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REVIEW ARTICLE

The microbiome of the neovagina: a systematic review and comparison of surgical techniques

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ABSTRACT

Introduction: Vaginoplasty is performed to create a neovagina for transgender individuals who seek surgical transition or for cisgender individuals with congenital or acquired absence of a vagina (or "cisvagina"). The current knowledge of the bacterial microenvironment of the neovagina is limited. The objective of this study was to conduct a systematic review of current knowledge regarding the microbiome of the neovagina in transgender women.

Methods: A systematic review of three medical databases (PubMed, MEDLINE, Web of Science) was performed in December 2021. Articles were included if they discussed the bacterial flora of the post-surgical neovagina in transgender women. Articles were excluded if their primary focus was pathogenic disease of the neovagina. Articles were summarized qualitatively and organized into a table.

Results: Ten articles were identified for the review. Surgical techniques included penile inversion vaginoplasty (PIV) and intestinal/sigmoid vaginoplasty. PIV neovaginas were most similar to cisvaginas with bacterial vaginosis, whereas intestinal vaginoplasty resulted in microbiomes comparable to that of the colorectum. Oral probiotic supplements may be able to encourage the growth of Lactobacillus in the neovagina. Maintenance protocols relating to cleaning are largely surgeon- and institution-dependent, and evidence regarding the use of estrogen and its effect on the neovagina is limited.

Conclusions: The microbiome of the neovagina is distinct from that of the cisvagina, and it differs based on surgical technique. Further research is warranted to better characterize the effect of different surgical techniques, patient characteristics, estrogen use, and cleaning habits on the health of the neovaginal microbiome.

KEYWORDS
Flora; microbiome; microbiota; neovagina; transgender; vaginoplasty

Introduction

Vaginoplasty is defined as the surgical procedure that either constructs or reconstructs a vagina by creating a neovagina. Indications for the procedure include gender dysphoria, congenital vaginal agenesis, acquired vaginal absence, vaginal contracture, or stenosis, which may be due to trauma or cancer (Horbach et al., 2015; McQuillan & Grover, 2014). Vaginoplasty techniques include penile inversion, intestinal, non-genital skin graft, buccal mucosa, peritoneal, and non-surgical dilation-based vaginoplasty (Dietrich, 2022). Penile inversion vaginoplasty (PIV) is the most frequently performed and extensively researched technique for gender vaginoplasty (Horbach et al., 2015).

The goal of vaginoplasty is to create a neovagina that is functionally and esthetically similar to the vagina of cisgender women (CW), which we will henceforth refer to as the "cisvagina". As noted in the "Standards of Care for the Health of Transgender and Gender Diverse People, Version 8" (SOC 8), vaginoplasty is one of the most frequently performed gender-affirming procedures (Coleman et al., 2022). A neovagina can provide patient satisfaction, identity affirmation, and acceptable functional, esthetic, and erogenous outcomes. In a systematic review and meta-analysis of 3716 TW who underwent vaginoplasty, a general satisfaction rate of 93% was reported (Manrique et al., 2018). High satisfaction specifically with

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sexual function has also been reported (Coleman et al., 2022). However, little is known about the biological composition of the neovagina in the post-surgical period and the changes that occur over time. Knowledge of the neovaginal microbiome may allow for improved health outcomes for patients who undergo vaginoplasty.

The goal of this systematic review is to identify and analyze the published medical literature regarding the microbiome of the neovagina. To provide context for the results of the review, the microbiome of the cisvagina will be briefly summarized. The results of the review will follow, organized by surgical technique. By aggregating all published patient data on the topic of the neovaginal microbiome in transgender women, we aim to provide a useful resource for physicians who perform vaginoplasty and engage in post-operative management of patients who undergo vaginoplasty and highlight areas for further research to improve patient care for this unique population.

