

## ORIGINAL STUDY

# Comparison of topical fractional CO<sub>2</sub> laser and vaginal estrogen for the treatment of genitourinary syndrome in postmenopausal women: a randomized controlled trial

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### Abstract

**Objective:** To compare the efficacy of fractional CO<sub>2</sub> laser therapy with topical estrogen therapy for the treatment of postmenopausal genitourinary syndrome of menopause.

**Methods:** We conducted a randomized controlled clinical trial involving 25 postmenopausal women. Participants were aged between 50 and 65 years with at least 1 year of amenorrhea and follicle-stimulating hormone levels of >40 IU/L. The women were randomized into two groups: the laser therapy group ( $n = 13$ ) and the vaginal topical estrogen therapy group ( $n = 12$ ). Changes in the vaginal epithelium thickness, Frost index, and cell maturation were analyzed in both the groups. The female sexual quotient of each woman was also evaluated. Subjective evaluation was performed through a physical examination.

**Results:** Histological analysis showed a significant increase in the vaginal epithelium thickness at the end of treatment in females in both the laser therapy ( $P < 0.001$ ) and topical estrogen therapy ( $P = 0.001$ ) groups. The topical estrogen therapy group tended to present a higher maturation index at the end of treatment when compared with that of the other group. Sexual function increased significantly over time in both the topical estrogen therapy ( $P < 0.001$ ) and laser therapy ( $P < 0.001$ ) groups. Subjective evaluation through physical examination showed a significant improvement in atrophy in both the groups.

**Conclusion:** Despite the nonequivalence with topical estrogen therapy, our data suggest that laser therapy is an effective method for the treatment of vulvovaginal atrophy.

**Key Words:** CO<sub>2</sub> laser – Dyspareunia – Estriol – Female urogenital disease – Postmenopause – Vulvovaginal atrophy.

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**H**ormonal changes have a great physiological impact on postmenopausal quality of life and sexual function in women.<sup>1-3</sup> Genitourinary syndrome of menopause (GSM) is associated with a decline in hormone levels, especially estrogens,<sup>4-7</sup> and affects at least 50% of postmenopausal women. GSM does not spontaneously decrease and commonly worsens in the fifth year after menopause.<sup>5,8-10</sup>

Estrogen, which has a large number of receptors, plays an important role in the genitourinary system physiology. Hormone deficiency or absence triggers morphological changes that lead to vulvovaginal atrophy (VVA),<sup>5,11,12</sup> which is the most common complaint among women.<sup>3,13</sup> Symptoms often include dyspareunia, vaginal dryness, pruritus, irritation, and dysuria,<sup>4,7,9,13-16</sup> with the prevalence of dyspareunia and vaginal dryness in approximately 55% and 44% of cases, respectively.<sup>4,7,9,14,16</sup> Among the many classification systems for hormonal influence on the vaginal epithelium, the maturation value or Frost index seems to be the most informative.<sup>17</sup>

The North American Menopause Society recommends the use of low-dose vaginal estrogen as the gold standard, rather than systemic hormonal therapy, to relieve GSM symptoms.<sup>6,7,15,18,19</sup> Although recent studies have reported on the safe use of estrogens with minimal doses, the effectiveness of the response to these levels does not meet the needs of participants with severe VVA, whose symptoms persist at these dosages.<sup>20</sup> Topical lubricants or moisturizers are recommended for cases where estrogen treatment is contraindicated or undesired; however, these treatments

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have less efficacy compared with topical estrogen (TE).<sup>5,7,11,21</sup> Microablative fractional CO<sub>2</sub> laser therapy (LT) is a potential alternative for VVA treatment in such cases.<sup>22,23</sup>

LT seems to restore the vaginal epithelium (VE),<sup>13</sup> by improving vascularization and angiogenesis, stimulating the production of collagen and elastic fiber production, and leading to thickening of VE.<sup>4,14,18,24,25</sup> Usually, LT is applied in three laser sessions at monthly intervals. Improvement symptoms of GSM are noted after the protocol or 4 weeks after the final therapy.<sup>13,14,23,26</sup> Some of these observations included superficial epithelium restoration, rugae, and lubrication, which are indistinguishable from estrogenized tissue at 3 weeks after the final treatment.<sup>25</sup>

An improvement in dyspareunia in participants with VVA was observed by Salvatore et al<sup>22</sup> at 12 weeks after the start of laser treatment, with a satisfaction rate of 84%. Previous studies have reported epithelial remodeling and an increase in the number of glycogen-rich cells in participants after treatment with LT.<sup>7,27,28</sup> However, most studies are open prospective, comparing vaginal effects before and after laser application, lacking a comparison group, or with small samples or short-term follow-ups.<sup>24,29</sup>

Recently, the efficacy of isolated LT as compared with that of local TE was evaluated.<sup>9,18,29</sup> Furthermore, LT alone or associated with TE was found to be an effective option for improvement of VVA,<sup>9</sup> but data on histological evaluation is still lacking.

