

ORIGINAL ARTICLE

The effect of microablative fractional CO₂ laser on vaginal flora of postmenopausal women

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ABSTRACT

Objectives: To assess the effect of microablative fractional CO₂ laser (MFCO₂-Laser) therapy on the vaginal microenvironment of postmenopausal women.

Methods: Three laser therapies at monthly intervals were applied in postmenopausal women with moderate to severe symptoms of genitourinary syndrome of menopause, pH of vaginal fluid >4.5 and superficial epithelial cells on vaginal smear <5%. Vaginal fluid pH values, fresh wet mount microscopy, Gram stain and aerobic and anaerobic cultures were evaluated at baseline and 1 month after each subsequent therapy. Nugent score and Hay-Ison criteria were used to evaluate vaginal flora.

Results: Fifty-three women (mean age 57.2 ± 5.4 years) participated and completed this study. MFCO₂-Laser therapy increased *Lactobacillus* ($p < 0.001$) and normal flora ($p < 0.001$) after the completion of the therapeutic protocol, which decreased vaginal pH from a mean of 5.5 ± 0.8 (initial value) to 4.7 ± 0.5 ($p < 0.001$). The prevalence of *Lactobacillus* changed from 30% initially to 79% after the last treatment. Clinical signs and symptoms of bacterial vaginosis, aerobic vaginitis or candidiasis did not appear in any participant.

Conclusion: MFCO₂-Laser therapy is a promising treatment for improving the vaginal health of postmenopausal women by helping repopulate the vagina with normally existing *Lactobacillus* species and reconstituting the normal flora to premenopausal status.

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Introduction

The vaginal microenvironment of postmenopausal women differs from that of women in premenopausal status mainly due to a lack of estrogens^{1,2}. In premenopausal women, estrogens are considered essential not only for the proliferation of the vaginal epithelial cells but also for the production of glycogen, an essential factor for the growth of vaginal lactobacilli. Vaginal lactobacilli play a key role for a healthy vaginal equilibrium due to their competitive activity to other pathogens but also because they are responsible for maintaining an acidic pH by producing lactic acid and H₂O₂^{3–6}. In postmenopausal women, a decrease in *Lactobacillus* species and a rise of vaginal pH values over 4.5 are observed, resulting in the loss of the local vaginal defense mechanisms against bacterial pathogens, which may predispose to local inflammation and/or infections^{7,8}.

The disturbance of Lactobacillary flora has been correlated with the presence of *Gardnerella vaginalis*, *Trichomonas vaginalis*, enterococci, group B streptococci, and *Escherichia coli*⁹. Available data indicate that pre-existing vaginal colonization with pathogenic enterobacteria is essential for the appearance and recurrence of urinary tract infections (UTIs)¹⁰.

Indeed, postmenopausal women are prone to UTIs with an incidence of about 8% per year and a 4% likelihood of recurrence, while asymptomatic bacteriuria has been estimated in up to 15% of women^{11,12}.

Various therapeutic strategies, hormonal or not, oral or local, have been proposed for the improvement of the vaginal microecosystem of postmenopausal women (e.g. estrogens, probiotics, combination of vaginal estrogens with live *Lactobacillus*)^{13–23}. Hormonal therapy (oral or local) has been associated with a healthier vaginal microecosystem by repopulating the *Lactobacillus* species to a premenopausal status and by reducing the pH of vaginal fluid^{12,14–24}. The use of vaginal estrogens compared to placebo decreased the incidence of UTIs in postmenopausal women²⁵. However, the ideal management for the achievement of the optimal benefit-risk balance is still under investigation. The choice of estrogen therapy should be guided by clinical experience and patient preference¹². Moreover, possible adverse events of estrogens and controversial guidance from physicians make the women reluctant to use them, particularly those with a history of estrogen-sensitive cancers, such as endometrial and breast cancers²⁶.

