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Microablative Fractional Radiofrequency on vaginal health, microbiota, and cellularity of postmenopausal women: protocol of randomized controlled trial

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ABSTRACT

Introduction: Menopause is a physiological and progressive phenomenon, secondary to decreased ovarian follicular reserve. These changes have as functional consequences: vaginal dryness, dyspareunia, sensations of discomfort, burning and irritation, vulvovaginal pruritus, dysuria, and increased frequency of genitourinary infections. The therapy more suitable for vaginal symptoms in post menopause yet is the use of topical hormone, once promote the renovation of the epithelium and vaginal flora, also improves the vulvovaginal atrophy symptoms. However, the prescription of topical estrogens should also be avoided in women with a history of breast cancer, estrogen-sensitive tumors, and thromboembolism, and this emphasizes the necessity for alternatives of treatment. Recently, physical methods, such as laser and radiofrequency (RF), in their non-ablative, ablative, and micro-ablative forms, have been used in the vaginal mucosa to promote neocolagenesis and neoelastogenesis. The objective of this randomized study is to compare the efficiency of microablative fractional radiofrequency (MAFRF) treatment with vaginal estrogens as well as no treatment.

Methods and analyses: The is a protocol of randomized, controlled clinical intervention trial with an open-label design comparing treatment of MAFRF, with vaginal estrogens as well as no treatment. Four important moments were considered for the evaluation of treatment results (T0, T1, T2, and T3). The primary endpoints will be vaginal microbiota, vaginal pH, and cell maturation. Secondary outcomes include the Vaginal Health Index (VHI), which will be applied only at times T0 and T3.

Ethics and dissemination: Due to the nature of the study, we obtained approval from the Ethics Committee. All participants must sign an informed consent form before randomization. The results of this study will be published in peer-reviewed journals. The data collected will also be available in a public repository of data.

Trial registration number: UTN - U1111-1212-5960

Keywords: Menopause; Radiofrequency; Laser; Lactobacillus; Therapeutics.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To the authors' knowledge, this is the first pilot randomized controlled trial (RCT) comparing the effect of Microablative Fractional Radiofrequency (MAFRF) with vaginal estrogens as well as no treatment for this patient population.
- Findings are likely to be clinically relevant and useful for treating postmenopausal women with a history of breast cancer, estrogen-sensitive tumors.
- Our strict inclusion criteria for participants will increase the likelihood of a more homogeneous postmenopausal women group and reduce selection bias.
- Blinding of participants, blinding of assessors of outcomes, detailed standardization of treatment protocols, and rigorous training for the researchers enhance this trial's internal validity.
- The study of postmenopausal women, and therefore its results may not be generalizable to other populations.
- This is a study of postmenopausal women, and therefore its results may not be generalizable to other populations.

INTRODUCTION

Menopause is a physiological and progressive phenomenon, secondary to decreased ovarian follicular reserve. Estrogen deficiency is responsible for the mucosa vulvovaginal thinning of the squamous epithelium, a decrease in the number of collagen and elastin fibers, impaired cell function, reduction in the number of vessel changes in vaginal pH, and in flora commensal.[1]

These changes have functional consequences, being vaginal dryness, dyspareunia, sensations of discomfort, burning and irritation, vulvovaginal pruritus, dysuria, and increased frequency of genitourinary infections.[1] The term Genitourinary Syndrome in Menopause (GSM) was defined in 2014 at a North American consensus conference, where all the disabling symptoms of the urogenital sphere secondary to menopause were more fully and objectively identified.[2]

The therapy more suitable for vaginal symptoms in post menopause yet is the use of the topical hormone, once promote the renovation of the epithelium and vaginal flora, also improves the vulvovaginal atrophy symptoms (VVA).[3] Besides that, the use of low-dose vaginal estrogen has demonstrated to be superior to systemic therapy for improvement VVA.[4] However, a major limitation is associated with low adherence to treatment, due to multiple and inconvenient self-applications and increased vaginal discharge. We cannot fail to emphasize that the prescription of topical estrogens should be avoided in women with a history of breast cancer, estrogen-sensitive tumors, and thromboembolism. For these reasons are necessary to search for alternatives to treatment.[5]

The use of fractional micro ablative CO2 laser therapy was approved in 2014, the Food and Drug Administration (FDA), but only for genitourinary surgery. Considering the context of the treatment of GSM, in order to avoid hormonal

interventions, the micro-ablative fractional CO2 laser, or the non-ablative vaginal erbium YAG laser can be considered. Beyond the laser, other non-ablative electromagnetic energy, such as radiofrequency, are being considered for this indication.[6]

Radiofrequency (RF) is a technique that involves cutting and coagulating biological tissues by using a high-frequency alternating current, which instantly raises the intracellular temperature to 100°C, thus determining cellular membrane expansion and rupture. By reaching the frequency of 4,000,000 cycles/second (4MHz), the FM radio frequency is obtained − this feature giving rise to the name RF electrosurgery. Microablative fractional radiofrequency (MAFRF) is a new procedure that uses random energy in a fractionation system that observes the thermal relaxation of the tissue at a certain time. Energy fractionation consists of energy distribution at equidistant points, producing microscopic columns of thermal injuries in the epidermis and upper dermis, resulting in microscopic columns of treated tissue and intervening areas of untreated skin, which in turn achieve faster reepithelialization. [7,8]

We can consider that the current literature lacks still needs research regarding the use of radiofrequency for the treatment of GSM, most publications evaluate only use the laser. The few studies that have been done on radiofrequency, are mostly restricted to results on sexual function and quality of life. There are no results about the clinical evaluations as pH, cellularity, and vaginal flora when using radio frequency, and that is exactly our proposal.

Objectives

We aim to investigate the therapeutic effect of vaginal MRFM in the genitourinary symptoms of climacteric women. We postulate that MAFRF promotes Cell Maturation by increasing superficial cells and decreasing parabasal cells. Furthermore, we believe that it causes 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.

METHODS AND ANALYSIS

This protocol will adhere to the Standard Protocol Items for Randomized Trials (SPIRIT)[9] and CONSORT statements.[10]

Trial design

The study is a randomized protocol, controlled clinical intervention trial with an open-label design comparing treatment with MAFRF with vaginal estrogens as well as no treatment.

Population

Participant recruitment is currently ongoing at a gynecological Unit of a public university hospital. Patients who fulfill the inclusion criteria and who sign the free and informed consent forms will enter the screening period. Patients who meet the exclusion criteria will be excluded before randomization.

Eligibility criteria and recruitment

The treatments will be conducted at one biggest gynecological unit of a public university Hospital Brazil. Participants will be sought via referrals from gynecologists physicians that attend in this hospital. The study will include healthy postmenopausal women (55 to 65 years old, with whom at least 12 months have passed since last menstrual period or bilateral oophorectomy), who are still sexually active, with GSM, plasma gonadotropin and presenting serum estradiol levels in the postmenopausal range (FSH >40 U/L; estradiol <25 pg/ml) as well as negative Papanicolaou (Pap) smear for cervical cancer precursor cells. 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 and any disease that would interfere following the protocol will be excluded. Figure1 shows the study flow.

Interventions

The microablative fractional radiofrequency (MAFRF) will be performed according to the technique described by Kamilos and Borelli.[6] For the procedure, the Wavetronic 6000 Touch device will be used with the Megapulse HF FRAXX system (Loktal Medical Electronics, São Paulo, Brazil), equipped with an electronic circuit of energy fractionation, connected to a vaginal pen with 64 microneedles, 200μ in diameter and 1mm in length, mounted on a Teflon body and divided into an eight-column matrix with eight needles each.[8]

In the vestibule and vaginal opening, 10% lidocaine spray will be applied 3 minutes before the procedure. Three applications will be realized in the vagina/vaginal introitus, with intervals of 30 days. A sequential application will be performed on the vaginal walls under direct vision. For the post-treatment care, the

use of 5% dexpanthenol solution in the vaginal opening will be recommended two to three times a day, for 2 to 5 days. With no intercourse for ten days.[8] The procedure will be performed in the outpatient clinic by an experienced gynecologist, and a single gynecologist supervises the carrying out of the whole process for the entire period of the research.

The patients from the group with estrogen will be instructed to use Estradiol 17β-based vaginal cream, 1g corresponding to the use of the filled applicator up to the ring mark, twice a week, for three months.[11-13]. The patients who did not receive any intervention will be instructed to attend the consultation for follow-up according to what was established in the study protocol.