Although LT is a new treatment option for genitourinary syndrome, more research is needed before its widespread use can be recommended.<sup>29</sup> Thus, for a better understanding of LT effects in postmenopausal women, we compared the efficacies of LT with TE therapy and assessed the changes of histological biopsies of the vaginal epithelium. We aimed to subjectively evaluate the symptoms of VVA before and after LT and to compare the same with those of TE by evaluating the hormonal influence in the VE (using the maturation indices of Frost and Meisel), sexual function, and histomorphometric assessment of the vaginal mucosa.

## METHODS

### Study details

This randomized controlled, unblinded, clinical trial was conducted between February 2017 and February 2018 in the Department of Gynecology at Universidade Federal de São Paulo.<sup>30</sup> It was approved by the institutional committee of the Federal University of São Paulo (protocol No. 101162/2017) and entered into the Brazilian Clinical Trials Registry (ReBEC) under ID RBR-228FSY. All participants who met the inclusion criteria and were asked to participate in the study gave their consent for participation before the start of the study. The investigator obtained hard copies of informed consent from all the women who participated in the study.

### Study population

First, we determined that the sample size would correspond to the number of participants who met the inclusion criteria over a 1-year period at the Gynecological Disease Prevention

Nucleus (NUPREV), Department of Gynecology. Twenty-six participants were initially selected for the study: one patient was excluded because she did not meet the inclusion criteria and hence, 25 women were randomized into two groups: 13 were allocated to receive LT and 12 were to receive TE. Randomization of participants was carried out through the Random.org website, which generates random numbers (<https://www.random.org/>).

The study included women aged 50 to 65 years with amenorrhea for at least 12 months, their serum FSH levels (>40 IU/L) were compatible with the postmenopausal stage, and they had a current clinical complaint of at least one of the following symptoms: dyspareunia or vaginal dryness. Exclusion criteria included women who had hormone therapy contraindications, used hormonal medication (systemic or topical) in the past 12 months before the study, or had cervical changes, autoimmune diseases, coagulopathies, immunosuppression, pelvic organ prolapse, and/or previous pelvic radiotherapy.

### Study interventions

Participants from both groups underwent vaginal hormonal cytology, which was performed using a plastic Ayres spatula, and the smear was collected from the vaginal walls and posterior vaginal fornix. Further, colposcopy of the cervix (when they did not undergo total hysterectomy) and the vagina, and biopsy of the proximal third of the right lateral vaginal wall was performed approximately 6.0 cm from the introitus. All procedures were performed by the same clinician. Transvaginal ultrasound was performed at the start of both treatments, and women with an endometrial echo <5 mm were included in the study. However, ultrasound control was performed since low-dose vaginal estrogen preparations were generally considered safe for vulvovaginal atrophy treatment.<sup>19</sup> Each woman was subjected to treatment and follow-up for 4 months.

The LT group was subjected to three sessions of fractional CO<sub>2</sub> laser application, 30 days apart, using the SmartXide2 system (Monalisa Touch, Deka Laser, Florence, Italy).<sup>13,14,23,26</sup> Manual rotation of the vaginal probe was performed along the length of the vaginal wall (360°). The parameters used were determined based on previous studies, with power of 30 W, dwell time of 1,000, dot spacing of 1,000, and smart stack 2.<sup>12,22</sup> Participants were informed about the possibility of minor bleeding and were recommended to avoid coital sexual activity and the use of tampon for at least 3 days.<sup>31,32</sup> There was no requirement of analgesia or anesthesia during any of the clinical procedures. The participants underwent a new biopsy and hormonal cytology 30 days after their last session.

The procedure in the case of treatment in the TE group included daily vaginal application of 1 mg of estriol cream for a period of 30 days, followed by a treatment regimen of twice a week application, for 2 months. Hormonal cytology and vaginal biopsy of each participant was performed 30 days after the last application of estriol. The study investigator (P.F.S.P.D.) at the obstetrics and gynecology clinic performed the procedures in both the groups, and cytological and histological evaluations were performed by two pathologists who

were blinded to the treatment groups and stage of the study at which they were involved.

