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The use of menstrual cups is associated with the maintenance of healthy vaginal microbiota: A prospective longitudinal study

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Abstract

Background: Menstrual cups are reusable flexible collectors adjustable to the body. Besides the economic and environmental advantages, it is possible that the use of menstrual cups influences modifications in the vaginal microbiota. We aimed to evaluate the influence of menstrual cups on the vaginal microbiota of reproductive-age women in comparison to sanitary pads and to determine its acceptance. **Methods:** A prospective longitudinal study was conducted with undergraduate/graduate volunteers, whose usual method of menstrual management was sanitary pads. In the first three menstrual cycles (M1 to M3), participants kept using sanitary pads. In the three consecutive cycles (M4 to M6), they used the menstrual cup offered by the research group. A questionnaire was used to obtain gynecological background and sexual behavior. In the first (M1) and third (M3) follow-up visits, participants underwent gynecological examinations including vaginal microbiota evaluation, oncotic cytology, diagnosis of Human Papillomavirus (HPV), *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*. At each visit, vaginal samples were collected for analysis of microbiota. A generalized linear model with binomial distribution for repeated measures was used and the model was adjusted for confounding factors, using the SAS software. **Results and Discussion:** We included 47 participants who completed the 6 follow-up examinations. The prevalence of HPV was 48.9% at M1 and 51.1% at M3 and the prevalence of *C. trachomatis* infection was 2.1% and 6.7%, respectively. None of the participants were infected by *N. gonorrhoeae* or *T. vaginalis*. One participant had altered cytology results and was referred to the colposcopy service. Patients with *C. trachomatis* and/or altered vaginal microbiota were treated. During the three months of sanitary pad usage, 27.7%, 12.8%, and 10.6% of women presented altered vaginal microbiota (bacterial vaginosis, intermediate microbiota, aerobic vaginitis, and/or vulvovaginal candidiasis), while only 8.5%, 12.8% and 8.5% of them had vaginal dysbiosis while using a menstrual cup (P = 0.04). Overall acceptance of menstrual cups among those who completed all the examinations was 93.6%. **Conclusion:** Our results show that menstrual cups are associated with the maintenance of healthy vaginal microbiota. Additionally, the acceptance of menstrual cups was high in our population.

Key words: menstrual cup, menstrual collector, sanitary pads, menstruation, vaginal microbiota, sexually transmitted infections

INTRODUCTION

The commonly used method for menstrual management of sanitary pads and tampons has an impressive environmental impact as these products take over a century to decompose.[1] A Canadian study has estimated that over 771

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million sanitary pads are used in the country every year.[2] A more sustainable alternative, such as menstrual collectors, could substantially reduce the environmental impact, since each collector can be used, on average, for more than 5 years depending on the quality and proper cleaning.

Regarding the economic aspect, menstrual cups are more cost-effective than other methods of menstrual management once they present a long lifespan. The cost of one menstrual cup is comparable to the expenses of one year using sanitary pads, thus the cost per menstrual cycle is significantly lower.[2] This fact can make the use of a collector more attractive for populations that use disposable pads, especially in countries with less economic development.[3]

Several studies carried out in different countries showed that these devices are usually well accepted after a couple of months of adaptation and the participants reported improved comfort during the menstrual period with the use of menstrual cups compared to disposable pads and tampons.[4–6] In Brazil, however, a country where a substantial number of women face menstrual poverty few studies have assessed the acceptance of menstrual collectors.[7,8]

Besides the environmental and economic advantages, it is possible that the use of menstrual cups influences modifications in vaginal microbiota, a parameter of great relevance in menstrual management, that lacks overall assessment in the literature. The healthy vaginal microbiota is predominately composed of *Lactobacillus* morphotypes over other bacterial taxa, which are part of the core of the vaginal microenvironment.[9] A lactobacilli-dominated microenvironment is pivotal for hydrogen peroxide production, a metabolite with antimicrobial action against other microorganisms.[10,11] It has been shown that the decrease or the depletion of vaginal lactobacilli promotes changes in the host's immune system and increases the risk for important gynecological and obstetric complications such as pelvic inflammatory disease, post-surgical infections, preterm birth, low birth weight and increased risk of acquiring sexually transmitted infections (STIs).[12–16]

