagnosis of BV in neovaginas
Lactobacillus focused evidence in penile skin-lined neovagina and rectum	16, 17	Petricevic et al detected *Lactobacillus* species in penile skin-lined neovaginas, especially *L. gasseri*, *L. crispatus*, *L. johnsonii* and *L. iners*. In the neovaginal-rectal study, the most frequent matching *Lactobacillus* species were *L. gasseri*, *L. johnsonii*, *L. crispatus*, *L. iners*, and *L. jensenii*	These studies are useful for *Lactobacillus*-specific interpretation, but they do not characterize the whole microbiome. Their results should not be interpreted as broad neovaginal microbiome profiles
Trans women after probiotic intervention	18	Oral administration of *Lactobacillus* strains increased lactobacilli and improved Nugent score compared with placebo. The probiotic preparation included *L. crispatus*, *L. rhamnosus*, *L. jensenii* and *L. gasseri*	This is an intervention study. It should be interpreted separately from studies describing spontaneous neovaginal microbiota
Rectosigmoid/ sigmoid-derived neovagina	13, 19, 20	In Birse et al, the neovaginal sample with sigmoid colon graft extension showed a more gut-like profile, including *Bacteroidaceae*, *Enterobacteriaceae* and *Escherichia*. Kim et al reported mainly *Escherichia coli*, *Proteus vulgaris*, *Streptococcus viridans* and *Streptococcus dysgalactiae* in the neovagina over postoperative follow-up. Van der Sluis et al identified discriminative taxa including *Sutterella* spp., *Bacteroides vulgatus*, *Alistipes finegoldii*, *Prevotella denticola*, *Faecalibacterium prausnitzii*, *Finegoldia magna*, *Bacteroides dorei*, *Alistipes putredinis*, and *Prevotella* spp.	The intestinal origin of the graft appears to influence the neovaginal microbiota. However, Kim et al involved a mixed population and Van der Sluis et al focused on fecal stream diversion; therefore, findings should be used cautiously for transgender-specific interpretation
Cytological and fungal findings in neovaginas	22, 23, 24, 25	Grosse et al reported *Doederlein bacilli* in a minority of samples and Candida colonization in one case. De Haseth et al described symptomatic neovaginal candidiasis in transgender women after penile inversion vaginoplasty. Other references provide contextual information on vulvovaginal candidiasis and vaginal microflora	Cytology is not equivalent to microbiome sequencing. Fungal components remain underexplored and should be discussed as a gap, rather than as a robust microbiome outcome

Note: Direct comparisons between studies were avoided or interpreted with caution because the studies differed in population, sample type, surgical technique, and microbiological method. Study protocols were excluded from the main corpus of the review
Source: Prepared by the authors, 2025

Vaginal microbiota and vaginal flora of trans men using testosterone and comparison with cisgender women

In this group, two studies[14,15] aimed to compare microbiome or cervicovaginal flora characteristics between transmasculine individuals on testosterone hormone therapy and cis women, or to describe the vaginal microbiota or flora of trans men using testosterone. These studies provide insights into potential differences in the microbiota of these populations, contributing to an in-depth understanding of transgender vaginal health and the hormonal impacts on the microbiome. These studies indicated that transmasculine individuals may present *Atopobium*, *Prevotella*, *Peptostreptococcus*, *Sneathia*, *Finegoldia*, *Gardnerella*, and *Lactobacillus*. Multiple microorganisms were found to be more abundant in transgender men than in cis women[14]. As for the identification techniques, McPherson et al[14] identified the bacterial community profiles using broad-range PCR primers targeting the 16S rRNA region. In contrast, Lin et al[15] used cytological and histological methods. Considering these methodological differences, particularly the distinction between molecular sequencing and cytological assessment of vaginal flora, the comparison between studies was interpreted cautiously.