Questionnaire

In the first query, the women will answer a standardized questionnaire with information on demographic characteristics including age, time menopause, skin color, schooling, and socioeconomic classification.

Outcomes

Four relevant time points were considered for the evaluation of treatment results based on a previous study [8] baseline (T0), 30 days after the first application (T1), 30 days after second application (T2), and 30 days after the third radiofrequency application (T3). Primary outcomes were vaginal microbiota, vaginal pH, and cell maturation. Primary outcomes will be vaginal microbiota, vaginal pH, and cell maturation. Vaginal smears will be obtained, which will be subsequently stained according to the standard Gram staining procedure for the classification of vaginal flora, following the criteria of Spiegel.[14] Vaginal pH can be measured in

different ways. Most studies describe the use of a pH indicator stripe on the lateral wall of the vagina.[15,16] Moreover, vaginal pH measurement is considered useful, practical, and inexpensive. For vaginal pH determination, the pH indicator strips will be applied against the vaginal wall. The pH will be measured by the gynecologist responsible for the procedure. For analysis of vaginal cytology, vaginal smears will be obtained from the upper third of the right lateral vaginal wall, at predefined times. The material collected from the vaginal sac will be distributed on the blade, adequately identified and fixed, and subsequently stained by the Papanicolaou technique for determining the degree of maturation of the vaginal epithelium by the Frost Index.[17,18]

The latter will be analyzed in the laboratory by two cytologists who are unaware of the women's identity and at what times of treatment the samples were obtained.

Secondary outcomes include the Vaginal Health Index (VHI). The vaginal health score consists of the clinical analysis during the specular examination of five parameters (elasticity, fluid volume, pH, the integrity of the epithelium, and humidity), and is graded from 1 to 5. The sum of the values of the parameters evaluated results in the total vaginal health score. When the overall rating is less than 15, the vaginal mucosa is considered atrophic.[19] The evaluation of the results is described in Table 1.

All possible adverse effects will be recorded and qualified during the period of treatment using questionnaires developed for this protocol. The adverse events will be reported in the results section of the manuscript and will be discussed. Any breaches of confidentiality, study protocol, or adverse events (AEs) attributable to this study will be reported to the research ethics committees.

Table 1. Outcome measurements

Outcome measurements	Explanation	Time point for assessment
Characterization of the vaginal ecosystem by Spiegel criteria	Vaginal flora will be classified, after gram stain according to the Spiegel Criteria, into type 1 (predominance of at least 85% lactobacilli), type 2 (balance between 50% lactobacilli and coccoid flora) and type 3 (0%, almost complete absence of lactobacilli with the presence of cocoidal flora).[14]	T0, T1, T2, and T3
Determination of vaginal pH	The vaginal pH values will be obtained with the application of a universal pH tape 4-7, produced by Merck (MColorpHastTM, Merck, Germany), directly on the right lateral vaginal wall at the allotted times. A pH less than or equal to 5.0 would be indicative of normal vaginal trophism and a pH greater than 5 would be indicative of vaginal atrophy.[1]	T0, T1, T2, and T3
Vaginal maturation	The slides will be examined by light microscopy, using a 10-magnification eyepiece and a 10X objective for the initial evaluation. Then, 100 cells will be analyzed with the same 40X objective and eyepiece in 5 randomly chosen fields. The percentage count of each cell type will be made, that is, parabasal, intermediate, and superficial cells (P / I / S), obtaining the cell maturation index or Frost index.[18] The maturation of the	T0, T1, T2, and T3

	vaginal epithelium (positive effect of the treatment) is evidenced by a decrease in parabasal cells and an increase in the proportion of superficial cells.[19]	
Vaginal Health Index (VHI)	VHI scores of vaginal moisture, vaginal fluid volume, vaginal elasticity, pH, and vaginal epithelial integrity on a scale of 1 (most inferior) to 5 (best) will be found. Vaginal moisture is an assessment of the appearance or consistency of the secretions that line the vagina. Vaginal elasticity is a measurement of the vaginal tissue's ability to stretch at the examiner's touch. Epithelial integrity takes into account color, thickness, and the absence of vaginal bleeding. The lower the score, the higher the atrophy.[4] The sum of the values of the evaluated parameters results in the total vaginal health score. When the overall score is less than 15, the vaginal mucosa is considered atrophic.[15]	T0 and T3

Follow-up

Data will be recorded during the follow-up period according to the multiple time points. The details are shown in Table 2.

Period 1: Screening time (day 0): before treatment.

Period 2: Intervention time (T1-T3): data will be recorded every seven days and 30 days during follow-up.

Period 3: The time after the intervention (within one year after treatment): follow-up at one year for long-term results.

Table 2. Schedule of enrollment, interventions, assessments and data collection

	Study Period				
	Enrollment / Baseline	Intervention			Follow-up
Time point	T0	T1	T2	T3	1 YEAR
Enrollment:	X				
Eligibility screen	X				
Informed consent	X				
Randomization	X				
Interventions:					
MAFRF		X	X	X	
vaginal estrogens		X	X	X	
no treatment		X	X	X	
Assessments:					
General condition					X

Sample size

Therefore, with an equal 1:1:1 allocation rate, according to the results of a pilot study, where data came from 55 volunteers,[20] accepting an alpha of 0.05 and a beta risk <0.2 in a bilateral contrast, assuming a patient attrition rate at follow-up of approximately 15%–20%. The total sample size needed will be established at patients 198, 66 in each group (66 in the MAFRF group, 66 in with vaginal estrogens, and 66 in the as no treatment).

Randomization and allocation concealment

Eligible participants who provide written consent will be randomized into the Software Research Randomizer® programme. Randomization will be by block (1:1:1). Patients will be randomized into three intervention groups (MAFRF group, vaginal estrogens group, and no treatment group). In order to ensure allocation concealment, an offsite randomization schedule will be used. The randomization schedule will be prepared in advance by a researcher at the Federal University of Rio Grande do Norte (UFRN), who will have no contact with any participants throughout the trial and will not be involved in the recruitment, screening, assessment, enrollment or treatment process. To enroll a participant, the primary researcher will email the consenting participant's name to the researcher at the Federal University of Rio Grande do Norte (UFRN). These details will be entered into the allocation spreadsheet, and the next treatment allocation and participant identification number with be emailed directly to the treatment.

Blinding

Personnel who analyze the data collected from the study are not aware of the treatment applied to any given group.

Data management

Researchers qualified are crucial that ensure the quality of a clinical trial. The researchers should understand the specific contents of the protocol. Data collection will be performed by experienced staff using an online electronic data system. The quality of the data management will be checked by the reliability, controlled access, and traceability of the system. Data management will include baseline characteristics (demographics, comorbidities, inclusion and exclusion criteria, and

blood test), potential confounder, and outcomes. Participants who withdraw from our study for any reason will be followed up, and data will be analyzed according to the intention-to-treat (ITT) principle. All randomized participants will be followed up until one year after randomization.

Data extraction and statistical analysis

Data will be analyzed on an intention-to-treat basis, including all participants enrolled in each group. Epidemiological and clinical characteristics data will be analyzed using the chi-squared test, nonparametric Kruskal–Wallis test, and analysis of variance. Analysis of percentages in each group (MAFRF, and no treatment) compared with the control group (vaginal estrogens), for vaginal maturation index, pH, vaginal health, and presence of lactobacilli will be performed with the chi-squared and Fisher's exact tests. $P < 0.05$ will be considered statistically significant. The software that will be used is SPSS for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

Patient and public involvement

Neither patients nor public were involved in the development of the research question, study design, outcome measures, recruitment to and conduct of the study or assessment of the burden of the intervention. The results of the study will be disseminated to study participants by means of lectures given by the investigators.

DISCUSSION

In this protocol, we described our randomized trial comparing the MAFRF to estrogen vaginal and placebo in order to evaluate radiofrequency as a new

treatment option for GSM. The strengths of this trial are the randomized design that allows control, by chance, of confounding factors, ease in forming the control group, and ability for the analysis of several clinical outcomes simultaneously. The main limitation is the loss of follow-up that occurs due to the extended treatment period.

