Clinical Trial Protocol

Faculty of Medicine Ramathibodi Hospital, Mahidol University (full version)

1. Research name

Efficacy of Vaginal 17β-Estradiol on the Urinary Storage Symptoms in Postmenopausal Women: A Randomized Double-blind, Placebo-controlled Study

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5. Background and Rationale

MENOPAUSE AND LOWER URINARY TRACT SYMPTOMS (LUTS)

The lower urinary tract is responsible for the storage and elimination of urine. The control of this phenomenon is attributed to the innervation of the autonomic, somatic, and sensory systems. The process of ageing is widely recognized as a significant determinant of the anatomical and functional changes that occur in the pelvic floor and lower urinary tract (LUT). The prevalence of pelvic floor diseases, such as pelvic organ prolapse, overactive bladder (OAB), urinary incontinence (UI), and sexual dysfunction, tends to rise as individuals age. While it is challenging to distinguish the specific impacts of reduced estrogen levels during menopause from the broader process of ageing, it is worth noting that the pelvic organs and their associated muscular and connective tissue support are influenced by estrogen. Epidemiological research suggests that menopause plays a significant role in the increased likelihood of developing pelvic floor disorders. Furthermore, the

severity of urinary symptoms associated with these disorders tends to escalate following menopause.[1]

Lower urinary tract symptoms (LUTS) encompass a variety of frequently co-occurring issues pertaining to storage symptoms. These symptoms include urinary incontinence, heightened frequency of urination during the day or night, a sense of urgency, as well as symptoms related to voiding and post-micturition. Voiding symptoms may manifest as hesitancy, a slow stream, and intermittent flow, difficulty initiating urination, spraying or splitting of the urinary stream, leakage, painful urination, and a sensation of incomplete bladder emptying (urine retention). The International Continence Society (ICS) has provided a definition for overactive bladder, which is considered the most troublesome subset of lower urinary tract symptoms (LUTS). According to the ICS, overactive bladder is characterized by a sense of urgency to urinate, with or without involuntary leakage, sometimes accompanied by increased frequency of urination and waking up at night to urinate (nocturia). Based on the provided definition, urgency is a fundamental element in the diagnostic criteria for Overactive Bladder (OAB), serving as the primary symptom that influences the manifestation of other OAB symptoms.[1,2,3] The symptoms of Overactive Bladder (OAB) have a significant impact on various dimensions of individuals' lives, including social, psychological, occupational, household, physical, and sexual aspects. Consequently, these symptoms impose a substantial burden on affected individuals, resulting in significant costs.[4]

ESTROGEN DEPLETION AND STORAGE SYMPTOMS

The Role of Estrogens and the Continence Mechanism

Estrogen plays an important role in the genital and lower urinary tract functions, with estrogen receptors found in the bladder, urethra, vagina, and pelvic floor musculature. Estrogen receptors contribute to the pelvic support mechanism by modulating the production and breakdown of collagen. Furthermore, the tissues of the female urinary continence mechanism are estrogen sensitive. Estrogens may disrupt continence by increasing the number of periurethral vessels, which account for one-third of urethral pressure. Furthermore, estrogens can diminish the frequency and amplitude of detrusor contractions, raising the bladder's sensory threshold and promoting detrusor muscle relaxation.[3,5,6,7,8,9]

Epidemiological studies have corroborated the importance of sex hormone insufficiency in the etiology of LUTS, with up to 70% of women attributing the onset of urine incontinence to the end of their menstrual cycle.

Pathophysiology of Estrogen Depletion and Storage Symptoms

Several mechanisms could explain the role of estrogen deprivation in the onset of urinary urgency such as increased detrusor contractility through Rho-kinase pathway activation, increased

acetylcholine release, changes in urothelial afferent signalling, or increased connexin-43 expression.[10,11] Previous study has been documented which demonstrates that the removal of ovaries in female mice can lead to a reduction in urethral tone in these mice. The administration of E2 supplementation in mice that underwent ovariectomy resulted in the restoration of urethral tone. The augmentation of urethral tone in ovariectomized mice through E2 mediation is associated with the upregulation of surviving motor neuron expression, reduction in proteolysis, facilitation of development, neurophysiological processes, and transcription within the urethra. This knowledge will provide insights into the pathophysiology of stress urine incontinence following menopause.[11]

The depletion of estrogen in the body leads to a reduction in blood supply to the pelvic floor tissues. Insufficient blood circulation leads to a reduction in collagen synthesis, hence compromising the process of tissue repair. These alterations result in a reduction in the capacity of the vagina and bladder, leading to symptoms such as dyspareunia and urgency. Animal studies suggest that estrogen inhibits bladder contractility, so one can speculate that estrogen loss might contribute to OAB symptoms. A loss of periurethral vascularity lowers the urethral closure pressure, in theory contributing to the development of stress incontinence.[12]

Estradiol has been demonstrated to reduce inflammatory cell infiltration and reverse bladder shrinkage via vascular endothelial growth factor, supporting estrogen's therapeutic capabilities.[13,14] In clinical trials, vaginal 17estradiol tablets (Vagifem) reduced OAB symptoms while improving urodynamic markers such as cystometric capacity, strong need to urinate, and unrestrained detrusor contractions.[15]

