International Journal of Women's Health

Dovepress
Taylor & Francis Group

Open Access Full Text Article

CLINICAL TRIAL REPORT

Vaginal Lactobacillus Alteration and Vaginal Symptom Relief After Carbon Dioxide Vaginal Laser Therapy in Postmenopausal Women

Teerapan Seehanantawong[1], Pisut Pongchaikul[2], Rujira Wattanayingcharoenchai[1], Komkrit Aimjirakul[1], Orawee Chinthakanan[1], Pitak Santanirand[3], Jittima Manonai[1]

[1]Department of Obstetrics & Gynaecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Ramathibodi Medical School, Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [3]Department of Microbiology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Correspondence: Jittima Manonai, Department of Obstetrics & Gynaecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, 270 Rama V Road, Thung Phaya Thai, Ratchathewi, Bangkok, 10400, Thailand, Tel +66-22012167, Email jittima.man@mahidol.edu; Pisut Pongchaikul, Ramathibodi Medical School, Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, 111 Suvarnabhumi Canal Road, Bang Pla Subdistrict, Bang Phli District, Samut Prakan, 10540, Thailand, Tel +66-28396000, Email pisut.pon@mahidol.edu

International Journal of Women's Health downloaded from https://www.dovepress.com/
For personal use only.

Objective: To assess changes in the prevalence and abundance of vaginal Lactobacillus after fractional micro-ablative carbon dioxide (CO_2) therapy in postmenopausal women with vulvovaginal atrophy (VVA).

Methods: This prospective, single-arm clinical study was conducted in postmenopausal women who underwent treatment with CO_2 laser therapy. Eligible participants were enrolled before the first session of therapy and evaluated at weeks 0, 8, 20, and 32. Vaginal fluid samples were collected using a standardized protocol and processed for Gram staining and expanded quantitative urine culture (EQUC). The level of Lactobacillus was assessed using the Nugent scoring system. The secondary outcomes included the prevalence of Lactobacillus, VVA symptoms, and vaginal pH. The results were analyzed using multilevel mixed-effects linear regression and paired *t*-tests.

Results: Forty-three postmenopausal women with at least one symptom of VVA, with a mean age of 67.3 ± 8.7 years, were recruited. The mean Nugent scores for Lactobacillus morphotypes gradually decreased from 2.84 (95% CI: 2.38–3.29) at baseline to 2.28 (95% CI: 1.79–2.77) at week 8, 2.09 (95% CI: 1.60–2.58) at week 20, and further to 1.84 (95% CI: 1.31–2.35) at week 32 ($p < 0.05$). The prevalence of Lactobacillus increased from 27.9% (12/43) at baseline to 48.8% (21/43) at week 8, 55.8% (24/43) at week 20, and 58.1% (25/43) at week 32 ($p < 0.05$). VVA symptom severity and vaginal pH consistently declined from baseline to week 32 ($p < 0.05$).

Conclusion: Fractional CO_2 laser treatment improved the prevalence and level of Lactobacillus after treatment and sustained improvements were observed at 3- and 6-month follow-ups. These positive effects were correlated with improvements in VVA symptoms.

Keywords: vaginal microbiota, Lactobacillus, CO_2 laser therapy, postmenopausal women, vulvovaginal atrophy, genitourinary syndrome of menopause

Introduction

Menopause is associated with many changes related to almost every organ in a woman's body, especially the urogenital organs. The symptoms commonly observed in the urogenital tract are vaginal irritation, itching, burning, dryness, dyspareunia, and urinary symptoms.[1] Genitourinary syndrome of menopause (GSM) is a term used to describe symptoms in the lower genital area or vulvovaginal atrophy (VVA) and lower urinary tract symptoms (LUTS) caused by a decrease in estrogen levels during the transition to menopause.[2] These symptoms also impact sexual function and the quality of life of women. Therefore, those experiencing GSM symptoms need to receive treatment to reduce the symptoms and restore the vaginal environment to a healthy premenopausal state.

Received: 29 April 2025
Accepted: 23 August 2025
Published: 22 September 2025

© 2025 Seehanantawong et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v4.0) License (http://creativecommons.org/licenses/by-nc/4.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

The vaginal microbiota, a complex ecosystem, plays an essential role in the health and disease of the female reproductive tract. Lactobacillus species dominate the healthy vaginal flora and are known to inhibit pathogenic colonization by producing lactic acid, hydrogen peroxide, and bacteriocins. An acidic vaginal pH, ideally between 3.5 and 4.5, creates an environment that is hostile to many pathogenic organisms. When there is a decline in the Lactobacillus count, the vagina becomes more susceptible to infections like bacterial vaginosis and can exacerbate vulvovaginal symptoms. Specifically, there is a decrease in the number of Lactobacillus species in postmenopausal women with VVA symptoms.[3]

A variety of treatment strategies for GSM are available to alleviate VVA symptoms and restore urogenital physiology. First-line treatment for symptomatic women with vulvovaginal symptoms is represented by local estrogen. If this therapy does not lead to satisfactory results or the patient is dissatisfied with the outcome, moisturizers and lubricants should be considered to mitigate the symptoms.[1] Laser therapy represents a non-pharmacological second-line option, particularly useful in women who are non-responsive and/or non-compliant and in those who have contraindications to hormones, such as breast cancer patients.[1]

Current data suggest that fractional micro-ablative carbon dioxide (CO_2) laser, via the vaginal remodeling pathway, alleviates GSM symptoms. In particular, improvement of dryness, dyspareunia, itching/burning, sexual function, dysuria, urinary frequency/urgency, and incontinence has been reported consistently in studies assessing the short-term efficacy of CO_2 laser therapy.[4] Numerous early-phase studies have reported significant short-term symptomatic relief following CO_2 laser treatment. However, the robustness of these findings has been questioned by recent randomized controlled trials (RCTs). Notably, studies by Li et al and Page et al demonstrated no statistically significant differences between laser and sham treatments, thereby challenging previous assumptions about its efficacy.[5,6] This underscores the need for further investigation, particularly into the microbiological and mechanistic effects of laser therapy. However, most prior studies have focused solely on symptom scores, with limited exploration of objective biological endpoints such as microbiota changes.

A significant knowledge gap exists regarding the effects of vaginal laser therapy on the vaginal microbiota, particularly the abundance of Lactobacillus species, and its correlation with improvements in vaginal symptom severity. Previous studies have typically featured short-term follow-up and lacked integration of culture-based techniques and cytological assessment. Investigating the impact of vaginal laser therapy on Lactobacillus alterations can provide insights into the therapy's mechanism of action and its potential benefits for postmenopausal women with VVA.

