PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Microablative Fractional Radiofrequency for the genitourinary syndrome of menopause: protocol of randomized controlled trial
AUTHORS	Sarmento, Ayane Cristine; Fernandes, Fabíola; Costa, Ana Paula; Medeiros, Kleyton; Crispim, Janaina; Gonçalves, Ana

VERSION 1 – REVIEW

REVIEWER	Abbott, Jason University of New South Wales, School of Women's and Children's Health
REVIEW RETURNED	06-Dec-2020

GENERAL COMMENTS	Microablative Fractional Radiofrequency on vaginal health, microbiota, and cellularity of postmenopausal women: protocol of randomized controlled trial This is a protocol of a randomised, nocebo controlled trial using vaginal fractional radiofrequency. The authors plan a 3 arm and non-blinded study: 1. Fractional radiofrequency 2. Topical vaginal estrogen 3. Nocebo The participants will not be blinded. It states early in the protocol (strengths and limitations page) that participants and assessors will be blinded, yet this is opposed to the open label nature of the intervention and it is unclear how any blinding will occur. The primary endpoint is a laboratory-based outcome. There are no patient reported outcomes. This is not appropriate for a condition where there are no mortal consequences (such as with malignancy). Reporting only laboratory outcomes with no clinical data has no role in GSM. The use of pH as a primary outcome and the VHI which also includes pH as the only objective assessment is unnecessary duplication of information. The use of the VMI and VHI are problematic and this has been demonstrated in similar studies using laser technologies. The variation in the technology from laser to radiofrequency vaginal skin damage is not sufficiently different to provide novelty to this technique to make those claims. There are 3 RCTs examining cellular outcomes and VHI using laser and none show superiority of the laser technology. Granted that this is different technology, but what is the biological plausibility for a different outcome?

	The sample size is considerable and will take effort on behalf of both the participant and the research team. Even in the event of a difference in primary outcome of pH, if this is not linked to any clinical data then there is no value in the study. It is critical that in a study such as this that patient-reported outcomes be the main (and possibly sole) focus. The research question being asked is insufficient to provide valuable information to women who present with a variety of symptoms, not a change in vaginal micriobiota. I strongly urge the authors to reconsider their research question and their approach to make this clinically relevant.

REVIEWER	Paszkowski, Tomasz 3rd Chair and Department of Gynecology, Medical University of Lublin, Poland
REVIEW RETURNED	15-Dec-2020

GENERAL COMMENTS	This protocol does not provide the information about the exact dose of estradiol to be applied in the local estrogen treatment group neither the name of the drug is mentioned.

REVIEWER	Luvero, Daniela Department of Obstetrics and Gynaecology, Campus Bio-Medico, University of Rome, Rome, Italy
REVIEW RETURNED	16-Dec-2020

GENERAL COMMENTS	The manuscript is well written, clear and the topic is very interesting but I have major revisions to underline: 1) in the introduction authors should better underlyne the presence of alternative methods such as the microablative Co2 laser with associated references (Filippini et al, Salvatore S, Athanasiou S) and YAG laser (Flint R) The discussion is too short, would be interesting compare these data about alternatives methods to the results of this research. 2)In addition the author should consider the possibility to add to the materials and methods if patients with a vaginal infection were enrolled or not, if not, how authors excluded them? (vaginal swab?, or other?)

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Prof. Jason Abbott, University of New South Wales
Comments to the Author:
Microablative Fractional Radiofrequency on vaginal health, microbiota, and cellularity of postmenopausal women: protocol of randomized controlled trial
This is a protocol of a randomised, nocebo controlled trial using vaginal fractional radiofrequency. The authors plan a 3 arm and non-blinded study:
1. Fractional radiofrequency
2. Topical vaginal estrogen
3. Nocebo

1- The participants will not be blinded. It states early in the protocol (strengths and limitations page)

that participants and assessors will be blinded, yet this is opposed to the open label nature of the intervention and it is unclear how any blinding will occur.
Response: We apologize for the due to the lack of clarification regarding this information. The latter information was clarified in the strengths and limitations, and the methodology.
" Blinding of assessors and standardization of protocols enhance this trial's internal validity."
"Participants and the group researchers cannot be blind to arm allocation because of the features of the interventions (MAFRF and vaginal estrogens). However, the researchers that will evaluate the outcomes will be blinded to which arm comprises each intervention and any other sociodemographic information that might facilitate the identification of the intervention group."

