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Use of fractional microablative CO2 laser in the treatment of urinary symptoms of Genitourinary Syndrome of Menopause: prospective cohort study protocol

Catarina Maria Mesquita Garcia Dalbem

catarinadalbem@unemat.br

Universidade Brasil Cassia Caroline Garcia Dalbem Teles, Universidade Brasil Matheus Rodrigues de Souza, Universidade do Estado de Mato Grosso Marina Dalbem Teles, Claudia Schmidt Moura, Universidade do Estado de Mato Grosso, Universidade

Cassia Caroline Garcia Dalbem Teles

Universidade Brasil Cassia Caroline Garcia Dalbem Teles, Universidade Brasil Matheus Rodrigues de Souza, Universidade do Estado de Mato Grosso Marina Dalbem Teles, Claudia Schmidt Moura, Universidade do Estado de Mato Grosso, Universidade

Matheus Rodrigues de Souza

Mato Grosso State University

Marina Dalbem Teles

Faculdade Morgana Potrich

Claudia Schmidt Moura

Mato Grosso State University

Nivaldo Antonio Parizotto

Universidade Brasil

Study protocol

Keywords: Genitourinary syndrome of menopause, CO_2 laser, vaginal atrophy, non-hormonal therapy, clinical trial

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Title

Use of fractional microablative CO2 laser in the treatment of urinary symptoms of Genitourinary Syndrome of Menopause: prospective cohort study protocol.

Names protocol contributors

Catarina Maria Mesquita Garcia Dalbem, Universidade Brasil

Cassia Caroline Garcia Dalbem Teles, Universidade Brasil

Matheus Rodrigues de Souza, Universidade do Estado de Mato Grosso

Marina Dalbem Teles, Faculdade Morgana Potrich (FAMP)

Claudia Schmidt Moura, Universidade do Estado de Mato Grosso

Nivaldo Antonio Parizotto, Universidade Brasil

Abstract

Background:

Stress urinary incontinence (SUI) is a common symptom among postmenopausal women, particularly in the presence of Genitourinary Syndrome of Menopause (GSM). Local hormonal therapies can be effective, but are not always feasible due to contraindications or limited adherence. In this context, the use of fractional microablative CO_2 laser has been proposed as a non-hormonal alternative. This study evaluates the efficacy and safety of this technology in the treatment of SUI in women with a clinical diagnosis of GSM.

Methods:

This is a prospective, open-label, non-randomized, single-center clinical trial. A total of 30 postmenopausal women with a clinical diagnosis of genitourinary syndrome of menopause and urinary incontinence symptoms were enrolled. All participants underwent three monthly sessions of fractional microablative CO_2 laser therapy. The primary outcome was the change in the International Consultation on Incontinence Questionnaire – Urinary Incontinence (ICIQ-UI) score. Secondary outcomes included the assessment of overactive bladder symptoms using the International Consultation on Incontinence Questionnaire – Overactive Bladder (ICIQ-OAB); urinary incontinence severity using the Incontinence Severity Index (ISI); and performance on the pad test, a diagnostic method recommended by the International Continence Society (ICS). Additional secondary outcomes included the impact of urinary incontinence on quality of life,

assessed with the King's Health Questionnaire (KHQ), and sexual function, evaluated with the Female Sexual Function Index (FSFI). Follow-up assessments were conducted at 3, 6, and 12 months after the first intervention. The study was approved by the Ethics Committee of the State University of Mato Grosso (approval number 5.292.046) and registered on the ReBEC platform: RBR-524vx5c. Data analysis remains blinded.

Discussion:

This study will contribute to the understanding of the effectiveness of fractional microablative CO_2 laser as a non-hormonal therapeutic strategy for the treatment of SUI in postmenopausal women with GSM, providing evidence to support clinical practice and future research.

Trial registration:

This trial is registered in the Brazilian Clinical Trials Registry (ReBEC): RBR - 524vx5c. Registered on December 2024 (16/12/2024) UTN: U1111-1315-6489. https://ensaiosclinicos.gov.br/rg/RBR-524vx5c .
This is a retrospectively registered trial.

Keywords

Genitourinary syndrome of menopause; CO_2 laser; vaginal atrophy; non-hormonal therapy; clinical trial.