Bacterial vaginosis (BV) is the most common dysbiosis among women of reproductive age, with a prevalence of 30%.[17,18] Such condition is characterized by the replacement of *Lactobacillus* species by other bacterial species, mostly anaerobic.[17] A study with adolescents in western Kenya showed that the use of a menstrual collector was associated with a lower prevalence of bacterial vaginosis and STIs among menstrual cup users when compared to women using external absorbents or reusable fabrics.[3]

Considering the possible health advantages of using menstrual collectors and the lack of information in Brazilian populations, we aimed to evaluate the influence of menstrual cups on the vaginal microbiota of reproductive-age women in comparison to sanitary pads and to determine its acceptance.

METHODS

Study design

A prospective longitudinal study was conducted with reproductive-age volunteers with regular menstrual cycles, whose usual method of menstrual management was disposable pads. We recruited undergraduate and graduate students from São Paulo State University - Botucatu, SP (UNESP) from July to December 2019. In the first three menstrual cycles (M1 to M3), participants were instructed to keep using sanitary pads (external disposable absorbents). In the three consecutive cycles (M4 to M6), they were oriented to use the menstrual cup offered by the research group, which was distributed during the third gynecological visit, when the participants were correctly instructed on how to use it and clean it. A questionnaire was used to obtain gynecological background and sexual behavior, and to assess the overall acceptance of menstrual cups at the end of the study. Participants were encouraged to keep a diary of any discomfort with each menstrual management method. Telephone contacts and e-mail addresses of the researchers were made available to participants.

Women who had not had their first sexual intercourse or were less than 18 years old were not eligible. Pregnant women and women with systemic (*e.g.* lupus, arthritis), gynecological diseases (*e.g.* endometriosis, ovarian cyst) or reproductive system abnormalities (*e.g.* abnormal vaginal opening) were excluded. The sample size calculation was based on a pilot study and assumed an α error of 5% and a test power of 80%, and a minimum of 40 participants were established for the study. The participants signed the Written Informed Consent. This project was approved by the Research Ethics Committee of the Faculty of Medicine of Botucatu, UNESP (CAAE 637273616.0.00005411).

Sample collection

We prospectively collected samples at six-time points (M1 to M6). In the first (M1) and third (M3) follow-up visits, participants underwent complete gynecological examinations including vaginal microbiota evaluation; oncotic cytology; collection of endocervical samples for molecular diagnosis of Human Papillomavirus (HPV), *Chlamydia trachomatis* and *Neisseria gonorrhoeae*; and research of *Trichomonas vaginalis* in Diamond's medium. At each visit, vaginal samples were collected for analysis of microbiota.

The samples were collected under the following conditions: at least 5 days after the end of the last menstrual period, sexual abstinence of more than 72 hours, no use of antibiotics in the last 4 weeks, and no vaginal examination or use of vaginal creams in the last 72 hours before the

gynecological exams.

After insertion of a non-lubricated Collins speculum, vaginal pH was measured with a tape (pH 4.0–7.0, Merck, Riverside, PA, USA) in the middle third of the vaginal wall. For evaluation of the vaginal microbiota, samples were collected with a swab from the middle third of the lateral wall and were evaluated under light and phase-contrast microscopy, gram stained and freshly stained. A whiff test was performed by adding 10% KOH solution to the vaginal contents and interpreted as positive, negative, or doubtful. Endocervical samples were collected at time points M1 and M3 with cytobrush for molecular analyses for HPV infection, *C. trachomatis*, and *N. gonorrhoeae*. An additional sample with a representation of squamous and glandular epithelium was collected at M1 for oncotic cytology. Finally, a sample of the vaginal pouch was collected with Ayre's spatula at M1 and M3 to investigate *T. vaginalis*. At each visit vaginal samples were recollected for evaluation of the vaginal microbiota pattern (Figure 1). Participants diagnosed with STIs or altered vaginal microbiota patterns were treated according to the protocol used at the Clinics Hospital from Botucatu Medical School (HC-FMB), UNESP.

