REC-MURA.04

Clinical Trial Protocol Template for Ramathibodi EC Submission

Based on SPIRIT 2013 checklist (https://doi.org/10.7326/0003-4819-158-3-201302050-00583)

(All information can be written in **Thai** or **English**. *Descriptions in red* must be deleted before submission)

***หมายเหตุ**: ห้ามตัดหัวข้อออก คงไว้ตามแบบฟอร์ม หากไม่มีข้อมูลที่เกี่ยวข้อง ให้ระบุว่า" ไม่มี" (ตัดเฉพาะคำอธิบายสีแดงออก) และสามารถระบุรายละเอียดเป็นภาษาไทยได้*

Study Title (English):

Effect of Vaginal Estrogen on Alterations in the Urine Microbiome of Menopausal Women with Overactive Bladder.

Study Title (Thai):

ผลของยาเอสโตรเจนชนิดเหน็บช่องคลอดต่อการเปลี่ยนแปลงแบคทีเรียไมโครไบโอมในปัสสาวะของสตรีวัยหมดประจำเดือนที่มีภาวะกระเพาะปัสสาวะบีบตัวไวเกิน

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Sponsor or planned sponsor, grant, scholarship <if applicable>:

The budget outlines a plan to request funding from the Faculty of Medicine Ramathibodi Hospital, Mahidol University and Menopausal fund.

Conflict of Interest:

None

Study sites (list all as planned):

- Outpatient department – Female Pelvic Medicine and Reconstructive Surgery clinic (FPMRS clinic), Department of Obstetrics & Gynaecology, Faculty of Medicine Ramathibodi Hospital

Trial registration:

The trial registration is planning to register on www.clinicaltrials.gov

Background and Significance:

Overactive bladder (OAB) is a prevalent condition characterized by urinary urgency, often associated with increased frequency and nocturia, with or without urgency urinary incontinence (UUI), and occurs in the absence of a urinary tract infection (UTI) or other apparent pathology (1). It affects about 12-20% of adults and increases incidence with age. It significantly impacts the quality of life, leading to physical discomfort, psychological stress, and social embarrassment with higher prevalence observed in women, particularly those in the menopausal transition or postmenopausal phase (2).

Menopause marks a significant hormonal shift in a woman's life, primarily characterized by the decline in estrogen production by the ovaries. Estrogen plays a crucial role in maintaining the structural and functional integrity of the lower urinary tract, vagina, and pelvic floor muscles. Due to these, the reduction in estrogen levels following menopause is considered a potential cause of pelvic floor disorders, including stress urinary incontinence (SUI), urgency urinary incontinence (UUI), and pelvic organ prolapse (POP), including OAB symptoms in menopausal women (3).

Estrogen therapy, particularly localized treatments like vaginal estrogen, has been utilized to alleviate urogenital symptoms associated with menopause (3). Vaginal estrogen has been shown to improve vaginal atrophy and increase urethral closure pressure (4). Moreover, it may alleviate OAB symptoms by restoring the health of the urogenital mucosa and improving bladder function. For clinical use, many studies have shown a significant reduction in urinary urgency symptoms, urinary incontinence, and increased maximal cystometric capacity (5, 6). However, the precise mechanisms by which estrogen influences bladder function and symptomatology remain not fully elucidated.

Recent advances in microbiome research have highlighted the significant role of the urinary microbiome (or urobiome) in urinary health and disease. Once considered sterile, the urinary tract is now known to host a diverse community of microorganisms that may influence urinary tract function and susceptibility to disorders like OAB (7). Alterations in the urinary microbiome composition, known as dysbiosis, have been associated with various urinary conditions, including overactive bladder, nocturia, interstitial cystitis and recurrent urinary tract infection (8). Understanding the impact of vaginal estrogen on the urinary microbiome of menopausal women with OAB could provide valuable insights into new therapeutic avenues and personalized treatment strategies. It may help clarify whether alterations in the urinary microbiome composition mediate the beneficial effects of estrogen on OAB symptoms.

This study aims to investigate the effect of vaginal estrogen therapy on changes in the urinary microbiome and its association with OAB symptom improvement in menopausal women. By exploring this relationship, we hope to enhance the understanding of OAB pathophysiology in menopausal women and contribute to developing more effective interventions targeting both hormonal and microbial factors.