This study aimed to extend current understanding by incorporating both Gram stain-based Nugent scoring and expanded quantitative urine culture (EQUC), enabling precise tracking of viable *Lactobacillus* populations over time. It aims to evaluate changes in vaginal *Lactobacillus* prevalence and levels using vaginal Gram stain methods and the EQUC technique following fractional CO_2 vaginal laser therapy in postmenopausal women presenting with vulvovaginal atrophy (VVA) symptoms. Additionally, the study seeks to assess the sustainability of symptom reduction post-therapy and to examine the relationship between changes in vaginal Lactobacillus and improvements in both the symptoms and clinical signs of VVA.

Materials and Methods

Study Setting

Participants were eligible women recruited consecutively from the Female Pelvic Medicine and Reconstructive Surgery Clinic at a university hospital between May 2024 and February 2025. Women were eligible for inclusion if they were aged 50–80 years, had no menses for at least 1 year for non-hysterectomized women, and had a clinical diagnosis of VVA. The diagnosis was based on the presence of at least one documented symptom of VVA in a medical record, including vaginal dryness, vaginal soreness, vaginal irritation/itching, or dyspareunia. Additionally, participants were required to have a clinical indication for and provide informed consent to receive micro-ablative fractional vaginal CO_2 laser treatment. Eligible participants must not have undergone vaginal laser therapy within the previous six months.

The following exclusion criteria were applied: use of products containing Lactobacillus within the past two months; use of antibiotics or antifungal agents, either orally or vaginally, within the past two weeks; vaginal intercourse or douching within 48 hours prior to sampling; and a history of immunosuppressive conditions.

Study Procedure

This study is a prospective, single-arm clinical study. Research assistants screened potential participants for eligibility, and after obtaining informed consent, participants completed screening questionnaires. Baseline assessments included the evaluation of VVA severity, specifically symptoms such as vaginal dryness, itching, pain, and dyspareunia, using a Visual Analog Scale (VAS) ranging from 0 to 10. Additionally, vaginal pH measurement and vaginal fluid collection were performed prior to vaginal laser therapy by the principal investigator (TS).

Participants received intra-vaginal therapy once per month for three consecutive months using the micro-ablative fractional CO_2 laser ($MFCO_2$-Laser) system (SmartXide2 V2LR, Monalisa Touch, DEKA, Florence, Italy). The laser treatment was administered with the following parameters: D-Pulse mode, dot power of 40 W, dwell time of 1000 microseconds, and dot spacing of 1000 micrometers.

Vaginal pH measurement was performed according to standard recommendations using a pH strip capable of detecting pH levels. The vaginal fluid collection was conducted through standardized vaginal rinsing with 5 mL of 0.9% NaCl, followed by aspiration of the fluid using a needle with a 0.9 mm diameter and 9 cm length from the left, central, and right upper vaginal walls. The collected vaginal fluid was transported to the microbiology laboratory within one hour for further analysis. The collected vaginal fluid was transported to the microbiology laboratory and processed as follows. It was centrifuged at 3000 RPM for 15 minutes, after which the supernatant was discarded, and the remaining sediment was thoroughly mixed. A sediment drop was then placed onto a glass slide, air-dried using a slide warmer, and subjected to Gram staining following the standardized procedure.[7]

The vaginal culture protocol, EQUC technique, involves specimen processing, culture inoculation, incubation, colony enumeration, and microbial identification. Upon receiving the specimen in the microbiology laboratory, it was centrifuged at 3000 RPM for 15 minutes.[8] The supernatant was discarded, and the sediment was mixed thoroughly. Using a calibrated loop (1 microliter), the sediment was streaked onto three culture media: Blood agar, MRS agar, and Chocolate agar, ensuring identical streaking procedures for all plates.[8] The inoculated plates were placed in an anaerobic jar and incubated at 35°C for 48–72 hours. After incubation, colony enumeration was performed for all detected microorganisms, and species identification was conducted using MALDI-TOF mass spectrometry. The final report included bacterial identification and colony counts, presented in logarithmic notation as 10^3, 10^4, and 10^5 CFU/mL.[8]

Study Outcomes

The primary objective was to assess changes in the prevalence and level of vaginal Lactobacillus using Gram stain analysis and EQUC at four-time points: Week 0 (baseline, prior to treatment), Week 8 (completion of laser sessions), Week 20 (three months post-treatment), and Week 32 (six months post-treatment).

Gram-stained vaginal smears were examined under light microscopy, beginning with the 10× objective lens and progressing to the 100× oil immersion objective for detailed morphological evaluation. The prevalence of Lactobacillus was interpreted using the Nugent scoring system, a validated and widely accepted method for assessing vaginal microbiota and diagnosing bacterial vaginosis (BV). This scoring system evaluates three bacterial morphotypes: Lactobacillus morphotypes (large Gram-positive rods; score range 0–4), Gardnerella vaginalis and Bacteroides spp. (small Gram-variable rods; score range 0–4), and Mobiluncus spp. (curved Gram-variable rods; score range 0–2).[5] Scores are assigned based on bacterial morphotype counts observed across multiple high-power fields under oil immersion. The individual morphotype scores are then summed to produce a total Nugent score ranging from 0 to 10. A score of 0–3 indicates normal vaginal flora, 4–6 represents intermediate flora, and 7–10 is indicative of BV. This system provides a standardized and reproducible approach for assessing the relative abundance of Lactobacillus and overall microbial balance within the vaginal environment.[7]

This study analyzed changes in Lactobacillus abundance by calculating the mean Lactobacillus score at each designated time point. In this system, the Lactobacillus score ranges from 0 to 4, reflecting the quantity of large Gram-

positive rods morphologically resembling Lactobacillus species. A lower score indicates a higher abundance of Lactobacillus, whereas a higher score suggests a reduced presence of these beneficial bacteria.[7] Two evaluators independently conducted the Gram stain interpretation: the principal investigator (TS), in coordination with the microbiologist (PP). In cases where interpretation differed, the evaluators conducted a joint review of the slides and reached a consensus through discussion. This procedure ensures high diagnostic accuracy and enhances inter-rater reliability in the assessment of vaginal flora.

For the culture-based analysis, vaginal swabs were cultured using the EQUC method. After a 72-hour incubation period, the presence or absence of Lactobacillus spp. was recorded to determine changes in prevalence over time. The prevalence of Lactobacillus was determined through culture when it was reported as $\geq 10^3$ CFU/mL.