Evaluation of the neovaginal and rectal microbiota or flora of trans women

Five studies[13,16-18,21] focused on characterizing the neovaginal and rectal microbiota (flora) of transgender women, including investigations of *Lactobacillus* species and one therapeutic intervention with oral lactobacilli. They explored neovaginal-rectal co-colonization and correlations with hormonal, behavioral, and surgical factors, providing valuable information for the management of these women's vaginal health. The main microorganisms described included *Lactobacillus* spp., *Prevotella*, *Porphyromonas*, *Peptostreptococcus*, *Mobiluncus*, *Jonquetella anthropi*, *Staphylococcus epidermidis*, *Streptococcus anginosus* group spp., *Enterococcus faecalis*, *Corynebacterium* sp., and *Bacteroides ureolyticus*. The identification techniques were: metaproteomics and 16S rRNA gene analysis on neovaginal, rectal, and vaginal secretions[13], molecular profiling by denaturing gradient gel electrophoresis (PCR-DGGE) on neovaginal smears[16], PCR-DGGE on neovaginal and rectal smears[17], culture and real-time PCR after probiotic intervention[18], and Gram stain, culture, tDNA-PCR, and species-specific PCR[21]. The study by Kaufmann et al[18] involved probiotic intervention; therefore, its results were interpreted separately from spontaneous microbiota characterization.

Evaluation of microbial changes after rectal/sigmoid graft vaginoplasty

This group of studies included evidence from Birse et al[13], Kim et al[19], and Van der Sluis et al[20], which investigated microbial findings associated with neovaginas constructed or extended with rectal/sigmoid tissue. These studies highlight the importance of the grafted tissue and its impact on the microbial profile of the neovagina. The studies from this group identified *Bacteroidaceae*, *Enterobacteriaceae*, *Escherichia*, *Escherichia coli*, *Proteus vulgaris*, *Streptococcus viridans*, *Streptococcus dysgalactiae*, *Sutterella* spp., *Bacteroides vulgatus*, *Alistipes finegoldii*, *Prevotella denticola*, *Faecalibacterium prausnitzii*, *Finegoldia magna*, *Bacteroides dorei*, *Alistipes putredinis*, and *Prevotella* spp. The identification techniques used by the authors were metaproteomics and 16S rRNA gene analysis[13], biopsy, pH tests, histology and cultures[19],

as well as interspacer profiling, a PCR-based bacterial profiling technique[20]. Because Kim et al[19] included a mixed population and lacked dedicated data for transgender women, we interpreted their findings as contextual evidence related to rectosigmoid grafts. Because these studies used different microbiological approaches, their findings were synthesized narratively and not compared directly.

Microbiome or microflora of the neovagina constructed with penile/scrotal flap

Three studies[13,21,22] characterized microbiological or cytological findings in neovaginas constructed with penile/scrotal tissue or skin-lined techniques. These studies suggest that the surgical method and the tissue used to construct the neovagina may influence the microbiome, microflora, and cytological characteristics of the neovaginal environment. These studies revealed mixed microbiota, with Gram-negative and Gram-positive cocci, *Bacteroides ureolyticus*, *Corynebacterium* sp., *Enterococcus faecalis*, *Mobiluncus curtisii*, *Staphylococcus epidermidis*, *Streptococcus anginosus* group spp., *Lactobacillus*, and *Candida* colonization in isolated cases. These authors identified the bacterial profiles through metaproteomics and 16S rRNA gene analysis[13], Gram stain, culture, tDNA-PCR and species-specific PCR[21], and cytology[22]. The use of cytology limits the characterization of the microbiota compared with molecular methods; we therefore interpreted the findings from Grosse et al[22] with caution and avoided direct comparison with sequencing-based or metaproteomic studies.