### Study outcomes

The Frost index represents the relative proportion of parabasal, intermediate, and superficial cells of the VE,<sup>13,33,34</sup> which is also calculated from the cell maturation index (Frost), and the number of cells in each category was multiplied by the point value (deep  $\times$  0 + intermediate  $\times$  0.5 + superficial  $\times$  1). A value of 0 to 49 indicated a low estrogen effect, a value of 50 to 64 indicated a moderate estrogen effect, and a value of 65 to 100 indicated a high estrogen effect.<sup>13,35,36</sup>

Epithelium biopsies were evaluated before the first treatment and at the fourth month follow-up after the third treatment, using a common optical microscope (Nikon Eclipse E200, Nikon Instruments Inc, NY), and the Breslow thickness of the mucosa was measured in millimeters (mm).<sup>37</sup> A 4 mm biopsy was performed from the proximal third of the right vaginal wall. The conventional histopathological method was performed as described by our group previously.<sup>38</sup> Hormonal and histological cytologies were measured by pathologists who were blinded to the treatment groups or time points of treatment.

Participants from both groups responded to the Brazilian SQ-F (female sexual quotient) questionnaire at baseline and at the end of their treatment. The SQ-F is a Brazilian validated instrument composed of 10 questions, each of which must be answered on a scale ranging from 0 to 5. Higher scores indicated better sexual performance and satisfaction. The sum of the 10 answers was multiplied by 2, resulting in a total index ranging from 0 to 100. The seventh question required a different calculation method; that was, the value of the answer given (from 0 to 5) had to be subtracted from 5 to achieve the final score for the question.<sup>35,39-41</sup>

### Statistical analysis

Statistical analysis was initially performed using the mean, median, minimum, and maximum values, standard deviation, and absolute and relative frequencies (percentages). Inferential analyses employed to confirm or refute the evidence found in the descriptive analysis were performed using the Shapiro–Wilk test to verify the assumption of normality of numerical information. Student's *t* test was used for independent samples when comparing groups (TE and LT) for numerical information with normal distribution (current age, body mass index, and number of deliveries). Chi-square or Fisher's exact tests were used to compare the groups (TE and LT) according to marital status and education. We used the Mann–Whitney *U* test to compare groups (TE and LT) according to age at the beginning of menopause (numerical information with skewed distribution). Analysis of variance with repeated measures was performed for the comparison of epithelium thickness (SQ-F), number of deep, intermediate, and superficial cells (Frost), and maturation index (Meisel),<sup>42</sup> between groups (TE and LT) and times (initial and final) in addition to multiple Bonferroni comparisons whenever needed. An alpha significance level

of 5% was used for the inferential analyses. Data were entered in Excel 2010 for Windows, and statistical analyses were performed using SPSS Statistics version 24 (IBM Corp., Armonk, NY) and R version 3.6.3 (R Core Team, 2016).<sup>43</sup>

## RESULTS

Of the 25 participants in this study, 12 (48.0%) were allocated to the TE group and 13 (52.0%) to the LT group; one participant was excluded from the study due to her altered cytology (Fig. 1). The mean age of the participants was  $55.3 \pm 4.3$  years, and the mean age at menopause was  $46.2 \pm 5.8$  years old. Clinical features such as age ( $P = 0.880$ ), body mass index ( $P = 0.591$ ), number of deliveries ( $P = 0.881$ ), and age at menopause ( $P = 0.366$ ) were homogeneous between the groups, with no significant differences.

We observed that 10 (83.3%) participants in the TE group had an increased epithelium thickness, 1 (8.3%) had a decreased thickness, and another (8.3%) had no change in thickness. In the LT group, 10 (76.9%) participants had an increase in epithelium thickness, whereas 3 (23.1%) did not show any change.

To compare the proportion of deep cells, we used the Frost index, and our findings showed that 1 (8.3%) participant in the TE group had an increase and 11 (91.7%) had a decrease in the deep cells. In the LT group, four (30.8%) participants showed an increase, five (38.5%) had a decrease, and four (30.8%) showed no changes in deep cells.

In the TE group, nine (75.0%) participants had an increase in intermediate cells, two (16.7%) had a decrease of the same, and only one (8.3%) had no change. In the LT group, five (38.5%) participants showed an increase in intermediate cells, and the other eight (61.5%) showed a decrease.

An increase in superficial cells was observed in eight (66.7%) participants, one (8.3%) showed a decrease in these cells, and the other three (25.0%) participants had no change in the TE group. We noted that six (46.2%) participants had an increase in the proportion of superficial cells, three (23.1%) had a decrease, and four (30.8%) had no change in the LT group (Table 1).