The secondary objectives focused on evaluating the long-term effects of fractional CO_2 vaginal laser therapy on vaginal health at three- and six months post-treatment. The VVA symptom severity—specifically vaginal dryness, itching, soreness, and dyspareunia—was assessed using a Visual Analog Scale (VAS) ranging from 0 to 10. Additional outcome measures included vaginal pH evaluations conducted at each follow-up visit, the proportion of participants exhibiting normal Nugent scores at both time points, and the self-reported percentage of subjective symptom improvement as perceived by each participant.

Sample Size

The sample size calculation was based on a previous study that evaluated changes in vaginal Lactobacillus prevalence as the primary outcome. A literature review, specifically the study "The effect of micro-ablative fractional CO_2 laser on vaginal flora of postmenopausal women" reported that the prevalence of Lactobacillus increased from 30% at baseline to 79% after the final treatment.[9] Based on this data and allowing for a 30% dropout rate, a sample size of 45 participants was determined to achieve 80% statistical power.

Ethics Approval and Trial Registration

This study was approved by Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (COA. MURA2023/946) affiliated with Faculty of Medicine Ramathibodi Hospital, Mahidol University, following a full board review on December 25, 2023. The ethics committee operates in accordance with international ethical standards, including the Declaration of Helsinki, the Belmont Report, the CIOMS Guidelines, and ICH-GCP. All participants provided written informed consent prior to enrollment. This trial was prospectively registered with the Thai Clinical Trials Registry (TCTR) on May 27, 2024, under the identification number TCTR20240527002. The full trial record is available at: https://www.thaiclinicaltrials.org/show/TCTR20240527002.

Statistical Analysis

Participant characteristics were summarized as mean ± standard deviation (SD) for continuous variables and frequency (proportion) for categorical variables, as appropriate. Changes in Lactobacillus prevalence were analyzed for the primary outcome using multilevel mixed-effects linear regression analysis. The model included participant ID as a random effect to account for intra-subject correlation resulting from repeated measures. The secondary outcomes, including the assessment of sustained vaginal symptom relief at three- and six-months post-treatment, were evaluated using mixed linear regression analysis. All statistical analyses were performed using Stata version 17 (StataCorp LLC), with a p-value < 0.05 considered statistically significant.

Data Sharing Statement

The authors do not plan to share individual deidentified participant data at this time. However, the full trial protocol and statistical code may be made available upon reasonable request from the corresponding author (jittima.man@-mahidol.edu). Data will be accessible beginning six months following publication and will remain available for one year.

Results

Participant Flow and Characteristics

The flow of participants through the study is detailed in the CONSORT diagram shown in Figure 1. Of 48 women who consented to participate, 45 met the study inclusion criteria. Two participants subsequently withdrew (loss to follow-up, n = 2), leaving data for 43 patients available for analysis.

The demographic characteristics of the study population, consisting of 43 participants who received vaginal CO_2 laser therapy, are summarized in Table 1. The mean age of the participants was 67.3 ± 8.7 years, with a mean body mass index (BMI) of 26.0 ± 4.7 kg/m². The mean age at menopause was reported as 50.4 ± 4.5 years, with an average duration of menopause of 16.2 ± 8.8 years. Regarding obstetric history, 11.6% of the participants (n = 5) were nulliparous, while the majority (88.4%, n = 38) were multiparous. The baseline vaginal pH was measured at 6.2 ± 0.9. Assessment of baseline VVA symptoms using the VAS revealed a mean vaginal dryness score of 7.7 ± 0.9, vaginal itching of 2.8 ± 2.2, vaginal pain of 5.5 ± 1.8, and dyspareunia of 1.8 ± 2.3.

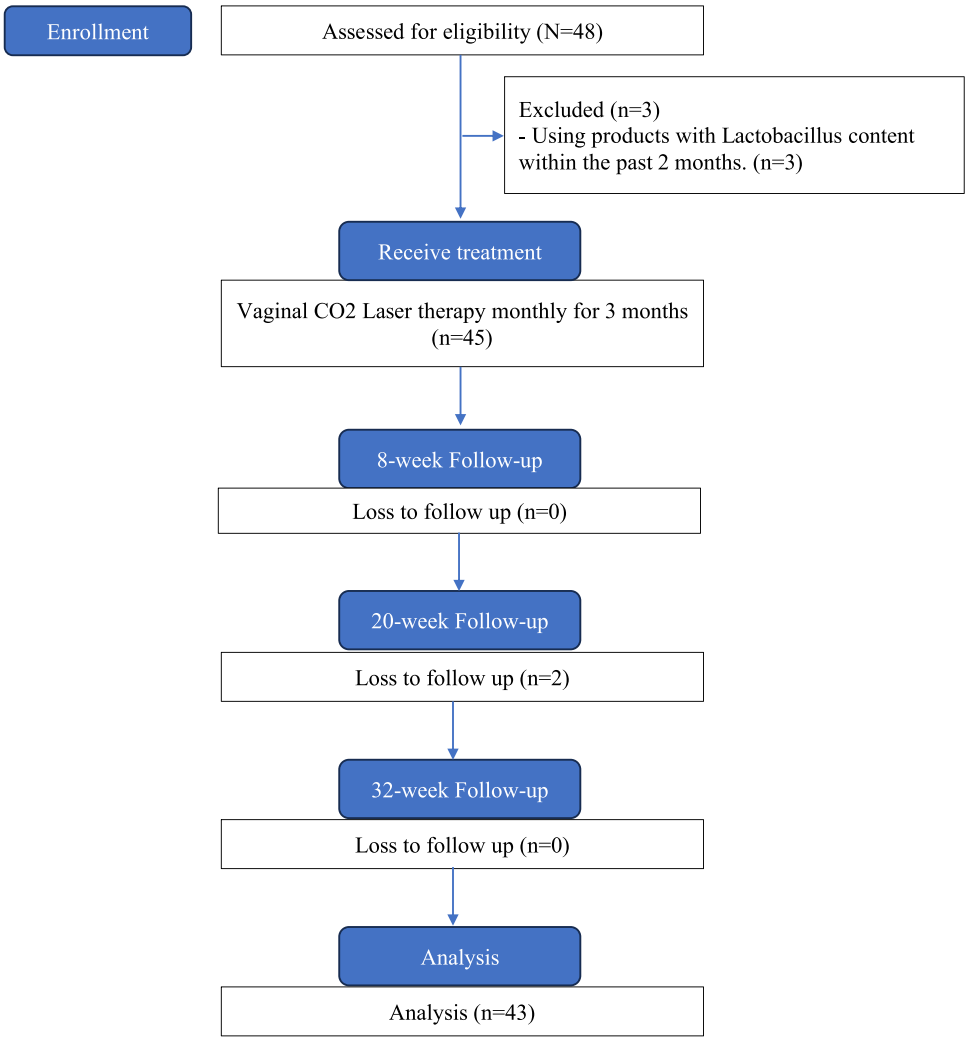

Figure 1 CONSORT flow diagram showing patient enrollment and the study protocol.