DISCUSSION

Trans men's vaginal microbiome

McPherson et al[14] conducted a cross-sectional study of 28 transgender men and nonbinary individuals who had not undergone sex reassignment surgery and had been taking exogenous testosterone for at least one year, with a control group of eight cisgender women. Vaginal swabs were collected from these patients, and bacterial community profiles were assessed using broad-range PCR primers targeting the V3-V4 region of the 16S bacterial rRNA gene, next-generation sequencing, and phylogenetic analysis. In this study, *Lactobacillus* was identified as the predominant bacterial genus (90%) in the vagina of all the cisgender women evaluated, even in one participant with symptomatic bacterial vaginosis (BV). In contrast, the majority of transgender men (20/28) had a very low relative abundance (2%) of *Lactobacillus* species; five trans men had a lower relative abundance (25%), and three had a higher relative abundance (90%) of *Lactobacillus* in the vagina. Scrutiny of the group with 90% *Lactobacillus* indicated that two of these trans men were prescribed vaginal estrogen to reduce tissue atrophy.

In this study[14], there was a significant correlation between vaginal estrogen therapy and the presence of a predominant *Lactobacillus* vaginal environment in trans men and no association between a history of sexually transmitted infections (STIs) and the presence of *Lactobacillus* or between receptive vaginal sex and the presence of *Lactobacillus*[14]. In addition, several bacteria found in relative abundance in transgender men, such as *Atopobium*, *Prevotella*, *Peptostreptococcus*, *Sneathia*, and *Finegoldia*, are traditionally associated with BV in cis women[14]. *Gardnerella*, which is commonly associated with BV, was detected in one-third (1/3) of transgender men and one-quarter of cisgender women. Notably, however, the relative abundance of

Gardnerella in cis women was lower than that observed in trans men, with 2 participants having a relatively high abundance (> 40%), 4 relatively low (1-2%), and 4 very low (< 1%)[14].

Although *Lactobacillus* was less predominant among trans men than among cisgender women, it was not completely absent in all trans men evaluated. The relative abundance of *Lactobacillus* differed greatly among these men, with three having more than 90%, three with 15-25%, 3 with 1-10%, and three with 1% *Lactobacillus*. The only covariate that accompanied the abundance of *Lactobacillus* in the transgender men was the use of an estrogen ring, which two of the three men with 90% lactobacilli used[14]. The decrease in estrogen reduces glycogen in cis women after menopause, making conditions less favorable for *Lactobacillus* species. This also induces epithelial thinning and, consequently, alters the vaginal microbiota[26,27]. Data from McPherson et al[14] suggest that a similar correlation between estrogen and vaginal *Lactobacillus* abundance may occur in transgender men.

Notably, changes in the microbiome may lead to BV, which is often observed in cisgender women[7,8]. However, in the cohort of Lin et al[15], none of the trans men presented clinical or cytological evidence of BV, suggesting that changes in the vaginal microbiota, secondary to testosterone, may have different implications in these individuals than in cisgender women of reproductive age. Lin et al[15] noted that lactobacilli were substantially reduced in 89% of their samples, and all patients with atrophic changes rarely had lactobacilli. Among the six patients without significant atrophic changes, two presented predominantly intermediate squamous cells, and a general decrease in *Lactobacillus*. In contrast, the remaining four patients presented with numerous superficial squamous cells, with abundant lactobacilli in three patients and none in one. Patients with virtually absent lactobacilli had been receiving testosterone for significantly longer periods than those with lactobacilli present, suggesting a prominent change in the vaginal microbiome in FTM individuals, with a significant decrease in lactobacilli associated with longer exposure to testosterone[15]. However, this interpretation must account for the fact that cytology and histology are not equivalent to molecular methods for microbiota characterization, thereby limiting direct comparison with sequencing-based studies.

Although we excluded study protocols from the main corpus of this review, we included the protocol by Muzny et al[28] as contextual support because it proposes a prospective investigation of vaginal microbiota changes in transgender men after starting testosterone. The protocol aims to evaluate changes in vaginal microbiota composition over time, identify variations that precede incident BV (iBV), and investigate behavioral and hormonal factors associated with these changes. The authors hypothesize that, in approximately 50% of these men, the baseline vaginal microbiota will migrate toward dysbiosis within 90 days of starting testosterone, based on previous evidence from McPherson et al[14]. Therefore, this reference was not interpreted as direct microbiological outcome evidence and was not included in the main corpus of the review. A similar study from 2024 showed that transgender men using testosterone were less likely to have resident *Lactobacillus* spp. in the vaginal environment compared to pre-testosterone transgender men, supporting previous findings[29].