PREVALENCE OF STORAGE SYMPTOMS

The overall prevalence of storage symptom in women was about 16% in US and Europe, but sex-specific prevalence differed substantially by severity of symptoms. Prevalence of urge incontinence increased with age from 2.0% to 19% with a marked increase after 44 years of age.[4,16] In Thailand, the prevalence of overactive bladder was 21.3%. Urgency, the main symptom, was found to occur in 32.8%.The common associated symptoms were frequency and urge incontinence which were found in 23.4% and 11.4% respectively.[17]

TREATMENT OF STORAGE SYMPTOMS

Bladder storage symptoms exhibit a clinical scenario and presentation that bears a striking resemblance to those observed in cases of an overactive bladder. In our forthcoming clinical trial, we intend to establish a treatment regimen by referencing the established standard guidelines for the management of overactive bladder as the foundational framework as will be mentioned in the following paragraphs.

Behavioral therapies, such as bladder training, bladder control strategies, pelvic floor muscle training, and fluid management, are commonly employed as first-line treatments. These therapies may be administered independently for patients experiencing mild symptoms that cause minimal distress. Alternatively, they may be combined with other more invasive treatment modalities for patients with severe symptoms. Pharmacologic management constitutes the subsequent therapeutic interventions for second-line treatments. Common pharmacological options for the treatment of overactive bladder (OAB) encompass the utilization of both anti-muscarinics and β3-adrenoceptor agonists.[18]

Vaginal estrogen therapy may be administered in the form of conjugated equine estrogen, estriol, or estradiol, utilizing vaginal pessaries, vaginal rings, or creams. This treatment aims to ameliorate symptoms of overactive bladder (OAB), including urgency linked to vaginal atrophy in postmenopausal women. The optimal period of treatment is undetermined.[19]

Estrogen has the potential to provide therapeutic advantages in managing irritative voiding symptoms, such as urgency, frequency, and urge urinary incontinence. The precise mechanism by which the medication induces the reversal of urogenital atrophy or its direct impact on the lower urinary tract remains uncertain. One challenge in comprehending the contradictory data now available on the subject pertains to the utilization of multiple estrogen formulations, varying dosages, diverse methods of administration, and the inconsistent use of concomitant progesterone. There exists substantial evidence supporting the efficacy of low-dose vaginal estrogen treatment in reversing urogenital atrophy, encompassing both the symptoms and cytological changes.[20, 21]

Review literatures

- **Comparison: Vaginal estradiol 25 mcg versus placebo**

Research title	country	year	Dosage	F/U	Outcome Measurement	Results
Low-dose 17beta-estradiol vaginal tablets in the treatment of atrophic vaginitis: a double-blind placebo-controlled study[14]	Denmark	Eriksen PS 1991	estradiol 25 mcg vs placebo	12 weeks	urological symptoms change	Vg E -> improve 62.8% placebo -> improve 32.4%
Local estrogen treatment in patients with urogenital symptoms[22]	Croatia	Simunic 2003	estradiol 25 mcg vs placebo	12 months	<u>symptoms/signs of urinary atrophy</u> Dysuria Frequency/nocturia Urinary tract infection Urinary incontinence Urinary atrophy	n=330->84 n=371->74 (Improve 80%) n=186->49 n=245->106 n=406->121
Vaginal estradiol for the treatment of lower urinary tract symptoms in postmenopausal women--a double-blind placebo-controlled study[23]	England	Cardozo 2001	estradiol 25 mcg vs placebo	12 weeks	N/A	A significantly greater reduction in the symptom of urinary urgency on visual analogue score was detected for women in stratum A (sensory urgency)

- **Weber MA et al. (2015)**

  Local Estrogen for Pelvic Floor Disorders: A Systematic Review

  Comparison: Vaginal estrogen versus placebo or no treatment

The group comprised of a total of eight research, out of which five studies employed a placebo-controlled design. Additionally, two studies compared the use of vaginal estrogen to no treatment, while one study compared the use of vaginal estrogen (in combination with physiotherapy and electrostimulation) to a group that received no treatment. In the present investigation, two separate investigations implemented the administration of vaginal estrogen via ovules, while an additional two studies employed estrogen vaginal tablets. Furthermore, three studies incorporated the utilization of an estrogen vaginal cream as part of their experimental protocol. The study lacked clarity regarding the manner of application.

In general, the subjective, semi-objective, and urodynamic results exhibited a favorable shift in the vaginal estrogen groups when compared to the placebo group. The study conducted by Simunic et al. observed that the occurrence of treatment adverse effects was evenly divided across

the vaginal estrogen and placebo groups. There were no instances of severe adverse effects reported. Three further investigations found no statistically significant differences in the occurrence of adverse events among the various groups. The researchers reached the conclusion that the application of topical estrogen appears to reduce the occurrence of overactive bladder (OAB) and urinary incontinence (UI) symptoms.[3]

- **J. A. Simon et al. (2013)**

Ultra-low-dose vaginal estrogen tablets for the treatment of postmenopausal vaginal atrophy

A pivotal study demonstrated that an ultra-low-dose 10-microgram E2 vaginal tablet effectively induces vaginal epithelial maturation. This change shifts the atrophic epithelium from a predominantly parabasal cell population to a superficial cell population, akin to premenopausal women. Significant improvements in vaginal cytology, evident after just 14 days of treatment, were maintained up to 52 weeks. This improvement in the cellular profile enhances the vaginal epithelium's resistance to bleeding and improves vaginal-cervical paracellular permeability, thereby increasing lubrication during intercourse.