It is recognized that vaginal estrogen may improve the symptoms of GSM, on the other hand, the non-hormonal approach can be useful in specific cases in which hormonal treatment is feared or not recommended (for instance, when there is breast cancer).[21,22] 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, and there is the use of 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 disappeared 3 to 5 days after the application.

ETHICS AND DISSEMINATION

All the procedures performed in this study involving human participants will be conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments, the Declaration of Madrid of the World Psychiatric Association and the established requirements for manuscripts submitted to biomedical journals or comparable ethical standards of good clinical practice. The trial was approved by the local Division Ethics Committee (date of approval: April 17, 2018; reference number: 81973618.2.0000.5292) and was registered in the Brazilian Clinical Trials Registry (ReBec) - (number registry UTN - U1111-1212-

5960) before enrollment of trial participants. Patients confidentiality will be assured through data anonymization.

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Authors' contributions: ACAS, AKG were involved in drafting the study protocol. KM and APFC were involved in statistical planning and drafting of the study protocol. JCC and FSF was involved in drafting and revising the study protocol. AKG developed the idea for this trial and was involved in drafting and revising the study protocol. ACAS conceived and developed the idea for this trial, was involved in

drafting and revising the study protocol and was the principal investigator of this trial. All authors are involved in data acquisition and approved the final version of the manuscript.

Conflict of interest statement: The authors report no conflict of interest.

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Disclaimer: These funding sources have no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Data sharing statement: All investigators will maintain full autonomy and involvement in the design, conduct and reporting of the trial with everyone having full access to the final data.

Confidentiality: The original documents and files will be kept at the trial sites for 15 years. The lead investigator is responsible for data and file storage. The lead investigator is responsible for data and files storage for 15 years.

Consent or assent: All participants will be asked to sign an informed consent form to join the trial. The form explicitly contains all stages of research.

Ethics approval: The trial was approved by the local Division Ethics Committee (date of approval: April 17, 2018; reference number: 81973618.2.0000.5292) and registered in the Brazilian Clinical Trials Registry - ReBec: (number registry UTN - U1111-1212-5960).

Figure legend:

Figure 1. CONSORT 2010 Flow Diagram

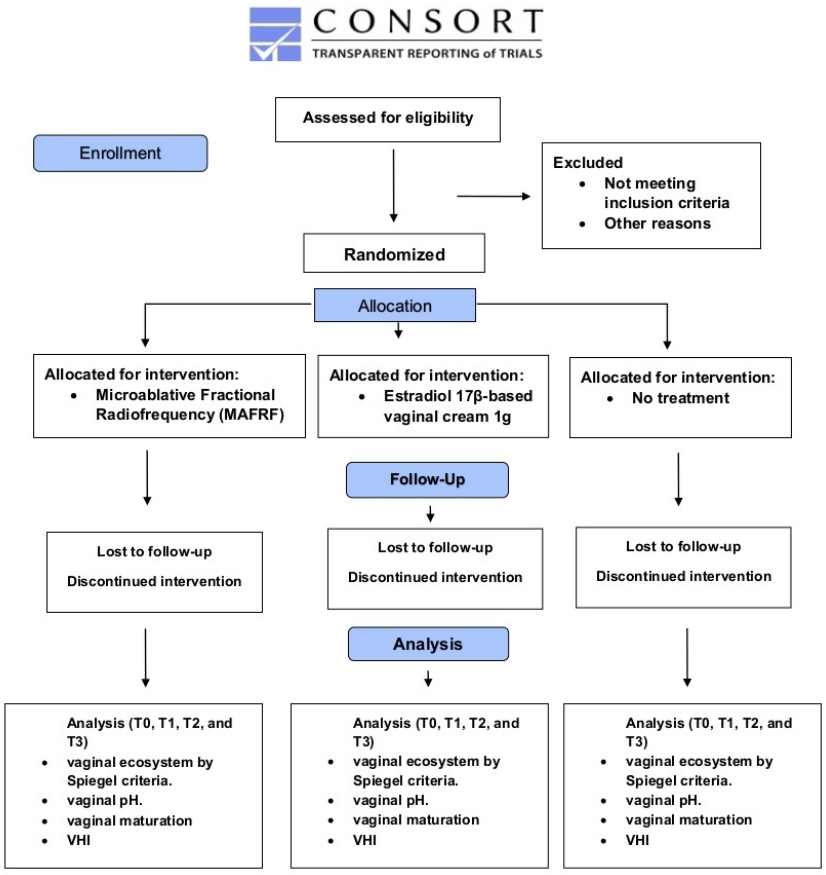

Figure 1. CONSORT 2010 Flow Diagram

239x309mm (96 x 96 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	01
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	01
	2b	All items from the World Health Organization Trial Registration Data Set	01
Protocol version	3	Date and version identifier	01
Funding	4	Sources and types of financial, material, and other support	01
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	01
	5b	Name and contact information for the trial sponsor	19
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	19
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	X

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	04-05
	6b	Explanation for choice of comparators	05
Objectives	7	Specific objectives or hypotheses	06
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	06
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	06
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	07
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	07-08
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	07
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	07-08
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	08
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	08-09
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10-12

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-15
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14-15

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	X
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	X
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	06
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	X
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	20
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	X
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	20
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	20
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	X
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	X
	31b	Authorship eligibility guidelines and any intended use of professional writers	X
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	X
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	X
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	X

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Microablative Fractional Radiofrequency on sexual function and vaginal health: protocol of a randomized controlled trial

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Microablative Fractional Radiofrequency on sexual function and vaginal health: protocol of a randomized controlled trial

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ABSTRACT

Introduction: Menopause is a physiological and progressive phenomenon secondary to decreased ovarian follicular reserve. These changes have consequences: vaginal dryness, dyspareunia, discomfort, burning and irritation, vulvovaginal pruritus, dysuria, and increased frequency of genitourinary infections. The therapy more suitable for vaginal symptoms in post-menopause yet is the use of a topical hormone. However, the prescription of topical estrogens should also be avoided in women with a history of breast cancer, estrogen-sensitive tumors, and thromboembolism, emphasizing the necessity of alternative treatments. Recently, physical methods, such as laser and radiofrequency (RF), in their non-ablative, ablative, and micro-ablative forms, have been used in the vaginal mucosa to promote neocolagenesis and neoelastogenesis. This randomized study aims to compare the efficiency of Microablative Fractional Radiofrequency (MAFRF) treatment with vaginal estrogens and no treatment.

Methods and analyses: This randomized, controlled clinical intervention trial with an open-label design comparing the treatment of MAFRF with vaginal estrogens and no treatment. Four important moments were considered to evaluate treatment results (T0, T1, T2, and T3). The primary outcomes include the female sexual function and 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.

Ethics and dissemination: Due to the nature of the study, we obtained approval from the Ethics Committee. All participants must sign an informed consent form before randomization. The results of this study will be published in peer-reviewed journals. The data collected will also be available in a public repository of data.

Trial registration number: Registered in REBEC (Brazilian Registry of Clinical Trials) under number RBR-94DX93. This study was approved by the Division Ethics Committee of University Hospital Onofre Lopes (UFRN), under CAAE 81973618.2.0000.5292.

Keywords: Menopause; Radiofrequency; Laser; Lactobacillus; Therapeutics.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The latter is the first randomized controlled trial comparing the MAFRF with the golden standard (vaginal estrogen).
- Inclusion criteria allow homogeneity of subjects and less risk of bias.
- Blinding of assessors and standardization of protocols enhance this trial's internal validity.
- The study will be performed among postmenopausal women; thus, its results may not be generalizable to other populations.