Review Literature:

Vaginal estrogen used in OAB treatment

Author/Year/ Country	Study	Aim	Results
Cardozo LD et al (5) 2001 London, UK	• Randomized controlled trial • N = 110 • Vaginal 17β Estradiol VS Placebo • 12 weeks follow up	Evaluate efficacy of 17β Estradiol in treatment of postmenopausal women with Frequency, Urgency or UUI	Significant reduction in symptom of urinary urgency for women with sensory urgency
Simunic V. et al (6) 2003 Croatia	• Multicenter RCT • N = 1612 • Vaginal 17β Estradiol VS Placebo • 12 months follow up	Determination of efficacy and safety of vaginal 17β Estradiol in patients with urogenital symptoms	In estrogen group • Improve urinary atrophy symptoms • Reduce urinary incontinence • Increase maximal cystometric capacity, first desire to void, strong desire to void

Overactive bladder/Urgency urinary incontinence and microbiome study

- **Pearce MM, et al., 2014** (9)
    - This study used Expand Quantitative Urine Culture (EQUC) techniques and 16s rRNA gene sequencing to analyze the trans-catheterized urine samples of patients with urgency urinary incontinence compared with the control group.
    - Results:
        - UUI microbiome was composed of increased Gardnerella and decreased Lactobacillus
        - Nine genera were more frequently cultured from the UUI group (Actinobaculum, Actinomyces, Aerococcus, Arthrobacter, Corynebacterium, Gardnerella, Oligella, Staphylococcus, Streptococcus)
        - More Lactobacillus gasseri in UUI group
        - More Lactobacillus crispatus in control group

- Conclusion: bacterial communities in the urinary tract may be linked to UUI. Data suggest that potential important differences exist in urine microbiomes of women with and without UUI

- **Curtiss N, et al., 2017** (10)
  - This study used 16s rRNA gene sequencing to analyze the urine samples from clean catch of patients with overactive bladder compared with the control group
  - Results:
    - Significant differences were found between the microbiomes of OAB patients and controls. *Lactobacillus* was more prevalent in controls (43%) compared to OAB patients (20%). Conversely, *Proteus* was more commonly found in OAB patients (23%) than controls (3%).
  - Conclusion: the female bladder has a diverse microbiome with significantly differences bacteria species present in overactive bladder patients and controls

Vaginal estrogen therapy in Overactive bladder and microbiome study

- **Thomas-White K, et al., 2020** (11)
  - The quasi-experimental study aimed to determine the effect of vaginal estrogen treatment (0.5 g of conjugated estrogen twice weekly for 12 weeks) on urobiome diversity (using Expand Quantitative Urine Culture (EQUC) techniques) in postmenopausal women with overactive bladder.
  - Results:
    - A significant increase in Lactobacillus was detected in the bladder (10.8 vs. 25.1; P= 0.037) but not in the vaginal (P = 0.33), perineum (P = 0.56) or void urine (P = 0.28)
    - The change in Lactobacillus levels in the bladder was associated with modest changes in urgency incontinence symptoms (P = 0.02)
  - Conclusion: vaginal estrogen therapy alters the microbiome in different pelvic niches, remarkably increasing Lactobacillus in the bladder, which is associated with symptom improvement in postmenopausal women with OAB.

Objectives:

Primary Objective:

To evaluate the effects of vaginal estrogen on the level of Lactobacillus in the urine of postmenopausal women with OAB.