https://doi.org/10.2147/IJWH.S537531

Table 1 Participants Characteristics

Demographic data	Patient Who Received Vaginal CO2 LASER Therapy N=43
Age, mean ± SD	67.3 ± 8.7
BMI, mean ± SD	26.0 ± 4.7
Menopausal age, mean ± SD	50.4 ± 4.5
Duration of menopause, mean ± SD	16.2 ± 8.8
Active SI, n(%)	
- Nulliparous	5 (11.6%)
- Multiparous	38 (88.4%)
Baseline vaginal pH, mean ± SD	6.2 ± 0.9
Baseline VVA symptoms (VAS), mean ± SD	
- Vaginal dryness	7.7 ± 0.9
- Vaginal itching	2.8 ± 2.2
- Vaginal pain	5.5 ± 1.8
- Dyspareunia	3.5 ± 2.3

Primary Outcome: Changes in the Prevalence and the Abundance of Vaginal Lactobacillus

Changes in Vaginal Lactobacillus Prevalence Using the Expanded Quantitative Urine Culture (EQUC)

The prevalence of Lactobacillus assessed by EQUC showed a significant increase following treatment, as shown in Figure 2. The prevalence rose from 27.9% (12/43) at baseline (week 0) to 48.8% (21/43) at week 8 and further increased to 55.8% (24/43) at week 20 and to 58.1% at week 32 (25/43) ($p < 0.05$).

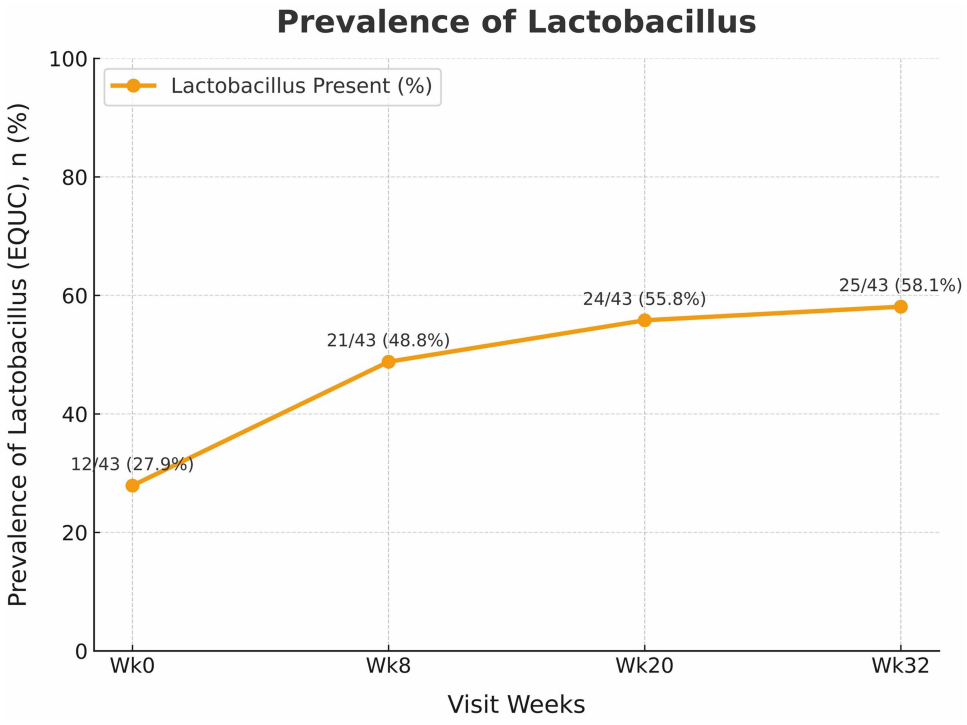

Figure 2 Changes in vaginal *Lactobacillus* prevalence using the expanded quantitative urine culture (EQUC) over 32 weeks.

Changes in the Levels of Vaginal Lactobacillus Using the Nugent Scoring System

The mean Nugent scores, indicative of Lactobacillus morphotypes, demonstrated a gradual and statistically significant reduction throughout the study period, as shown in Figure 3. The scores decreased from 2.84 (95% CI: 2.38–3.29) at baseline to 2.28 (95% CI: 1.79–2.77) at week 8, 2.09 (95% CI: 1.60–2.58) at week 20, and further to 1.84 (95% CI: 1.31–2.35) at week 32 ($p < 0.05$).

Secondary Outcomes

Vaginal Symptoms Severity Scale (VAS)

Regarding clinical symptoms, vulvovaginal symptom severity assessed using the VAS revealed a significant reduction across all symptom categories (Figure 4). The mean vaginal dryness score declined from 7.76 (95% CI: 7.49–8.04) at

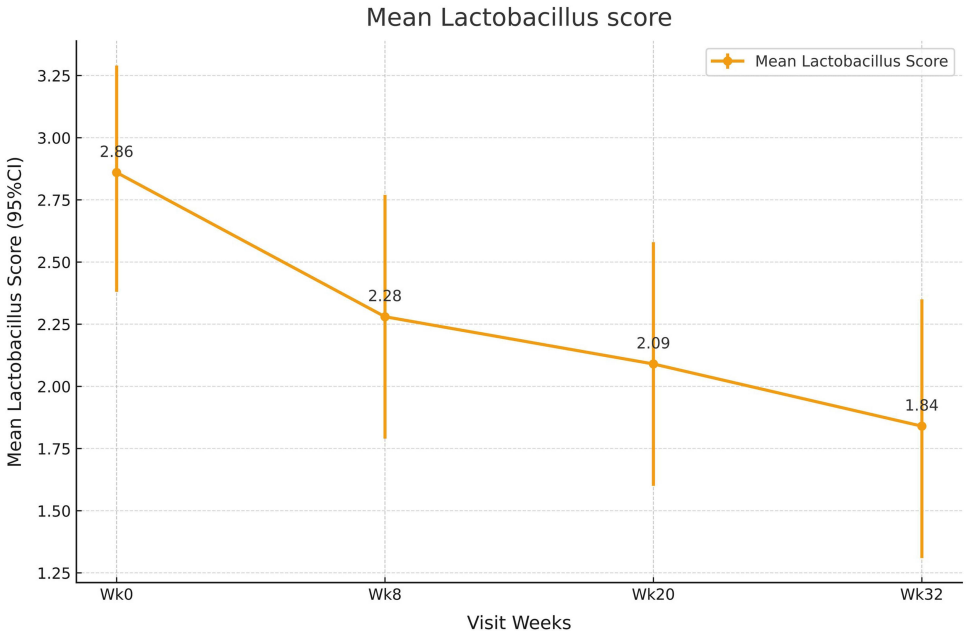

Figure 3 Changes in vaginal *Lactobacillus* abundance (Nugent score 0–4 scale) using the Nugent scoring system across study time points.