In the inferential comparison between the two groups, the epithelial thickness (in mm) was statistically the same at both the baseline ( $P = 0.19$ ) and at the end of the treatment ( $P = 0.316$ ), increasing over time in both the TE ( $P = 0.001$ ) and LT ( $P < 0.001$ ) groups (Fig. 2A). Figure 2B to E shows an increase in glycogen-rich cells as well as an increase in the intermediate and superficial layers of the VE in both groups.

To evaluate the Frost index, we performed an analysis of epithelial layer cells. At baseline, the number of parabasal ( $P = 0.100$ ), intermediate ( $P = 0.253$ ), and superficial cells ( $P = 0.595$ ) was statistically similar between the groups. The TE group showed a decrease in parabasal cells at the end of treatment ( $P < 0.001$ ); however, the same was not observed in the other LT group ( $P = 0.948$ ). With regard to the intermediate cells, we noted an increase in the TE group ( $P = 0.002$ ) but not in the LT group ( $P = 0.432$ ). In addition, the TE group showed an increasing trend for superficial cells ( $P = 0.092$ ),

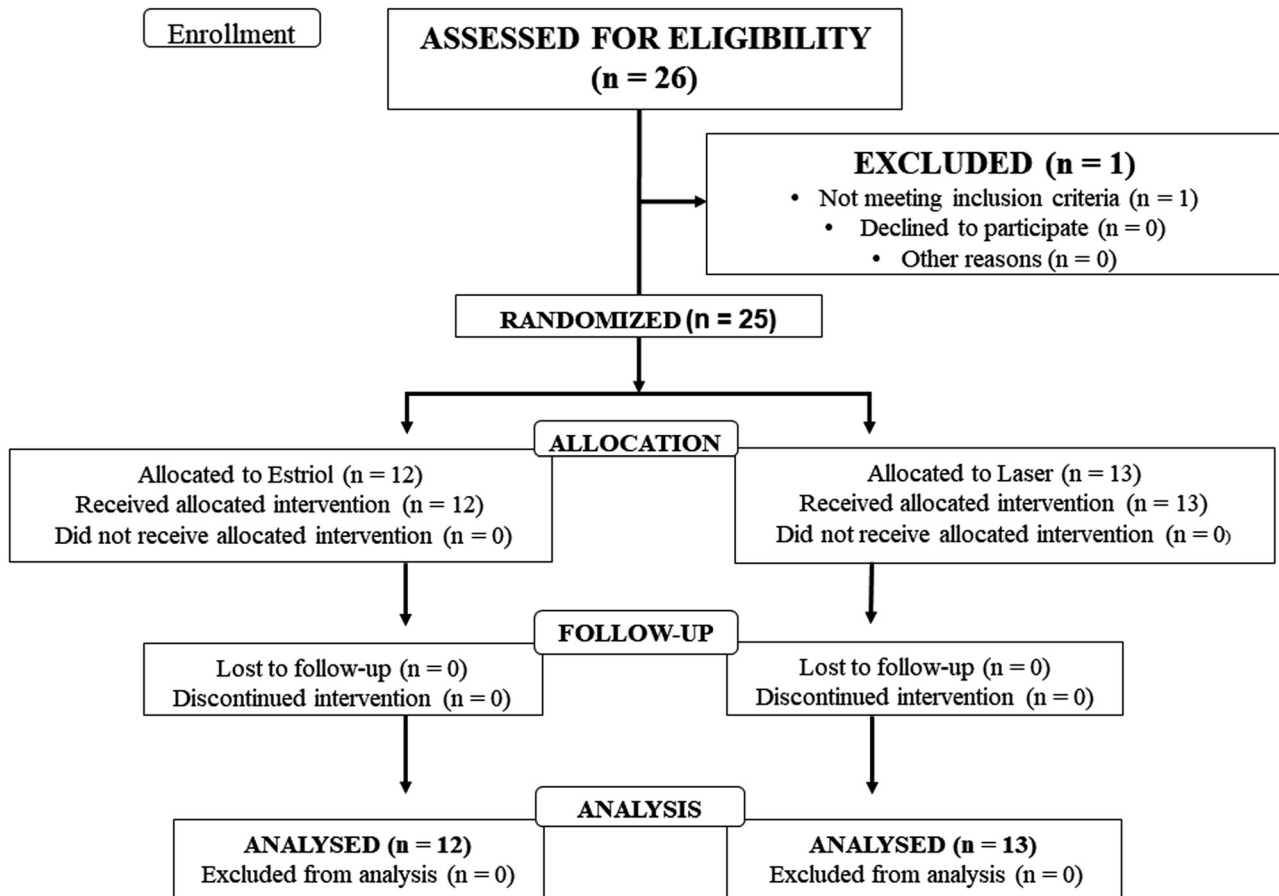


FIG. 1. Flow diagram of study design with details of number of participants.