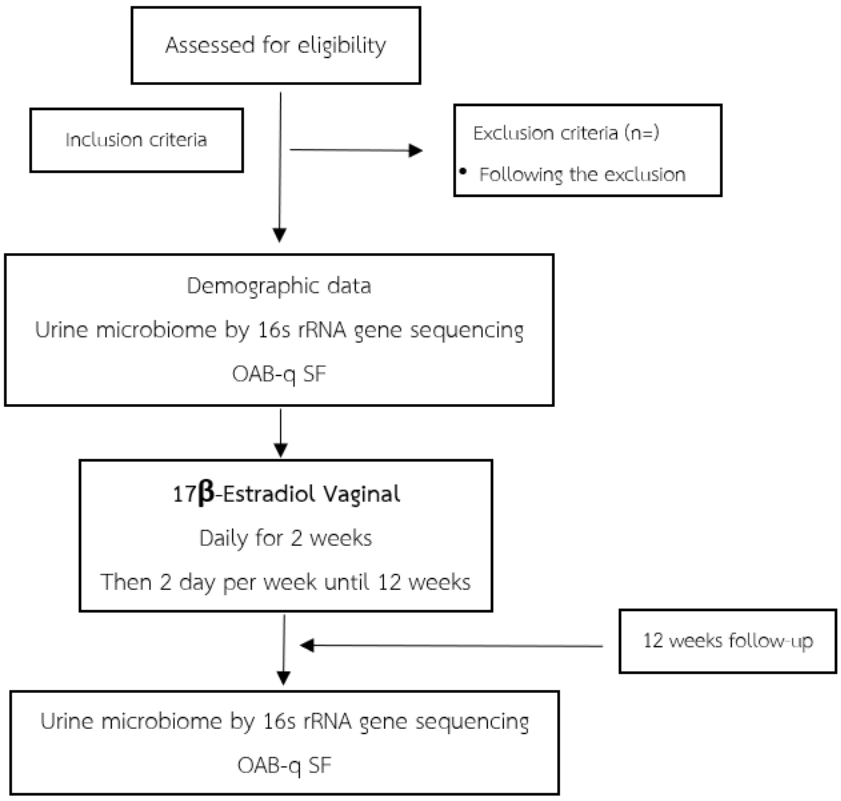
Secondary Objectives (if any):

1. To evaluate the effects of vaginal estrogen on urine microbiome in postmenopausal women with OAB.
2. To identify the association of alterations of urine microbiome after vaginal estrogen treatment with overactive bladder symptoms.

Study design/methodology:

Study design: Pre-post quasi-experimental study

Study flow diagram



Note: The validated Thai version of OAB-q SF (12)

Study Population

Postmenopausal women with overactive bladder

Inclusion Criteria:

1. Natural or surgical menopause at least 1 year

2. Present with overactive bladder symptoms by using validated Thai version of OABSS (13) (score in No.3 ≥ 2 and overall score ≥ 3)
3. Absence of urinary infection
4. Post-void residual urine less than 100ml

Exclusion criteria

1. Currently or prior use of systemic HRT or vaginal estrogen within the past 3 months
2. Contraindication or allergy to estrogen therapy
3. Use of antibiotics, prebiotics and probiotics within the past 2 weeks
4. Currently on antimuscarinic or β3 agonists medication or within the past 3 months
5. Pelvic organ prolapse greater than stage II

Study drug /Interventions <if applicable>:

17β-estradiol 10 mcg (Femiest®, HAUPT PHARMA MUNSTER GMBH, Muenster, Germany)

1 tab daily for 2 weeks vaginally before bed, then 1 tab twice a week vaginally before bed until 12 weeks (Participants will be asked to bring the blister pack for pill count at the 12-week follow-up (checking compliance)

Comparator/Control <if applicable>:

None

Treatment Allocation and concealment:

None

Blinding (masking):

None

Participant timeline and Procedures:

Time	Study Visit	Procedures
Week 0	Screening and Enrollment	Eligibility screening, informed consent
Week 0	Baseline assessment	Demographic data, baseline symptom by OAB-q SF questionnaire, baseline urine sample collection for microbiome analysis by 16s rRNA gene sequencing.
Week 1-2	Intervention period	17β-Estradiol Vaginal: Daily
Week 3-12	Intervention period	17β-Estradiol Vaginal: 2 day per week
Week 12	Post-intervention assessment	Symptom evaluation by OAB-q SF questionnaire, urine sample collection for microbiome analysis by 16s rRNA gene sequencing.

Urine sample collection

Sterile urine collection procedures will be done by well-trained investigators on catheterization urine. The urine will be transferred immediately to the laboratory and placed at 4°C.

