Study Protocol: NCT05551949

Study title: Mechanisms of Successful Vaginal Estrogen Prophylaxis for Postmenopausal Women With Recurrent Urinary Tract Infections: Urogenital Microbiota and Host Immune Responses

PI: Victoria Handa, MD

Date of this final protocol: 2/13/24

JHM IRB - eForm A – Protocol

- **Use the section headings to write the JHMIRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.**
- **When submitting JHM IRB eForm A (new or revised), enter the date submitted to the field at the top of JHM IRB eForm A.**

****

1. Abstract

Postmenopausal women are disproportionately impacted by urinary tract infection (UTI), particularly chronic recurrent UTI (rUTI), defined as UTIs that recur ≥2 times per 6 months. Presently, the only evidence-based prevention options are daily low dose antibiotics and topical vaginal estrogen therapy (VET). Given concerns related to chronic antibiotic use, VET is preferred for postmenopausal women. Unfortunately, 50% of women experience recurrence despite VET. It is unknown why some women benefit from VET while others do not. Also, the mechanism by which VET, when effective, prevents rUTI is uncertain. This study seeks to identify mechanisms that explain how VET reduces rUTI among postmenopausal women for whom VET is a preferred therapy.

The purported mechanism is via changes to the vaginal microbiome, possibly related to restoration of Lactobacilli. This may be due to production of D-Lactic acid, which suppresses vaginal growth of uropathogens such as E. coli. (This increased production of lactic acid is reflected by vaginal pH, which is typically reduced with VET use.) It has also been suggested that the beneficial effects of VET are mediated by changes in the *urinary* microbiome. Finally, recent studies (mostly in mice) suggest that host immune responses in the bladder and urogenital tract are critical to rUTI susceptibility, symptoms, and outcomes.

To investigate the mechanisms by which VET reduces rUTI, we will conduct a single-arm, open-label clinical trial of conventional therapy with vaginal estrogen therapy for postmenopausal women with rUTI. These are women who would be recommended (as a part of routine clinical care) to receive VET for rUTI prevention. Among 50 women with confirmed rUTI, we will investigate (a) the effects of vaginal estrogen therapy on the bladder and vaginal microbiota (as well as D- and L-lactic acid concentrations in the vagina) and (b) the effects of vaginal estrogen therapy on immune responses in both compartments (including soluble mediators of inflammation (SMI) associated with neutrophil and macrophage activity and those associated with Th1, Th2 and Th17 pathways). Over 24 weeks, we will then identify the subset of participants who continue to experience rUTI versus those who respond to vaginal estrogen therapy. Comparing these two groups, we will consider (c) the extent to which changes in microbiota and immune responses are associated with successful UTI prevention.

2. Objectives (include all primary and secondary objectives)

The three aims of this proposal are:

1. **To compare the vaginal and urinary microbiota before and after VET in women with rUTI.** *Hypothesis: After VET, vaginal and urinary microbiota will exhibit*

significantly increased relative abundance of key Lactobacillus spp. (L. crispatus, L. gasseri, L. jensenii, L. iners). Vaginal D-lactic acid concentration will increase and pH will decrease.

2. **To compare host immune responses, by measuring vaginal and urinary soluble mediators of inflammation (SMIs), before and after VET in women with rUTI.** *Hypothesis: after VET, vaginal and urinary samples will show decreased SMIs associated with neutrophil and macrophage activity and Th2-associated cytokines, with concomitant increases in Th1 and Th17 related pathways.*

3. **To acquire preliminary data regarding vaginal and urinary markers that distinguish women who remain UTI-free after VET versus those who continue to experience UTI recurrence**

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Each year in the U.S., urinary tract infection (UTI) is the cause of 8 million ambulatory and 1 million ED visits. Postmenopausal women are disproportionately impacted: 13% of female Medicare beneficiaries experience ≥1 UTI annually. Futhermore, among older women who experience UTI, >40% experience chronic recurrent UTI (rUTI) (defined as UTIs that recur ≥2 times per 6 months or ≥3 per 12 months). Prevention of UTI recurrence is crucial for older women with rUTI, yet effective options are limited. **American Urologic Association guidelines recommend two options to reduce recurrence: vaginal estrogen therapy (VET) or daily low dose antibiotics. While daily antibiotic prophylaxis is effective, concerns regarding side effects and emergence of antibiotic resistance limit its use. Thus, VET prophylaxis is first line for rUTI prevention in postmenopausal women.** Several clinical trials demonstrate that 50% of women will continue to experience rUTI recurrence despite VET. At present, it is unknown why some women benefit while others do not and the mechanisms for treatment success are unknown.