Furthermore, the study revealed that local administration of 10 micrograms E2 significantly alleviated the most bothersome both urinary and genital symptoms, such as vaginal dryness, irritation, itching, dysuria, and dyspareunia. This symptom relief was more consistent and effective compared to systemic hormone therapy. While systemic absorption of estrogen does occur with all vaginal estrogen therapy products, studies have shown that absorption from low-dose vaginal estrogen therapy is minimal. Overall, the results firmly establish the efficacy of the ultra-low-dose 10-microgram E2 vaginal tablet in treating symptoms of estrogen deficiency-induced urogenital atrophy. The treatment effectively normalizes vaginal pH and cytology profiles and provides significant relief from the most bothersome urogenital symptoms soon after therapy initiation.[24]

- **Placebo and nocebo**

The administration of placebo has been observed to have a discernible impact on the amelioration of signs and symptoms associated with Overactive Bladder (OAB). Furthermore, the collective placebo responses observed across several examined outcomes exhibit statistical significance, and in certain cases, may also possess potential therapeutic significance. A recent systematic review (SR) encompassing 57 studies and involving a total of 12,901 patients revealed several noteworthy findings. Specifically, the standardized mean difference (SMD) for various urinary parameters were as follows: -0.45 for daily micturition episodes, -0.33 for daily nocturia episodes, -0.46 for episodes of urgency urinary incontinence (UUI), -0.50 for daily urgency episodes, -0.51 for daily incontinence episodes, and 0.25 for volume voided per micturition.[25,26]

The same group published a SR with meta-analysis of data retrieved from 57 RCTs comprising 15,446 patients were included in this systematic review. They selected 13 commonly reported adverse events for the meta-analysis. Nocebo effect of pharmacotherapy in patients with OAB (up to 80% females). They reported dry mouth as the most common reported adverse event with mean rate of 4.9%, followed by constipation 2.6%. The authors concluded that HCPs should appreciate the possible positive and negative patient expectation regarding pharmacotherapy for OAB in order to optimize the individual outcomes.[26,27]

The placebo response seems to be non-negligible in OAB, supporting the requirement for placebo control in RCTs.[25,26]

Knowledge gap

To date, no prior investigations have been conducted employing robust methodological approaches to assess the efficacy of 17β-Estradiol at a dosage of 10 mcg in the treatment of Lower Urinary Tract Symptoms (LUTS) in postmenopausal women, particularly in comparison to a placebo control group. This notable gap in the existing literature underscores the need for a comprehensive and rigorously designed study to investigate the potential therapeutic benefits of 17β-Estradiol in alleviating LUTS among postmenopausal women, while concurrently establishing its superiority or non-inferiority relative to a placebo intervention. Such an investigation would not only contribute significantly to our understanding of this therapeutic avenue but also provide valuable insights into the management of storage symptoms of LUTS in this specific population.

6. Objective

Primary objective

To determine the efficacy of vaginal 17β-estradiol treatment in alleviating storage symptoms of LUTS in comparison to placebo.

Secondary objective

- To determine the efficacy of vaginal 17β-estradiol treatment on urethral maturation index and vaginal pH in comparison to placebo.
- To assess the impact of vaginal 17β-estradiol treatment on the overall quality of life and the patient's global improvement (PGI) perception.

7. Study Objectives and Study Design Overview

7.1) Study design

The clinical study is a two arm, randomized, double-blind, placebo-controlled trial study of postmenopausal women who suffer from storage symptom of LUTS.

7.2) Population

Postmenopausal women who suffer from storage symptom of LUTS.

7.3) Outcomes

Primary outcome

Storage symptom improvement: will be evaluated using "the International Consultation on Incontinence Questionnaire-Female Lower Urinary Tract Symptoms: ICIQ-FLUTS" (Validated Questionnaire in Thai version)[28]

It comprises of 5 items, each represent the storage symptoms in the following items;

- Item 2a) nocturia
- Item 3a) urgency
- Item 5a) daytime frequency
- Item 9a) UUI
- Item 11a) SUI

Each item will have 2 parts of sub-categories questions are severity and bothersome.

- In severity categories: the questionnaire has established ranging from 0, denoting the absence of any severity, to 4, indicating the highest level of symptom severity. The cumulative point range across all questionnaire items spans from 0 to 20.
- In bothersome categories: the questionnaire employ ranging from 0, signifying the absence of any bothersome effect, to 10, representing the utmost degree of symptom bother. The cumulative point range across all questionnaire items encompasses 0 to 50.

The primary outcome interpretation will be predicated on the assessment of storage symptom improvement as follows.

- Improve outcome mean ICIQ-FLUTS post-treatment score less than pre-treatment score.
- Not improve outcome mean ICIQ-FLUTS post-treatment score more than or equal to pre-treatment score.