INTRODUCTION

Menopause is a physiological and progressive phenomenon, secondary to decreased ovarian follicular reserve. Estrogen deficiency is responsible for the mucosa vulvovaginal thinning of the squamous epithelium, a decrease in the number of collagen and elastin fibers, impaired cell function, reduction in the number of vessel changes in vaginal pH, and in flora commensal.[1]

These changes have functional consequences, being vaginal dryness, dyspareunia, sensations of discomfort, burning and irritation, vulvovaginal pruritus, dysuria, and increased frequency of genitourinary infections.[1] The term Genitourinary Syndrome of Menopause (GSM) was defined in 2014 at a North American consensus conference, where all the disabling symptoms of the urogenital sphere secondary to menopause were more fully and objectively identified.[2]

The therapy more suitable for vaginal symptoms in post menopause yet is the use of the topical hormone, once promote the renovation of the epithelium and vaginal flora, also improves the vulvovaginal atrophy symptoms (VVA).[3] Besides that, the use of low-dose vaginal estrogen has demonstrated to be superior to systemic therapy for improvement VVA.[4] However, a major limitation is associated with low adherence to treatment, due to multiple and inconvenient self-applications and increased vaginal discharge. We cannot fail to emphasize that the prescription of topical estrogens should be avoided in women with a history of breast cancer, estrogen-sensitive tumors, and thromboembolism. For these reasons are necessary to search for alternatives to treatment.[5]

The use of fractional micro ablative CO2 laser therapy was approved in 2014, the Food and Drug Administration (FDA), but only for genitourinary surgery. Considering the context of the treatment of GSM, in order to avoid hormonal

interventions, the micro-ablative fractional CO2 laser, or the non-ablative vaginal erbium YAG laser can be considered. 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] Beyond the laser, other non-ablative electromagnetic energy, such as radiofrequency, are being considered for this indication.[11]

Radiofrequency (RF) is a technique that involves cutting and coagulating biological tissues by using a high-frequency alternating current, which instantly raises the intracellular temperature to 100°C, thus determining cellular membrane expansion and rupture. By reaching the frequency of 4,000,000 cycles/second (4MHz), the FM radio frequency is obtained − this feature giving rise to the name RF electrosurgery. Microablative fractional radiofrequency (MAFRF) is a new procedure that uses random energy in a fractionation system that observes the thermal relaxation of the tissue at a certain time. Energy fractionation consists of energy distribution at equidistant points, producing microscopic columns of thermal injuries in the epidermis and upper dermis, resulting in microscopic columns of treated tissue and intervening areas of untreated skin, which in turn achieve faster reepithelialization.[12,13]

We can consider that the current literature lacks still needs research regarding the use of radiofrequency for the treatment of GSM, most publications evaluate only use the laser. The few studies that have been done on radiofrequency, are mostly restricted to results on sexual function and quality of life. There are no results about the clinical evaluations as pH, cellularity, and vaginal flora when using radio frequency, and that is exactly our proposal.

Objectives

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. Additionally, 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.

METHODS AND ANALYSIS

This protocol will adhere to the Standard Protocol Items for Randomized Trials (SPIRIT)[14] and CONSORT statements.[15]

Trial design

The study is a randomized protocol, controlled clinical intervention trial with a single-blind design comparing treatment with MAFRF with vaginal estrogens as well as no treatment.

Population

Participant recruitment is currently ongoing at a gynecological Unit of a public university hospital. Patients who fulfill the inclusion criteria and who sign the free and informed consent forms will enter the screening period. Patients who meet the exclusion criteria will be excluded before randomization.

Eligibility criteria and recruitment

The treatments will be conducted at one biggest gynecological unit of a public university Hospital Brazil. Participants will be sought via referrals from gynecologists physicians that attend in this hospital. The study will include healthy postmenopausal women (55 to 65 years old, with whom at least 12 months have passed since last menstrual period or bilateral oophorectomy), who are still sexually active, with GSM, plasma gonadotropin and presenting serum estradiol levels in the postmenopausal range (FSH >40 U/L; estradiol <25 pg/ml) as well as negative Papanicolaou (Pap) smear for cervical cancer precursor cells. 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. Figure1 shows the study flow.

Interventions

The microablative fractional radiofrequency (MAFRF) will be performed according to the technique described by Kamilos and Borelli.[13] for the procedure, the Wavetronic 6000 Touch device will be used with the Megapulse HF FRAXX system (Loktal Medical Electronics, São Paulo, Brazil), equipped with an electronic

circuit of energy fractionation, connected to a vaginal pen with 64 microneedles, 200μ in diameter and 1mm in length, mounted on a Teflon body and divided into an eight-column matrix with eight needles each.[13]

In the vestibule and vaginal opening, 10% lidocaine spray will be applied 3 minutes before the procedure. Three applications will be realized in the vagina/vaginal introitus, with intervals of 30 days. A sequential application will be performed on the vaginal walls under direct vision. For the post-treatment care, the use of 5% dexpanthenol solution in the vaginal opening will be recommended two to three times a day, for 2 to 5 days. With no intercourse for ten days.[13] The procedure will be performed in the outpatient clinic by an experienced gynecologist, and a single gynecologist supervises the carrying out of the whole process for the entire period of the research.

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.[16-18]

The patients who did not receive any intervention will be instructed to attend the consultation for follow-up according to what was established in the study protocol.

Questionnaire

In the first query, the women will answer a standardized questionnaire with information on demographic characteristics including age, time menopause, skin color, schooling, and socioeconomic classification.

Outcomes

Two relevant time points will be considered for evaluating female sexual function and vaginal health: baseline (T0) and 30 days after the third radiofrequency application (T3). In addition, for vaginal microbiota and cell maturation, four relevant time points will be considered based on a previous study [13]: baseline (T0) 30 days after the first application (T1), 30 days after second application (T2), and 30 days after the third radiofrequency application (T3). Primary outcomes consists of assessing female sexual function using the validated Portuguese version of the Female Sexual Function Index (FSFI). The FSFI is a brief scale for assessing sexual function in women. The latter is a written test with six subscales and one sum of scores that measure the degree of desire, excitement, lubrication, orgasm, satisfaction, and pain (dyspareunia).[19-21]

The secondary outcome will be vaginal health that will be evaluated per Vaginal Health Index (epithelial integrity, vaginal elasticity, moisture, fluid volume, pH vaginal), beyond the vaginal microbiota, and cell maturation. The Vaginal Health Score (VHI) consists of the clinical analysis during the specular examination of five parameters and is graded from 1 to 5. The sum of the values of the parameters evaluated results in the total vaginal health score. When the overall rating is less than 15, the vaginal mucosa is considered atrophic.[22]

Vaginal pH can be measured in different ways. Most studies describe the use of a pH indicator stripe on the lateral wall of the vagina.[23,24] Moreover, vaginal pH measurement is considered useful, practical, and inexpensive. For vaginal pH determination, the pH indicator strips will be applied against the vaginal wall. The pH will be measured by the gynecologist responsible for the procedure.

Vaginal smears will be obtained, which will be subsequently stained according to the standard Gram staining procedure for the classification of vaginal flora, following the criteria of Spiegel.[25] For analysis of vaginal cytology, vaginal smears will be obtained from the upper third of the right lateral vaginal wall, at predefined times. The material collected from the vaginal sac will be distributed on the blade, adequately identified and fixed, and subsequently stained by the Papanicolaou technique for determining the degree of maturation of the vaginal epithelium by the Frost Index.[26-28] The latter will be analyzed in the laboratory by two cytologists who are unaware of the women's identity and at what times of treatment the samples were obtained. The evaluation of the results is described in Table 1.

All possible adverse effects will be recorded and qualified during the period of treatment using questionnaires developed for this protocol. The adverse events will be reported in the results section of the manuscript and will be discussed. Any breaches of confidentiality, study protocol, or adverse events (AEs) attributable to this study will be reported to the research ethics committees.

Table 1. Outcome measurements

Outcome measurements	Explanation	Time point for assessment
FSFI	The FSFI evaluates six subscales and one sum of scores that measure the degree of desire, excitement, lubrication, orgasm, satisfaction, and pain (dyspareunia). The scores of the subscales are corrected and added up, resulting in a final score. Final scores can range from 2 to 36. Higher scores	T0 and T3

	indicate a better degree of sexual function[19,20]	
VHI	VHI scores of vaginal moisture, vaginal fluid volume, vaginal elasticity, pH, and vaginal epithelial integrity on a scale of 1 (most inferior) to 5 (best) will be found. Vaginal moisture is an assessment of the appearance or consistency of the secretions that line the vagina. Vaginal elasticity is a measurement of the vaginal tissue's ability to stretch at the examiner's touch. Epithelial integrity takes into account color, thickness, and the absence of vaginal bleeding. The lower the score, the higher the atrophy. [4] The sum of the values of the evaluated parameters results in the total vaginal health score. When the overall score is less than 15, the vaginal mucosa is considered atrophic.[22]	T0 and T3
Characterization of the vaginal ecosystem by Spiegel criteria	Vaginal flora will be classified, after gram stain according to the Spiegel Criteria, into type 1 (predominance of at least 85% lactobacilli), type 2 (balance between 50% lactobacilli and coccoid flora) and type 3 (0%, almost complete absence of lactobacilli with the presence of cocoidal flora).[25]	T0, T1, T2, and T3
Vaginal maturation	The slides will be examined by light microscopy, using a 10-magnification eyepiece and a 10X objective for the initial evaluation. Then, 100 cells will be analyzed with the same 40X objective	T0, T1, T2, and T3

	and eyepiece in 5 randomly chosen fields. The percentage count of each cell type will be made, that is, parabasal, intermediate, and superficial cells (P / I / S), obtaining the cell maturation index or Frost index.[26] The maturation of the vaginal epithelium (positive effect of the treatment) is evidenced by a decrease in parabasal cells and an increase in the proportion of superficial cells.[27]	

Follow-up

Data will be recorded during the follow-up period according to the multiple time points. The details are shown in Table 2.