Assessment of vaginal microbiota and diagnosis of vulvovaginal candidiasis

Vaginal microbiota was microscopically evaluated at attendance using gram-stained smears of vaginal content. Deviation from healthy vaginal microbiota were classified according to the criteria of Nugent *et al.*, Donders *et al.*, and Cybley & Cybley[19–21] in bacterial vaginosis (scores 7–10), intermediate microbiota, aerobic vaginitis, and cytolytic vaginosis, respectively. The diagnosis of vulvovaginal candidiasis was made by visualization of blastoconid and/or pseudohyphae in the presence of inflammatory response on microscopic examination of gram-stained smears of vaginal contents.

Treatment of vaginal dysbiosis

Women diagnosed with bacterial vaginosis were treated with metronidazole 250 mg, 2 tablets every 12 hours for 7 days, or 0.75% metronidazole gel formula, one application at night for 7 days. Those diagnosed with intermediate microbiota were treated with metronidazole 250 mg, 1 tablet every 8 hours for 5 days, or 0.75% metronidazole gel formula, one application at night for 5 days. Patients with vulvovaginal candidiasis were treated with a single dose of fluconazole 150 mg and those with cytolytic vaginosis were orientated to prepare a sodium bicarbonate bath (30–40 g/1 tablespoon of baking soda in 1 liter of filtered water) daily for 2 weeks. No women were diagnosed with aerobic vaginitis. Patients were instructed not to consume alcohol and to abstain from sexual intercourse during the treatment period.

Detection of Trichomonas vaginalis infection

Vaginal content was seeded in Diamond's medium and incubated at 37℃. Microscopic evaluations were performed every 24 hours for 3 consecutive days. *T. vaginalis* infection was diagnosed by the presence of the protozoan in circular movements on any day of the incubation period.

Detection of Chlamydia trachomatis and Neisseria gonorrhoeae

DNA from endocervical samples was obtained using AmpliLute Liquid Media DNA extraction kit (Roche Molecular Systems Inc., Basel, Switzerland) and verified by amplification of the constitutive beta-globin gene using GH20 and PCO4 primers.[22] The research of *C. trachomatis* was also performed by polymerase chain reaction (PCR), using the primers CTP1 (5'-TAGTAACTGCCACTTCATCA-3') and CTP2 (5'-TTCCCCTTGTAATTCGTTGC-3') for a final product of 201 bp, using a standardized amplification protocol.[23] Negative (water) and positive (plasmid DNA from infected McCoy cells) controls were included.

For the detection of *N. gonorrhoeae*, the duplex real-time PCR technique was used, targeting the pseudogene porA (primers: 5'-CAGCATTCAATTTGTTCCGAGTC-3', 5'-GAACTGGTTTCATCTGATTACTTTCCA-3' and probe: Fam-CGCCTATACGCCTGCTACTTTCACGC-BHQ1) and the opa genes (primers: 5'-TTG AAACACCG