Secondary outcomes

Subjective assessment

- Assess the impact of urinary storage symptoms on quality of life with reference to social effects by Thai version of International Consultation on Incontinence Questionnaire Urinary Incontinence Quality of Life (ICIQ-LUTSqol), which comprises 20 items. The ranges from 19 to 76, with greater values indicating increased impact on quality of life. ICIQ-LUTSqol is a validated Thai adaptation of King's Health Questionnaire (KHQ).[29]
- Global Improvement score is evaluated by Thai version of Patient Global Impression of Improvement (PGI-I).[30]

Objective assessment

- Urethral maturation index (UMI) and maturation value (MV)
- Vaginal pH

7.4) Study procedure

I. First Visit and Recruitment

Participants will be recruited from the urogynecology clinic, menopause clinic, and urology clinic using hospital and public advertisement. An information sheet in Thai language will be provided to health care providers in the designated OPD which can ensure the providers convey the correct study information to potential participant. Participants will only be able to enroll into this study one time.

Informed Consent

Individuals with at least one storage symptom of LUTS presenting to clinics in selected OPD will undergo the informed consent process by principal investigator prior to initiating screening procedures. Consent form in Thai language describing in detail the study procedures will be given to the participant privately in a separate room, and extensive discussion of risks and possible benefits of participation in this study will be provided. Participation will be informed that they may withdraw consent at any time throughout the course of this study.

Screening

After documentation of informed consent, principal investigator will interview the participant and perform vital signs and physical examination. The urinalysis will be performed by urinalysis test strip before the start of the study. Screening procedures are as follows;

- Pelvic exam
- Pelvic floor muscle strength measured as Brink scale
- POP-Q, Stress test
- Gynecologic ultrasonography with post-void residual volumes (PVR)
- Urinalysis by urinalysis test strip
- Bladder diary: consecutive three-day diary which the time of each micturition and the volume voided, fluid intake, incontinence episodes, episodes of urgency and sensation will be recorded, as might be the activities performed during or immediately preceding the involuntary loss of urine.[31]

After screening procedure, the appointment will be made for the next visit, which is one or two weeks after first visit.

Bladder diary

The study will incorporate the use of essential diagnostic tool, namely the bladder diary, to assess and monitor the outcomes. These assessments will be administered under the careful supervision of the study investigators. These diagnostic tests play a pivotal role in our research, serving as indispensable instruments for evaluating the progression and ultimate success of the treatment interventions.

II. Second Visit and Randomization

At one or two weeks after 1st visit, the following study procedure will be performed;

- Bladder diary interpretation
- ICIQ-FLUTS
- ICIQ-LUTSqol
- Vaginal pH
- Urethral maturation index (UMI)

After evaluating all the data collected during the screening process at the initial visit and combining it with the procedures conducted during the second visit, if the woman does not meet all study inclusion or if the woman has an exclusion criterion, she will not be enrolled into this study. A screening log will be kept of all who were evaluated for participation to document who is and is not enrolled and reason for not enrolling in this study.

Women who meet all the following criteria will participate the study.

Inclusion criteria

1. Presenting with storage phase symptom score more than/equal to 1 evaluated by the ICIQ-FLUTS questionnaire in these items
    - Item 2a) nocturia and/or
    - Item 3a) urgency and/or
    - Item 5a) daytime frequency and/or
    - Item 9a) UUI and/or
    - Item 11a) SUI
2. Being natural or surgical menopause for more than 1 year
3. Absence of urinary tract infection or other identifiable cause
4. Not using hormone replacement therapy or any route of estrogen within 4 weeks
5. Never undergone onabotulinumtoxinA therapy, PTNS, or neuromodulation for OAB treatment
6. Willing to adhere to the research protocol and actively participate in the scheduled follow-up appointments as delineated within the framework of this study

Exclusion criteria

1. Contraindication for estrogen therapy: undiagnosed abnormal vaginal bleeding, previous thromboembolic event, breast cancer, gynecologic/genitourinary malignancy, active liver disease
2. Pelvic organ prolapse of anterior compartment stage III and IV
3. Immunocompromised patient or taking immunosuppressant drug
4. History of antibiotics drug use within the past 7 days
5. History of bladder outlet obstruction
6. History of using anti-muscarinics, β3-adrenoceptor agonists, vaginal energy-based devices (laser and radiofrequency) or electromagnetic energy-based therapies within the past 2 weeks
7. History of documented positive urine culture in the past 6 weeks
8. Have an allergic reaction to study's drug
9. Any other significant finding that in the opinion of the investigator would increase the risk of having an adverse outcome from participating in this study

Women who meet inclusion criteria will be randomized with block randomization technique and a ratio of treatments of 1:1. The sequence generation will be performed under supervision of a senior statistician at the section of Clinical Epidemiology and Biostatistics in Ramathibodi Hospital.

The random sequences will be generated using Stata version 18. This is an automated process with no interfere from the investigators.

Upon a subject's successful enrollment in the clinical trial following eligibility assessment, principal investigator or research personnel shall provide participants with a comprehensive Thai guidebook for behavior modification which is designed to ensure the harmonization of knowledge and behaviors among all trial participants. This guidebook was developed and published by IUGA and will be distributed by research assistants.