Period 1: Screening time (day 0): before treatment.

Period 2: Intervention time (T1-T3): data will be recorded every seven days and 30 days during follow-up.

Period 3: The time after the intervention (within one year after treatment): follow-up at one year for long-term results.

Table 2. Schedule of enrollment, interventions, assessments and data collection

	Study Period				
	Enrollment / Baseline	**Intervention**			**Follow-up**
Time point	T0	T1	T2	T3	1 YEAR
Enrollment:	X				
Eligibility screen	X				
Informed consent	X				

Randomization	X				
Interventions:					
MAFRF		X	X	X	
Vaginal estrogens		X	X	X	
no treatment		X	X	X	
Assessments:					
General condition					X

Sample size

Therefore, with an equal 1:1:1 allocation rate, according to the results of a pilot study, where data came from 55 volunteers,[29] accepting an alpha of 0.05 and a beta risk <0.2 in a bilateral contrast, assuming a patient attrition rate at follow-up of approximately 15%–20%. The total sample size needed will be established at patients 198, 66 in each group (66 in the MAFRF group, 66 in with vaginal estrogens, and 66 in the as no treatment).

Randomization and allocation concealment

Eligible participants who provide written consent will be randomized into the Software Research Randomizer® programme. Randomization will be by block (1:1:1). Patients will be randomized into three intervention groups (MAFRF group, vaginal estrogens group, and no treatment group). In order to ensure allocation concealment, an offsite randomization schedule will be used. The randomization schedule will be prepared in advance by a researcher at the Federal University of Rio Grande do Norte (UFRN), who will have no contact with any participants throughout the trial and will not be involved in the recruitment, screening, assessment, enrollment or treatment process. To enroll a participant, the primary

researcher will email the consenting participant's name to the researcher at the Federal University of Rio Grande do Norte (UFRN). These details will be entered into the allocation spreadsheet, and the next treatment allocation and participant identification number with be emailed directly to the treatment.

Blinding

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.

Data management

Researchers qualified are crucial that ensure the quality of a clinical trial. The researchers should understand the specific contents of the protocol. Data collection will be performed by experienced staff using an online electronic data system. The quality of the data management will be checked by the reliability, controlled access, and traceability of the system. Data management will include baseline characteristics (demographics, comorbidities, inclusion and exclusion criteria, and blood test), potential confounder, and outcomes. Participants who withdraw from our study for any reason will be followed up, and data will be analyzed according to the intention-to-treat (ITT) principle. All randomized participants will be followed up until one year after randomization.

Data extraction and statistical analysis

Data will be analyzed on an intention-to-treat basis, including all participants enrolled in each group. Epidemiological and clinical characteristics data will be analyzed using the chi-squared test, nonparametric Kruskal–Wallis test, and analysis of variance. Analysis of percentages in each group (MAFRF, and no treatment) compared with the control group (vaginal estrogens), for vaginal maturation index, pH, vaginal health, and presence of lactobacilli will be performed with the chi-squared and Fisher's exact tests. $P < 0.05$ will be considered statistically significant. The software that will be used is SPSS for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

Patient and public involvement

Neither patients nor public were involved in the development of the research question, study design, outcome measures, recruitment to and conduct of the study or assessment of the burden of the intervention. The results of the study will be disseminated to study participants by means of lectures given by the investigators.

DISCUSSION

In this protocol, we described our randomized trial comparing the MAFRF to estrogen vaginal and placebo in order to evaluate radiofrequency as a new treatment option for GSM. The strengths of this trial are the randomized design that allows control, by chance, of confounding factors, ease in forming the control group, and ability for the analysis of several clinical outcomes simultaneously. The main limitation is the loss of follow-up that occurs due to the extended treatment period. It is recognized that vaginal estrogen may improve the symptoms of GSM.[21,28]

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]

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, and there is the use of 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 disappeared 3 to 5 days after the application.

ETHICS AND DISSEMINATION

All the procedures performed in this study involving human participants will be conducted following the ethical standards of the 1964 Declaration of Helsinki and its later amendments, the Declaration of Madrid of the World Psychiatric Association, and the established requirements for manuscripts submitted to biomedical journals

or comparable ethical standards of good clinical practice. The trial was approved by the local Division Ethics Committee of University Hospital Onofre Lopes (UFRN), under the number CAAE 81973618.2.0000.5292 (date of approval: April 17, 2018; reference number: CAAE 81973618.2.0000.5292) and was registered in REBEC (Brazilian Registry of Clinical Trials) under number RBR-94DX93. Before enrollment of trial participants. Data confidentiality will be assured through data anonymization.

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Authors' contributions: ACAS, AKG were involved in drafting the study protocol. KM and APFC were involved in statistical planning and drafting of the study protocol. JCC and FSF were involved in drafting and revising the study protocol. AKG developed the idea for this trial and was involved in drafting and revising the study protocol. ACAS conceived and designed the concept for this trial, was involved in

drafting and revising the study protocol and was the trial's principal investigator. All authors are involved in data acquisition and approved the final version of the manuscript.

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Disclaimer: These funding sources have no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Data sharing statement: All investigators will maintain full autonomy and involvement in the design, conduct and reporting of the trial with everyone having full access to the final data.

Confidentiality: The original documents and files will be kept at the trial sites for 15 years. The lead investigator is responsible for data and file storage. The lead investigator is responsible for data and files storage for 15 years.

Consent or assent: All participants will be asked to sign an informed consent form to join the trial. The form explicitly contains all stages of research.

Ethics approval: The trial was approved by the local Division Ethics Committee (date of approval: April 17, 2018; reference number: 81973618.2.0000.5292) and registered in the Brazilian Clinical Trials Registry - REBEC: RBR-94DX93.

Figure legend:

Figure 1. CONSORT 2010 Flow Diagram

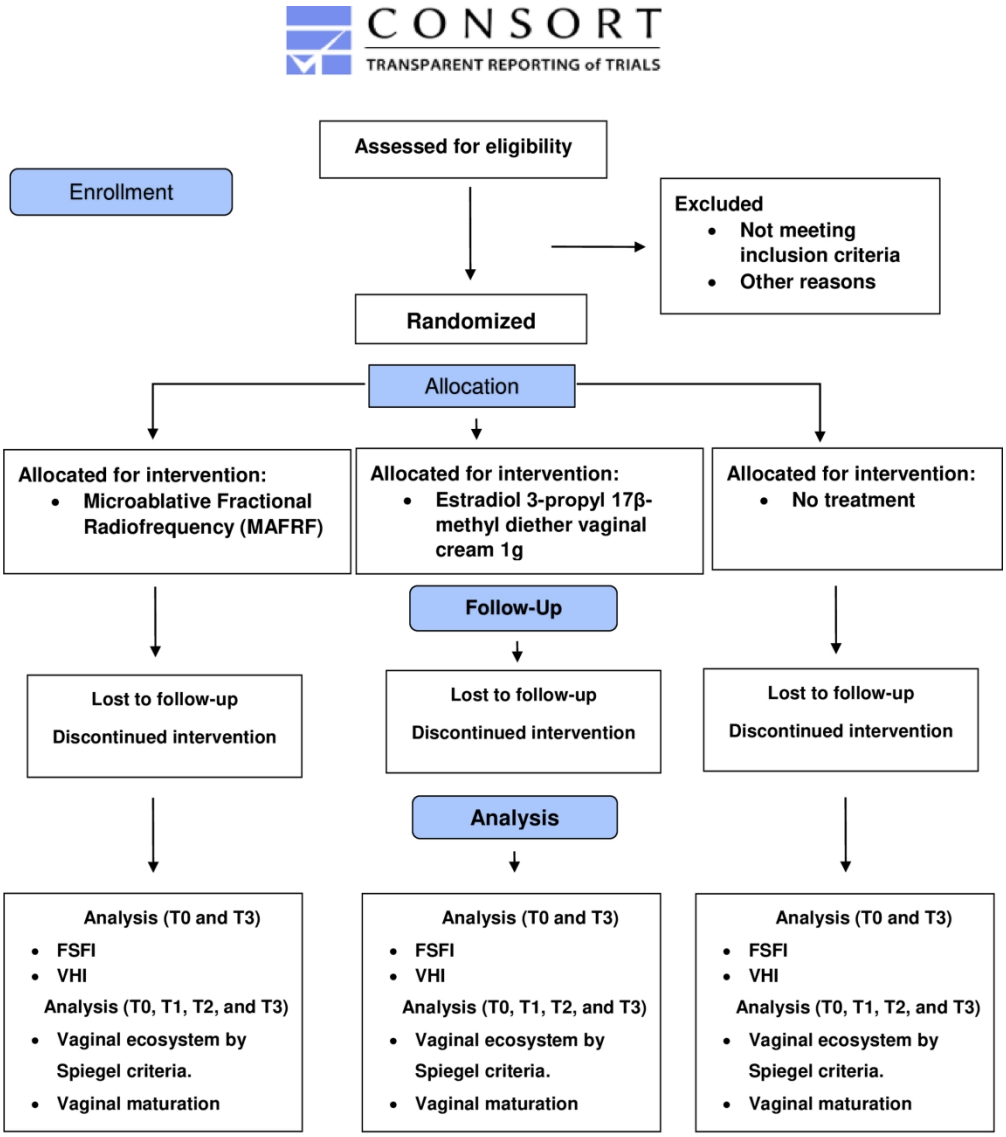

Figure 1. CONSORT 2010 Flow Diagram

Figure 1. CONSORT 2010 Flow Diagram

189x222mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	01
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	01
	2b	All items from the World Health Organization Trial Registration Data Set	01
Protocol version	3	Date and version identifier	01
Funding	4	Sources and types of financial, material, and other support	01
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	01
	5b	Name and contact information for the trial sponsor	X
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	X
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	X

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	04-05
	6b	Explanation for choice of comparators	05
Objectives	7	Specific objectives or hypotheses	06
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	06
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	06
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	07
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	07-08
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	07
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	07-08
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	09
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	09-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12-13

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	14
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	15-16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	15-16

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	X
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	X
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	X
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	23

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	23
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	X
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	X
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	X
	31b	Authorship eligibility guidelines and any intended use of professional writers	X
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	X
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	✓
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	X

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Microablative Fractional Radiofrequency for the genitourinary syndrome of menopause: protocol of randomized controlled trial

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Microablative Fractional Radiofrequency for the genitourinary syndrome of menopause: protocol of randomized controlled trial

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ABSTRACT

Introduction: Menopause is a physiological and progressive phenomenon secondary to decreased ovarian follicular reserve. These changes have consequences: vaginal dryness, dyspareunia, discomfort, burning and irritation, vulvovaginal pruritus, dysuria, and increased frequency of genitourinary infections. The therapy more suitable for vaginal symptoms in post-menopause yet is the use of a topical hormone. However, the prescription of topical estrogens should also be avoided in women with a history of breast cancer, estrogen-sensitive tumors, and thromboembolism, emphasizing the necessity of alternative treatments. Recently, physical methods, such as laser and radiofrequency (RF), in their non-ablative, ablative, and micro-ablative forms, have been used in the vaginal mucosa to promote neocolagenesis and neoelastogenesis. This randomized study aims to compare the efficiency of Microablative Fractional Radiofrequency (MAFRF) treatment with vaginal estrogens and no treatment.

Methods and analyses: This randomized, controlled clinical intervention trial with an open-label design comparing the treatment of MAFRF with vaginal estrogens and no treatment. Four important moments were considered to evaluate treatment results (T0, T1, T2, and T3). The primary outcome includes vulvovaginal atrophy (vaginal pain, burning, itching, dryness, dyspareunia, and dysuria), and the secondary outcomes will be sexual function, vaginal health (epithelial integrity, vaginal elasticity, moisture, fluid volume, and pH vaginal), and quality of life.

Ethics and dissemination: Due to the nature of the study, we obtained approval from the Ethics Committee. All participants must sign an informed consent form before randomization. The results of this study will be published in peer-reviewed journals. The data collected will also be available in a public repository of data.

Trial registration number: Registered in REBEC (Brazilian Registry of Clinical Trials) under number RBR-94DX93. This study was approved by the Division Ethics Committee of University Hospital Onofre Lopes (UFRN), under CAAE 81973618.2.0000.5292.

Keywords: Menopause; Radiofrequency; Laser; Lactobacillus; Therapeutics.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The latter is the first randomized controlled trial comparing the MAFRF with the golden standard (vaginal estrogen).
- Inclusion criteria allow homogeneity of subjects and less risk of bias.
- Blinding of assessors and standardization of protocols enhance this trial's internal validity.
- The study will be performed among postmenopausal women; thus, its results may not be generalizable to other populations.

INTRODUCTION

Menopause is a physiological and progressive phenomenon, secondary to decreased ovarian follicular reserve. Estrogen deficiency is responsible for the mucosa vulvovaginal thinning of the squamous epithelium, a decrease in the number of collagen and elastin fibers, impaired cell function, reduction in the number of vessel changes in vaginal pH, and in flora commensal.[1]

These changes have functional consequences, being vaginal dryness, dyspareunia, sensations of discomfort, burning and irritation, vulvovaginal pruritus, dysuria, and increased frequency of genitourinary infections.[1] The term Genitourinary Syndrome of Menopause (GSM) was defined in 2014 at a North American consensus conference, where all the disabling symptoms of the urogenital sphere secondary to menopause were more fully and objectively identified.[2]

The therapy more suitable for vaginal symptoms in post menopause yet is the use of the topical hormone, once promote the renovation of the epithelium and vaginal flora, also improves the vulvovaginal atrophy symptoms (VVA).[3] Besides that, the use of low-dose vaginal estrogen has demonstrated to be superior to systemic therapy for improvement VVA.[4] However, a major limitation is associated with low adherence to treatment, due to multiple and inconvenient self-applications and increased vaginal discharge. We cannot fail to emphasize that the prescription of topical estrogens should be avoided in women with a history of breast cancer, estrogen-sensitive tumors, and thromboembolism. For these reasons are necessary to search for alternatives to treatment.[5]

The use of fractional micro ablative CO2 laser therapy was approved in 2014, the Food and Drug Administration (FDA), but only for genitourinary surgery. Considering the context of the treatment of GSM, in order to avoid hormonal

interventions, the micro-ablative fractional CO2 laser, or the non-ablative vaginal erbium YAG laser can be considered. 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] Beyond the laser, other non-ablative electromagnetic energy, such as radiofrequency, are being considered for this indication.[11]

Radiofrequency (RF) is a technique that involves cutting and coagulating biological tissues by using a high-frequency alternating current, which instantly raises the intracellular temperature to 100°C, thus determining cellular membrane expansion and rupture. By reaching the frequency of 4,000,000 cycles/second (4MHz), the FM radio frequency is obtained − this feature giving rise to the name RF electrosurgery. Microablative fractional radiofrequency (MAFRF) is a new procedure that uses random energy in a fractionation system that observes the thermal relaxation of the tissue at a certain time. Energy fractionation consists of energy distribution at equidistant points, producing microscopic columns of thermal injuries in the epidermis and upper dermis, resulting in microscopic columns of treated tissue and intervening areas of untreated skin, which in turn achieve faster reepithelialization.[12,13]

We can consider that the current literature lacks still needs research regarding the use of radiofrequency for the treatment of GSM, most publications evaluate only use the laser. The few studies that have been done on radiofrequency, are mostly restricted to results on sexual function and quality of life. There are no results about the clinical evaluations as pH, cellularity, and vaginal flora when using radio frequency, and that is exactly our proposal.

Objectives

We aim to investigate the therapeutic effect of vaginal MARFM in the GSM. We postulate that MAFRF could promote the improvement of vulvovaginal atrophy. Furthermore, it could improve sexual function, vaginal health, and quality of life in postmenopausal women.