2- The primary endpoint is a laboratory-based outcome. There are no patient reported outcomes. This is not appropriate for a condition where there are no mortal consequences (such as with malignancy). Reporting only laboratory outcomes with no clinical data has no role in GSM.
Response: We agree with your considerations. Thus, the primary outcome will be sexual function. The women will be evaluated using the Questionnaire Female Sexual Function Index (FSFI) (Thiel; et al., 2008). The secondary outcome will be vaginal health that will be evaluated per Vaginal Health Index (epithelial integrity, vaginal elasticity, moisture, fluid volume, and pH vaginal); beyond the vaginal microbiota, and cell maturation according to Lustosa et al. (2002); Lee et al. (2011) and Athanasiou; et al. (2017).

3- The use of pH as a primary outcome and the VHI which also includes pH as the only objective assessment is unnecessary duplication of information. The use of the VMI and VHI are problematic and this has been demonstrated in similar studies using laser technologies.
Response: We agree with your suggestion, and the primary outcome was adjusted. Thus, pH is not the main aim of this research. Despite the demonstrated problem of using the VMI and VHI, at the moment, there is no consensus and definition about the assessment of VA or GSM. Thus, the current recommendation is to combine a subjective assessment (FSFI in our case), and objective assessments for the atrophy vaginal (for example, VMI and VHI). (Gandhi; et al., 2016; Fillipini; et al., 2019; Athanasiou; et al., 2017; Gambacciani; et al., 2017)

4- The variation in the technology from laser to radiofrequency vaginal skin damage is not sufficiently different to provide novelty to this technique to make those claims. There are 3 RCTs examining cellular outcomes and VHI using laser and none show superiority of the laser technology. Granted that this is different technology, but what is the biological plausibility for a different outcome?
Response: Although the laser is the most well-known and used physical method, using radiofrequency presents advantages, such as the application is realized under direct vision, using a vaginal speculum, facilitating treatment along the vaginal walls, and preventing overlapping of shots. As well as this, the method is easy to learn and less costly. The procedure features a useful tolerance index, the patients recovered quickly, and the microablation disappear 3 to 5 days after the application. Radiofrequency is based on radiation between 30KHz and 300MHz, within the electromagnetic spectrum that generates heat. This type of heat reaches the deepest tissues, creating energy and strong heat in the deepest layers of the skin, keeping the surface fresh and protected, causing the contraction of existing collagen fibers and stimulating the formation of new fibers, making them more efficient in supporting the skin. The thermal effects of radiofrequency cause collagen denaturation, promoting the immediate and effective contraction of its fibers, activating fibroblasts and leading to neocolagenesis, the reorganization of collagen fibers, and the subsequent remodeling of the tissue. The energy fractionation through the distribution of energy in equidistant points, producing microscopic columns of thermal lesions in the epidermis and the upper dermis, allows a faster re-epithelialization. (Kamilos MF and Borelli CL, 2017; Leibaschoff G, et al, 2016; Vicariotto F and Raichi M, 2016; Bloom BS, et al, 2012; Carvalho GF, et al, 2011; Sarmento AC, et al, 2020)

5- The sample size is considerable and will take effort on behalf of both the participant and the research team. Even in the event of a difference in primary outcome of pH, if this is not linked to any clinical data then there is no value in the study.
Response: We agree with your placement. We have a collaborative team to carry out the clinical trial. Furthermore, we have adjusted the outcome; and the primary outcome will be sexual function.

6- It is critical that in a study such as this that patient-reported outcomes be the main (and possibly sole) focus. The research question being asked is insufficient to provide valuable information to women who present with a variety of symptoms, not a change in vaginal microbiota. I strongly urge the authors to reconsider their research question and their approach to make this clinically relevant.
Response: We understand your concern and, we agree on the suggested adjustments. Our research question was revised, and we aim to investigate the therapeutic effect of vaginal MARFM in the genitourinary symptoms of climacteric women. We postulate that MAFRF could promote the improvement of sexual function and vaginal health. Furthermore, could occur cell maturation based on increasing superficial cells and decreasing parabasal cells. Furthermore, could appear alteration of the microbiota vaginal, with an increased number of vaginal lactobacilli, and decreases the vaginal pH. Thus, it is possible to hypothesize that the MAFRF treatment is as safe and effective as standard vaginal estrogen treatment.