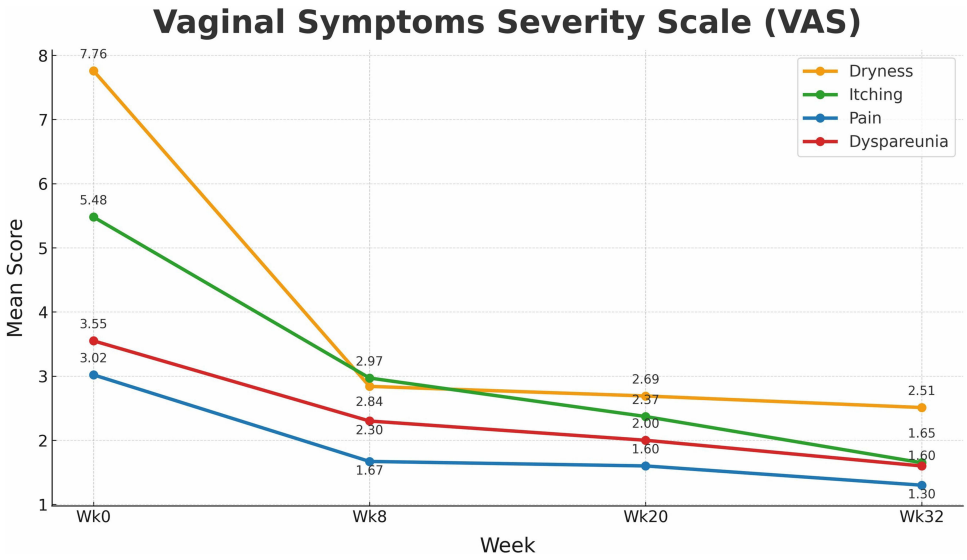

Figure 4 Changes in Visual Analog Scale (VAS) scores for vaginal symptoms, including dryness, itching, and dyspareunia.

https://doi.org/10.2147/IJWH.S537531

baseline to 2.84 (95% CI: 2.53–3.14) at week 8, 2.69 (95% CI: 2.40–2.99) at week 20, and 2.51 (95% CI: 2.23–2.71) at week 32 ($p < 0.05$). Similarly, the mean vaginal itching score decreased from 5.48 (95% CI: 4.92–6.05) at baseline to 2.97 (95% CI: 2.48–3.47) at week 8, 2.37 (95% CI: 1.88–2.86) at week 20, and 1.65 (95% CI: 1.32–1.97) at week 32 ($p < 0.05$). Furthermore, dyspareunia severity declined from 3.55 (95% CI: 2.48–4.61) at baseline to 2.30 (95% CI: 1.49–3.10) at week 8, 2.00 (95% CI: 1.27–2.72) at week 20, and 1.60 (95% CI: 0.93–2.26) at week 32 ($p < 0.05$).

Vaginal pH

The study also observed a substantial reduction in vaginal pH, suggesting a shift toward a more favorable vaginal environment Figure 5. The mean vaginal pH decreased from 6.23 (95% CI: 5.94–6.49) at baseline to 5.30 (95% CI: 5.01–5.59) at week 8, 5.06 (95% CI: 4.80–5.33) at week 20, and 4.90 (95% CI: 4.71–5.07) at week 32 ($p < 0.05$).

Normal Vaginal Flora Using Nugent Scoring System (Score 0-3)

Figure 6 demonstrates the proportion of participants with normal vaginal flora (Nugent score 0–3), which significantly increased over time. At baseline, 20.9% (9/43) of women had normal Lactobacillus levels, which increased to 34.9% (15/43) at week 8, 44.2% (19/43) at week 20, and 51.1% (22/43) at week 32 ($p < 0.05$).

Percentage of Subjective Improvement

The self-reported percentage of subjective improvement indicated that 80% of patients experienced at least a 50% improvement in VVA symptoms, with the effects persisting for up to six months post-treatment.

Safety

Among the 45 postmenopausal women who underwent vaginal CO_2 laser therapy, mild discomfort during the procedure was reported in 8 patients (17.8%), all of whom experienced immediate resolution upon completion of the session without requiring analgesics. Post-procedural spotting or minor bleeding occurred in 3 patients (6.7%), resolving