Recently, an intravaginal microablative fractional CO₂ laser (MFCO₂-Laser) procedure has been introduced for the treatment of postmenopausal women with vulvovaginal atrophy (VVA)^{27–29}. Initial reports indicated that the MFCO₂-Laser treatment improves significantly the VVA symptoms, sexual function and quality of life of postmenopausal women, as well as the vaginal health index^{27–29}. Other studies found that MFCO₂-Laser therapy restores the thickness of the squamous stratified epithelium of the vaginal mucosa with a significant storage of glycogen in the epithelial cells and remodels the vaginal connective tissue with the production of neocollagen and ground substance molecules^{30,31}.

Genitourinary syndrome of menopause (GSM) is a new terminology more accurate than the terms of VVA/atrophic vaginitis, because it describes the clinical signs and symptoms of the vulva, vagina and lower urinary system. In contrast, VVA/atrophic vaginitis describes the appearance of the vulvovaginal structures only³². The GSM has a prevalence of more or less 50%, depending on country of origin, with a negative impact on sexuality, quality of life and well-being^{33–37}. There are many different strains of lactobacilli (e.g. *L. crispatus*, *L. Jensenii*, *L. iners*) and to study such a population is relevant for GSM. However, as the status of vaginal glycogen is indicative of the estrogenic effect, the general population of lactobacilli, as assessed in the routine practice, provides indirect information regarding the estrogenic status. To our knowledge there is currently no evidence regarding the potential impact that the intravaginal MFCO₂-Laser therapy may have on the vaginal microenvironment in women with symptoms of GSM.

The aim of the current study was to assess the effect of MFCO₂-Laser laser therapy on the vaginal microenvironment of postmenopausal women.

Material and methods

Participants and study design

This prospective study has been conducted at the Urogynecologic Unit of a tertiary referral hospital. The local Ethics Committee approved the study protocol. All women eligible for inclusion in the study provided written informed consent before initiating the therapeutic protocol.

Postmenopausal women with at least one moderate to severe symptom of GSM, as defined by the International Society for the Study of Women's Health and The North American Menopause Society³⁸, were eligible to participate in this study. Women were also required to have an objective evidence of menopause; the percentage of superficial vaginal epithelial cells, in the maturation index of vaginal smear (MI) and the vaginal fluid pH had to be $\leq 5\%$ and > 4.5 , respectively. The typical proportion of superficial epithelial cells in postmenopausal women with VVA is less than 5%⁷, whereas vaginal fluid with a pH > 4.5 has a sensitivity of 84.9% for menopausal diagnosis, better than follicle stimulating hormone (sensitivity of 77.4%)⁸.

We excluded from the study women who had used any form of hormone therapy (systemic or local) within the previous 6 months, lubricants or vaginal moisturizers within the

last month, suffering from active genital infections (e.g. bacterial vaginosis, genital herpes), with prolapse stage $\geq II$ according to the pelvic organ prolapse quantification (POP-Q) system³⁹, and any disease that would interfere with compliance to the protocol.

The selection procedure for participation in the study involved: a questionnaire in which women were asked to report the intensity of each individual GSM symptom (dyspareunia, vaginal dryness, vaginal itching, vaginal burning, dysuria, frequency and urgency) measured by a 10-cm visual analogue scale, in which zero applied to 'absence of symptom' and ten to 'symptom as bad as could be'. A score of 4–7 was considered moderate and a score of 8–10 severe. Women with moderate to severe symptom/s were then clinically examined in order to assess signs of VVA and pelvic organ prolapse. A week after the vaginal examination, vaginal samples for microbiological and cytological processing were obtained from all potential participants in the study protocol. Eligible postmenopausal women for participating in the therapeutic protocol were assigned a randomly generated number and entered in a database. In each subsequent therapy, a new number, different from those previously used, was randomly assigned to each participant. In this way the microbiologist was blind to all information regarding participants' clinical findings and treatment status. In addition, an experienced nurse, who was blind to all information regarding participants' clinical findings and treatment status, performed the evaluation of pH indicator strips.