Administrative information

Title {1}	Use of Fractional Microablative CO_2 Laser in the Treatment of Urinary Symptoms of Genitourinary Syndrome of Menopause: Protocol for a Prospective Cohort Study
Trial registration {2a and 2b}.	This clinical trial was registered in the Brazilian Registry of Clinical Trials (ReBEC): RBR-524vx5c UTN code: U1111-1315-6489 Registration in Plataforma Brasil (CAAE): 55003522.9.0000.5494 Ethics Committee Approval Number: 5.292.046 The registration was performed retrospectively, in December 2024. Although the study received ethical approval in March 2022 and recruitment began in July 2022, the clinical interventions and data collection only started in March 2023. The protocol was developed and followed prospectively. This is the first formal submission of the complete protocol.
Protocol version {3}	Version 1.0, Date: 28/11/2025

Funding {4}	This study did not receive external funding. The equipment used in the intervention was provided by the principal investigator, who has no financial or institutional ties to the device manufacturer (Deka, Italy). Logistical support—including clinical infrastructure, human resources, and technical assistance for performing the procedures—was provided by the Hospital Regional de Cáceres Dr. Antônio Fontes.
Author details {5a}	*Catarina Maria Mesquita Garcia Dalbem*, State University of Mato Grosso and Universidade Brasil; Physician. Principal investigator. Responsible for study conception, protocol development, and for conducting the interventions and follow-up. *Cássia Caroline Garcia Dalbem Teles*, State University of Mato Grosso and Universidade Brasil; Physician. Co-author. Contributed to protocol development and conducted the clinical interventions alongside the principal investigator. *Matheus Rodrigues de Souza*, State University of Mato Grosso. Member of the Data Monitoring Committee (DMC). Participated in the continuous safety assessment and monitoring of the integrity of clinical data. *Marina Dalbem Teles*, Faculdade Morgana Potrich (FAMP); Physician. Organized clinical follow-up and collaborated with the Data Monitoring Committee (DMC) in monitoring data integrity. *Claudia Schmidt Moura*, State University of Mato Grosso; Medical student. Co-responsible for drafting this protocol article and for supporting the organization of the data used in the manuscript. *Gabriela Fonseca Arenhart, Guilherme Bezerra, Leticia Andrioli da Cunha, Rayanni Freire Alves Pedroso, Viviane Ribeiro*, State University of Mato Grosso; Medical students. Provided technical support to the study, assisted in data organization, and maintained contact with participants throughout the study timeline. *Nivaldo Antonio Parizotto*, Universidade Brasil; PhD. Academic advisor. Responsible for the methodological development of the study.
Name and contact information for the trial sponsor {5b}	Primary Sponsor: Catarina Maria Mesquita Garcia Dalbem Contact: catarinadalbem@unemat.br

Role of sponsor {5c}	The principal investigator, Catarina Maria Mesquita Garcia Dalbem, conceived and conducted the study and provided the equipment used in the intervention, which belonged to her personal property. The Regional Hospital of Cáceres Dr. Antônio Fontes supplied logistical support, clinical infrastructure, and technical personnel to carry out the procedures. Universidade Brasil served as the investigator's academic affiliated institution, providing scientific guidance and approving the study. None of the sponsoring or supporting institutions had any role in the study design, data collection or analysis, manuscript preparation, or the decision to submit the article for publication. All scientific and methodological decisions were the sole responsibility of the principal investigator and the study team.

Introduction

Background and rationale {6a}

Stress urinary incontinence (SUI) is one of the most prevalent clinical manifestations among postmenopausal women, negatively affecting their quality of life, self-esteem, and sexuality. The estrogen deficiency characteristic of this period is associated with structural changes in the lower genitourinary tract, such as vaginal mucosal atrophy, reduced local vascularization, and weakening of periurethral support (1). These factors compose the condition known as Genitourinary Syndrome of Menopause (GSM) (1).

Topical estrogen therapy remains the treatment of choice for GSM, demonstrating efficacy both in relieving urogenital symptoms and improving bladder function (2, 3). However, this therapy presents limitations, such as the need for continuous use and its restricted action on the epithelial surface, as well as contraindications in patients with hormone-dependent neoplasms (4, 5). Meanwhile, surgical or invasive options—indicated for patients with moderate to severe UI symptoms—are more effective, particularly sling procedures, which, although effective, are not free from risks, leading to an increasing search for minimally invasive and effective alternatives (6, 7, 8).

Among these emerging approaches, fractional microablative CO_2 laser therapy has gained attention for the treatment of GSM. This technology, already used in other medical specialties, has been increasingly applied in regenerative gynecology. Studies suggest that the controlled thermal stimulus generated by the laser promotes functional regeneration of the vaginal mucosa, with increased collagen production, neovascularization, and structural reorganization, resulting in improvement of atrophic and urinary symptoms associated with GSM (9, 10, 11). Systematic reviews support these findings, indicating significant improvement in urinary incontinence scores and evidence of sustained results over time, in comparison with the effects reported for topical estrogens (12, 13).