Given the sparse literature concerning the effects of testosterone on the vaginal microbiome, it is impossible to infer a consensus. However, it is suggested that the vaginal microbiota of trans men is similar to that of cisgender women during menopause, given the atrophy of the mucosa, although it differs in composition. The prevalence of *Lactobacillus* in the vaginas of trans men subjected to testosterone appears to be reduced, suggesting a possible correlation between vaginal estrogen levels, suppressed by testosterone,

and the abundance of lactobacilli. Taken together, these findings suggest a recurrent pattern of reduced *Lactobacillus* and increased abundance of anaerobic bacteria in trans men using testosterone, although the available evidence remains limited and heterogeneous. Thus, the presence of microorganisms traditionally associated with BV in cisgender women should not be automatically interpreted as the same clinical condition in trans men, since hormonal environment, epithelial maturation, and diagnostic criteria may differ.

The trans women's neovagina

Penile inversion technique/scrotal graft

Birse et al[13] collected rectal and neovaginal secretions of nine transgender women and compared them to vaginal secretions of cisgender women. However, only five neovaginal samples and seven rectal samples from transgender women yielded measurable bacterial proteins for microbiological analysis; the authors compared these 32 vaginal samples from 30 cisgender women. Using mass spectrometry, a total of 541 unique bacterial proteins from 38 genera across seven phyla were identified: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Synergistetes*, and *Tenericutes*. On average, eight species were identified per neovaginal sample, six species per rectal sample, and five species per cis vaginal sample. The most prevalent taxa by protein abundance in the neovaginal samples were *Porphyromonas* (30.2%), *Peptostreptococcus* (9.2%), *Prevotella* (9.0%), and *Mobiluncus* (8.0%), along with a large, indistinguishable proportion (16.9%). In rectal secretions, the most abundant bacterial taxa were *Prevotella* (52.0%), *Roseburia* (20.7%), *Firmicutes* (6.8%), *Eubacterium* (3.6%), and *Lysinibacillus* (3.1%). In cis vaginal secretions, the most abundant taxa were *Lactobacillus* (64.8%), *Gardnerella* (18.2%), *Lysinibacillus* (8.2%), and *Prevotella* (2.7%)[13]. The authors identified *Jonquetella anthropi* exclusively in 60% of the neovaginal samples. Genes from the *Synergistaceae* family were detected in 80% of the neovaginal samples. Other unique taxa identified in at least one neovaginal sample included several *Proteobacteria* (*Escherichia*, *Campylobacter*, and *Eikenella*), *Firmicutes* (*Anaerosphaera*, *Anaeroglobus*, and *Pseudoramibacter*), *Fusobacteria* (*Fusobacterium*), and *Actinobacteria* (*Actinomyces*)[13].

In summary, this study revealed that anaerobic bacterial species dominated the neovaginal microbiome and indicated that patients who underwent sex reassignment surgery using the surgical method of penile inversion/scrotal graft had bacteria compatible with the tissue used in the construction of the neovagina, including high levels of *Prevotella*, *Porphyromonas*, and *Peptoniphilus*, which is congruent with other studies that evaluated the circumcised penile microbiome and uncircumcised microbiome[29,30] and the trans vaginal microbiome[17,21]. Finally, several taxa associated with BV in cisgender women have been detected in neovaginas, including elevated levels of *Prevotella*, *Mobiluncus*, *Porphyromonas*, and *Peptostreptococcus*[7,8]. These findings suggest that neovaginas constructed with penile/scrotal tissue may exhibit a microbiota distinct from that of cisgender women, with frequent predominance of anaerobic microorganisms. However, this comparison must be interpreted as contextual, because the cisgender vagina and the neovagina differ in tissue origin, epithelial structure, and local substrates for microbial colonization.