Trial Treatment: Dosage Form, Regimen, Route of Administration

- **17β-Estradiol 10 mcg** (Femiest® Haupt Pharma Munster GMBH, Muenster, Germany (ESTRADIOL HEMIHYDRATE 0.0103 milligram, pH 6.94)

17β-Estradiol will be administered to group A treatment arm intravaginally. The assigned dosage will be one vaginal tablet daily for two weeks then one vaginal tablet twice a week for 10 weeks. Participants will be carefully monitored for any changes in clinical condition in each follow-up visits using logbook. It is important to clarify that we are not using 17β-Estradiol (Vagifem®) 25 mcg as the study drug. Although 17β-Estradiol 25 mcg is frequently mentioned in literature reviews and has been historically used for treating vaginal dryness, this drug is currently not available in Thailand. Therefore, we are using 17β-Estradiol (Femiest®) 10 mcg as an alternative for our study drug. Notably, 17β-Estradiol 10 mcg has been shown to provide a therapeutic dose equivalent to that of 17β-Estradiol 25 mcg for treatment, as stated by the FDA. This decision ensures that participants receive an effective and appropriate dosage while adhering to the availability of medications in our region.

- **Placebo**

Placebo will be administered to group B treatment arms intravaginally. The assigned dosage will be one vaginal tablet daily for two weeks then one vaginal tablet twice a week for 10 weeks. Participants will be carefully monitored for any changes in clinical condition in each follow-up visits. Placebo is produced by Chulalongkorn University Drug and Health Products Innovation & Promotion Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University. Details for presentation and packing are as follows;

Appearance	White, round, biconvex with "E" imprint one side
Weight	106 - 110 mg
Hardness	4.1 - 4.8 kP

Thickness	3.26 - 3.3 mm
Diameter	6.02 - 6.03 mm
Disintegration time	4 min 58sec - 7min 42sec
pH	7.18
Placebo contains	1. Agglomerated Lactose
	2. Microcrystalline cellulose PH 102
	3. Magnesium stearate

Both subject groups will be counseled on potential signs/symptoms of adverse events and post-marketing experience, such as breast pain, peripheral edema, and postmenopausal bleedings. Due to the vaginal administration and minimal systemic absorption, it is unlikely that any clinically relevant drug interactions will occur with 17β-Estradiol. However, interactions with other locally applied vaginal treatments will be considered and advised to the patients. If a dose is forgotten, participants will be advised that it should be taken as soon as he/she remembers. A double dose should be avoided.

Random allocation

Each participant in the study will receive a prescription for the research drug, an adequate supply to cover a 12-week treatment duration. This drug will be uniformly packaged within identical containers, featuring a unique alphanumeric code and detailed usage instructions on the label, thereby avoiding the inclusion of the original medication name. The data analysis will be performed by a statistician who will be the sole individual privy to the allocation of drug A or drug B to their respective groups. It is essential to note that the statistician will remain unaware of which of the two is the active drug and which is the placebo. Additionally, an independent statistician will maintain a sealed randomization list as a contingency. Allocation will be as follows;

Group A

17β-Estradiol 10 mcg (Femiest® Haupt Pharma Munster GMBH, Muenster, Germany (ESTRADIOL HEMIHYDRATE 0.0103 milligram)

Group B

Placebo

III. Third Visit

At four weeks after 2nd visit, the following study procedure will be performed;

- Bladder diary, the participant will be reminded to provide a three-day diary prior to schedule visit date.
- ICIQ-FLUTS
- ICIQ-LUTSqol
- PGI-I
- Vaginal pH
- Urethral maturation index (UMI)

Participants will also have clinical evaluation and vital signs measurements evaluated. Compensation for participants will be provided.

IV. Fourth and Final Visit

At eight weeks after 3rd visit, the following study procedure will be performed;

- Pelvic exam
- Pelvic floor muscle strength measured as Brink scale
- Bladder diary, the participant will be reminded to provide a three-day diary prior to schedule visit date.
- ICIQ-FLUTS
- ICIQ-LUTSqol
- PGI-I
- Vaginal pH
- Urethral maturation index (UMI)

Participants will also have clinical evaluation and vital signs measurements evaluated. Compensation for participants will be provided. Overall study procedures and flow diagram for study are outlined in topic No.8 Protocol flow chart.