Microbiota of the cisvagina

The cisvaginal flora is a complex ecosystem that changes throughout life, from birth to menopause. The vaginal microbiota resides in and on the outermost layer of the vaginal epithelium and consists of species which typically do not cause infections in women with normal immunity (Amabebe & Anumba, 2018). The prepubertal vaginal microbiome is predominantly anaerobic. After puberty, increased estrogen levels promote the accumulation of glycogen in vaginal epithelial cells. This encourages the proliferation of Lactobacillus, which metabolizes glycogen to produce lactic acid and create an acidic pH (< 4.5) which is protective against the growth of opportunistic bacteria. Lactobacillus also produces hydrogen peroxide that further deters the growth of potentially harmful bacteria (Collins & Aramaki, 1980). Under the influence of hormones, such as estrogen, progesterone, and FSH, the vaginal ecosystem undergoes cyclic changes with the menstrual cycle. The composition of the microbiota can also be affected by sexual activity, cleaning habits, antibiotic therapy, pregnancy, lactation, stress, and underlying diseases, e.g. diabetes mellitus.

Disruption of the normal vaginal flora (vaginal dysbiosis) can have significant clinical consequences (Amabebe & Anumba, 2018). Bacterial vaginosis (BV) is characterized by an increase in mixed anaerobic bacteria, such as Bacteroides, Ureaplasma, Mycoplasma, Streptococcus, Prevotella, Mobiluncus, and Gardnerella, and a decrease in Lactobacillus. While BV can be asymptomatic, it can cause abnormal discharge and reduce the acidity of the vaginal environment. Aerobic vaginitis (AV) is a similar condition due to an overgrowth of aerobic bacteria, such as Escherichia coli, Enterococci, Group B Streptococcus, and Staphylococcus, that tends to cause more inflammation than BV. Both BV and AV are associated with increased risk of sexually transmitted infection (STI) acquisition and pregnancy complications.

Methods

The objective of the systematic review was to identify all published articles that studied the microbiome of the post-surgical neovagina in transgender women. It was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. Due to the expected small amount of literature on the subject, there were no restrictions on publication year. Studies were to be included if they discussed the topic of the post-surgical neovaginal microbiome, reported novel human patient data, were written in English, and if the full text of the article was available. Studies were to be excluded if the primary focus of the article discussed neovaginal pathology (e.g. bacterial or viral infection, cancer, or another disease process), if they did not discuss the neovaginal microbiome, if they did not contribute any novel patient data to the literature (e.g. a meta-analysis or review article), if they were animal studies or only involved cisgender women, if not written in English, or if the full text was not available. The references of the selected articles were reviewed for inclusion of potential articles which would fit the inclusion criteria.

Medical databases (MEDLINE, PubMed, and Web of Science) were queried with relevant search terms (Figure 1). Articles were initially screened

by title and abstract. If it was unclear whether an article met the inclusion criteria, the full text of the article was obtained. Full text articles of all included studies were then obtained. Data from each article, including title, authors, publication year, Level of Evidence, vaginoplasty technique, number of patients, surgical indications, microbiome findings, cleaning habits, estrogen use, and conclusions were extracted and summarized in a table. Articles were summarized qualitatively by technique.

Group 1 Terms		Group 2 Terms
neovagina		microbiota
vaginoplasty	And	microbiome
neovaginoplasty		bacteria
		flora

Figure 1. Visualization of search terms. Full search term was entered into each database search engine as follows: "(neovagina and microbiota) or (neovagina and microbiome) or (neovagina and bacteria) or (neovagina and flora) or (vaginoplasty and microbiota) or (vaginoplasty and microbiome) or (vaginoplasty and bacteria) or (vaginoplasty and flora) or (neovaginoplasty and microbiota) or (neovaginoplasty and microbiome) or (neovaginoplasty and bacteria) or (neovaginoplasty and flora)."