Recent evidence also suggests that fractional microablative CO_2 laser therapy may induce relevant

biological changes in the vaginal microbiota, including increased Lactobacillus colonization, reduction of vaginal pH, and sustained improvement in symptoms such as dryness, itching, and dyspareunia (14). These findings reinforce the hypothesis regarding the effects of laser therapy and support the need for clinical trials to more broadly investigate the impacts of this intervention, including its application in the management of stress urinary incontinence.

Objectives {7}

Primary Objective: To evaluate the effectiveness of fractional microablative CO_2 laser therapy in reducing urinary incontinence symptoms in women with genitourinary syndrome of menopause, based on changes in the total score of the ICIQ-UI questionnaire.

Secondary Objectives:
To assess overactive bladder symptoms using the total score of the ICIQ-OAB.
To measure the severity of urinary incontinence using the ISI index.
To evaluate objective urinary loss through the one-hour pad test.
To investigate the impact of urinary incontinence on quality of life using the King's Health Questionnaire (KHQ).
To assess potential changes in sexual function after treatment using the FSFI.
To conduct subgroup analyses according to the type of urinary incontinence (stress, mixed, or overactive bladder), body mass index (BMI), and use of topical estrogen.
To describe the epidemiological characteristics of the sample and baseline urinary symptoms prior to intervention.

Trial design {8}

This is a prospective, open-label cohort study with a single therapeutic intervention (three sessions of fractional microablative CO_2 laser). It will be conducted with structured clinical follow-up and the assessment of multiple outcomes at different time points throughout the study.

Methods: Participants, interventions and outcomes
Study setting {9}

The study was conducted at the gynecology outpatient clinic of the Regional Hospital of Cáceres Dr. Antônio Fontes (HRCAF), a secondary and tertiary public reference institution linked to the Brazilian Unified Health System (SUS), located in the municipality of Cáceres, Mato Grosso, Brazil. It is a tertiary-level public hospital affiliated with the SUS, providing specialized outpatient care and serving as a center for teaching and research.

Eligibility criteria {10}

Inclusion Criteria

- Women aged >45 years and <80 years, with a clinical diagnosis of menopause (absence of menstruation for at least 12 months) and laboratory confirmation (FSH > 40 mIU/mL and estradiol < 25 pg/mL);
- Presence of clinical symptoms of GSM and mandatory presence of clinical symptoms of urinary incontinence;
- Diagnosis of stress urinary incontinence, overactive bladder, or mixed urinary incontinence confirmed by urodynamic testing;
- Cervical cytology performed within the past 6 months with negative results for neoplasia;
- Urinalysis showing no evidence of urinary tract infection.

Exclusion Criteria

- Explicit refusal to participate in the study;
- Patients who have undergone any surgical intervention for urinary incontinence correction at any time;
- Patients with urinary incontinence not directly or indirectly related to GSM, such as those with neurogenic bladder;
- Presence of active genital infections (clinical and colposcopic diagnosis);
- Patients with a history of genital herpes who do not agree to receive oral acyclovir prophylaxis;
- Women with ulcerative colitis, Crohn's disease, or diarrhea secondary to intestinal disease; positive serology for HIV, hepatitis B or C; or genital condylomatosis;
- Immunosuppressed patients, chronic corticosteroid users, or those with scleroderma, collagen diseases, or neoplasms of the genitourinary tract;
- Patients with thrombophilias or chronic users of anticoagulants or antiplatelet agents;
- Patients presenting with second- or third-degree vaginal prolapse according to the Pelvic Organ Prolapse Quantification System (POP-Q);
- Patients with a history of photosensitivity;
- Patients who have undergone previous CO_2 endovaginal laser treatment at any time;
- Patients who have used vaginal estrogen therapy (creams) in the past three months.

Who will take informed consent? {26a}

Informed consent was obtained by the research team prior to the inclusion of participants in the study, through a printed document signed by both the participant and the principal investigator. The process took place during the clinical visit in the outpatient setting, in a private room, with a detailed explanation of the study objectives, the procedures involved, the potential risks and benefits, and a clear emphasis on the participant's right to refuse or withdraw from the study at any time.

Participants received a printed copy of the consent form and were allowed to take it home, reflect on the proposal, and return to the clinic with the requested examinations for confirmation of eligibility. At that time, they reviewed the document, clarified any remaining questions, and, after signing, immediately received a copy of the form.