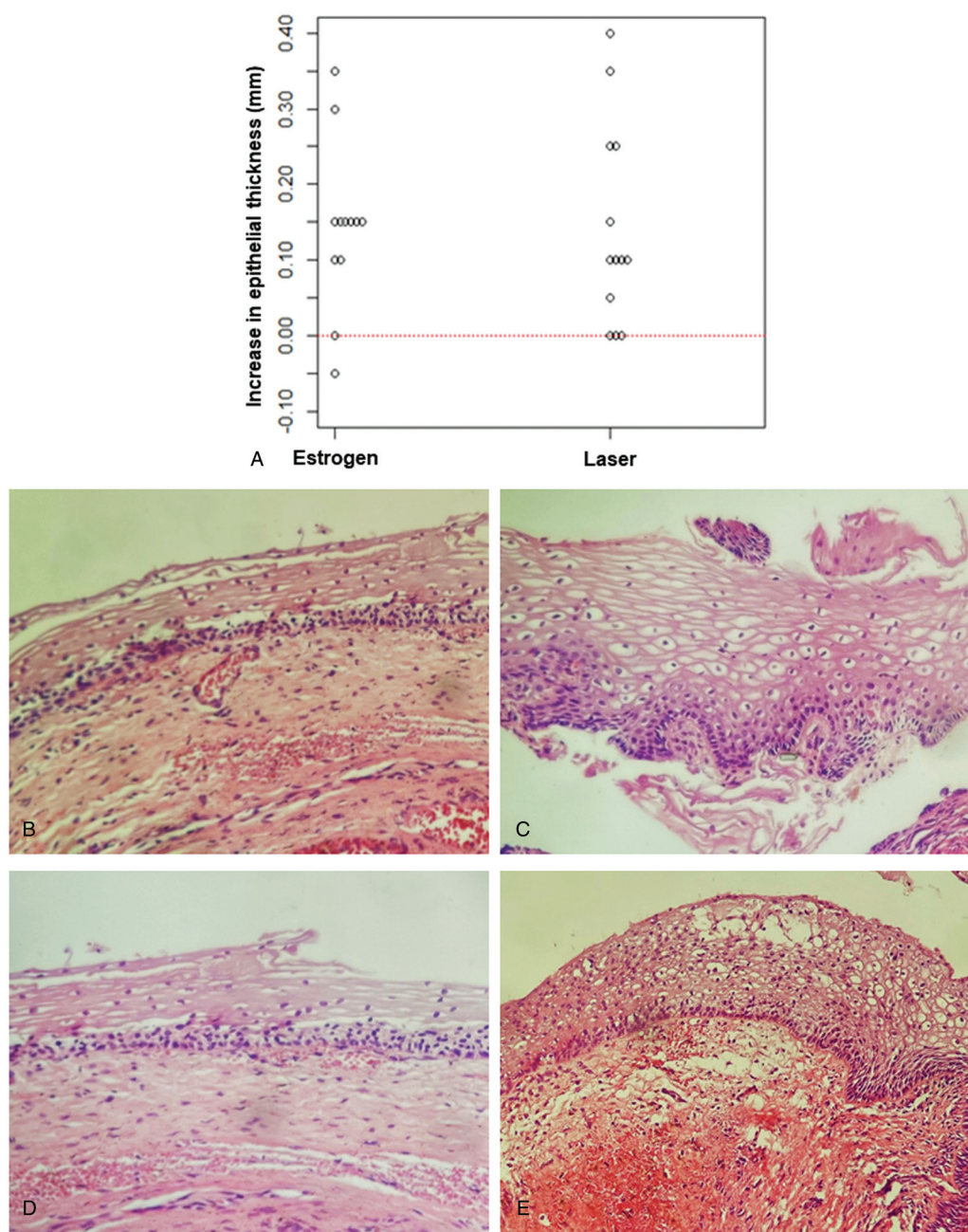
whereas the LT group showed no statistically significant changes ( $P = 0.471$ ; Fig. 3A).

The Meisel index was also compared between the two groups. We found that 11 (91.7%) participants had an increase

in this index in the TE group, whereas 8 (61.5%) of the participants had an increase in the LT group ( $P = 0.160$ ). The inferential results revealed that, at the beginning, the maturation index was statistically similar in both groups ( $P = 0.379$ ).