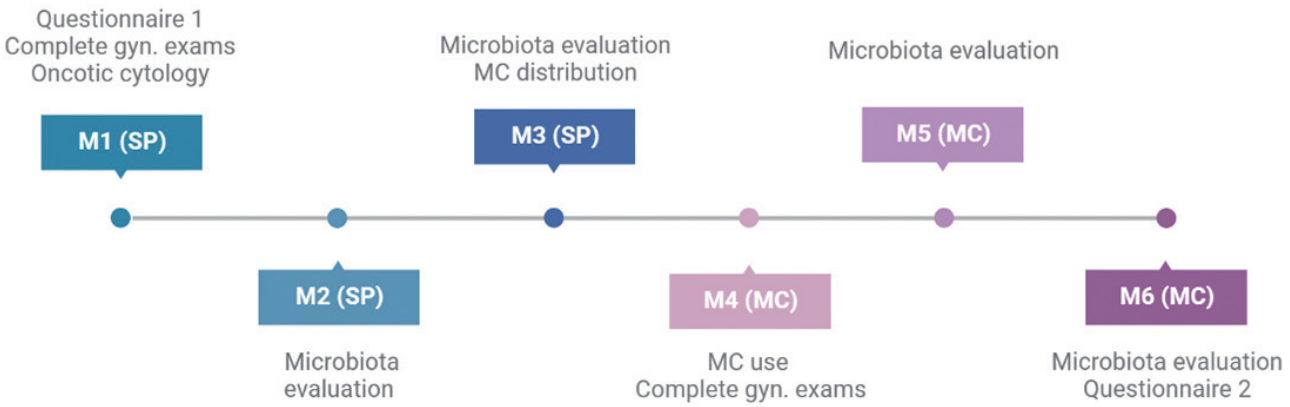

Figure 1. Evaluations performed by moment/month during the follow-up study. This figure was created with BioRender.com. SP: sanitary pads; MC: menstrual cup.

CCCGGAA-3', 5-TTTCGGCTCCTTATTCGGTTTAA-3' and probe: Yak-CCGATATAATC+CGTC+CTTCAA+CATCAG-BHQ1).[24] Amplification and detection were performed in the Line-Gene K equipment (Hangzhou Bioer Technology Co., Ltd, Hangzhou, China). A negative and a positive control containing *N. gonorrhoeae* DNA were used.

Detection and HPV genotyping

HPV infection was tested using the nested PCR technique. Initially, the DNA sample was amplified with the MY9/11 primers. The second set of primers used was GP5+/6+.[25] The amplification cycling followed the following parameters: 95℃ for the first 45 seconds for denaturation, 47.7℃ for 45 seconds for primer annealing, and 72℃ for 1 minute for amplification, followed by 44 cycles, ending with a 7-minute extension at 72℃. Negative and positive controls were used (DNA-HPV from HeLa cells). The products were subjected to electrophoresis in 1.5% agarose gel and visualized with Gel Red TM (New England Biolabs Inc., Ipswich, MA, USA).

Positive HPV samples were genotyped using the Xgen Multi HPV Chip - HS12 genotyping Kit by reverse hybridization, a qualitative *in vitro* test for genotyping 35 types of high-risk (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and low-risk HPV (6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81 and 84). Genotyping was performed according to the manufacturer's instructions.

Oncotic cytology

The oncotic cytology exams were performed in the Cytology Laboratory of HC-FMB, where they were evaluated according to the Bethesda System criteria.[26]

STATISTICAL ANALYSIS

Data obtained were evaluated by descriptive data analysis. For continuous variables, the mean followed by standard deviation and/or median followed by total or interquartile range were calculated.

For categorical data frequency and percentage calculations were performed. The outcomes of microbiota alteration were considered binary (normal vaginal microbiota *versus* altered vaginal microbiota) and a generalized linear model with binomial distribution for repeated measures was fitted for the six-time moments, followed by Wald's multiple comparison test. For moments 1 and 4, the same model was adjusted including confounding variables (presence or absence of HPV infection). In addition, some moments were grouped for the absorbent and collector group (M2 and M3 *vs.* M5 and M6) and compared using the same binomial model in repeated measures. We also evaluated the alterations in vaginal microbiota while using the same menstrual management method (M1 *vs.* M3 and M4 *vs.* M6).