7.5) <u>Sample Preparation, Processing, Handling, Storage</u>

- **Vaginal pH**

To assess pH, a piece of litmus paper (MQuant® pH-indicator stripe) will be placed on the lateral vaginal wall until moistened. A pH of 4.6 or greater indicates vulvovaginal atrophy

(VVA)[32]. According to a preliminary double-blinded study in 67 symptomatic postmenopausal women confirmed that atrophic vaginitis is associated with an increase in the lateral wall vaginal pH and this finding is paralleled by similar changes in pH in the urethra. Also, locally applied vaginal conjugated estrogen cream can normalizes the pH in the vagina and urethra Thus, the testing of the vaginal pH serves both as a surrogate for evaluating urethral pH and as a monitor of compliance with treatment.[33,34]

- **Urethral maturation index**

Cytohormonal urethral analysis serves as the definitive diagnostic approach for female urogenital atrophy in both clinical and research contexts, primarily owing to the pivotal role of estrogen in facilitating the maturation of the urethral epithelium. The urethral maturation index (UMI) emerges as a promising indicator of the estrogenic status within the body, derived from the ratio assessment of parabasal, intermediate, and superficial cells within a set of 100 cells. The interpretation of UMI values is closely associated with distinctive physiological conditions, wherein a decline in estrogen levels or the onset of menopause is characterized by elevated proportions of parabasal and intermediate cells compared to the reproductive age phase.[34]

Procedure: We will use wet cotton buds coated with NSS solution to gently swab the distal urethra for cytologic preparation. Smear slides will be prepared for cytologic evaluation by immediately immersing them in 95% alcohol for 24 hours and staining them with a Papanicolaou stain. The urethral epithelial cell will be examined under a microscope and classified into three types: parabasal cells, intermediate cells, and superficial cells by an independent cytologist.[35] Two hundred cells were counted at each high-power field which was randomly chosen. Six high-power fields were evaluated and the mean of the cell numbers was calculated.[36]

Maturation value (MV)[37]

MV = (% parabasal cells × 0) + (% intermediate cells × 0.5) + (% superficial cells × 1.0)

Interpretation: [34,38]

MV = 0-49 will indicate low estrogen effect

MV = 50-64 = moderate estrogen effect

MV = 65-100 = high estrogen effect

- **Urinalysis (dipstick test strip)**

The procedure commonly incorporates the utilization of Cybow^{TM} 10-urinalysis test strip, which are advanced diagnostic tools designed to analyze multiple urine parameters simultaneously. Initially, a fresh clean-voided midstream urine sample is collected and visually inspected for its

color, clarity, and odor. Subsequently, the Cybow strip is immersed into the urine specimen for a specific duration, enabling the strip's chemical pads to react with the components in the urine. These pads, strategically embedded with reagents, undergo color changes in response to the presence and concentration of specific analytes, including but not limited to pH, protein, glucose, ketones, nitrites, leukocytes, bilirubin, and urobilinogen. The strip is then carefully removed and placed on a flat surface, allowing for accurate color comparison with standardized color charts to quantify the concentration of each parameter. For this research purpose, exclusion of urinary tract infection is imperative for eligible criteria.

Interpretation: In the event of leukocytes displaying a concentration of one plus (1+) or higher, or a positive nitrite result, these findings could be indicative of a urinary tract infection (UTI). It is imperative to note that the presence of a UTI serves as an exclusion criterion during the evaluation process.[39]

7.6) Statistical analysis

Demographics for enrolled participants will be tabulated and described for each study group including age, ethnicity, occupation etc. All clinical and laboratory data related to objectives will be summarized and compared for each group. Appropriate statistics will be calculated to summarize and compare the range, means, medians, etc., and variability of numerical data. Confidence limits (95%) for means, geometric means and proportions will be calculated. Graphical summaries (e.g., box plots, histograms) will be used to describe and compare distributions of numeric variables. Frequency tables will be used to summarize distributions for discrete data. All clinical and laboratory data related to outcomes will be summarized and compared in the two regimens. T-tests and chi-square tests will be used to assess the statistical significance of differences in two means (possibly log-transformed) or proportions. Descriptive summaries of serious adverse events, subject discontinuations due to adverse events, and potentially clinically significant abnormal values (clinical laboratory or vital signs) will also be provided.

7.7) Study Subjects

Collect data and publicizing invitations to participate in research "Efficacy of Vaginal 17β-Estradiol on the Urinary Storage Symptoms in Postmenopausal Women: A Randomized Double-blind, Placebo-controlled Study" in Outpatient departments, Department of Obstetrics & Gynecology, Faculty of Medicine Ramathibodi Hospital