Results

Search of three medical databases (MEDLINE, PubMed, and Web of Science) in December 2021 identified ten articles for inclusion in the review (Figure 2, Table 1) (Birse et al., 2020; Grosse et al., 2017; Kaufmann et al., 2014; Kim et al., 2011; Petricevic et al., 2014a, 2014b; Toolenaar et al., 1993; van der Sluis et al., 2019; Weyers et al., 2009; 2010). Most studies were case series (Level of Evidence 4), with the exceptions of one

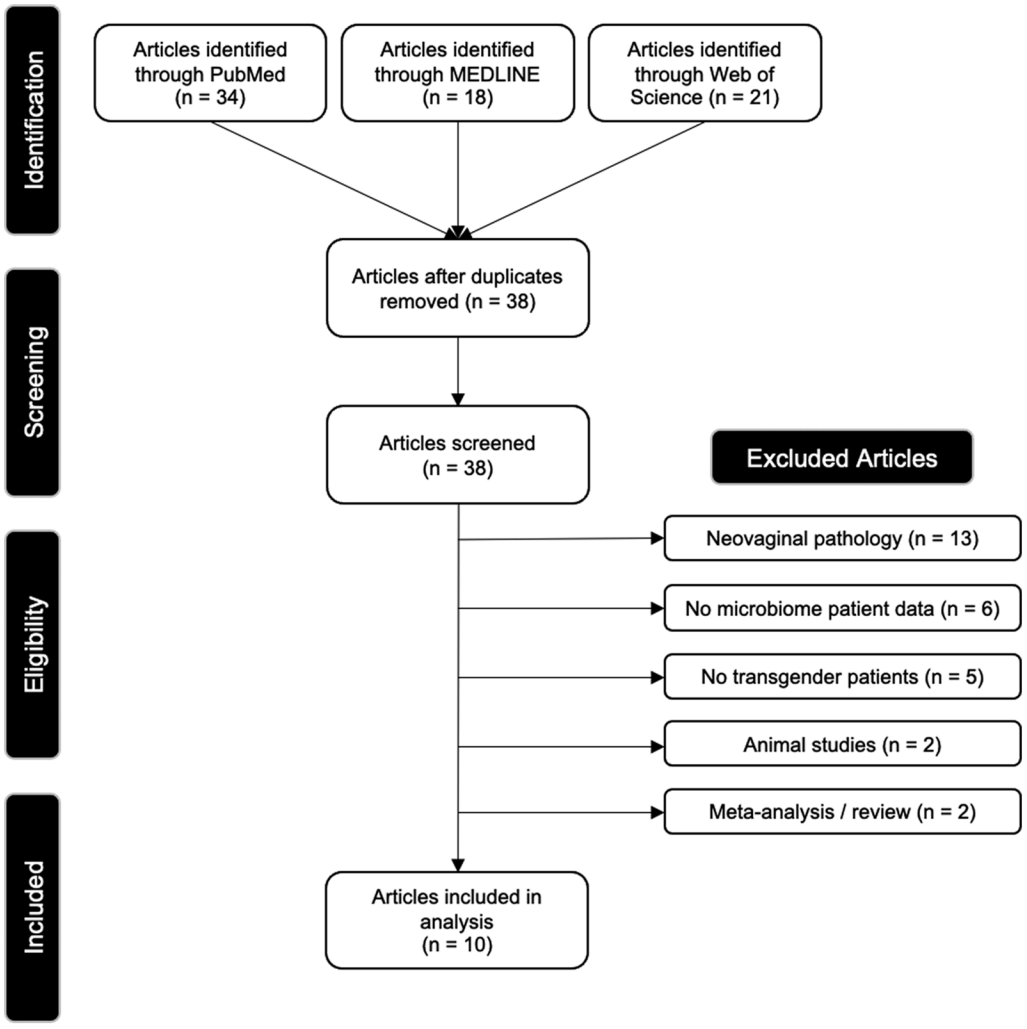

Figure 2. Flow diagram depicting results of systematic review of the literature on the topic of the neovaginal microbiome.

Table 1. Results of systematic review of neovaginal microbiome.