Outcomes/endpoints:

Primary Outcome

- Change in Lactobacillus abundance in urine after vaginal estrogen therapy

Secondary Outcome

- Change in urine microbiome diversity after vaginal estrogen therapy
- Association of change in urine microbiome with overactive bladder symptoms

Discontinuation/withdrawal criteria:

1. Adverse events or medical reasons
    a. Severe Adverse Reactions: If a participant experiences severe or unmanageable side effects related to vaginal estrogen use (for example unexplained vaginal bleeding, severe irritation, or allergic reactions), they should be withdrawn from the study.
    b. New Medical Conditions: development of new medical conditions that could interfere with the study outcomes for example cardiovascular issues
2. Non-Adherence to Treatment Protocol: Missed Follow-Up Assessments
3. Voluntary Withdrawal

Adverse Event Reporting:

1. Adverse events (AEs)
    a. Definition: any unintended, unfavorable medical occurrence in a participant during the study, whether it is related to the study intervention (vaginal estrogen therapy) or not.
    b. Examples: vaginal irritation, vaginal discomfort, vaginal discharge, bleeding, allergic reaction and possible systemic absorption of estrogen could lead to side effects such as breast tenderness or headaches.
    c. Participant Responsibilities: Participants are instructed to immediately report any discomfort, symptoms, or health changes they experience during the study, regardless of severity.
    d. Investigator Responsibilities: AEs and compliance will be inquired by follow-up phone calls from the investigator. Mild or moderate AEs will be managed by providing symptomatic treatment or observing the participant closely.

2. Serious adverse events
    a. Definition: Any event that results in hospitalization, life-threatening conditions, permanent disability, or death.
    b. Investigator Responsibilities: If a serious adverse event occurs, the participant will be withdrawn from the study, and immediate medical care will be provided. SAEs must be reported to the Ethics Committee and relevant regulatory bodies within 24 hours.

Statistical Analysis Plan:

- Descriptive statistics for baseline demographics and clinical characteristics were presented as mean and standard deviation or medians and interquartile ranges (IQRs) for continuous variables and frequency and percentage for categorical variables.
- Comparisons between pre-and post-intervention were tested using pair t-test or Wilcoxon signed-rank tests for continuous variables and chi-square or the Fisher exact tests for categorical variables.
- Association between changes in urine microbiome and changes in the OAB symptom were calculated using the Spearman rho test.

Microbiome study

- Alpha Diversity (within-sample diversity): measures provide insights into the richness (number of species) and evenness (distribution of species) within a sample.
    - Observed species, Chao1, Shannon, Simpson using QIIME software
    - Richness was calculated using Chao1 and observed species indices
    - Shannon and Simpson indices combined the interaction between richness and evenness
    - Wilcoxon rank sum test was applied to identify significant differences
- Beta Diversity (between-sample diversity): evaluates differences in microbial composition between samples.
    - Bray-Curtis and Uni-Frac (weighted and unweighted)
- Linear Discriminant analysis effect size (LEfSe): investigate microbial biomarkers between groups at the genus level

P-values <0.05 were considered statistically significant.

Sample size determination:

Using 2 dependent means formula for sample size calculation.

$$n = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \sigma^2}{\Delta^2}$$

- From Pearce MM's study (2014), the median (IQR) percentage of lactobacillus in women with OAB was 13.6 (0-61.6); we expected the difference in mean percentage of lactobacillus after vaginal estrogen treatment was 25.
- Alpha (α) = 0.05, Beta (β) = 0.2
- The sample size was calculated = 27.
- Then, 10% data loss was added, the required sample size was 30.

Recruitment procedure:

- Menopausal women with a clinical diagnosis of overactive bladder (OAB) who meet the eligibility criteria will be recruited from the outpatient department – FPMRS clinic, Ramathibodi hospital.
- Interested patients will be directed appointed by research nurse to meet with the investigator.
- The investigator will give a detailed explanation, confirm eligibility through a detailed review of medical history and informed consent procedures.

Informed Consent Process:

1. Recruitment: Interested patients will be directed to meet with an investigator at the outpatient department – FPMRS clinic.
2. Detailed Explanation: The study's purpose, procedures, risks, and benefits are explained clearly.
3. Voluntary Participation: Emphasis is placed on the voluntary nature of participation and the right to withdraw at any time.
4. Signing the Consent Form: Participants sign the form after understanding the study and asking questions.
5. Ongoing Consent: Participants are reminded of their rights and informed of any protocol changes during the study.

Privacy and confidentiality (Data Management Plan):

1. The principal investigator and research collaborators will manage data and maintain patient confidentiality.
2. Assign Participant IDs to de-identify data and samples. The principal investigator will record information such as hospital numbers or names in a codebook.