TABLE 1. Behavior of epithelial thickness (mm) according to group

	Estrogen (n = 12)		Laser (n = 13)		Total (n = 25)	
Initial epithelial thickness (mm)						
Mean		0.19		0.14		0.17
Median		0.18		0.15		0.15
Minimum		0.10		0.05		0.05
Maximum		0.35		0.25		0.35
Standard deviation		0.09		0.06		0.08
Final epithelial thickness (mm)						
Mean		0.33		0.28		0.31
Median		0.35		0.25		0.30
Minimum		0.25		0.10		0.10
Maximum		0.45		0.55		0.55
Standard deviation		0.07		0.15		0.12
Increase in epithelial thickness (mm)						
Mean		0.14		0.14		0.14
Median		0.15		0.10		0.15
Minimum		-0.05		0.00		-0.05
Maximum		0.35		0.40		0.40
Standard deviation		0.11		0.13		0.12
Change in epithelial thickness						
Decreased	1	8.3%	—	—	1	4.0%
None	1	8.3%	3	23.1%	4	16.0%
Increased	10	83.3%	10	76.9%	20	80.0%



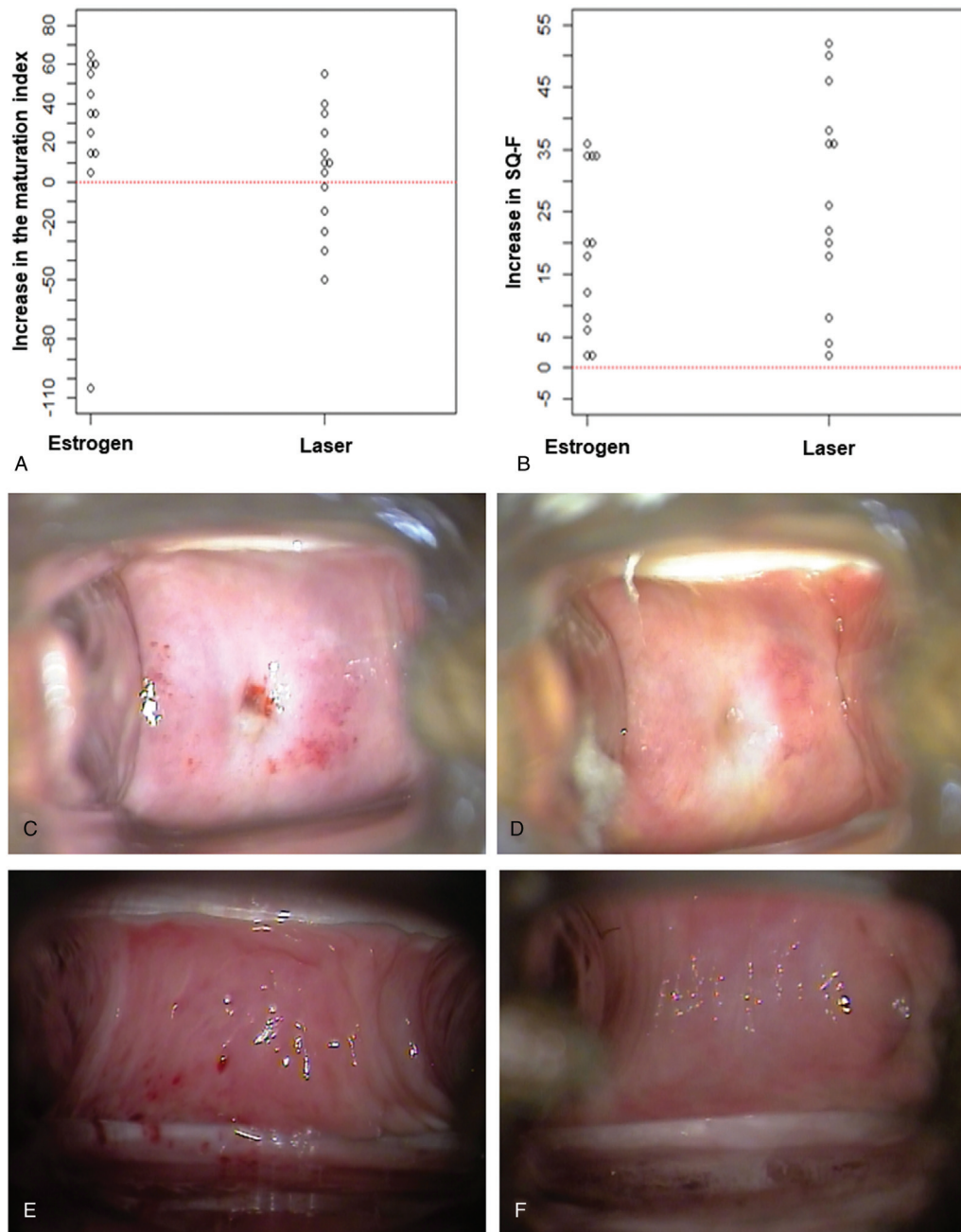
**FIG. 2.** Comparison of epithelial thickness between participants of TE and LT groups: (A) One-dimensional scatter plot showing increase in epithelial thickness (mm) in both the groups. Photomicrographs of histological sections of the vaginal wall biopsies of a participant who underwent fractional LT. Photomicrograph of biopsies from the proximal third of the right lateral vaginal wall: (B) before treatment, and (C) after treatment with fractional LT. Photomicrographs of histological sections of vaginal wall biopsies of a participant who underwent treatment with TE. Photomicrograph of biopsies from the proximal third of the right lateral vaginal wall: (D) before treatment, and (E) after treatment with TE therapy. Hematoxylin and eosin staining was performed for histology sections. (Magnification:  $\times 200$ ). LT, laser therapy; TE, topical estrogen.