Technique	Author	Year	LoE	N	Indication	Bacterial Findings	Cleaning	Estrogen Use	Conclusions
PIV	Weyers	2009	4	50	50 GD	8.6 species / sample; most common: Bacteroides ureolyticus, Mobiluncus curtisii, Corynebacterium, Enterococcus faecalis, Staphylococcus epidermidis, Streptococcus anginosus	Multiple rinsing products reported, not associated with pH, malodorous discharge, or presence of certain bacteria	47/50 using ERT	PIV neovagina harbors bacteria of skin, intestine, and cisvagina with BV
	Weyers	2010	4	50	50 GD	4% with Doderlein bacilli	Not discussed	47/50 using ERT, no associations	Majority have different cytology than cisvagina
	Petricevic	2014	4	63	63 GD	75% with Lactobacilli; 13 different Lactobacillus species (most common: L. gasseri, L. crispatus, L. johnsonii, and L. iners)	Not discussed	Not discussed	PIV neovagina supports Lactobacilli
	Petricevic	2014	4	61	61 GD	11 different Lactobacillus species (most common: L. gasseri, L. johnsonii, L. crispatus, L. iners, and L. jensenii); 70.5% with same lactobacillus in neovagina and rectum	Not discussed	Not discussed	Rectum serves as a reservoir for neovaginal Lactobacilli
	Kaufmann	2014	2	60	60 GD	30% with Lactobacilli and normal Nugent score prior to intervention	Not discussed	Not discussed	Oral probiotic supplementation can increase Lactobacilli in neovagina
	Grosse	2017	3	9 (PIV) 8 (Intestinal) 3 (Skin graft)	9 GD 4 MRKHS, 3 DSD, 1 GD 2 GD, 1 VA	0% (0/9) with Doderlein bacilli 38% (3/8) with Doderlein bacilli 33% (1/3) with Doderlein bacilli	Not discussed	65% using ERT, associated with nucleated squamous cells	Cytology largely dissimilar from cisvagina
	Birse	2020	4	5	5 GD	Most common species: Porphyromonas, Peptostreptococcus, Prevotella, and Mobiluncus	20% reported vaginal washing (no specific details)	60% of TW using transdermal ERT	Neovaginal microbiome similar to uncircumcised penis, cisvagina with BV
Intestinal	Toolenaar	1993	4	15	10 GD, 5 MRKHS	E. coli, Bacteroides, Lactobacilli most common; pH 8	Not discussed	Not discussed	Sigmoid neovagina retains similar pH and flora as colon *in situ*, lower bacterial counts
	Kim	2011	4	12	9 VA 2 GD, 1 DSD	E. coli, Proteus, Strep viridans, Strep disgalactiae identified; no lactobacillus; pH 6	Not discussed	Not discussed	Similar flora as colon *in situ*
	van der Sluis	2019	4	28	26 GD, 2 VA	Sutterella, Bacteroides, Alistipes, Prevotella, Fecalibacterium, and Finegoldia; no discussion of Lactobacilli	Iodine solution for 1 week, water after; progressive decreasing schedule of rinsing	Not discussed	Similar flora as colon *in situ*, but relative differences emphasize diet dependence of bacteria

LoE = Level of Evidence; GD = Gender dysphoria; PIV = Penile inversion vaginoplasty; VA = Vaginal absence (congenital or acquired); MRKHS = Mayer-Rokitansky-Küster-Hauser syndrome; ERT = Estrogen replacement therapy; BV = Bacterial vaginosis.

retrospective cohort study (Level of Evidence 3) and one prospective, double-blind randomized clinical trial (Level of Evidence 2) (McNair & Lewis, 2012).

Penile inversion vaginoplasty

Seven articles studied the microbiome of the neovagina created using the PIV technique. Weyers et al. (2009) conducted one of the first studies to examine the microbiota of the neovagina. The authors collected neovaginal swabs from 50 TW who underwent PIV. The mean interval since vaginoplasty was 6.3 years (SD 6.4 years). Clinically, 23.5% reported malodorous vaginal discharge, 22% reported vaginal irritation, and 8% had frequent dysuria. The neovaginal pH was elevated (mean 5.88) in comparison to the cisvagina (pH < 4.5). An average of 8.6 bacterial species were isolated per sample, the most common of which were Bacteroides ureolyticus, Mobiluncus curtisii (both associated with bacterial vaginosis), Corynebacterium, Enterococcus faecalis, Staphylococcus epidermidis, and Streptococcus anginosus. Only one sample contained Lactobacillus. There were no associations between certain species, dilatation/cleaning habits, or malodorous discharge. There was a significant correlation between the presence of E. faecalis and coitus with a male partner. The authors concluded that PIV neovaginas harbored a complex microflora comprised of species found on the skin, in intestine, and in cisvaginas with bacterial vaginosis.