Participants in the study protocol received intravaginal therapy monthly for 3 months, with MFCO₂-Laser system (SmartXide² V²LR, Monalisa Touch, DEKA, Florence, Italy). The following settings of the MFCO₂-Laser were used: D-Pulse mode, dot power, 40 W; dwell time, 1000 μ s; and dot spacing, 1000 μ m. The smart stack parameter from 1 to 3 was used for the treatment of the vaginal canal and the dot power was reduced to 24 W; dwell time, 400 μ s; and dot spacing, 1000 μ m for the treatment of the vaginal introitus. The procedure of MFCO₂-Laser was performed as previously described²⁹. The participants were not allowed to use any lubricants and/or moisturizers during the study protocol.

Before each laser application, a sterile swab was inserted into the vaginal canal and a sample was obtained from the posterior fornix of the vagina. Vaginal samples were placed in a transport gel (Stuart). pH indicator strips (MColorpHastTM, Merck, Germany) were applied against the lateral vaginal wall using sterile forceps, followed by a vaginal lavage for wet mount. Smears from vaginal samples were placed on a glass slide, observed under microscope for *Trichomonas vaginalis* and *Candida* species and stained afterwards according to standard Gram stain procedure. Cultures for aerobic and anaerobic bacteria species were also performed, using MacConkey agar, blood agar, Sabouraud dextrose agar, chocolate agar as culture media, focusing on bacteria with potential clinical impact (e.g. aerobic vaginitis³⁹, UTIs, vaginal candidiasis). Mycoplasma, ureoplasma and/or corynebacterium species were not assessed. Lactobacilli, *Mobiluncus*, *Gardnerella* and *Bacteroides* identified by Gram stain were not further evaluated by cultures. All samples were collected at baseline and at each subsequent therapy.

The vaginal flora was evaluated according to the European guidelines on the management of vaginal discharge, using the Nugent score and Hay-Ison criteria^{39–42}. Predominant bacteria were defined as a single bacterium prevailing from all visible bacteria under Gram staining at baseline and subsequent laser therapies. The bacteria that could be identified by microscopy of Gram-stained smears were categorized as gram-positive or gram-negative.

Normal vaginal epithelial cells and leukocytes were evaluated using Gram stain in a similar manner to Nugent scoring for morphotypes, receiving scores of 0, 1, 2, 3 and 4. When vaginitis was assessed based only on laboratory findings without any clinical signs and symptoms, pharmaceutical therapy was not recommended according to the Centers for Disease Control and Prevention guidelines⁴³.

Statistical analysis

Statistical analysis was performed comparing baseline and subsequent therapies. The distribution of data was assessed using the Shapiro–Wilk test. For abnormally distributed variables, Wilcoxon signed rank test for related paired samples and Spearman correlation coefficient were used. For categorical variables, χ^2 test was used. Logistic regression analysis was performed using as dependent variable the threshold of 4.5 for pH values of vaginal fluid at the final visit. All tests were based on a significance level of 5% ($p < 0.05$). Data were presented as mean \pm standard deviation and as percentages (%). SPSS statistical software was used for the analyses.

Results

Eighty-four postmenopausal women with symptoms of GSM sought treatment in our department. Thirty-one women were excluded as they did not meet the inclusion criteria; 18 had pH < 4.5 , two had a POP-Q stage \geq II, and 11 reported mild symptoms of GSM. Thus, 53 postmenopausal women (mean age 57.2 ± 5.4 years, mean body mass index 26.0 ± 4.8 kg/m², and mean years since the last menstrual period 8.9 ± 6.6) were eligible for inclusion in the study protocol. The majority of the participants had natural menopause (77%), were non-smokers (70%), and married (85%), with dyspareunia and dryness being the most common symptoms (77% and 87%, respectively). Seven participants had a history of hysterectomy; in six participants hysterectomy was the cause of menopause and in one woman the uterus and adnexals were removed after the onset of menopause due to endometrial cancer stage Ia. All participants completed the study protocol. The baseline demographic characteristics of the study participants are shown in Table S1 (see Supplementary Table 1 at <http://dx.doi.org/10.1080/13697137.2016.1212006>).