3. Physical Storage: Any physical copies of data (e.g., paper forms and consent documents) will be securely stored in locked cabinets, with access limited to authorized personnel.
4. Biological Samples: Use urine samples only for outlined study purposes, store samples securely and label them with study ID and destroy samples according to biohazard regulations after use.
5. Digital Storage: All electronic data will be stored in encrypted databases, and any data transfers will occur over secure, encrypted networks. Regular backups will be performed to prevent data loss. Data will be stored for at least five years.
6. Participants can request access to or withdraw their data.

Ethical consideration:

- ***Risks to participants and how to minimize the risks:***

1. Risks Related to Vaginal Estrogen Therapy
    a. Potential risks: vaginal discomfort, allergic reaction, vaginal discharge
    b. Risk minimization: Screen participants carefully to exclude those with contraindications to estrogen therapy, monitoring allergic reactions and educating participants
2. Risks Related to Urine Sample Collection by transurethral catheterization
    a. Potential risks: discomfort, urinary tract infection and urinary tract injury
    b. Risk minimization: Sterile urine collection procedures by the investigator who is well-trained, monitoring symptoms of urinary tract infection and educating participants

- ***Direct Benefits to Participants***

Participants receive vaginal estrogen therapy as part of the study intervention at no cost. Vaginal estrogen therapy has been shown to alleviate symptoms associated with overactive bladder, such as urinary urgency, frequency, nocturia (nighttime urination), and urge incontinence.

- ***Scientific or social value***

Supporting evidence of vaginal estrogen used in overactive bladder.

Understanding the changes in the urine microbiome caused by vaginal estrogen could allow for more personalized treatment strategies.

- ***Justification if enrolling potentially vulnerable subjects.***

The study will not be conducted on vulnerable subjects.

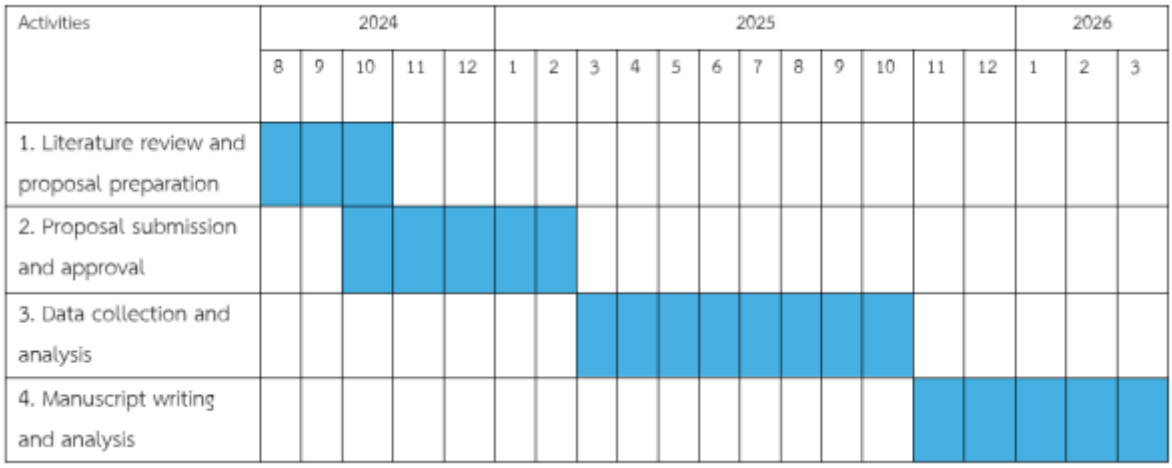
- *Travel compensation and compensation for injury*

Participants will receive 1,000 Thai Baht per visit to compensate for travel expenses incurred while attending study appointments. This compensation is provided after each completed visit.

- **Plan of board consent**

None

Study Timeline:



Budget:

Expense	Estimated cost/unit	Estimated cost
Participant Compensation	1000 Baht/visits	60,000 Baht
Intervention (Femiest®)	1197 Baht/box (18 tablets)	71,820 Baht
Urine catheterization	78 Baht/set	4,680 Baht
Urine microbiome analysis	5000 Baht/specimen	300,000 Baht
Total budget		436,500 Baht

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Date.....

Signature..... Major Advisor

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Date.....