The same study team also used Pap smears and evaluated the neovaginal cytology of the same cohort of 50 TW who underwent PIV (Weyers et al., 2010). Evidence of bacterial vaginosis was found in 50% of specimens, and Doderlein bacilli (a type of Lactobacillus) were present in 4% of specimens. One sample had combined Candida and Gardnerella colonization and koilocytes, and further testing diagnosed HPV infection. Weyers and colleagues concluded that only a minority of neovaginas demonstrated cytology similar to "'normal'" cervical cytology in CW.

Petricevic et al. (2014a) studied the prevalence of Lactobacillus species in 63 TW who underwent PIV in order to determine whether penile skin could support Lactobacillus colonization. The mean interval since vaginoplasty was 5.2 years (SD 4.8 years). The authors used the PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) technique, which allows for the detection of bacteria that are difficult to culture or not detectable through other methods. In contrast to the earlier study by Weyers et al. (2009), the presence of Lactobacillus was detected in 75% of samples. There were 13 different species detected, and the most common species were L. gasseri, L. crispatus, L. johnsonii, and L. iners, which have also been isolated in CW. Ninety percent (90%) of samples had two or more species, 60% harbored the most common four species simultaneously, and 25% had up to 8 different species. The authors conclude that neovaginas created by the PIV technique support the growth of Lactobacillus.

After demonstrating the prevalence of Lactobacillus in the neovagina of TW, the same research team sought to characterize the types of Lactobacillus present in the neovaginas and rectums of 61 TW who underwent PIV (Petricevic et al., 2014b). The mean interval since vaginoplasty was 4.9 years (SD 0.5 years). They found that 70.5% of the TW harbored the same Lactobacillus species in their neovagina and rectum, and a majority (61%) had two or more matching species. The most common matching species were L. gasseri, L. johnsonii, L. crispatus, L. iners, and L. jensenii. The authors noted that co-colonization of the vagina and rectum has been reported in healthy pregnant and post-menopausal CW and associated with a protective effect against BV (Antonio et al., 2005; El Aila et al., 2009; Petricevic et al., 2012). As the neovagina does not communicate with any internal pelvic organs, the authors concluded that the rectum acts as a reservoir for neovaginal Lactobacillus. This finding supports the concept that intestinal microbiota contributes to the maintenance of vaginal microbiota in both TW and CW.

In order to determine the feasibility of increasing Lactobacillus in the PIV neovagina with oral probiotic supplementation, Kaufmann et al. (2014) conducted a clinical trial in which they randomized TW who underwent PIV to receive either oral probiotics twice daily for seven days or

placebo. The mean interval since vaginoplasty was 5.2 years (SD 4.8 years). The probiotics included four lyophilized Lactobacillus species (L.crispatus, L.rhamnosus, L.jensenii and L.gasseri). Neovaginal swabs were evaluated using the "Nugent scoring system", used for evaluating bacterial vaginosis, and culture (Nugent et al., 1991). More TW in the intervention group showed improvement in their Nugent scores (48.5% vs. 14.8%, $p < 0.05$), and their culture results also demonstrated higher amounts of Lactobacillus. It was also noted that 30% of participants had normal Lactobacillus flora present at the start of the study. The authors concluded that oral probiotic supplementation can improve neovaginal health by increasing the presence of Lactobacillus and reducing the risk of developing bacterial vaginosis in the TW.