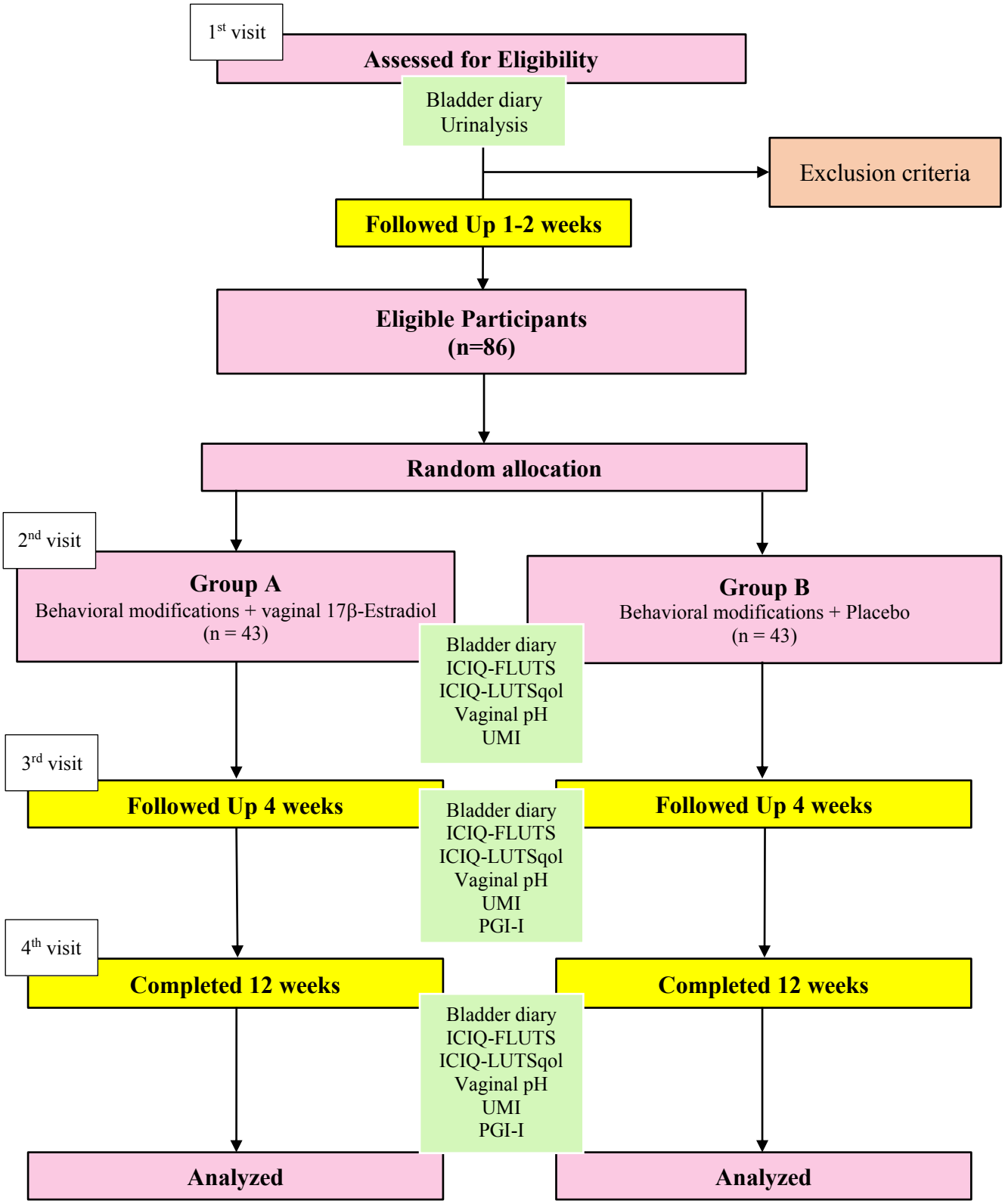
7.8) Research site

Outpatient departments, Department of Obstetrics & Gynecology, Faculty of Medicine Ramathibodi Hospital

7.9) Research plan

Activities	2023					2024												2025		
	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3
1. Literature review and proprosal preparation																				
2. Proposal submission and approval																				
3. Data collection and analysis																				
4. Manuscript writing and submission																				

8. Protocol Flow Chart



9. Sample size calculation and inclusion/exclusion criteria

- **Sample size calculation**

The sample size is calculated using two independent population proportions formula.

$$n_1 = \left[\frac{z_{1-\frac{\alpha}{2}} \sqrt{\bar{p}\bar{q}\left(1+\frac{1}{r}\right)} + z_{1-\beta}\sqrt{p_1 q_1 + \frac{p_2 q_2}{r}}}{\Delta} \right]^2$$

$$\Delta = p_1 - p_2, \quad \bar{p} = \frac{p_1 + p_2 r}{1+r}, \quad r = \frac{n_2}{n_1}$$

$$q_1 = 1 - p_1, \quad q_2 = 1 - p_2, \quad \bar{q} = 1 - \bar{p}$$

Proportion in group1 (p1) = 0.63

Proportion in group2 (p2) = 0.32

alpha(α) = 0.05 , Beta(β) = 0.2

According to Eriksen PS, Rasmussen H (1991)[14] , after 12 weeks, 62.8% of the women with urological symptoms underwent a change for the better in the estradiol (Vagifem®) group compared to 32.4% in the placebo group. ($P < 0.001$). With sample size (n) equal to 39 subjects per group and add drop out at rate of 10%, which is equal to 43 subjects per group. The total number of sample size will be 86 subjects.

- **Inclusion criteria**

1. Presenting with storage phase symptom score more than/equal to 1 evaluated by the ICIQ-FLUTS questionnaire in these items
    - Item 2a) nocturia and/or
    - Item 3a) urgency and/or
    - Item 5a) daytime frequency and/or
    - Item 9a) UUI and/or
    - Item 11a) SUI
2. Being natural or surgical menopause for more than 1 year
3. Absence of urinary tract infection or other identifiable cause
4. Not using hormone replacement therapy or any route of estrogen within 4 weeks
5. Never undergone onabotulinumtoxinA therapy, PTNS, or neuromodulation for OAB treatment
6. Willing to adhere to the research protocol and actively participate in the scheduled follow-up appointments as delineated within the framework of this study