A significant progressive reduction of vaginal pH between baseline and 1 month after the third laser therapy was observed (Figure 1). At the end of the therapeutic protocol, the pH of the vaginal fluid decreased in 47 (89%) participants. After the completion of the therapeutic protocol, 43 (81%) participants had pH < 5 and 17 (32%) pH < 4.5 . The pH

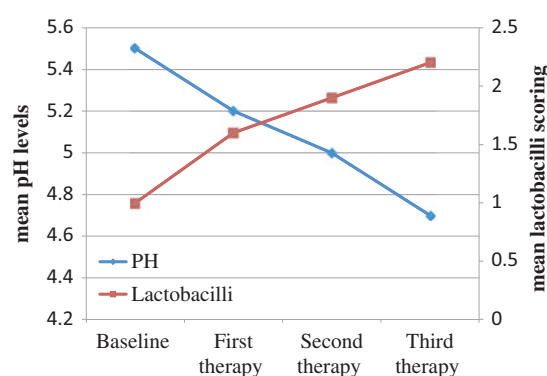


Figure 1. Decrease in the mean vaginal pH levels and increase in the growth of lactobacilli at baseline, and after subsequent therapies. The mean values of pH at baseline and after the first, second and third laser therapy were 5.5 ± 0.8 , 5.2 ± 0.5 ($p = 0.002$), 5.0 ± 0.6 ($p < 0.001$) and 4.7 ± 0.5 , respectively ($p < 0.001$). Increase in the growth of Lactobacilli was observed after the first ($p = 0.001$), the second ($p < 0.001$) and the final laser therapy ($p < 0.001$).

decreased < 4.5 independently of the participant's baseline demographic characteristics (e.g. years since the last menstrual period) and the characteristics of vaginal flora at baseline (pH, vaginal flora according to Nugent scoring and to Hay-Ison criteria). A significant progressive increase of *Lactobacillus* morphotypes was observed (Figure 1).

At baseline, the predominant bacteria identified were *Lactobacillus* (30%), *Bacteroides* (2%), *Mobiluncus* (2%), *Gardnerella* (6%), gram-positive (6%) and gram-negative (11%). The bacteria were evenly grown in 28% of participants while 15% did not have morphotypes of any bacteria species (i.e. complete absence of vaginal flora). In contrast, 1 month following the last therapy, all treated women had some growth of morphotypes. Specifically, 79% of women had *Lactobacillus* as the predominant bacteria species, 2% had *Gardnerella*, 8% gram-positive, 4% gram-negative, 2% had *Candida* and in 6% no predominant bacteria were present.

The changes observed in the vaginal flora according to the Nugent scoring system and Hay-Ison criteria are presented in Table 1. The presence of microorganisms in Gram stain and cultures are presented in Table 2. The detailed changes of *Lactobacillus*, *Gardnerella*, *Mobiluncus* and *Bacteroides* based on the Nugent scoring system are presented in Table S2 (see Supplementary Table 2 at <http://dx.doi.org/10.1080/13697137.2016.1212006>).

Normal vaginal epithelial cells significantly increased after the second and third therapies compared to baseline ($p = 0.007$ and $p < 0.001$, respectively). At baseline, 33% of participants' epithelial cells had a score of 3 or 4, 21% a score of 2, 40% a score of 1, and 6% a score of 0. After three laser therapies, 58% of participants had a score of 3 or 4, while 26% a score of 2 and 16% a score of 1. Leukocyte proportion remained unchanged through the therapeutic protocol.