METHODS AND ANALYSIS

This protocol will adhere to the Standard Protocol Items for Randomized Trials (SPIRIT)[14] and CONSORT statements.[15]

Trial design

The study is a randomized protocol, controlled clinical intervention trial with a single-blind design comparing treatment with MAFRF with vaginal estrogens as well as no treatment.

Population

Participant recruitment is currently ongoing at a gynecological Unit of a public university hospital. Patients who fulfill the inclusion criteria and who sign the free

and informed consent forms will enter the screening period. Patients who meet the exclusion criteria will be excluded before randomization.

Eligibility criteria and recruitment

The treatments will be conducted at one biggest gynecological unit of a public university Hospital Brazil. Participants will be sought via referrals from gynecologists physicians that attend in this hospital. The study will include healthy postmenopausal women (55 to 65 years old, with whom at least 12 months have passed since last menstrual period or bilateral oophorectomy), who are still sexually active, with GSM, plasma gonadotropin and presenting serum estradiol levels in the postmenopausal range (FSH >40 U/L; estradiol <25 pg/ml) as well as negative Papanicolaou (Pap) smear for cervical cancer precursor cells. 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. Figure1 shows the study flow.

Interventions

The microablative fractional radiofrequency (MAFRF) will be performed according to the technique described by Kamilos and Borelli.[13] for the procedure, the Wavetronic 6000 Touch device will be used with the Megapulse HF FRAXX system (Loktal Medical Electronics, São Paulo, Brazil), equipped with an electronic circuit of energy fractionation, connected to a vaginal pen with 64 microneedles, 200μ in diameter and 1mm in length, mounted on a Teflon body and divided into an eight-column matrix with eight needles each.[13]

In the vestibule and vaginal opening, 10% lidocaine spray will be applied 3 minutes before the procedure. Three applications will be realized in the vagina/vaginal introitus, with intervals of 30 days. A sequential application will be performed on the vaginal walls under direct vision. For the post-treatment care, the use of 5% dexpanthenol solution in the vaginal opening will be recommended two to three times a day, for 2 to 5 days. With no intercourse for ten days.[13] The procedure will be performed in the outpatient clinic by an experienced gynecologist, and a single gynecologist supervises the carrying out of the whole process for the entire period of the research.

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.[16-18]

The patients who did not receive any intervention will be instructed to attend the consultation for follow-up according to what was established in the study protocol.

Questionnaire

In the first query, the women will answer a standardized questionnaire with information on demographic characteristics including age, time menopause, skin color, schooling, and socioeconomic classification.

Outcomes

Four relevant time points will be considered for evaluating the results based on a previous study [13]: baseline (T0) 30 days after the first application (T1), 30

days after the second application (T2), and 30 days after the third radiofrequency application (T3).

The primary outcome includes vulvovaginal atrophy using the 11-point Visual Analog Scale (VAS). The VAS associate symptoms (vaginal pain, burning, itching, dryness, dyspareunia, and dysuria).[19-22]

Secondary outcomes will be sexual function, vaginal health (epithelial integrity, vaginal elasticity, moisture, fluid volume, and pH vaginal), and quality of life. Female sexual function will be evaluated using the validated Portuguese version of the Female Sexual Function Index (FSFI). FSFI is a brief scale for assessing sexual function in women. The latter is a written test with six subscales and one sum of scores that measure the degree of desire, excitement, lubrication, orgasm, satisfaction, and pain (dyspareunia).[23-25].

The Short Form 12 (SF-12) is a self-reported outcome measure assessing the impact of health on an individual's everyday life. The Sf-12 assesses the physical (PCS12), and mental (MCS12) component summary scores of It is often used as a quality of life measure.[20-22,26] Vaginal health will be evaluated per Vaginal Health Score (VHI). The VHI consists of the clinical analysis during the specular examination of five parameters and is graded from 1 to 5. The sum of the values of the parameters evaluated results in the total vaginal health score.[19,27] The evaluation of the results is described in Table 1.

All possible adverse effects will be recorded and qualified during the period of treatment using questionnaires developed for this protocol. The adverse events will be reported in the results section of the manuscript and will be discussed. Any breaches of confidentiality, study protocol, or adverse events (AEs) attributable to this study will be reported to the research ethics committees.

Table 1. Outcome measurements

Outcome measurements	Explanation	Time point for assessment
VAS	VAS evaluates the change in 6 categories of symptoms commonly associated with VVA: vaginal pain, burning, itching, dryness, dyspareunia, and dysuria. VAS will be scored on an 11-point scale for each symptom with 0 being the lowest level (none) and 10 being the highest (extreme).[19-22]	T0, T1, T2, and T3
FSFI	The FSFI evaluates six subscales and one sum of scores that measure the degree of desire, excitement, lubrication, orgasm, satisfaction, and pain (dyspareunia). The scores of the subscales are corrected and added up, resulting in a final score. Final scores can range from 2 to 36. Higher scores indicate a better degree of sexual function.[23-25]	T0, T1, T2, and T3
VHI	VHI scores of vaginal moisture, vaginal fluid volume, vaginal elasticity, pH, and vaginal epithelial integrity on a scale of 1 (most inferior) to 5 (best) will be found. Vaginal moisture is an assessment of the appearance or consistency of the secretions that line the vagina. Vaginal elasticity is a measurement of the vaginal tissue's ability to stretch at the examiner's touch. Epithelial integrity	T0, T1, T2, and T3

	takes into account color, thickness, and the absence of vaginal bleeding. The lower the score, the higher the atrophy. [4] The sum of the values of the evaluated parameters results in the total vaginal health score. When the overall score is less than 15, the vaginal mucosa is considered atrophic.[19,27]	
SF-12	The SF-12 provides accurate and efficient information to assess physical and mental health. It includes 8 dimensions as the initial SF-36 instrument: general health perceptions (1 item), physical functioning (2 items), role limitations due to physical problems (2 items), bodily pain (1 item), vitality (1 item), social functioning (1 item), role limitations due to emotional problems (2 items), and mental health (2 items). The composite physical (PCS) and mental health (MCS) scores are computed using the scores of the 12 items, ranging from 0 to 100, where zero reflects the lowest health level and 100 the highest level.[20-22,26]	T0, T1, T2, and T3

Follow-up

Data will be recorded during the follow-up period according to the multiple time points. The details are shown in Table 2.

Period 1: Screening time (day 0): before treatment.

Period 2: Intervention time (T1-T3): data will be recorded every seven days and 30 days during follow-up.

Period 3: The time after the intervention (within one year after treatment): follow-up at one year for long-term results.

Table 2. Schedule of enrollment, interventions, assessments and data collection

	Study Period				
	Enrollment / Baseline	**Intervention**			**Follow-up**
Time point	T0	T1	T2	T3	1 YEAR
Enrollment:	X				
Eligibility screen	X				
Informed consent	X				
Randomization	X				
Interventions:					
MAFRF		X	X	X	
Vaginal estrogens		X	X	X	
no treatment		X	X	X	
Assessments:					
General condition					X

Sample size

Therefore, with an equal 1:1:1 allocation rate, according to the results of a pilot study, where data came from 55 volunteers,[28] accepting an alpha of 0.05 and a beta risk <0.2 in a bilateral contrast, assuming a patient attrition rate at follow-up of approximately 15%–20%. The total sample size needed will be established at patients 198, 66 in each group (66 in the MAFRF group, 66 in with vaginal estrogens, and 66 in the as no treatment).

Randomization and allocation concealment

Eligible participants who provide written consent will be randomized into the Software Research Randomizer® programme. Randomization will be by block (1:1:1). Patients will be randomized into three intervention groups (MAFRF group, vaginal estrogens group, and no treatment group). In order to ensure allocation concealment, an offsite randomization schedule will be used. The randomization schedule will be prepared in advance by a researcher at the Federal University of Rio Grande do Norte (UFRN), who will have no contact with any participants throughout the trial and will not be involved in the recruitment, screening, assessment, enrollment or treatment process. To enroll a participant, the primary researcher will email the consenting participant's name to the researcher at the Federal University of Rio Grande do Norte (UFRN). These details will be entered into the allocation spreadsheet, and the next treatment allocation and participant identification number with be emailed directly to the treatment.

Blinding

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.