No intervention was initiated prior to the formal obtainment of informed consent.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Not applicable, as no biological material was collected and no ancillary studies were planned.

Interventions

Explanation for the choice of comparators {6b}

Not applicable. This is a cohort study with a single group.

Intervention description {11a}

Participants included in the study underwent three monthly sessions of fractional microablative CO_2 laser application, with a fixed interval of 30 days between each session. The intervention was performed using the MonaLisa Touch® CO_2 system (SmartXide2 V2LR, DEKA, Italy), employing three distinct configuration modes according to the clinical objective and the area treated:

Urinary Incontinence Mode: Intravaginal application focused on the anterior vaginal wall using a 90° probe. For patients with dry vaginal mucosa, the parameters were: 20 W power, 1500 µs dwell time, 1000 µm DOT spacing, Stack 4. For patients with moist vaginal mucosa: 20 W power, 1600 µs dwell time, 800 µm DOT spacing, Stack 5.

Vaginal Atrophy Mode: Intravaginal application with a 360° probe using the Deka Pulse mode. Parameters: 40 W power, 1000 µs dwell time, 1000 µm DOT spacing. Ablation depth was adjusted according to the clinical severity of atrophy: Stack 2 (severe), Stack 3 (moderate), and Stack 4 (mild).

External Mode: Application to the vestibule and vaginal introitus using the vulvar handpiece in Deka Pulse mode. Parameters: 20 W power, 1000 µs dwell time, 1000 µm DOT spacing, Stack 2.

Criteria for discontinuing or modifying allocated interventions {11b}

The intervention could be discontinued or modified under the following conditions:

- Participant request to stop treatment for any reason.
- Adverse events such as severe pain, abnormal bleeding, local infection, or tissue injury.
- Emergence of a health issue during follow-up (e.g., infection, hospitalization, acute condition) that made continuation unsafe.
- Detection of hormonal therapy use (local or systemic) during the study, violating protocol criteria.
- Recommendation from the Data Monitoring Committee (DMC) due to safety concerns or unexpected

effects.

Strategies to improve adherence to interventions {11c}

Participants were contacted by telephone calls or WhatsApp messages in order to reinforce the scheduling of therapeutic sessions and follow-up visits. Communication was conducted individually, using clear and supportive language, with the aim of emphasizing the importance of full adherence to the therapeutic protocol.

The research team remained available throughout the follow-up period to answer questions and provide additional support whenever necessary. Attendance at the sessions was recorded in a password-protected electronic spreadsheet, and absences were followed by a new contact attempt to reschedule the appointment.
This active monitoring model enabled the early identification of barriers to adherence and the implementation of adaptive strategies according to the characteristics and needs of each participant.

Relevant concomitant care permitted or prohibited during the trial {11d}

During the conduct of the study, the use of any form of hormonal therapy—either topical or systemic—was prohibited, as well as the use of creams, vaginal lubricants, or other non-hormonal products that could interfere with the effects of the proposed interventions. The use of systemic medications, such as antibiotics, was permitted exclusively in situations of clinical necessity, provided that they did not impact the primary or secondary outcomes of the research. Medications with potential influence on genitourinary health were evaluated by the clinical team, and in cases where significant interference was identified, the exclusion of the participant from the study protocol was considered.

Provisions for post-trial care {30}

Participants remained under follow-up for up to 12 months after the final intervention, with scheduled visits at months 6 and 12. The purpose of this monitoring was to assess the durability of therapeutic effects and to identify any late adverse events. Should any participant experience harm related to the intervention, appropriate medical care was guaranteed at no cost. No additional follow-up was planned beyond this period, considering the established safety profile of the technologies employed. Although no financial compensation was provided, clinical support was ensured for any adverse event directly associated with the study.

Outcomes {12}

Primary Outcome: The reduction of stress urinary incontinence (SUI) symptoms was defined as the primary outcome, assessed by the change in the total score of the International Consultation on Incontinence Questionnaire – Short Form (ICIQ-SF).

Secondary Outcomes: The secondary outcomes included: changes in quality of life related to SUI, assessed through the ICIQ-SF subscale on interference with daily activities; changes in genitourinary symptoms of menopause, measured by the total score of the Female Sexual Function Index (FSFI); patient satisfaction with the treatment, evaluated through a structured form; and the safety and tolerability of the intervention, monitored through the reporting of adverse events.

All outcomes were assessed at three time points: before the first session (baseline), and at 6 and 12 months after the beginning of treatment. Statistical analysis has not yet been performed, as the data remain blinded. The analysis will be conducted after acceptance of this protocol for publication, in order to preserve the impartiality and integrity of the study.