Reviewer: 2
Dr. Tomasz Paszkowski
Comments to the Author:
1-This protocol does not provide the information about the exact dose of estradiol to be applied in the local estrogen treatment group neither the name of the drug is mentioned.
Response: This information was added: “The patients from the group with estrogen will be instructed to use Promestriene (Estradiol 3-propyl 17β-methyl diether) vaginal cream, 1g corresponding to the use of the filled applicator up to the ring mark, twice a week, for three months”

Reviewer: 3
Dr. Daniela Luvero
Comments to the Author:
The manuscript is well written, clear and the topic is very interesting but I have major revisions to underline:
1- in the introduction authors should better underlyne the presence of alternative methods such as the microablative Co2 laser with associated references (Filippini et al, Salvatore S, Athanasiou S) and YAG laser (Flint R)
Response: We agree with your suggestion. The references were added in the introduction.
“Recently, studies showed that the use of fractional CO2 laser in the treatment of VVA was beneficial, effective, and safe. The latter positive effects on VVA symptoms can be improved not only the quality of life; but also the aspect of sexual pain; and other dimensions of women’s sexual response, such as desire, initiative, and receptivity to their sexual partner. [6-8]. Similar results have been observed in the use of YAG laser treatment. Application of Er: YAG laser is associated with an improvement in vaginal atrophy, and such treatment induced a significant decrease in Visual Analog Scale (VAS), an increase of VHI, and a significant improvement in urinary incontinence.[9,10]”

2- The discussion is too short, would be interesting compare these data about alternatives methods to the results of this research.
Response: We agree with your suggestion; the latter information were added. “Some systematic reviews have already been published on the subject.[30-33] A recent study assessing the physical methods for the treatment of SGM showed that, among physical methods, the CO2 laser continues one of the most commonly used methods, as it has the largest body of scientific evidence. The CO2

laser has been demonstrated to be an efficacious therapy for managing all GSM symptoms up to 12 months after treatment. [33]. The VHI score improved concerning elasticity, fluid volume, pH, epithelial integrity, vaginal moisture, and VAS scores improved considerably for sensitivity, vaginal dryness, itching/stinging, dyspareunia, and dysuria. The studies about the Er: YAG treatment showed that this method is effective, practical, and safe too, and the effects are rapid and sustained for at least 12 months. Application of Er: YAG laser is associated with an improvement in vaginal atrophy, and such treatment induced a significant decrease in VAS, an increase of VHIS, and a substantial improvement in the urinary incontinence (UI).[33]
Additionally, the RF method could be a safe and effective non-surgical option for treating mild to moderate UI and other symptoms related to GSM. Significant improvements were observed in the mean VAS score and for complaints of VVA. However, little is known about the actual effectiveness of RF in the treatment of GSM/UI since, as we have already reported in this review, the current literature is still sparse for this topic. For this reason, new research about this topic is necessary.[33]
We can also quote a prospective study[29] conducted at a public university hospital to evaluate the effectiveness of MAFRF in the non-hormonal treatment of GSM. In this research, 55 postmenopausal women were examined before and after the treatment about the VHI, vaginal microbiota, vaginal pH, and cell maturation. The latter study observed after treatment an increase in the percentage of Lactobacillus spp. Consequently, occurred a progressive decrease in vaginal pH. Regarding cell maturation, there was a decrease in the percentage of parabasal cells and an increase in the rate of superficial cells. Additionally, there was an improvement in the VHI index. In conclusion, the results showed that the therapy of MAFRF restored the vaginal balance, as would usually be expected with sufficient estrogen levels. The predominance of Lactobacillus species and acidic pH of the vaginal fluid achieved after radiofrequency therapy could protect postmenopausal women from vaginal infections, inflammation, and infections of the urogenital tract. Therefore, the MAFRF treatment was considered well-tolerated and promoted significant improvement in the vaginal microenvironment; therefore, radiofrequency could be an option for GSM symptoms.[29]"

3- In addition the author should consider the possibility to add to the materials and methods if patients with a vaginal infection were enrolled or not, if not, how authors excluded them? (vaginal swab?, or other?)
Response: The patients with a vaginal infection diagnosis by GRAM stain and Multiplex-PCR were excluded. This information was added to on eligibility criteria on a section of materials and methods.
"Women who have used any form of hormonal (systemic or local) therapy in the last six months, lubricants or vaginal moisturizers in the past month, suffering from active genital infections (diagnosis by GRAM stain and Multiplex-PCR), and any disease that would interfere following the protocol will be excluded."