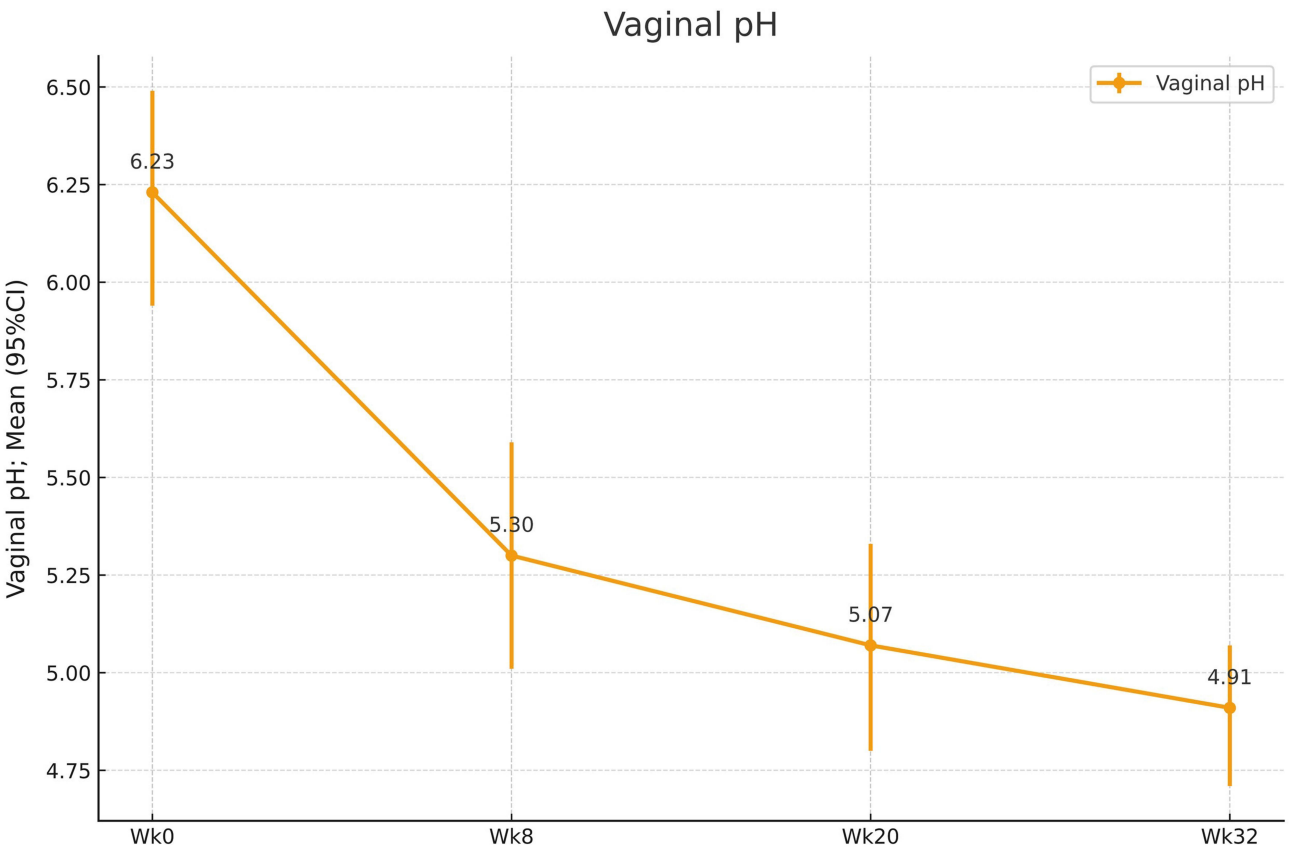

Figure 5 Change in vaginal pH measured using standardized pH strips from baseline through week 32.

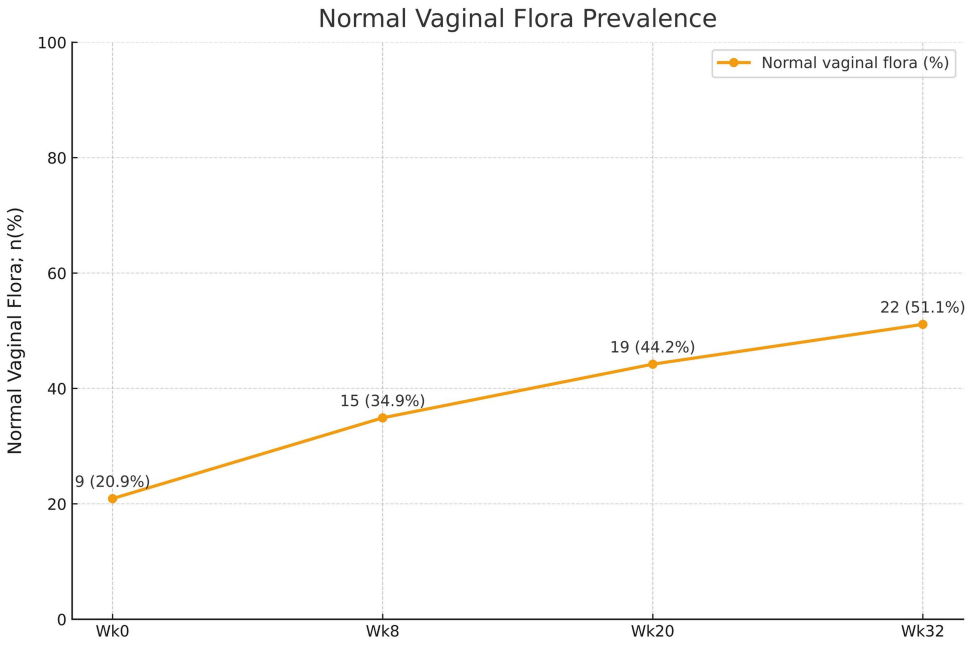

Figure 6 Proportion of participants with normal vaginal flora (Nugent score 0–3), indicating Lactobacillus-dominant microbiota.

spontaneously within 1–2 days without the need for medical intervention. Additionally, no participants experienced complications severe enough to warrant treatment discontinuation.

Discussion

This study is a prospective, single-arm clinical trial, aimed to evaluate the effects of fractional micro-ablative CO_2 vaginal laser therapy on vaginal Lactobacillus and sustained symptom improvement in postmenopausal women. The results revealed a statistically significant and sustained increase in the prevalence and abundance of Lactobacillus spp., maintained up to 32 weeks following fractional micro-ablative CO_2 vaginal laser therapy. This microbiological improvement was accompanied by a significant enhancement in overall vaginal health, including a marked reduction in clinical symptoms of VVA, a significant decrease in vaginal pH, and a notable increase in the proportion of participants achieving normal Nugent scores. In parallel, the patient-reported outcomes indicated that approximately 80% of patients experienced at least a 50% improvement in VVA symptoms, with these effects persisting for up to six months post-treatment.

These findings are consistent with previous literature. Dutra et al reported that a substantial proportion of postmenopausal women experienced persistent symptomatic improvement for up to six months following CO_2 laser treatment, particularly in vaginal dryness and sexual discomfort.[10] Similarly, Woźniak and Woźniak (2025) found that most patients continued to report relief from GSM symptoms well beyond the initial treatment phase, reinforcing the growing consensus regarding the long-term utility of laser therapy.[11] However, given the absence of a control or sham group, these findings should be interpreted as exploratory. Placebo effects, patient expectations, and regression to the mean may partially account for the observed improvements. The lack of blinding is another important factor that could have influenced symptom reporting.

This study demonstrates that a statistically significant increase in the prevalence of Lactobacillus detected by EQUC was observed from 27.9% at baseline to 58.1% at week 32. Our findings align with those of Athanasiou et al, who reported increased Lactobacillus presence post-CO_2 laser using Gram stain,[9] and with Qi et al, who demonstrated microbiota remodeling using 16S rRNA sequencing.[12] However, our study extends this knowledge by offering culture-based evidence of microbial recovery over 32 weeks, an area that remains underexplored in prior work.