Participant timeline {13}

The follow-up schedule comprised six time points: T0 (clinical screening, application of eligibility criteria, signing of the informed consent form, and performance of urodynamic testing); T1 (first treatment session and baseline assessment using clinical instruments and the pad test); T2 (30 days); T3 (60 days); T4 (6 months); and T5 (12 months). The validated questionnaires (ICIQ-UI SF, ICIQ-OAB, ISI, FSFI, and KHQ) and the one-hour pad test were administered from T1 through T5. Urodynamic testing was repeated at T5. Figure 1 presents this schedule in detail, following the SPIRIT 2013 guidelines.

Figure 1. SPIRIT Figure: Schedule of enrolment, interventions, and assessments.

STUDY PERIOD						
	Recruitment	**Intervention**				
Time in months		***D1***	***D30***	***D60***	***D180***	***D365***
Time	T0	T1	T2	T3		
Eligibility criteria application	X					
ICF (Informed Consent Form)	X					
Gynecological consultation	X					
Urodynamic study	X					X
Treatment session – CO_2 Laser group		X	X	X		
Clinical instrument application		X	X	X	X	X
One-hour pad test		X	X	X	X	X

Note: The line “Administration of clinical instruments” refers to the application of the following validated tools: Vaginal Health Index Score (VHIS), Female Sexual Function Index (FSFI), Visual Analogue Scale (VAS) for

genitourinary symptoms, and the International Consultation on Incontinence Questionnaire – Urinary Incontinence Short Form (ICIQ-UI Short Form). Vaginal biopsy was performed in a subgroup of participants.

Sample size {14}

The sample size calculation was performed with the aim of detecting a minimum difference of 8 points in the primary outcome, measured using the International Consultation on Incontinence Questionnaire – Urinary Incontinence (ICIQ-UI), considering an estimated standard deviation of 3 points. These parameters were based on previous studies that demonstrated similar clinical improvement after fractional microablative CO_2 laser intervention for urinary incontinence symptoms (13, 15, 16).

The calculation was conducted using the G*Power software, version 3.1, employing a paired t-test for intra-individual pre- and post-intervention comparison, with a type I error (alpha) of 5% and statistical power (1– beta) of 95%. The resulting effect size was 2.66, indicating that a minimum sample of 10 patients would be sufficient to detect the expected difference in the primary outcome.

However, considering the methodological design of the study and the intention to perform subgroup analyses, the sample was expanded to 30 participants. The planned analyses included comparisons among different types of urinary incontinence (stress, overactive bladder, and mixed), as well as an assessment of the impact of body mass index (BMI) on clinical outcomes (obese vs. non-obese patients) and the influence of concomitant use of topical estrogen therapy. The expansion of the sample aimed to provide greater robustness to exploratory analyses, while acknowledging the limitations of a single-center study with a relatively small number of participants.

Recruitment {15}

Recruitment was conducted through consecutive screening in the gynecology outpatient clinic of the Hospital Regional de Cáceres Dr. Antônio Fontes, beginning in July 2022. Eligible patients were approached in person by the research team during scheduled consultations and were provided with comprehensive explanations about the study, including the option of taking the informed consent form (ICF) home for further review. A total of 30 participants were enrolled, and recruitment was concluded once the sample size required to ensure the planned statistical power was reached.

The main challenges encountered included the participants' limited prior knowledge regarding the procedures and difficulties in traveling to the research center. Nevertheless, adherence was considered satisfactory, allowing the study to be conducted according to the original design.

Assignment of interventions: allocation

Sequence generation {16a}

Not applicable – this is an open-label, non-randomized study.

Concealment mechanism {16b}

Not applicable – no group allocation requiring concealment.

Implementation {16c}

Not applicable – this is an open-label, non-randomized study.

Assignment of interventions: Blinding

Who will be blinded {17a}

Not applicable – open-label study.

Procedure for unblinding if needed {17b}

Not applicable.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Eligible participants were recruited at the Gynecology Outpatient Clinic of Hospital Regional Dr. Antônio Fontes, in Cáceres (MT), after confirmation of the clinical diagnosis of stress urinary incontinence associated with genitourinary syndrome of menopause. After signing the Informed Consent Form (ICF), the patients underwent systematic collection of clinical and sociodemographic data, obtained through a structured interview and completion of validated questionnaires.