In the research of Petricevic et al[17] on lactobacilli in the neovagina, the most frequently detected *Lactobacillus* species were *L. gasseri*, *L. crispatus*, *L. johnsonii*, and *L. iners*. More than 90% of these 47 *Lactobacillus*-positive women had two or more species, and approximately 60% of the transgender women

had the species *L. gasseri*, *L. crispatus*, *L. johnsonii*, and *L. iners* concomitantly. The authors reported that the use of the technique affects the accuracy of *Lactobacillus* identification, as detection is challenging with methods other than PCR-DGGE. Furthermore, such unreliability of culture and phenotypic techniques for the enumeration and identification of species constitutes a distinct problem in the characterization of the vaginal microbiota of *Lactobacillus*[17]. In previous studies[31,32], the authors noted that in the microbiota of pregnant cis women, the most frequent species were *L. crispatus* and *L. gasseri*, followed by *L. jensenii* and *L. rhamnosus*. In this sense, there is an intersection between the first two species detected in the microbiomes of trans women and cis pregnant women, which is also congruent with other studies on the regular microbiota of cis women during reproductive age[4,33]. Nevertheless, this comparison should be understood only as contextual support and not as equivalence between cisgender vaginal microbiota and neovaginal microbiota.

Thus, the authors highlight[17] that further research is needed on the possible recto-neovaginal connection to explain the origin of lactobacilli in transsexual women with a neovagina formed with penile skin – also justifying the subsequent study published in the same year[18], which aimed to characterize the species of *Lactobacillus* present in the neovagina and rectum of transsexual women to determine the degree of neovaginal-rectal co-colonization. In this last study[18], the most frequently detected *Lactobacillus* species in both the neovagina and rectum were *L. gasseri*, *L. johnsonii*, *L. crispatus*, *L. iners*, and *L. jensenii*. Additionally, 43 of the 61 transsexual women simultaneously harbored the same lactobacilli in the neovagina and rectum, providing evidence that the gut may be a reservoir for colonization of the neovagina with *Lactobacillus* species in trans women[18].

Kaufmann et al[18] aimed to determine whether an orally administered preparation of four lactobacilli strains exerted any measurable effect on the neovaginal microbiota of transsexual women. Transgender women in the intervention group received oral probiotic capsules twice daily for seven days. Each capsule contained four lyophilized strains of *Lactobacillus*: *L. crispatus*, *L. rhamnosus*, *L. jensenii*, and *L. gasseri*. The first neovaginal swabs were obtained before the intervention, whereas the second and third neovaginal swabs were obtained the day after the administration of the last capsule and two weeks later[19]. The results demonstrated that oral administration significantly improved the neovaginal microbiota, reduced the Nugent score, and enriched the vaginal microbiome of these women with lactobacilli compared with the placebo group[19]. However, this finding should be interpreted with caution, as it is based on an interventional study and may not reflect the spontaneous microbiota profile of the neovagina.

In this context, other studies have reported that the microbiome of the neovagina is distinct from that of the cisgender vagina and may differ according to the surgical technique used[22,23]. Similarly, Weyers et al[21] reported that colonization of the neovagina with lactobacilli is minimal, and that this absence is associated with BV in cisgender women in the presence of Gram-negative bacteria. This may explain why neovaginas are more similar to cis vaginas with bacterial vaginosis[13,23]. In view of the study by Kaufmann et al[18], probiotics may constitute a possible resource for the vaginal health of trans women, since even in asymptomatic cases, vaginal microbiomes deficient in lactobacilli are associated with impaired epithelial maturation, increased mucosal inflammation, changes in epithelial barrier function, and greater susceptibility to sexually transmitted infections such as Chlamydia, Gonorrhea, HIV, and HPV[27]. Nevertheless, the available evidence remains insufficient to support clinical recommendations for probiotic use in this population.