Additional correlations between vaginal microbiota patterns and behavioral habits were evaluated using Pearson's correlation test for categorical variables and the student T-test for continuous variables. Analyses were done with the SAS software version 3.0 (Cary, North Carolina, USA). A *P*-value of 0.05 was considered significant.

RESULTS

Characterization of the study participants

We initially recruited 84 participants, and 47 of them completed the 6 follow-up examinations. Reasons for study withdrawal included missing appointments, noncompliance with conditions for gynecological examination, and inconsistent use of the menstrual cup.

The mean age of the women included in the study was 24.0 ± 4.7 years. Most of them were single (89.4%), white (78.7%), and nonsmokers (87.2%). Since the volunteers recruited were undergraduate and graduate students from the Bioscience Institute and the Medical School – UNESP Botucatu, all had at least 12 years of education. Of the participants, 21.3% reported having been previously vaccinated against HPV. The main contraceptive method of choice was oral male condoms (51.1%), followed by contraceptive pills (46.8%). Only five participants reported knowledge of previous STIs, three reported HPV-induced lesions (condyloma acuminata), and the other two reported endocervicitis by *C. trachomatis*. Of the total number of participants, 14.9% had previous oncotic cytology alterations. At the time of recruitment (M1), 42.6% of the participants reported vaginal discharge. The sociodemographic and gynecological data of the patients included in the study are presented in Table 1.

Menstrual cup acceptability

Practicality, hygiene, comfort, and effectiveness in controlling menstruation were the main benefits reported. Initial adaptation difficulties and leaks were the main complaints. Despite these complaints, 93.6% of the participants who reached the end of the study intended to continue using the menstrual cup, and the evasion of only one participant was related to lack of adaptation to the device. During the time of this study, we had no reports of adverse effects related to the use of menstrual cups.

Sexual behavior and hygiene habits

The questionnaire applied also contained questions about sexual habits and lifestyle since it is already known how sexual and hygiene practices are directly related to changes in vaginal microbiota and STI transmission. To evaluate behavioral habits, participants were dichotomized at M1 in those who presented normal microbiota *vs.* dysbiosis. Data on the sexual habits of the participants included in the study are presented in Table 2.

Anal sex was associated with healthy vaginal microbiota ($P = 0.004$) while smoking was associated with vaginal dysbiosis ($P = 0.020$). The number of lifetime sexual partners was also associated with altered vaginal microbiota. Women with healthy vaginal microbiota reported 6.0 ± 5.8 sexual partners during their lifetime, while those with dysbiosis referred 11.5 ± 11.9 sexual partners ($P = 0.047$).

Table 1: Sociodemographic and gynecological data of the study participants

Variables	Participants (*N* = 47)
Age (years)	24.0 ± 4.7
Menarche (years)	12.3 ± 1.4
1st sexual relationship (years)	17.1 ± 2.2
Marital status	
Single	89.4%
Stable union /Married	10.6%
Reported ethnicity	
White	78.7%
Non-White	21.3%
Smoking habit	
Smoker	12.8%
Non smoker	87.2%
HPV vaccine	21.3%
Preferred contraceptive method*	
Condom	51.1%
Contraceptive pill	46.8%
Rhythm method/withdrawal/IUD	17.0%
Does not use	8.5%
Previous STI	10.6%
Previous altered oncotic cytology	14.9%
Presence of vaginal discharge at M1	42.6%
Previous use of a menstrual collector	19.1%

Data represented as mean ± standard deviation or percentage. *Due to the combined use of contraceptive methods, the sum for this category exceeds 100%. HPV: Human Papillomavirus; IUD: intrauterine device; STI: sexually transmitted infections (Chlamydia trachomatis or condyloma).