The variables of interest included age, menopausal status, obstetric history, body mass index, lifestyle habits, and prior use of hormone therapy. Clinical outcomes were assessed using validated instruments, with emphasis on the ICIQ-UI Short Form (primary outcome) and the ICIQ-OAB, ISI, FSFI, and KHQ questionnaires (secondary outcomes). All instruments were administered electronically using the Google Forms platform, with self-administered completion on a tablet provided by the research team during on-site visits at time points T1, T2, T3, T4, and T5.

The one-hour pad test was performed immediately before each intervention session (T1, T2, and T3), as well as during clinical follow-up visits (T4 and T5). The urodynamic study was conducted at two time points: during the screening phase (T0) and twelve months after the beginning of treatment (T5), with the aim of comparing functional parameters.

All steps of data collection and recording were conducted by the principal investigator, ensuring standardized procedures and minimizing bias. Data were stored in a secure electronic system with restricted access to the research team. All collection procedures followed methodological recommendations to ensure the quality and reproducibility of the information.

Important: Data collection was completed in December 2024. Statistical analysis of the outcomes has not yet been performed and will be conducted in due course as described in the analytical plan.

Plans to promote participant retention and complete follow-up {18b}

Adherence to the protocol was promoted through close follow-up by the healthcare team, under the supervision of the principal investigator, who was responsible for the clinical assessments and the longitudinal follow-up of the participants. To facilitate adherence, reminders were sent by telephone and WhatsApp messages, with flexible scheduling of the sessions according to each participant's availability. Participants also had direct access to the principal investigator via mobile phone and could report any intercurrent events at any time.

As an additional active monitoring measure, the research team conducted standardized contact five days after each session, via WhatsApp, with the purpose of verifying participant well-being and investigating potential adverse events. All reported adverse events were documented in a specific form and monitored by the research team. No serious adverse events were identified during the study.

All clinical and outcome assessments were maintained even for participants who, for any reason, did not attend all scheduled sessions of the protocol. Data from these participants were preserved according to the intention-to-treat principle, ensuring completeness of follow-up.

Data management {19}

Data collection of clinical variables and outcomes was conducted using two complementary methods. The validated questionnaires (ICIQ-UI Short Form, ICIQ-OAB, ISI, FSFI, and King's Health Questionnaire) were completed directly by the participants in digital format through the Google Forms platform, accessed using electronic devices provided at the time of data collection. The system was configured to prevent submission of forms with incomplete fields, ensuring data completeness.

Clinical assessments, such as the gynecological examination with a vaginal speculum and the one-hour pad test, were performed in a standardized manner by the principal investigator. All information collected was stored in a secure digital database with restricted access throughout the study period, ensuring participant confidentiality.

Periodic verification of data consistency and integrity was carried out during follow-up. As this was an open cohort study conducted at a single center, blinding of the investigator to the intervention was not feasible, as all participants received the same treatment. Access to the database remained restricted to the research team until the completion of data analysis and submission for publication.

All team members involved underwent prior training to ensure standardized application of the instruments and proper completion of records, in accordance with the scheduled timeline for clinical visits.

Confidentiality {27}

All information collected from participants was kept strictly confidential, in accordance with ethical standards for research involving human subjects. Each participant was assigned a unique numerical code at the time of enrollment, and no identifying information was recorded in the case report forms or databases. Data were stored in a secure digital platform (Google Forms), with access restricted to authorized team members and limited to operational information such as contact lists and pre-intervention clinical records.

Individual information was used solely for scientific purposes, and no participant was identified in any presentations or publications. After study completion, all data will be stored in a secure location for five years, as required by regulations, and subsequently destroyed in a secure manner.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Not applicable – no biological specimens will be collected for genetic or molecular analysis in this trial or for future use.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Statistical analysis has not yet been performed. The data are currently undergoing processing and, as planned, the analytical stage will be conducted prospectively using the following methods:

Initially, a descriptive analysis of the clinical and epidemiological characteristics of the participants will be performed, presenting absolute and relative frequencies for categorical variables, and measures of central tendency (mean and standard deviation, or median and interquartile range) for numerical variables, according to the distribution of the data assessed using the Shapiro–Wilk test.

Categorical variables will be compared using the chi-square test. For numerical variables, pre- and post-intervention comparisons will be conducted using the paired Student's t-test (for normally distributed data) or the Wilcoxon signed-rank test (for nonparametric data). All tests will be two-tailed, with a significance level of $p < 0.05$ and a 95% confidence interval.