All women had no clinical signs and symptoms of urogenital infections and did not receive pharmaceutical therapy at any stage of the protocol. At baseline, 16 participants were not sexually active, due to the intensity of the GSM symptoms. After treatment, 15 of these women resumed sexual activity. No serious adverse events occurred during the

Table 1. The vaginal flora assessed according to the Nugent scoring and the Hay-Ison criteria during the study period with the fractional microablative CO₂ laser system. Data are given as n (%)

	Baseline (n = 53)	After 1 laser therapy (n = 52)	After 2 laser therapies (n = 53)	After 3 laser therapies (n = 53)	p Value ^c
<i>Nugent flora^a</i>					
Normal	25 (47.2)	39 (75)	45 (84.9)	46 (86.7)	<0.001
Intermediate	23 (43.4)	13 (25)	7 (13.2)	6 (11.3)	
Bacterial vaginosis	5 (9.4)	0 (0)	1 (1.9)	1 (1.9)	
<i>Hay-Ison flora^b</i>					
Grade 0	17 (32.1)	5 (9.6)	7 (13.2)	0 (0)	<0.001
Grade 1	24 (45.3)	37 (71.2)	40 (75.5)	48 (90.6)	
Grade 2	3 (5.7)	6 (11.5)	2 (3.8)	4 (7.6)	
Grade 3	7 (13.2)	1 (1.9)	1 (1.9)	1 (1.9)	
Grade 4	2 (3.8)	3 (5.8)	3 (5.7)	0 (0)	

^a, Nugent flora refers to vaginal flora according to the Nugent scoring system. It was obtained by using the sum of scores resulting from the synthesis of *Lactobacillus*, *Gardnerella*, *Bacteroides* and *Mobiluncus* morphotypes. A score of 0–3 indicated normal flora, a score of 4–6 was considered intermediate and a score of ≥ 7 was defined as bacterial vaginosis^{40,41}; ^b, Hay-Ison Flora refers to vaginal flora according to Hay-Ison criteria. Grade 0: absence of bacterial species (*Lactobacilli*, *Gardnerella*-like and gram-positive species) and presence of only epithelial cells; Grade 1 (normal flora): predominantly *Lactobacillus* morphotypes; Grade 2 (intermediate): mixed flora with some *Lactobacilli* present, but *Gardnerella* or *Mobiluncus* morphotypes also present; Grade 3 (bacterial vaginosis, BV): predominantly *Gardnerella* and/or *Mobiluncus* morphotypes, clue cells, with few or absent *Lactobacillus* morphotypes; Grade 4 (aerobic vaginitis flora): growth of gram-positive cocci only without *Lactobacillus* morphotypes, not related to BV^{41,42}; ^c, p value at level <0.05 is significant. The p value was calculated comparing the number of women with normal flora (according to Nugent and Hay-Ison) before and after the completion of the therapeutic protocol

Table 2. Presence of microorganisms in the vaginal fluid of the women included in this study. Data are given as n (%)

Microorganisms ^a	Baseline (n = 53)	After 1 laser therapy (n = 52)	After 2 laser therapies (n = 53)	After 3 laser therapies (n = 53)	p Value ^b
<i>Lactobacillus</i>	36 (67.9)	45 (86.5)	46 (86.8)	53 (100)	<0.001
<i>Gardnerella vaginalis</i>	5 (9.4)	2 (3.8)	4 (7.5)	4 (7.5)	0.7
<i>Bacteroides</i>	5 (9.4)	5 (9.6)	4 (7.5)	2 (3.8)	0.2
<i>Mobiluncus</i>	4 (7.5)	2 (3.8)	2 (3.8)	0 (0)	0.04
<i>Streptococcus agalactiae</i>	5 (9.4)	2 (3.8)	1 (1.9)	2 (3.8)	0.2
<i>Enterococcus faecalis</i>	12 (22.6)	8 (15.1)	7 (13.2)	6 (11.3)	0.1
<i>E. coli</i>	20 (37.7)	10 (19.2)	6 (11.3)	9 (16.9)	0.02
<i>Klebsiella</i>	3 (5.7)	2 (3.8)	1 (1.9)	0 (0)	0.08
<i>Proteus</i>	1 (1.9)	2 (3.8)	3 (5.7)	1 (1.9)	1
<i>Candida spp.</i>	1 (1.9)	1 (1.9)	1 (1.9)	1 (1.9)	1