Grosse et al. (2017) evaluated cytological samples of 20 neovaginas to investigate their cellular composition and microbiology. The mean interval since vaginoplasty was 11.6 years (range 4.8 to 29 years). Multiple surgical techniques were utilized to create the neovaginas, including PIV ($n = 9$), intestinal ($n = 3$), and non-genital skin grafts ($n = 8$). Indications for vaginoplasty included gender dysphoria ($n = 12$), Mayer-Rokitansky-Kuster-Hauser syndrome (MRKHS, $n = 4$), differences/disorders of sex development (DSD, $n = 3$), and vaginectomy secondary to squamous cell carcinoma ($n = 1$). The primary study objective was to draw conclusions about HPV and cancer risk, but they also reported on the presence of Doderlein bacilli. While approximately 1/3 of the neovaginas created with intestinal or non-genital skin grafts demonstrated the presence of Doderlein bacilli, none of the PIV neovaginas did. These results are in accordance with the earlier study by Weyers et al. (2010), which reported a low presence of Doderlein bacilli in a larger cohort of TW. A formal statistical comparison was not made. The authors concluded that while neovaginal cytology is different than cisvaginal cytology, patients with neovaginas should still undergo routine cancer screening.

Most recently, Birse et al. (2020) utilized meta-proteomic techniques to investigate the neovaginal and rectal microbiome in 5 TW who underwent PIV, and then compared the results to the vaginal microbiome of 30 CW. The mean interval since vaginoplasty was 9.5 years (range 3.7-35.7 years). They identified on average 8 species of bacteria per neovaginal sample, of which the most prevalent taxa were Porphyromonas, Peptostreptococcus, Prevotella, and Mobiluncus. The neovaginal samples demonstrated higher overall bacterial diversity compared to the cisvaginal and rectal samples. The neovaginal samples were more similar to non-Lactobacillus dominant/polymicrobial cisvaginas than Lactobacillus dominant cisvaginas. Differential protein analysis demonstrated that neovaginas had immune responses similar to cisvaginas with BV, such as increased immune activation and reduced barrier proteins. One of the TW had a sigmoid colon graft in addition to PIV, and it was noted that the sample had a microbiome more similar to an intestinal microbiome, including bacteria such as Bacteroidaceae, Enterobacteriaceae, and Escherichia. The authors concluded that the neovaginal bacterial profiles were most similar to the bacterial compositions of uncircumcised penises and cisvaginas with BV, which may be due to insufficient estrogen in the neovagina.

Intestinal vaginoplasty

Three articles specifically studied the microbiome of the neovagina created using the intestinal or sigmoid colon technique. The bacterial flora of the sigmoid neovagina was first described in 1993 in a study by Toolenaar et al. The mean interval since vaginoplasty was 52 months (range 23-87 months). Indications for vaginoplasty in the study included gender dysphoria ($n = 10$) and MRKHS ($n = 5$). The average pH in the studied individuals was 8. The average number of species per sample was 6, and the most prevalent species were Escherichia coli, Bacteroides, and Lactobacillus, which was found in 67% of samples. The authors concluded that the intestinal neovagina retains a similar pH to the pH of a healthy colorectum. It also retains much of the same flora, although the bacterial counts are lower than that of a normal sigmoid colon, and more facultative anaerobes were present, as opposed to strict anaerobes.

The microbiome of the intestinal neovagina was also briefly noted in a case series of

laparoscopic sigmoid vaginoplasty by Kim et al. (2011). Twelve patients underwent the procedure, and neovaginal characteristics were described at 6-12 months post-operatively. Indications for the procedure included congenital vaginal agenesis ($n = 9$), gender dysphoria (2), and pseudo-hermaphroditism ($n = 1$). Intravaginal pH was 6, and in one patient, columnar epithelium had transformed into squamous epithelium. Culture studies demonstrated the presence of different bacteria at different time points: E. coli at 6 months, E. coli and Proteus vulgaris at 12 months, and E. coli, Streptococcus viridans, and Streptococcus disgalactiae at 18 months after the vaginoplasty surgery. No Lactobacillus species were identified.

Most recently, van der Sluis et al. (2019) studied samples from 28 patients who underwent sigmoid vaginoplasty to analyze the microbiome. The median interval since vaginoplasty was 24 months (range 8-90 months). The majority of patients ($n = 26$) were TW; the other two patients were CW with congenital or acquired vaginal absence. Samples were collected from the skin, neovagina, and rectum. The bacterial species of the neovagina were more similar to native rectum than to skin and cisvagina microbiota. Species identified included Sutterella, Bacteroides dorei, Bacteroides vulgatus, Alistipes finegoldii, Alistipes putredinis, Prevotella denticola, Fecalibacterium prausnitzii, and Finegoldia magna. However, there were differences in the abundance of the populations between the neovagina and the rectum, most notably in bacteria from the phylum Bacteroidetes. There was no mention of Lactobacillus. The authors concluded that these differences may distinguish which intestinal bacteria are dependent or independent of diet.