Analogous statistical methods will be applied to the secondary outcomes. Given that data were collected at multiple follow-up points (1, 2, 3, 6, and 12 months), longitudinal evaluation will be performed using

repeated-measures analysis of variance (ANOVA), provided that parametric assumptions are met; otherwise, the Friedman test will be applied.

Subgroup analyses are planned to explore the impact of the intervention across different types of urinary incontinence (stress, overactive bladder, or mixed), as well as according to body mass index and concomitant use of topical hormonal therapy. In these cases, comparisons of numerical variables between subgroups will use the Student's t-test or the Mann–Whitney U test, depending on data distribution and the results of Levene's test for homogeneity of variances.

Interim analyses {21b}

Not applicable – no interim analyses or stopping guidelines were planned for this study.

Methods for additional analyses (e.g. subgroup analyses) {20b}

Although the initial study protocol included the enrollment of participants with overactive bladder–related incontinence and mixed urinary incontinence, only one participant presented a clinical profile compatible with overactive bladder, and none met criteria for mixed urinary incontinence, which made these comparisons unfeasible. Likewise, although subgroup analysis based on concomitant use of topical estrogen therapy had been planned, none of the participants reported current use of this treatment at the time of enrollment. Therefore, subgroup analyses will focus on comparisons across different body mass index profiles, using a cutoff point of 30 kg/m² to define obesity. These analyses will be exploratory in nature and are not linked to predefined specific hypotheses.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

Statistical analysis will follow the intention-to-treat principle, including all participants who initiated the study, even in the presence of incomplete adherence to the protocol, such as absence from one or more intervention sessions. Participants who did not attend all follow-up visits were retained in the database, and their information will be considered in the analyses according to the availability of collected data.

The platform used for questionnaire collection (Google Forms) was configured to prevent submission of forms with incomplete fields, minimizing the occurrence of missing data for self-reported variables. If missing data are identified in outcome variables or specific clinical variables, the statistical approach will be defined according to the proportion and pattern of missingness, and may include complete-case analysis, simple imputation, or variable-specific exclusion, respecting the appropriate technical criteria. All decisions will be documented at the time of analysis.

Plans to give access to the full protocol, participant level-data and statistical code {31c}

After completion of the study, the full protocol, the anonymized participant data, and the statistical code may be made available upon formal request from researchers affiliated with recognized institutions, provided that the request has a clear scientific purpose and includes an explicit commitment to the ethical and confidential use of the information. Data sharing will be contingent upon prior approval from the Research Ethics Committee, when applicable, and will follow all current ethical and regulatory standards regarding privacy and data confidentiality in human subject research.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

The coordinating team was composed of the principal investigator, responsible for the study design and clinical assessments, together with healthcare professionals from the participating center, including physicians, nurses, nursing technicians, and volunteer students. This team performed the interventions, collected the data, and provided clinical follow-up to participants, ensuring the methodological integrity of the trial. Professor Nivaldo Antonio Parizotto, PhD, served as the academic supervisor of the project.
As this was an open-label, single-center, non-randomized, low-risk study, no separate Trial Steering Committee was established.

Composition of the data monitoring committee, its role and reporting structure {21a}

No Data Monitoring Committee (DMC) was established due to the study design (open-label, single-center, non-randomized, and low-risk). Participant safety and data integrity were continuously monitored by the coordinating team.

Adverse event reporting and harms {22}

All adverse events were to be recorded and assessed for severity, relationship to the intervention, and the need for additional management. Any serious adverse events (SAEs) would be promptly reported to the Research Ethics Committee (CEP) and registered in Plataforma Brasil, in accordance with national and international guidelines.

No serious adverse events were identified during the study; therefore, no reporting to the Ethics Committee was required.

Frequency and plans for auditing trial conduct {23}

No independent external audit was planned for this study. Compliance with the protocol, ethical guidelines, and operational procedures was continuously monitored by the coordinating team throughout the conduct of the trial.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Any relevant changes to the protocol — such as methodological adjustments, modifications to study outcomes, revisions to sample size, updates to inclusion or exclusion criteria, or matters related to research ethics — would be reported in advance to the Research Ethics Committee (CEP) and recorded in Plataforma Brasil and ReBEC, when applicable. Implementation of such changes would require prior formal ethical approval.

Dissemination plans {31a}

The results of this clinical trial will be disseminated through scientific publications in peer-reviewed journals, presentations at relevant medical events, and updates to public registries, including ClinicalTrials.gov and ReBEC. Participants will be able to request a lay summary of the findings.
There will be no restrictions on data publication, ensuring transparency in communication with the scientific community, healthcare professionals, and study participants. Full dissemination will occur only after this protocol has been accepted for publication.