Weyers et al[21] also identified a mixed microbiota similar to the bacterial microflora of the cis vagina, containing varying numbers of polymorphous Gram-negative and Gram-positive cocci, often with spindle-shaped and comma-shaped rods, and sometimes with spirochetes. We identified an average of 8.6 species per woman, most commonly *Bacteroides ureolyticus*, *Corynebacterium* sp., *Enterococcus faecalis*, *Mobiluncus curtisii*, *Staphylococcus epidermidis*, and *Streptococcus anginosus* group spp. Only one woman had lactobacilli in the neovaginal microbiota. Interestingly, the authors identified a significant correlation between the presence of *E. faecalis* and sexual orientation: in heterosexual MTF, *E. faecalis* was present in 78.6%, whereas it was present in 14.2% of lesbian trans women and 12.5% of bisexual women[22]. Similarly, there was a significant correlation between the bacteria and the occurrence of coitus with a male partner. In those who had regular intercourse with penetration, *E. faecalis* was present in 75%, whereas it was present in only 25% of those without. This may indicate that the migration of this uropathogen to the vagina is intensified by sexual intercourse, which is relevant to the health of these women, since vaginal colonization with uropathogens is known to precede urinary tract infections (UTIs), partially explaining why one in five transsexual women reported the frequent occurrence of dysuria in the study[22].

Although only one of these women had lactobacilli, the authors noted that although these women have estradiol levels comparable to those of postmenopausal women on hormone replacement, the environment of the neovagina covered with penile skin may not support the growth of lactobacilli[22], which may be attributed to the absence of glycogen-rich epithelial cells, which are substrates necessary for the colonization of *Lactobacillus*. However, the relevance of the detection method used should be emphasized, as evidenced above[17]. This divergence between studies may be related not only to biological differences, but also to methodological heterogeneity in microbial identification. Therefore, the researchers must compare studies based on PCR-DGGE, metaproteomics, Gram stain, cytology, and culture with caution.

Sigmoid intestinal graft technique

Unlike the other women in the study by Birse et al[13], a single neovaginal sample constructed with a sigmoid colon graft for vaginal extension had a microbiome in which *Bacteroidaceae* and *Enterobacteriaceae* were the main taxa detected, along with *Escherichia* proteins. This woman's corresponding rectal sample also showed elevated levels of *Bacteroidaceae* (41%). Furthermore, this finding supports the theory that the organs used to generate and/or modify the neovagina are the primary sources of bacteria, which may contribute to the neovaginal microbiome[13].

Kim et al[19] investigated neovaginas constructed with laparoscopic rectosigmoid grafts in 12 patients, including nine with congenital vaginal agenesis, two with sexual identity disorder and one with female pseudohermaphroditism. Therefore, this study does not represent an exclusively transgender population and was interpreted only as contextual evidence regarding rectosigmoid grafts. Curiously, a biopsy of the neovaginal wall at the 12th postoperative month revealed that the normal intestinal columnar epithelium grafted into the patient's neovagina had transformed into squamous cell metaplasia of the vaginal mucosa. For the microbiota, microbiological studies revealed mainly *Escherichia coli* in the neovagina at 6 months postoperative, and *E. coli* and *Proteus vulgaris* at 12 months. At 18 months after surgery, the following bacteria were found: *E. coli*, *Streptococcus viridans*, and *Streptococcus dysgalactiae*; however, no *Lactobacillus* was identified[19]. These findings suggest that the environment to which the epithelium and microbiota are

exposed influences the epithelial and microbiological composition of the neovagina. Still, they should not be interpreted as isolated evidence of the microbiota of transgender women.