Prevalence of altered vaginal microbiota pattern and STIs

During the months of external absorbent pad use (M1 to M3), the prevalence of altered microbiota (*i.e.* bacterial vaginosis, intermediate microbiota, cytolytic vaginosis, and/or vaginal candidiasis) was 27.7%, 12.8%, and 10.6%, respectively. During the use of menstrual collectors (M4 to M6) only 8.5%, 12.8%, and 8.5% of the participants had vaginal dysbiosis, respectively. BV was the most prevalent dysbiosis. The overall rate of cure within one month after dysbiosis treatment was 66.6%. The results of the vaginal microbiota patterns obtained at each moment are presented in Figure 2.

By Wald's multiple comparison test, the analysis of the percentages of healthy microbiota *versus* altered microbiota at all time points shows a statistically significant difference ($P = 0.044$). When we compare time points M1 *vs.* M4 this difference remained statistically significant even after adjusting for confounding factors such as HPV infection. When we excluded the M1 (time of inclusion) and M4 (transition to collector use) and compared M2 + M3 *vs.* M5 + M6, however, there was no statistically significant difference. Considering only the use of pads (M1 to M3) we report a higher prevalence of dysbiosis in the first screening ($P = 0.003$), but no difference between M2 and M3. Considering only the use of menstrual cups (M4 to M6) we report no statistical difference after three months using the device ($P = 0.795$).

Regarding oncotic cytology evaluations, only one patient presented altered results (atypical squamous cells of undetermined significance) and was referred for follow-up at the colposcopy outpatient clinic at the HCFMB, UNESP. No cases of *T. vaginalis* or *N. gonorrhoeae* infections were detected. The prevalence of *C. trachomatis* endocervicitis was 2.3% and 6.4% at M1

Table 2: Sexual behavior and hygiene habits of the study participants at M1

Variables	Normal microbiota (*N* = 34)	Dysbiosis (*N* = 13)	*P*
N. sexual partners/life	6.0 ± 5.8	11.5 ± 11.9	0.047
N. sexual partners/year	2.1 ± 1.9	2.0 ± 1.4	0.919
Freq. intercourse/week	2.0 ± 1.2	1.8 ±1.3	0.731
Active sex life	91.2%	92.3%	0.216
Smoker	5.9%	30.8%	0.020
Use of male condom	50.0%	53.9%	0.873
Use of vaginal douche	32.4%	30.8%	0.819
Washing genitals before sex	58.8%	92.3%	0.059
Washing genitals after sex	76.5%	100%	0.107
Oral sex practice	85.3%	100%	0.367
Anal sex practice	50.0%	7.7%	0.004

Data represented as mean ± standard deviation or percentage. N.: number, Freq.: frequency.