Data management

Researchers qualified are crucial that ensure the quality of a clinical trial. The researchers should understand the specific contents of the protocol. Data collection

will be performed by experienced staff using an online electronic data system. The quality of the data management will be checked by the reliability, controlled access, and traceability of the system. Data management will include baseline characteristics (demographics, comorbidities, inclusion and exclusion criteria, and blood test), potential confounder, and outcomes. Participants who withdraw from our study for any reason will be followed up, and data will be analyzed according to the intention-to-treat (ITT) principle. All randomized participants will be followed up until one year after randomization.

Data extraction and statistical analysis

Data will be analyzed on an intention-to-treat basis, including all participants enrolled in each group. Epidemiological and clinical characteristics data will be analyzed using the chi-squared test, nonparametric Kruskal–Wallis test, and analysis of variance. Data presented in the text and tables will be reported as mean and standard deviation, median, and percentage (%). Continuous variables will be analyzed by using the paired t-test and the signed-rank test accordingly to data distribution. $P < 0.05$ will be considered statistically significant. The software that will be used is SPSS for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

Patient and public involvement

Neither patients nor public were involved in the development of the research question, study design, outcome measures, recruitment to and conduct of the study or assessment of the burden of the intervention. The results of the study will be disseminated to study participants by means of lectures given by the investigators.

DISCUSSION

In this protocol, we described our randomized trial comparing the MAFRF to estrogen vaginal and placebo in order to evaluate radiofrequency as a new treatment option for GSM. The strengths of this trial are the randomized design that allows control, by chance, of confounding factors, ease in forming the control group, and ability for the analysis of several clinical outcomes simultaneously. The main limitation is the loss of follow-up that occurs due to the extended treatment period. It is recognized that vaginal estrogen may improve the symptoms of GSM.[21,28]

Some systematic reviews have already been published on the subject.[29-32] 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.[32] 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).[32]

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.[32]

We can also quote a prospective study[28] 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.[28]

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, and there is the use of 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 disappeared 3 to 5 days after the application.

ETHICS AND DISSEMINATION

All the procedures performed in this study involving human participants will be conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments, the Declaration of Madrid of the World Psychiatric Association and the established requirements for manuscripts submitted to biomedical journals or comparable ethical standards of good clinical practice. The trial was approved by the local Division Ethics Committee of University Hospital Onofre Lopes (UFRN), under the number CAAE 81973618.2.0000.5292 (date of approval: April 17, 2018; reference number: CAAE 81973618.2.0000.5292) and was registered in REBEC (Brazilian Registry of Clinical Trials) under number RBR-94DX93. before enrollment of trial participants. Patients confidentiality will be assured through data anonymization.

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Authors' contributions: ACAS, AKG were involved in drafting the study protocol. KM and APFC were involved in statistical planning and drafting of the study protocol. JCC and FSF was involved in drafting and revising the study protocol. AKG developed the idea for this trial and was involved in drafting and revising the study protocol. ACAS conceived and developed the idea for this trial, was involved in drafting and revising the study protocol and was the principal investigator of this trial. All authors are involved in data acquisition and approved the final version of the manuscript.

Conflict of interest statement: The authors report no conflict of interest.

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Disclaimer: These funding sources have no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Data sharing statement: All investigators will maintain full autonomy and involvement in the design, conduct and reporting of the trial with everyone having full access to the final data.

Confidentiality: The original documents and files will be kept at the trial sites for 15 years. The lead investigator is responsible for data and file storage. The lead investigator is responsible for data and files storage for 15 years.

Consent or assent: All participants will be asked to sign an informed consent form to join the trial. The form explicitly contains all stages of research.

Ethics approval: The trial was approved by the local Division Ethics Committee (date of approval: April 17, 2018; reference number: 81973618.2.0000.5292) and

registered in the Brazilian Clinical Trials Registry - ReBec: (number registry RBR-94DX93).

Figure legend:

Figure 1. CONSORT 2010 Flow Diagram

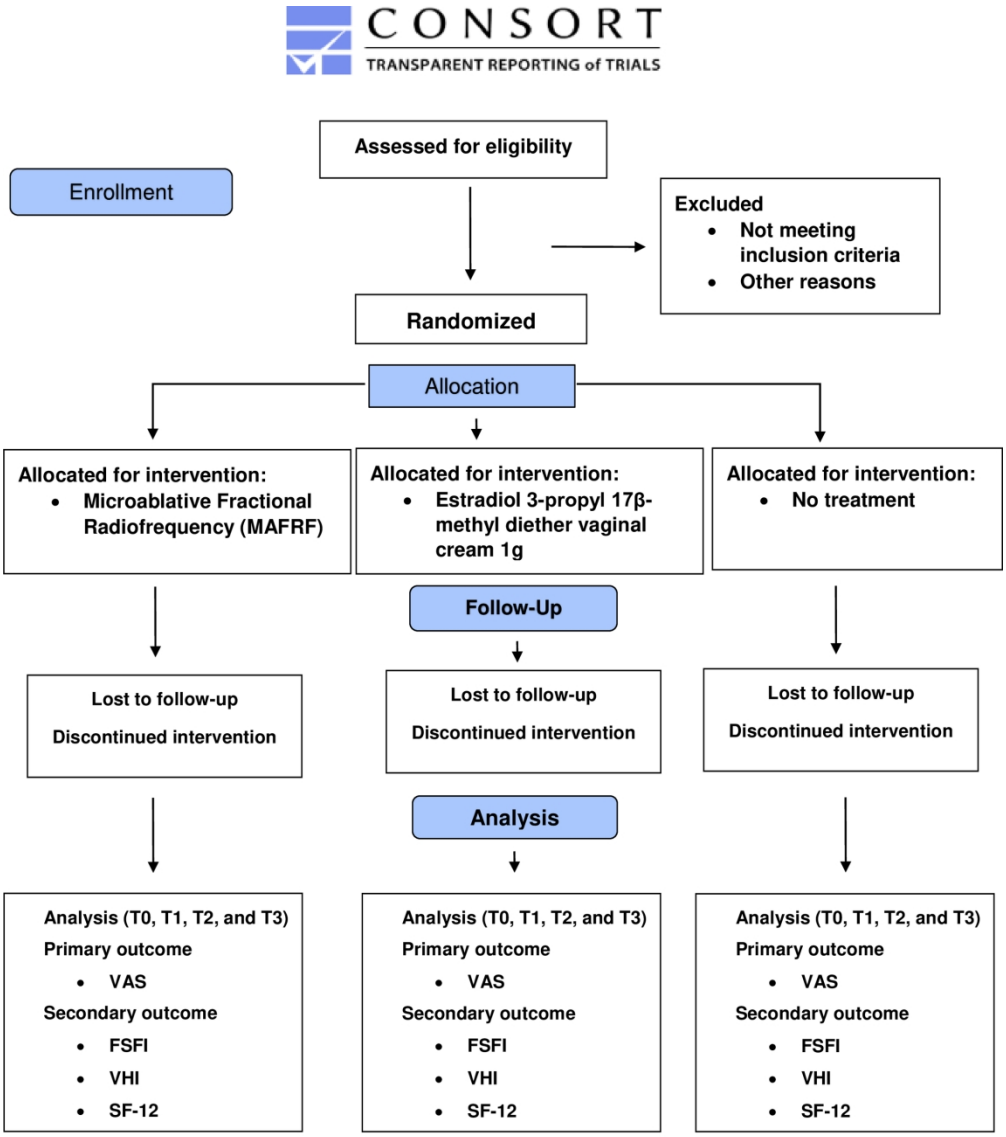

Figure 1. CONSORT 2010 Flow Diagram

Figure 1. CONSORT 2010 Flow Diagram

189x222mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	01
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	01
	2b	All items from the World Health Organization Trial Registration Data Set	01
Protocol version	3	Date and version identifier	01
Funding	4	Sources and types of financial, material, and other support	01
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	01
	5b	Name and contact information for the trial sponsor	X
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	X
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	X

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	04-05
	6b	Explanation for choice of comparators	05
Objectives	7	Specific objectives or hypotheses	06
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	06
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	06
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	07
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	07-08
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	07
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	07-08
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	08
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	08-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11-12

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	13
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13-14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13-14

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	X
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	X
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	X
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	22

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	22
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	X
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	X
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	X
	31b	Authorship eligibility guidelines and any intended use of professional writers	X
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	X
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	✓
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	X

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.