The work by Van der Sluis et al[20] included a sample of patients with sigmoidal grafts in the neovagina to investigate differences in bacterial profiles in terms of abundance and phyla diversity. To do so, they relied on samples of the sigmoid-derived neovagina and multiple biological sites: rectum, skin, vaginal, and vulvar skin. The correlation between samples was greater within patients than between patients, observed for all phyla combined and for the phylum analyses of *Bacteroidetes*, *Verrucomicrobia*, and *Proteobacteria*. The most discriminative bacterial species between the colonic and neovaginal microbiota were *Sutterella* spp., *Bacteroides vulgatus*, *Alistipes finegoldii*, *Prevotella denticola*, *Faecalibacterium prausnitzii*, *Finegoldia magna*, *Bacteroides dorei*, *Alistipes putredinis*, and *Prevotella* spp., which are notorious for fermenting complex carbohydrates and producing butyrate[21]. This finding suggests that the sigmoid microbiota is partially conserved even when dissociated from fecal content[21].

Together, these studies suggest that the type of graft used in vaginoplasty influences the neovaginal microbiota. In neovaginas constructed with intestinal tissue, microorganisms commonly associated with intestinal microbiota appear to be more frequent. In contrast, neovaginas constructed with penile/scrotal tissue tend to show microorganisms related to skin, penile, or anaerobic environments[13,20-22]. However, the evidence remains limited due to small sample sizes, heterogeneous surgical techniques, and varied microbiological identification methods.

In another analysis, Grosse et al[22] reported cytological findings in 20 patients with neovaginas, including 12 with gender dysphoria and eight with congenital or acquired absence of a natural vagina. Thus, despite including transgender women, this study also involved a heterogeneous population. Cytological findings compatible with superficial, intermediate, and parabasal cells, along with Döderlein flora similar to that in normal cervical cytology, were present in only a minority of cases. Although few articles included in this review mention fungal components[21,22], the case series described by Haseth et al[23] reported the presence of candidiasis in the neovagina of five transsexual patients, who were treated with miconazole and responded to treatment. Vulvovaginal candidiasis (VVC) is an infection caused by *Candida* species and is a common condition in cisgender women; it is usually triggered by changes in the microbiota, such as hormonal oscillations, diseases that affect the immune system, antibiotics/medications, hygiene habits, clothing, and sexual practices[24]. In line with this, it has been reported that vaginal colonization by *Lactobacillus* may effectively reduce the risk of VVC[25], thus reinforcing the relevance of this genus to the neovaginal microbiota and the potential of probiotics. This point also indicates that fungal components remain underexplored in the literature on neovaginal health. However, because Grosse et al[22] used cytology and evaluated a heterogeneous population, we advise interpreting its microbiological findings with caution and avoiding direct comparisons with sequencing-based studies.

In summary, it is noteworthy that the genus *Lactobacillus* is the most frequently studied across all included studies, with particular attention to its apparent drastic reduction in transgender populations, a phenomenon intrinsically linked to estrogen and glycogen levels, as mentioned above. In both trans women and trans men, the absence of lactobacilli behaves similarly to what is observed in cisgender women; that is, it allows for the growth of anaerobic bacteria, which are commonly associated with BV in cis women, such as *Prevotella*, *Gardnerella*, and *Peptostreptococcus*[8]; however, further studies are needed to clarify the prevalence of BV in transgender populations. Furthermore, while the microbiota of trans men appears to be

shaped predominantly by endocrine factors, that of trans women is determined by endocrine factors, surgical techniques, and sexual behavior, a factor not observed as a determining influence on the microbiome of the trans men studied. Thus, the findings suggest convergence across studies, particularly regarding the reduction or absence of *Lactobacillus* and the presence of anaerobic bacteria, but also divergences in tissue origin, surgical technique, hormonal exposure, sexual behavior, and microbiological methods.

The study by Hayashida et al[34], although useful for contextualizing the influence of surgical technique and tissue characteristics on neovaginal structure and biology, was not included in the main corpus of this review because it does not directly align with the target population defined by the eligibility criteria. Therefore, it was used only as contextual support in the discussion, not as direct evidence about the vaginal or neovaginal microbiota of transgender people.