^a, *Lactobacillus*, *Gardnerella vaginalis*, *Bacteroides*, *Mobiluncus* and *Candida* were identified by Gram stain. *Streptococcus agalactiae*, *Enterococcus faecalis*, *E. coli*, *Klebsiella*, *Proteus* and *Candida* were identified by cultures using MacConkey agar, blood agar, Sabouraud dextrose agar, chocolate agar as cultures media. Microscopy of wet mount was also used for *Candida*. *Candida albicans* was identified at baseline and after three laser therapies, while *Candida glabrata* was identified after one and two laser therapies; ^b, p values presented were calculated at a level of significance of 0.05 comparing baseline with 1 month after the three laser therapies

period of the laser treatment, but only a temporary mild irritation of the introitus was noted. This irritation started immediately after the laser treatment, lasted up to 2 h and resolved spontaneously.

Discussion

The intravaginal MFCO₂-Laser has recently been introduced to ameliorate symptoms of GSM without the adverse events of other modes of therapy, especially the hormonal ones^{26–28}. Other laser technologies are available (e.g. erbium laser)^{44,45}. Erbium has a thermal effect while the MFCO₂-Laser has both ablative and thermal effects, stimulating heat shock proteins and other factors (e.g. TGF- β), prompting neocollagenesis and neoangiogenesis, resulting in tissue rejuvenation^{45,46}. The results of the present study show that MFCO₂-Laser therapy improved the vaginal flora with a significant reduction of the pH of the vaginal fluid, and a significant increase of *Lactobacillus* flora. To our knowledge, this is the first study to assess the effect of MFCO₂-Laser on the vaginal microenvironment of menopausal women in a clinical aspect.

In the present study, the vaginal fluid pH values gradually declined after each laser therapy, as would be expected if

the women had used estrogens. Although these results were not compared to a control group and should thus be considered with caution, this effect was observed as early as the first treatment session with a further reduction of pH after each session. After completion of the treatment protocol, the mean pH value was 4.7 with the vast majority of participants (81%) having pH <5 . In the literature, the reported mean pH values after 12 weeks of vaginal estrogen ranges from 4.6 to 4.9, depending on the type of estrogen, modes of delivery and dosage^{16–20,22,23}. In a randomized, controlled trial comparing hyaluronic acid to estradiol vaginal tablets, 14.3% of participants receiving estradiol had pH <5 after the end of the therapeutic protocol and 86% of participants that received estradiol had pH 5.0–5.49²¹.

The progressive reduction of the pH during the study protocol was concomitant with a statistically significant increase in normal vaginal epithelial cells after each monthly treatment. Moreover, the observed increase of the normal vaginal epithelial cells in the current study confirms the results of the histological study of Zerbinati and colleagues³⁰. In this study, it was demonstrated that one of the effects of the MFCO₂-Laser therapy on the vaginal mucosa was a high degree of epithelial exfoliation, with superficial cells filled

with glycogen shedding at the epithelial surface³⁰. In the present study, although only 32% of the participants reduced pH levels to <4.5, the statistically significant increase of normal vaginal epithelial cells between baseline and the third laser therapy possibly indicates that, at the end of the study protocol, the re-establishment of vaginal health was still in progress and that the pH may have not reached its lowest value.