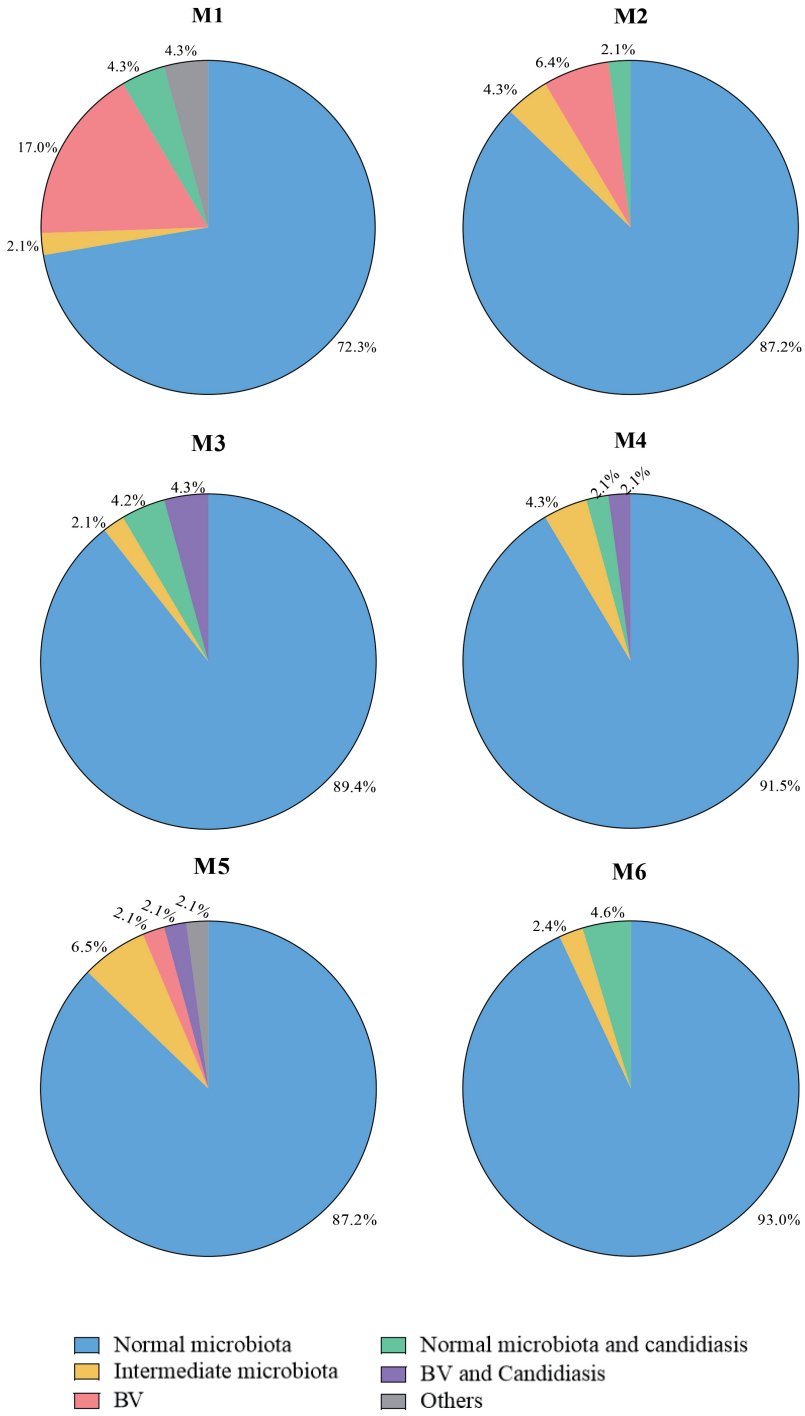

Figure 2. Fluctuation of vaginal microbiota from M1 to M6. These graphs were created with Prism 8.0 software (GraphPad, San Diego, CA, USA). BV: bacterial vaginosis.

and M3, respectively. These patients were treated with a single dose of azithromycin 1 g and were oriented towards safe sexual practices.

The prevalence of genital HPV infection was 48.9% at M1 and 51.1% at M3. Of HPV-positive women, 73.9% in M1 and 82.6% in M3 presented high-risk HPV types. Coinfection by more than one HPV type was also high, reaching 79.1% in M1 and 82.6% in M3. Nevertheless, the presence of HPV was not associated with vaginal dysbiosis.

DISCUSSION

This is the first Brazilian study to evaluate how menstrual cups interfere with vaginal microbiota and its acceptance. Our results show that the use of menstrual cups is associated with the maintenance of healthy vaginal microbiota when compared to the use of disposable sanitary pads, an observation that corroborates with the literature.[3] Such observation makes sense, considering the great number of chemical substances present in external pads may cause alterations in vaginal pH, which plays an important role in

the maintenance of the healthy vaginal microbiota, while the menstrual collector is composed of nontoxic silicone. Opposing our observations, a French study reported no difference in bacterial vaginal microbiota and increased reported fungal genital infection among menstrual cup users compared to women using tampons. Nevertheless, the participants of the French study did not exclusively use one menstrual management method at a time.[27]

Reinforcing the safety of this method, we report no side effects of menstrual cup usage during the time of the study. Despite eventual case reports in the literature of complications associated with menstrual collectors, a robust set of recent evidence reinforces our observations, indicating their safety.[3,27–33]

Additionally, we report a great acceptance of this method by most participants. Claims of initial adaptation difficulties and leaks are consistent with other studies.[2–5] Despite these complaints, 93.6% of the participants who reached the end of the study intended to continue using the menstrual cup, a higher acceptability rate than reported by most studies.[2,4–8] The high educational level of the participants and the academic environment may have influenced the high acceptability reported herein.