The integration of these findings reveals that trans vaginal health requires a personalized approach that goes beyond diagnostic criteria applied to cisgender women, including routine gynecological care focused on dysbiosis, the migration of uropathogens, and sexually transmitted infections such as HPV. This emphasizes the need for protocols and care guidelines focused on transgender gynecology. Overall, the studies reviewed suggest convergent evidence that the microbiota of trans men and trans women differs from the pattern classically described in cisgender women. However, the magnitude and clinical implications of these differences remain uncertain.

The significance of this study lies in its ability to consolidate an emerging, fragmented body of evidence on the health of populations often marginalized in clinical research. By synthesizing data on microbiomes as distinct as those of trans men and trans women, the study goes beyond mere biological description, offering a scientific basis for personalized health care. This review contributes to existing knowledge by challenging the cisnormative view of vaginal health, enriching the scientific literature with the distinction between microbiome profiles resulting from different vaginoplasty techniques and gender-affirming therapy, and providing a comprehensive overview of this under-explored topic.

Despite these contributions, the interpretation and generalization of the results should be approached with caution due to the inherent limitations of the methodologies used in the studies analyzed, particularly the heterogeneity of microbial identification techniques, which may lead to underestimation of lactobacilli and other critical species. Furthermore, the scarcity of available research is reflected in the small number of participants in several of the analyzed studies, which limits the statistical power of the conclusions. Comparing studies with different methodological designs, including cross-sectional, retrospective, prospective, and interventional studies, introduces a complexity that may hinder the establishment of direct causality. In addition, some included studies used microbiota characterization methods with limited comparability, such as cytology, whereas others involved interventional designs or heterogeneous populations, which may also affect the interpretation of the findings. In this context, comparisons between studies based on genotyping, metaproteomics, PCR-DGGE, culture, Gram stain, cytology, and histology should be made with caution, since these methods do not share the same objectives, sensitivities, or capacity to characterize microbial communities.

Therefore, the results of this review should be interpreted as a narrative synthesis of available evidence, rather than as a direct comparison of prevalence or abundance between studies. These factors, coupled with the lack of consensus on what constitutes a "healthy" microbiome for a neovagina or a vagina under prolonged testosterone exposure, indicate that current results are preliminary indicators and still

in the refinement stage, reinforcing the need for further studies with methodological rigor and a broader sample size to contribute more substantially to the topic.

CONCLUSION

This systematized narrative review suggests that the vaginal bacterial ecology of transgender individuals exhibits characteristics that distinguish it from the pattern observed in cisgender women. In trans men, testosterone hormone therapy has been associated with changes in the mucosa and with a reduction in the *Lactobacillus* population. In contrast, in trans women, the microbiological profile of the neovagina appears to vary according to the surgical method and the type of biological graft used (penile, scrotal, rectal, sigmoid, etc.). However, these findings should be interpreted with caution, given the limited number of studies and the heterogeneity of the methodologies used. Studies involving mixed populations, cytological methods, or interventional approaches should not be interpreted as directly comparable to molecular microbiome studies. Studies to determine the vaginal and neovaginal microbiota of transgender individuals are scarce, reinforcing the necessity for further studies with methodological rigor, longitudinal design, standardized microbiological methods, and a broader sample size.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this manuscript.

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AUTHOR'S CONTRIBUTIONS

Narciso DiLorenzo Machado Dias: study conception and design, analysis and interpretation of results, manuscript drafting, and critical revision of the content. Jeferson Bastos Santos, Michelli Christina Magalhães Novais, and Nilo Manoel Pereira Vieira Barreto: data analysis and interpretation, manuscript drafting, and critical revision of the content. All authors approved the final version and assume public responsibility for the content of the work.

USE OF ARTIFICIAL INTELLIGENCE

During the preparation of this manuscript, the authors used DeepL to translate selected studies and to draft the English version of the text. Following the use of this service, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

NOTE

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