- **Exclusion criteria**

1. Contraindication for estrogen therapy: undiagnosed abnormal vaginal bleeding, previous thromboembolic event, breast cancer, gynecologic/genitourinary malignancy, active liver disease
2. Pelvic organ prolapse of anterior compartment stage III and IV
3. Immunocompromised patient or taking immunosuppressant drug
4. History of antibiotics drug use within the past 7 days
5. History of bladder outlet obstruction
6. History of using anti-muscarinics, β3-adrenoceptor agonists, vaginal energy-based devices (laser and radiofrequency) or electromagnetic energy-based therapies within the past 2 weeks
7. History of documented positive urine culture in the past 6 weeks
8. Have an allergic reaction to study's drug
9. Any other significant finding that in the opinion of the investigator would increase the risk of having an adverse outcome from participating in this study

10. Duration of data collection

It will take about 12 months for data collection. analyses and manuscript writing. All steps will start after the IRB ethical approval. If the research continues beyond the specificized time or Ethics committee License expired, the investigators will request for renewal every year.

11. Risk of the study

There will be no physical, psychological or legal risks related to this study. Estradiol 10 mcg has a low dose and is safe. The adverse events observed with a higher frequency in patients treated with Estradiol 10 micrograms as compared to placebo and which are possibly related to treatment are presented as follows.[40]

stem organ class	Common ≥1/100 to <1/10	Uncommon ≥1/1,000 to <1/100	Rare ≥1/10,000 to <1/1,000
Infections and infestation		Vulvovaginal mycotic infection	
Nervous system disorders	Headache		
Gastrointestinal disorders	Abdominal pain	Nausea	
Reproductive system and breast disorders	Vaginal haemorrhage, vaginal discharge or vaginal discomfort		
Skin and subcutaneous tissue disorders		Rash	
Investigations		Weight increased	
Vascular disorders		Hot flush Hypertension	

During the course of the research, participants will be required to undergo pelvic examinations. This critical procedural step is imperative for the comprehensive evaluation of storage symptoms, aiming to assess the severity and progression of symptoms. The eligibility of research participants is also contingent upon the results derived from these pelvic assessments, ensuring the selection of suitable candidates and the appropriate alignment of the study's objectives with the participants' health profiles. Such meticulous monitoring and assessment not only uphold the integrity of the research but also prioritize the well-being and safety of the participants, reinforcing the ethical and scientific standards governing the study.

Distal urethral cell sampling utilizing a cotton bud will be conducted on the study participants. This non-invasive procedure is anticipated to last approximately three to five seconds and might induce mild discomfort. The sampling process is scheduled for the second, third and fourth visits, primarily to facilitate a comprehensive evaluation of the cellular composition in the distal urethra. Despite the potential discomfort, this meticulous sampling approach is instrumental in gathering crucial data, enabling a deeper understanding of the cellular dynamics and potential changes within the urethral microenvironment over the course of the study.

12. Benefit of the study

This research will be the foundation of knowledge in the future by using objective and subjective assessments results to determine the relationship between low dose vaginal estrogen and storage symptoms of LUTS in postmenopausal women. This study will also increase efficiency in the care of patients with overactive bladder or storage symptoms of LUTS, especially in postmenopausal women, by applying the knowledge gained from this research to provide patients with the best and most appropriate treatment, supported by research outcome and information from this research as well.

13. Ethic consideration

1. *Respect for person*

Participants will receive all information about the research from an information sheet and will be asked to consent for the research, giving them time to make their own decisions. Then sign it in writing and have the right to refuse to participate in the research at any time. And the information in the research will not be personally identifiable. Instead, it will be assigned a code for research to maintain patient confidentiality.

2. *Beneficence/Non-maleficence*

Treatment of the participants in this study is standard treatment following steps from a safe and easy method for the patients first, namely receiving advice on lifestyle modification that causes

storage symptoms of LUTS. The intervention in the study has been certified by the Thai FDA, which contains a low dosage and fewer side effects than other drugs used to treat storage symptoms of LUTS.

3. Justice

Research has clear inclusion and exclusion criteria. All patients have an equal chance of receiving treatment using a systematic random sampling method. There is no discrimination against any individual resulting in equal benefits.

14. Compensation to research participants in the event of harm or adverse effects on the subject.

The research participants will receive a compensation of 200 THB during the initial visit, which is intended to cover the time and effort invested in the informed consent process and preliminary screening procedures. Following this, during the second visit, once the participant qualifies for enrollment in the trial, an additional compensation of 300 THB will be provided. Subsequently, for both the third and final visits, each participant will be remunerated with 300 THB for every scheduled appointment. These monetary reimbursements not only acknowledge the participants' valuable contribution to the study but also serve to offset any inconveniences or expenses associated with their active involvement in the research. Such compensation strategies are designed to uphold ethical considerations and ensure that the participants' dedication and engagement in the research are duly recognized and appreciated.

Medical care in case of research-related injury or serious event on either an emergency or routine basis will be provided free of charge according to the local standard of care by qualified medical personnel at Ramathibodi Hospital.

15. Conflict of Interests and Budget

No conflict of interest is anticipated, as this research study does not engage private enterprises. The entire budget allocation is projected to be sourced through a funding application submitted to the research grant provided by the Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

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