There was a tendency for the TE group to present a higher maturation index than the LT group at the end of treatment ( $P = 0.073$ ). At the beginning of the current study, the TE group had a lower maturation index than that noted at the end of the study ( $P = 0.003$ ). On the other hand, the LT group did not demonstrate a significant change in the index between the two time points ( $P = 0.734$ ).

All participants in both groups had an increase in the SQ-F. The LT group had an average increase of 27.84, whereas the TE

group showed an average increase of 18.83 of SQ-F. We observed that the SQ-F was statistically similar between the two groups at both the baseline and the end of the study ( $P = 0.368$ ). In addition, SQ-F was found to increase over time in both the TE ( $P < 0.001$ ) and LT ( $P < 0.001$ ) groups (Fig. 3B).

Subjective evaluation through physical examination, as shown in Figure 3C to F, revealed clear signs of atrophy initially, such as pallor in the vaginal mucosa and petechiae. However, after LT, there was a marked improvement in the



**FIG. 3.** Comparisons between different parameters of TE and LT groups: (A) One-dimensional scatter plot showing increase in maturation index at the end of treatment, by group; (B) One-dimensional scatter plot showing increase in female sexual quotient (SQ-F) at the end of treatment, by group. Images of the cervix obtained by an imaging system (Diagnose) before treatment (C) and 30 days after the last fractional LT session (D). Images of the vaginal mucosa obtained by an imaging system (Diagnose) before (E) and after (F) treatment with TE.

brightness and color of the vaginal mucosa, increased exudate, and absence of petechiae.

### DISCUSSION

In our subjective analysis, it was possible to verify the changes in the vaginal mucosa features, where improvement of pallor and absence of petechiae was noted in both the LT and TE groups. None of the women subjected to LT experienced any harmful thermal effects on the vaginal epithelium.

In contrast to the findings of Cruz et al<sup>9</sup> and Politano et al,<sup>29</sup> who observed a significant increase in the maturation index in the participants undergoing LT, we did not notice any such significant effect in our study. Regarding the Frost index, we observed a significant reduction in deep cells only in the group subjected to TE. The women in this group were associated with an increase in the number of cells in the intermediate layer and a tendency toward an increase in the number of superficial cells. Thus, there was a tendency for the TE group to present a higher

maturation index than the LT group at the final time point. In a previous study, it was observed that participants treated with fractional CO<sub>2</sub> laser showed some evidence of recovery in hormonal activity at the level of VE by the maturation of parabasal cells. At the end of the first month, there was a marked decrease in these cells, while the number of intermediate and superficial cells had increased accordingly.<sup>33</sup>

Despite the complexity of sexual function, which depends not only on local but also on hormonal vaginal factors,<sup>31,44</sup> we observed that the use of LT is an effective method for vaginal atrophy treatment, corroborating with the improvement in sexual function, as seen in previous studies.<sup>3,9,14,23,27,31</sup>

Using objective measurements, we observed that both treatments resulted in a significant increase in the thickness of the VE, similar to the findings obtained in previous studies.<sup>9,12,21,45,46</sup> Based on similar protocols<sup>12</sup> for the application of LT and totaling approximately 240 women, some authors demonstrated the effectiveness of fractional CO<sub>2</sub> laser in the treatment of VVA, presenting it not only as another option to be offered to the patient, but also as an alternative option as viable as TE.<sup>9,12,21,45,46</sup> Until now, TE was considered more effective for these symptoms. However, it is worth mentioning that LT is an onerous procedure, since it is necessary to purchase the machine by the professional as well as it requires adequate training, and thus far, it has not been paid for by health insurers.