Concurrently, the increase in Lactobacillus abundance based on the Nugent scoring following CO_2 laser therapy observed in this study is in alignment with previous investigations. For instance, Jacobsen et al conducted a randomized controlled trial evaluating CO_2 vaginal laser therapy in breast cancer survivors with GSM. Their findings revealed

https://doi.org/10.2147/IJWH.S537531

a significant increase in the relative abundance of Lactobacillus spp. after treatment, alongside a reduction in dysbiosis-associated taxa.[13] Similarly, Qi et al demonstrated that CO_2 laser therapy was associated with a transition toward a Lactobacillus-dominant microbiota, accompanied by an improved vaginal epithelial environment in postmenopausal women, compared to estrogen treatment alone.[12]

Building upon previous studies, the present work contributes additional perspectives by integrating Gram stain and culture-based quantification, enabling a more precise evaluation of viable Lactobacillus populations over time. Furthermore, the extended follow-up period of 32 weeks allowed for an assessment of the persistence of microbial restoration. This aspect has not been well characterized in previous laser therapy studies. By focusing on an older postmenopausal cohort, the present study also extends the applicability of CO_2 laser therapy to populations often underrepresented in clinical trials.

In addition to the fractional CO_2 laser therapy's impact on vaginal microbiota, this study provides evidence of significant improvements across multiple indicators of vaginal health. Treatment was associated with notable reductions in VVA-related symptoms and a significant decline in vaginal pH over the 32-week follow-up period. The marked decrease in VAS scores indicated substantial alleviation of discomfort and improved quality of life. These results align with prior reports by Salvatore et al and Cruz et al, which demonstrated significant improvement in VVA symptoms following CO_2 laser therapy, including among hormone-sensitive populations such as breast cancer survivors.[14,15] The observed reduction in mean vaginal pH reflects a shift toward the optimal acidic range, which supports Lactobacillus colonization and inhibits the growth of pathogenic bacteria.

These findings are further supported by previous studies showing consistent reductions in vaginal pH following laser treatment, as documented by Gambacciani et al and Athanasiou et al, highlighting the reproducibility of this effect.[9,16] While this pH shift may promote a more favorable microbial environment, we emphasize that proposed mechanisms—such as epithelial remodeling and glycogen deposition—remain hypothetical within the scope of our study. These biological processes were not directly measured and should therefore be interpreted as plausible explanations rather than confirmed outcomes. Morphological changes in the vaginal microbiota were indirectly assessed via Nugent scoring, which reflected microbial profile shifts potentially associated with therapy.

However, it is important to interpret these findings in the context of recent randomized controlled trials that have reported conflicting outcomes. In particular, Li et al and Page et al found no statistically significant differences between CO_2 laser and sham treatment groups in terms of symptom improvement, raising questions about the clinical efficacy of laser therapy.[5,6] Differences in study design, population characteristics, outcome measures, and follow-up duration may account for these discrepancies. Unlike these trials, our study incorporated longitudinal microbiological endpoints using both Nugent scoring and culture-based detection of viable Lactobacillus species, offering additional insight into the biological effects of laser therapy beyond subjective symptom reporting. Nevertheless, given the absence of a control arm in our study, causality cannot be established, and our results should be considered exploratory. These inconsistencies across studies highlight the need for further high-quality, comparative research to determine which subgroups may benefit most from laser-based interventions.

These findings are consistent with Jacobsen et al and Qi et al, who demonstrated similar transitions toward a healthier microbiota composition post-laser therapy.[12,13] In addition, no severe complications or treatment discontinuations were observed. These findings align with previous studies demonstrating the safety of CO_2 vaginal laser therapy.[17,18]

In addition to CO_2 laser therapy, other vaginal laser platforms such as Er:YAG and diode lasers have been explored for the treatment of GSM, each demonstrating distinct tissue interactions and microbiological outcomes. The Er:YAG laser, which operates via a non-ablative photothermal mechanism, has been shown to induce epithelial remodeling with minimal thermal penetration. Lin et al reported significant improvements in both vulvovaginal atrophy symptoms and sexual function following Er:YAG laser treatment, alongside reductions in vaginal pH and enhanced Lactobacillus colonization.[19] Similarly, a recent prospective study by Vitale et al demonstrated the efficacy of non-ablative dual-wavelength diode laser therapy, noting marked symptom relief and improvements in vaginal health indices without adverse effects.[20] Compared to CO_2 lasers, which ablate superficial epithelial layers to stimulate regeneration, Er:YAG and diode lasers may exert their therapeutic effects through subtler thermal mechanisms, potentially leading to differing degrees of microbiota remodeling and epithelial thickening. These platform-specific biological responses underscore the importance of selecting appropriate

laser modalities based on individual patient profiles and therapeutic goals. Future head-to-head comparative studies are warranted to delineate the relative advantages and microbiological implications of each technology.

The results of this study highlight the potential of fractional CO_2 laser therapy as an effective and durable non-hormonal intervention for the treatment of GSM. However, we refrain from drawing direct comparisons to estrogen therapy in terms of superiority, as our study was not designed for such comparison and lacked an active control group. Our intention is to emphasize the potential of CO_2 laser therapy as a non-hormonal alternative in estrogen-contraindicated populations.

This study possesses several strengths, including its prospective design, integration of microbiological and clinical endpoints, and extended follow-up duration. However, it also has limitations. The single-arm design and lack of blinding preclude definitive conclusions about causality. The absence of a comparator arm and the relatively small sample size (n = 43) may limit statistical power and the ability to detect subtle or subgroup-specific effects.

Additionally, while participant ID was included as a random effect in the multilevel mixed-effects regression model to account for intra-subject correlation, further clarity in model specification may enhance interpretability.

Potential confounding variables such as hormone use, sexual activity, and prior antibiotic exposure were minimized through inclusion/exclusion criteria but were not quantitatively assessed or included as covariates in the model. This omission should be considered when interpreting associations between microbiological and clinical outcomes.

Future research should address these limitations through randomized, sham-controlled trials with extended follow-up periods and include direct comparisons with other treatment modalities.

In addition, future studies would benefit from incorporating molecular-based approaches such as 16S rRNA gene sequencing or metagenomic analysis to provide deeper insight into microbiota diversity and species-level shifts beyond culture-based methods.

Conclusion

This prospective, single-arm clinical study suggests that fractional micro-ablative CO_2 vaginal laser therapy may enhance vaginal Lactobacillus prevalence and abundance, reduce vaginal pH, and improve both microbiota composition and clinical symptoms in postmenopausal women with VVA. While the observed improvements were sustained at 3- and 6-month follow-ups.