The decline of pH described above was in accordance with the increase of *Lactobacillus* morphotypes, to almost a premenopausal status. In a randomized, controlled trial, the prevalences of *Lactobacillus* after the use of vaginal estriol cream and after the application of hyaluronic acid were 55% and 43%, respectively (baseline prevalences were 34% and 31%, respectively)²⁰. The prevalence of lactobacilli in the present study was 30% before treatment and reached 79% after the three laser therapies. The lactobacilli predominance is of great importance and is considered as one of two criteria that distinguishes bacterial community groups; the other criterion is considered to be the particular *Lactobacillus* species present⁴⁷. Even though the assessment of the particular *Lactobacillus* species was not conducted in this study, the significant increase of *Lactobacillus* predominance implies that the laser therapy restores the vaginal mucosa in a beneficial manner for the vaginal microecosystem.

After the completion of the therapeutic protocol, the prevalences of gram-negative, gram-positive, *Gardnerella*-like species and *Candida* were 4%, 7.5%, 2%, and 2%, respectively. Although significant decreases were observed only for *E. coli* and *Mobiluncus*, all *Lactobacillus* antagonists had a trend of lowering their growth. Asymptomatic bacterial vaginosis with vagina colonized with pathogens occurs more often than previously thought in postmenopausal women, but is usually not recognized⁴⁸. The prevalences of *Gardnerella*-like species and *Candida* in postmenopausal women without receiving hormone therapy (HT) have been reported as 10–40% and 1%, respectively, while, when HT was administered, the prevalences were 8.3–33.3% and 23.3%, respectively²⁴. However, there are no data regarding the prevalence of *Lactobacillus* antagonists in Greek healthy postmenopausal women with or without receiving HT. In the studied population, at the baseline, the colonization of *Gardnerella*-like species was present in 6% of participants, while after the end of the therapeutic laser protocol it was 2%. The low percentage of *Gardnerella*-like species at baseline could be explained by the hygiene habits and the sexual intercourse behavior of the women. Most importantly, the decrease from 6% to 2% of *Gardnerella*-like species shows that MFCO₂-Laser improves the vaginal equilibrium status and does not predispose to vaginal infections.

Although our study was not designed to include postmenopausal women with a history of recurrent UTIs and/or vaginitis, these women could potentially benefit from the improvement of vaginal flora resulting from treatment with the MFCO₂-Laser technique. Vaginal colonization with uropathogens is believed to predispose to UTIs and/or recurrences. Hormonal therapy with or without probiotics,

according to the current data, is the only option for maintaining vaginal health in menopausal women and reducing the incidence of UTIs^{12,14–25}. Future studies could therefore evaluate whether the MFCO₂-Laser represents a valid alternative to vaginal estrogens alone or in combination with lactobacilli for relapse prevention, particularly for women with contraindications to hormonal therapies.

A weakness of this study is the lack of a control group. The findings could thus be due to a placebo effect, or other factors (e.g. time). Furthermore, the follow-up period was relatively short and it remains to be seen whether the above-described effect will be persistent in a longer time interval. Another potential weakness is that the study was not designed to evaluate comprehensively the whole vaginal microbiota. However, the microbiological evaluation was based on diagnostic procedures that are performed in the daily clinical practice and was focused on common pathogens related with urogenital infections. On the other hand, the strengths of the study include its prospective design and the use of clearly defined objective and subjective criteria. Moreover, the assessors of all samples, parameters and data were blinded to all the details related to the study participants.

Conclusion

The novel non-pharmaceutical therapeutic approach of MFCO₂-Laser in women with symptoms of GSM was found to have a beneficial effect on the vaginal microenvironment. The laser therapy restored the vaginal equilibrium to a healthier status, as would normally be expected if estrogen levels were sufficient. The predominance of *Lactobacillus* species and the more acidic pH of vaginal fluid achieved after the MFCO₂-Laser therapy could protect postmenopausal women from vaginal infections and inflammation and possibly from UTIs. However, studies with larger sample sizes and longer follow-up period, focusing on the comparison of MFCO₂-Laser to other therapeutic modalities or placebo, regarding the impact on the prevention of vaginal infections and UTIs, are needed.

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