In a recent publication using collagen analysis and subjective assessment of VE layers, Politano et al<sup>29</sup> compared treatments with CO<sub>2</sub> laser, promestriene, and vaginal lubricant and found a similar response between promestriene and CO<sub>2</sub> laser.

Zerbinati et al<sup>28</sup> observed in their histological findings the recovery of the whole structures supporting full functionality in the epithelial and connective tissue of vaginal mucosa after fractional CO<sub>2</sub> laser therapy, similar to a premenopausal structure. LT was found to promote profound regenerative effects on the vaginal wall.<sup>23</sup> Our histomorphometric evaluation and comparison add to the histological basis of the laser application as a therapeutic option for postmenopausal women. In a study carried out by Salvatore et al in 2014,<sup>22</sup> the use of the LT on vaginal tissue of postmenopausal women was demonstrated to provide important remodeling with an increase in the thickness of the VE and no associated tissue damage. Furthermore, a recent study published by Samuels et al<sup>27</sup> showed that the epithelial thickness increased in participants subjected to LT, and there was an increase in the number of cell layers and a better degree of superficial maturation. These studies are in synergy with our findings that demonstrated an improvement in the Meisels indices in both groups. In the present study, the thickness of the epithelium increased over time in both the groups. Our study adds new data showing that LT can be used in a different way and is as safe and effective as TE. Few studies have been conducted in the literature in which histological evaluation of VE was performed.

We also observed regression of the signs of vaginal atrophy on colposcopy, as mentioned by Simões et al.<sup>46</sup> Fractional CO<sub>2</sub> laser has been shown to be a useful and minimally

invasive alternative for the treatment of GSM,<sup>45,47</sup> and it provides advantages over currently available treatments.<sup>46</sup> In particular, it has the convenience of not using creams and produces a longer-lasting effect. The great advantage of this treatment is the absence of immediate or late complications. It is important to consider that LT is a painless, fast technique with an almost immediate effect and has a prolonged duration when compared with other therapies.<sup>46</sup>

Our histomorphometric evaluation demonstrated that the significant increase in the thickness of the VE in both groups equates the efficiency of the LT to the current topical estrogen therapy standard that is recognized as the “gold standard” for treating VVA. The increase in SQ-F at the end of treatment in all women demonstrates the impact of VVA on quality of life as well as the importance of its treatment and recovery of physiological conditions of the vagina. None of the participants showed any signs of side effects or adverse events during the study with both treatments, which was in line with a multicenter study performed with a large sample size (645 women) by Filippini et al.<sup>11</sup> LT was well tolerated by most studied women, and only a few studies reported discontinuation or withdrawal from LT.<sup>48</sup> The treatment recommendations and follow-up time discussed or proposed in earlier studies of the three laser sessions showed improvement in the quality of life of the participants. Eder<sup>14</sup> and Tovar-Huamani et al<sup>33</sup> emphasized that the effectiveness of fractional CO<sub>2</sub> LT was noted even after a single session.

Nevertheless, it is important to note that our study had some limitations, including the fact that it was conducted in a single center, had a short-term follow-up, had a small number of the participants, and involved the cost of LT. LT is not covered by insurance and it is not available in the public health system yet. The age range of the women included in the study was based on the hypothesis that vaginal atrophy is progressive and that there could be a discrepancy between the studied populations. It is essential to highlight that new prospective and controlled studies evaluating a wider range of women are needed to obtain a greater understanding of the duration and long-term effects of vaginal LT as well as the functioning of this remodeling process, so that LT can be disseminated and used routinely and safely in the treatment of menopausal genitourinary syndrome.

## CONCLUSIONS

In conclusion, histomorphometric analysis showed a significant increase in vaginal mucosa thickness in both groups, demonstrating that laser treatment is an effective option for the treatment of VVA. However, considering the proportion of increase in each treatment, we cannot state that there is an equivalence between the two treatments.

The increase in the sexual quotient of all participants at the end of their treatment demonstrates the impact of VVA on quality of life and the importance of its treatment and recovery of the normal physiology of the vagina.

Thus, the results presented suggest that fractional CO<sub>2</sub> LT may be a viable therapeutic option, especially in situations where the use of topical hormones is not desired or contraindicated.

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