Discussion

The results of this systematic review present the small body of literature that has studied the microbiome of the neovagina. While few articles directly compared techniques and many used different methodologies, some conclusions can be drawn to compare the resultant microbiomes of the techniques.

Penile inversion vaginoplasty

Regarding PIV, most studies found a low prevalence of Lactobacillus, and thus dissimilarity from the normal cisvagina, and instead found bacterial populations more similar to the skin, intestine, and cisvagina with bacterial vaginosis (Birse et al., 2020; Grosse et al., 2017; Kaufmann et al., 2014; Weyers et al., 2009; 2010). However, studies using more advanced detection methods and specifically searching for the presence of Lactobacillus found greater presence of Lactobacillus, implying that while it may not be present in as high abundance as in the cisvagina, it is still able to survive in the PIV neovagina (Petricevic et al., 2014a, 2014b). The only prospective trial in the review demonstrated that oral probiotic supplementation can improve the abundance of Lactobacillus in the neovagina (Kaufmann et al., 2014). This finding is corroborated by similar research in premenopausal and postmenopausal CW demonstrating that oral probiotic supplementation can also improve Lactobacillus presence in cisvaginas (Petricevic et al., 2008; Reid et al., 2003).

Intestinal vaginoplasty

Regarding intestinal vaginoplasty, most studies demonstrated that the neovagina retains a similar pH and flora as the colon *in situ* (Kim et al., 2011; Toolenaar et al., 1993; van der Sluis et al., 2019). The lower total bacteria counts and slight differences in bacterial populations highlight the effect of diet on the bacterial composition of the microbiome (Toolenaar et al., 1993; van der Sluis et al., 2019). Two studies reported the presence of Lactobacillus, though only in a minority of patients (Grosse et al., 2017; Toolenaar et al., 1993). While both techniques (PIV and intestinal) have been demonstrated to create functional neovaginas that harbor diverse bacterial populations, including some Lactobacillus, neither seem to be inherently conducive to supporting a Lactobacillus-predominant microbiome. Future research could explore whether oral probiotics could similarly improve the presence of Lactobacillus in intestinal neovaginas, as they have been shown to do in PIV neovaginas.

Cleaning regimens

While maintenance habits of the neovagina were not the primary focus of this review, a minority of the included articles discussed cleaning regimens of the neovagina (Birse et al., 2020; van der Sluis et al., 2019; Weyers et al., 2009). Cleaning the neovagina, which also may be referred to as rinsing, washing, or douching, is an important component of neovaginal care that is not required of the cisvagina. Cleaning protocols discussed in the review articles included the use of different products, such as an iodine solution, low pH lactic acid serum, body douche gel, and tap water (van der Sluis et al., 2019; Weyers et al., 2009). Only one study published a detailed protocol of their preferred post-operative cleaning schedule (van der Sluis et al., 2019). In another study, the authors found that the type of cleaning product was not associated with the composition of the microbiome, pH, or malodorous discharge (Weyers et al., 2009). Beyond the studies of the review, some authors have emphasized the importance of rinsing in the setting of symptomatic bacterial dysbiosis and wound dehiscence (van der Sluis et al., 2020). The preferred cleaning protocol of the senior author (AH) involves alternating use of diluted vinegar, salt, betadine, and/or probiotic solutions, depending on the presence or absence of granulation tissue, time from surgery, and amount or characteristics of vaginal discharge. In the presence of copious, thick vaginal secretions that are resistant to changes in the cleaning regimen, a course of oral antibiotics, such as Flagyl (Metronidazole), can be used. To the best of our knowledge, there has been no published direct comparison of vaginoplasty cleaning protocols. Cleaning protocols seem to be largely anecdotal and surgeon- or institution-dependent, and further research may be warranted in this area to determine the effect of cleaning protocols on the composition of the neovaginal microbiome.