Discussion

This clinical trial faced operational challenges, particularly in maintaining participant follow-up over the 12-month period. Difficulties were greater among those with logistical limitations or transportation barriers.
A decline in adherence to the 6- and 12-month evaluations was observed, possibly due to the perception that the treatment had ended after the intervention sessions. To minimize losses, the team implemented active follow-up, with individual contact and reinforcement of the importance of continued monitoring for participant safety and data quality.
Careful planning of the interventions and systematic follow-up were essential to avoid delays and ensure adherence to the study protocol.

Trial status

This protocol corresponds to version 1.0, dated November 28, 2025.
Ethical approval was granted in March 2022.
Participant screening began in July 2022.
The study was registered in ReBEC (RBR-524vx5c) in December 2024, retrospectively. At the time, the research team was not fully aware that prospective registration was required as an essential criterion for publication in international journals, which resulted in the delay.
Clinical interventions were initiated in March 2023, and the study was formally concluded in September 2024.
At the time of submission, the database is finalized.

Abbreviations

The following abbreviations are used throughout the manuscript:
UI: Urinary Incontinence
GSM: Genitourinary Syndrome of Menopause
ICIQ-UI - *International Consultation on Incontinence Questionnaire – Urinary Incontinence*
ICIQ-OAB - *International Consultation on Incontinence Questionnaire – Overactive Bladder*
ISI – *Incontinence Severity Index*
FSFI – *Female Sexual Function Index*
KHQ - *King's Health Questionnaire*

Declarations

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Authors' contributions {31b}

CMMGD was the principal investigator, responsible for the study conception, protocol development, pre-intervention clinical assessments, and manuscript drafting.

NAP served as academic supervisor, contributing to the methodological design and performing the critical revision of the manuscript.

CCGDT contributed to protocol development, assisted in the clinical interventions according to the study design, and participated in manuscript drafting.

MRS served monitoring the integrity of the clinical data and collaborating in the technical review of the manuscript.

MDT and CS contributed to manuscript drafting and revision.

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Availability of data and materials {29}

The raw datasets generated and/or analyzed during the current study are available from the corresponding

author on reasonable request, provided that ethical principles and participant confidentiality are respected. Data sharing will be subject to approval by the Research Ethics Committee, when applicable

Ethics approval and consent to participate {24}

This study was approved by the Research Ethics Committee (Comitê de Ética em Pesquisa – CEP) of Universidade Brasil, Brazil (approval number 5.357.602). Written informed consent was obtained from all participants prior to any study procedure, in accordance with national and international ethical guidelines. The trial was also registered in the Brazilian Clinical Trials Registry (Registro Brasileiro de Ensaios Clínicos – ReBEC) as required by current regulations.

Consent for publication {32}

Not applicable. This study does not involve the publication of any individual participant's data, images, or other identifying information.

Competing interests {28}

The authors declare that they have no financial or non-financial interests that could be interpreted as conflicts of interest related to this study. C.M.M.G.D., C.C.G.D.T., M.R.S., M.D.T., C.S.M., N.A.P., confirm the absence of competing interests.

Authors' information (optional)

Catarina Maria Mesquita Garcia Dalbem is a gynecologist, holds a master's degree, and is a professor at the State University of Mato Grosso (UNEMAT).

Nivaldo Antonio Parizotto is a physical therapist, holds a PhD in Electrical Engineering from the University of Campinas (UNICAMP), and completed postdoctoral training at the Wellman Center for Photomedicine at Harvard Medical School. He is currently a Senior Full Professor at the Federal University of São Carlos (UFSCar) and a faculty member of the Graduate Program in Biomedical Engineering at Universidade Brasil.

Cássia Caroline Garcia Dalbem Teles is a gynecologist and obstetrician, holds a master's degree, and is a faculty member in the School of Medicine at the State University of Mato Grosso (UNEMAT), with a specialization in Lower Genital Tract Pathology and Colposcopy.

Matheus Rodrigues de Souza (UNEMAT) is a physician specializing in statistical analysis and the conduct of clinical trials, affiliated with the State University of Mato Grosso (UNEMAT).

Marina Dalbem Teles (FAMP) is a physician trained at the Morgana Potrich Faculty (FAMP).

Claudia Schmidt Moura (UNEMAT) is a medical student at the State University of Mato Grosso (UNEMAT).

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Corresponding author:

Catarina Maria Mesquita Garcia Dalbem, Universidade Brasil – UB, Fernandópolis, São Paulo, Brasil.

E-mail: catarinadalbem@unemat.br