We also evaluated the presence of STIs, and we reported a higher rate of HPV infection than described by studies with similar populations.[34,35] Nevertheless, the presence of such infection did not influence changes in the vaginal microbiota patterns. We reported a low rate of chlamydial infection in our population and the absence of *N. gonorrhoeae* and *T. vaginalis* infections.

Regarding behavior factors, we report an association between the number of sexual partners and smoking habits with alterations in vaginal microbiota patterns. Indeed, while dysbioses such as BV are not primarily considered to be sexually transmitted infections, failure to treat sexual partners has been associated with higher recurrence rates.[36] Smoking is associated with changes in the metabolomic vaginal profile that leads to the depletion of *Lactobacillus* species, therefore increasing the predisposition to altered vaginal microbiota.[37] Differently from expected, the practice of anal sex was associated with a high percentage of healthy vaginal microbiota in our sample. We deduce that this practice prompts women towards more careful genital hygiene.

There are possible confounding factors for our analysis. An expressive percentage of women presented vaginal dysbiosis at the time of study inclusion when they received treatment and guidance towards healthy intimate hygiene habits. Therefore, is rather difficult to infer how much the new instructions and treatments received after M1 interfered with the much-improved following gynecological results and how much is a consequence of the menstrual method *per se*. Moreover, although our study design minimizes bias, it is not possible to completely rule out if patients' pre-existing habits could have biased the results. On the other hand, the strength of our study relies not only on the microscopic and clinical evaluation of vaginal dysbiosis during the use of menstrual cups but also on the investigation of such issue in our population and the adequate study design that allowed us to aggregate scientific information to a field that needs awareness.[38]

Considering the cost-efficiency of menstrual cups, the compromised environmental situation we are currently living, and the results presented here, we believe this to be a suitable method for menstrual hygiene management, not only for women from developing countries but for women on a global level. Indeed, the UN's goals for sustainable development include gender equality, sustainable communities, and responsible consumption.[39] Based on the data obtained from the questionnaires, only during the development of this project, over 1500 absorbents were no longer used and discarded. Much is to be done towards these goals, nevertheless, the path to reaching them includes fighting menstrual poverty and empowering women with safe menstrual management options.

We conclude that, in addition to the economic and environmental advantages of menstrual collectors, upon the intended use, this method of menstrual management is safe and efficient, as it is associated with the maintenance of normal vaginal microbiota. Longer-term longitudinal studies should be conducted to confirm the results presented here.

DECLARATIONS

Author contributions

Beatriz Cassolatti Graciolli: Data collection, Methodology, Draft writing. Mariana Alice de Oliveira Ignácio: Sample collection. Jeniffer Sena Baptista Ferreira: Data collection, Methodology, Writing. Júlia Abbade Tronco: Data collection, Methodology. Mariana de Castro Silva: Data collection, Methodology, Writing. Giovana Fernanda Cosi Bento: Methodology. Andréa da Rocha Tristão: Clinical practice; Marli Teresinha Cassamassimo Duarte: Sample collection and discussion. Márcia Guimarães da Silva: Study design, Funding acquisition, Supervision, Writing. Bruna Ribeiro de Andrade Ramos: Conceptualization, Study design, Project administration, Supervision, Writing. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

This project was approved by the Research Ethics Committee of the Faculty of Medicine of Botucatu, UNESP (CAAE 637273616.0.00005411). The participants signed the Written Informed Consent.

Conflict of interest

None declared.

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