Estrogen replacement therapy

The use of estrogen replacement therapy (ERT), through oral, transdermal, or intravaginal delivery, could also play a role in the composition of the microbiome of the neovagina. ERT has been demonstrated to improve Lactobacillus colonization and reduce the risk of bacterial vaginosis in post-menopausal CW (Heinemann & Reid, 2005). Four of the included studies reported the use of ERT (Weyers et al., 2009, Weyers et al., 2010, Grosse et al., 2017, Birse et al., 2020). Only one study specified the delivery method as transdermal (Birse et al., 2020), and it is likely that the patients in the other studies were taking oral ERT. None reported the use of intravaginal ERT. Some authors found an association between ERT and the presence of nucleated squamous cells, while others did not (Grosse et al., 2017, Weyers et al., 2010). ERT was also not associated with inflammatory cells or BV (Weyers et al., 2010), although these results are limited because most study participants were using ERT. In two of the studies, ERT use was reported, but no comparisons were made between participants who were and were not using estrogen (Weyers et al., 2009, Birse et al., 2020). Thus, there is limited evidence regarding the effect of ERT on the neovagina, and no studies have specifically evaluated the effect of intravaginal estrogen. One study has reported that intravaginal estrogen improved Lactobacillus colonization in the vaginas of transgender men (Winston McPherson et al., 2019). Thus, future research efforts may aim to determine whether there is a role for intravaginal estrogen in improving the flora of the TW neovagina.

Future directions

While the research included in this review has begun to contribute to the understanding of the neovaginal microbiome, it is evident that more work needs to be done to better understand it. The microbiome of the neovagina is associated with its origin tissue, which produces a microbiome dissimilar from the cisvagina. Thus, the care of the neovagina, including hygiene and preventing infection, cannot be assumed to be similar to the cisvagina. As recommended by the SOC 8, it is important that patients who undergo vaginoplasty receive appropriate follow up care with either their primary surgeon, or with a gynecologist or primary care provider that provides gender-affirming care (Coleman et al., 2022).

Ongoing care allows for the detection of early complications and prompt management, as well as the opportunity for research. Future research could explore the effect of different post-surgical interventions, such as cleaning, intravaginal estrogen, and probiotics, on the health of different types of neovaginas.

Many patients with neovaginas belong to the LGBTQ+community, a population which has been historically underserved both in medical care and medical research (Baldwin et al., 2018; Howard et al., 2019; Matthews et al., 2018). There has been a call-to-action for increased study in this specific area by the transgender population, as evidenced by a recent editorial titled "The Neglected Science of Neovaginas," (Pearlman, 2021) and the creation of the TransBiome project, a crowd-funded study based at Université de Paris that seeks to study the bacterial diversity of neovaginas and directly compare different surgical techniques and post-operative protocols (*Transbiome: Understanding the neovaginal microbiome of transwomen*). The scientific community should heed this call by continuing to increase access to gender-affirming care and research efforts that benefit both transgender women with neovaginas and the LGBTQ+community at large.

Conclusion

The microbiome of the neovagina varies based on surgical technique. Bacterial composition is related to the origin of the neovaginal lining. PIV neovaginas harbor complex microbiomes consisting of bacteria found on skin, in the rectum, and cisvaginas with bacterial vaginosis, while the microbiome of intestinal neovaginas share more similarities with the colorectum. Oral probiotic supplements may be able to encourage the growth and future survival of Lactobacillus in the neovagina, although further study is warranted. The effects of estrogen replacement therapy and different cleaning protocols on the neovagina are not well established and may be worthy topics for future research inquiry. Through these efforts, we can contribute to the scientific knowledge of the neovagina and different vaginoplasty techniques, further elucidate the interactions between skin, the gut, and the vaginal microbiomes, and improve patient care for all patients with neovaginas.

Disclosure statement

The authors declare that they have no conflicts of interest and there are no competing interests to declare.

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