These findings support the potential role of CO_2 laser therapy as a non-hormonal option in estrogen-contraindicated populations. However, future randomized controlled trials, ideally incorporating sham interventions and molecular microbiota profiling, are necessary to validate these results and inform broader clinical adoption.

Acknowledgments

The authors would like to thank Mr. Chanatpon Aonnuam Department of Clinical Epidermology and Biostatistics, Faculty of Medicine Ramathibodi Hospital, Mahidol University for assistance with the statistical analyses. Thank you to Mrs. Sirirat Sarit-apirak Mrs. Jittrat Dechdee, Ms. Nuttanan Panthong, Ms. Peeranuch Mangmeesri, and Ms. Natwalan Petkongthong and Ms. Sirikanya Phaikaew members of the research assistance team, for their assistance and management, including the preparation of research documents and data preparation.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the Faculty of Medicine, Ramathibodi Hospital, Mahidol University. No specific grant number was assigned.

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Faubion SS, Kingsberg SA, Clark AL, et al. The 2020 genitourinary syndrome of menopause position statement of The North American menopause society. *Menopause*. 2020;27(9):976–992.
2. Portman DJ, Gass ML; Vulvovaginal Atrophy Terminology Consensus Conference Panel. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the international society for the study of women's sexual health and the North American menopause society. *Menopause*. 2014;21(10):1063–1068. doi:10.1097/GME.0000000000000329
3. Muhleisen AL, Herbst-Kralovetz MM. Menopause and the vaginal microbiome. *Maturitas*. 2016;91:42–50. doi:10.1016/j.maturitas.2016.05.015
4. Hutchinson-Colas J, Segal S. Genitourinary syndrome of menopause and the use of laser therapy. *Maturitas*. 2015;82(4):342–345. doi:10.1016/j.maturitas.2015.08.001
5. Li FG, Maheux-Lacroix S, Deans R, Nesbitt-Hawes E, Kaur H, Abbott JA. Effectiveness of fractional CO_2 laser treatment compared to sham for women with genitourinary syndrome of menopause: a randomized controlled trial. *Climacteric*. 2021;24(3):243–249.
6. Page AS, Verbakel JY, Verhaeghe J, van den Bosch T, Weyers S, Deprest J. Laser versus sham for genitourinary syndrome of menopause: a randomised controlled trial. *BJOG*. 2023;130(13):1416–1423. doi:10.1111/1471-0528.17335
7. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 1991;29(2):297–301. doi:10.1128/jcm.29.2.297-301.1991
8. Deen NS, Ahmed A, Tasnim NT, Khan N. Clinical relevance of expanded quantitative urine culture in health and disease. *Front Cell Infect Microbiol*. 2023;13:1210161. doi:10.3389/fcimb.2023.1210161
9. Athanasiou S, Pitsouni E, Antonopoulou S, et al. The effect of microablative fractional CO_2 laser on vaginal flora of postmenopausal women. *Climacteric*. 2016;19(5):512–518. doi:10.1080/13697137.2016.1212006
10. Dutra SG, Pinho LG, Vieira CS, Costa-Paiva L, Pinto-Neto AM. Safety and efficacy of fractional CO_2 laser therapy in the treatment of genitourinary syndrome of menopause: a randomized controlled trial. *Menopause*. 2021;28(5):505–512. doi:10.1097/GME.0000000000001797
11. Woźniak D, Woźniak MK. Long-term outcomes of CO_2 laser therapy in postmenopausal women with vaginal atrophy: a prospective cohort study. *Maturitas*. 2025;173:29–35.
12. Qi Y, Mo K, Wang A, He Y. Different effects of CO2 laser and estrogen treatment on vaginal mucosa microbiota and function in genitourinary syndrome of menopause patients. *J Obstet Gynaecol Res*. 2024;50(4):671–681. doi:10.1111/jog.15876
13. Jacobsen S, Glavind-Kristensen M, Jensen AB, Forman A, Bor P. Vaginal CO_2 laser therapy for genitourinary syndrome in breast cancer survivors—VagLaser study protocol: a randomized blinded, placebo-controlled trial. *BMC Cancer*. 2023;23:1164. doi:10.1186/s12885-023-11656-x
14. Salvatore S, Nappi RE, Zerbinati N, et al. A 12-week treatment with fractional CO_2 laser improves symptoms and signs of vulvovaginal atrophy in postmenopausal women. *Climacteric*. 2015;18(2):219–225. doi:10.3109/13697137.2014.975197
15. Cruz VL, Steiner ML, Pompei LM, et al. Efficacy of fractional CO_2 laser treatment in vulvovaginal atrophy: a randomized controlled trial. *Menopause*. 2021;28(3):269–275.
16. Gambacciani M, Palacios S. Laser therapy for the restoration of vaginal function. *Maturitas*. 2017;99:10–15. doi:10.1016/j.maturitas.2017.01.012
17. López V, Suárez Garcia ME, Rivera KS, Rivera A, Romero C. Experience in the management of genitourinary syndrome of menopause with fractional CO2 laser at Dr. Alejandro Dávila Bolaños Military Teaching Hospital in Nicaragua. *Cureus*. 2024;16(11):e74667. doi:10.7759/cureus.74667
18. Pessoa LLMN, de Souza ATB, Sarmento ACA, et al. Laser therapy for genitourinary syndrome of menopause: systematic review and meta-analysis of randomized controlled trial. *Rev Bras Ginecol Obstet*. 2024;46:e–rbgo38. doi:10.61622/rbgo/2024rbgo38
19. Lin KL, Chou SH, Loo ZX, Liu YY, Cheng JC, Long CY. The Er:YAG vaginal laser for management of women with genitourinary syndrome of menopause (GSM). *Lasers Med Sci*. 2022;37(2):1063–1071. doi:10.1007/s10103-021-03484-x
20. Vitale SG, Saponara S, Succu AG, et al. Efficacy and safety of non-ablative dual wavelength diode laser therapy for genitourinary syndrome of menopause: a single-center prospective study. *Adv Ther*. 2024;41(6):2450–2462. doi:10.1007/s12325-024-03004-7

International Journal of Women's Health

Dovepress
Taylor & Francis Group

Publish your work in this journal

The International Journal of Women's Health is an international, peer-reviewed open-access journal publishing original research, reports, editorials, reviews and commentaries on all aspects of women's healthcare including gynecology, obstetrics, and breast cancer. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/international-journal-of-womens-health-journal