The first aim of this protocol is to investigate the effect of VET on urogenital microbiota. The rationale is that changes to the vaginal microbiome are purportedly critical to rUTI susceptibility. The vagina is a reservoir for uropathogens (e.g. E. coli). Vaginal lactobacilli may protect against rUTI through a variety of mechanisms, including displacement of uropathogens by competitive binding and direct inhibition of bacterial growth, likely through the bactericidal action of lactic acid. There may be other important functional parameters of the microbiome that influence UTI risk (e.g., features that can be best understood through metabolomics, proteomics, etc.).

Measures for Aim 1 are as follows:

Vaginal microbiota: After menopause, vaginal colonization by lactobacilli declines. There is some evidence that VET restores vaginal Lactobacilli. Prospective studies linking a lactobacillus-dominant microbiome to UTI prevention are lacking. Also, new data from studies using contemporary molecular techniques (e.g., 16S rRNA gene amplicon sequencing, shot gun or whole genome metagenomics, RNA-seq) suggest that some Lactobacillus species (and certain vaginal “community state types”) may be more likely that others to reduce UTI recurrences.

Vaginal D-Lactic acid and pH: Differences in the vaginal microbiota may be linked to production of d-lactic acid. This isomer is produced more effectively by certain lactobacillus species (most notably *L. crispatus*). Therefore, for aim 1, we propose to

compare the vaginal microbiota and d-lactic acid concentrations before and after 12 weeks of VET among women with rUTI. Vaginal pH will also be examined, as this bedside test may prove to be a valuable (bedside) diagnostic test, reflecting d-Lactic acid and other markers. Vaginal pH >5 is typical for menopause and a restoration of acidic pH provides additional evidence of adequate estrogenization.

In addition, we hope to expand our analyses to complement these microbiota analyses with additional functional (e.g., metabolomic, proteomic) analyses.

Vaginal estrogen status: The vaginal maturation index (VMI) is a measure of vaginal "estrogenization", which we will collect at enrollment and after 12 weeks of VET. This is based on a cytologic examination of vaginal epithelial cells, obtained via a swab of the vaginal wall. The VMI is a widely used objective and quantitative measure of vaginal estrogen status. Collection of this marker will allow us to establish how these conventional measures of estrogenization compare to other study outcomes, such as changes to urogenital microbiota and immune response.

Urinary microbiota: In addition, there is some evidence that the composition of urinary microbiota may influence rUTI. For example, a preoperative urinary microbiome deficient in lactobacilli and high in uropathogens has been associated with postoperative UTI. For aim 1 we also plan to compare the urinary microbiota before and after 12 weeks of VET among women with rUTI.

The second aim of this research is to investigate the effect of VET on the localized immune response in the vagina and bladder. Finally, there is evidence (primarily from animal models) that the host immune response is critical to rUTI susceptibility. For example, bladder invasion by uropathogens is 10–12 fold greater in Toll like receptor 4 mutant mice versus wild type. Also, in a murine model, severe acute immunopathology (with elevated IL-6 and IL-8 in response to UTI), predicts chronic cystitis and rUTI susceptibility. Also, importance of Th1 versus Th2 pathway activation is demonstrated by IFN-ɣ knockout mice (unable to mount a Th1 response): these mice have an increased incidence and severity of UTI, are vulnerable to re-infection, and are less able to clear bacteria when re-challenged after initial infection. In contrast IL-4 knockout mice (Th2 impaired, Th1 uninhibited), appear to be protected from re-infection. Given the apparent importance of the host immune response to UTI susceptibility, it is relevant that some aspects of immune response may be modified by estrogen. For example, consider gender differences in UTI susceptibility: among mice infected via transurethral catheter, males develop higher bacterial burdens than females, possibly because males lack a robust increase in inflammatory cytokines (notably IL-17). Additional evidence of estrogen's protective effect comes from ovariectomized mice, which show increased bacteriuria, IBC's, and pro-inflammatory (Th2 associated) IL-6 compared with sham-operated females. Human data are limited, although it has been shown that serum IL-6 increases with menopause and decreases with systemic estrogen therapy. Also, in non-rUTI populations vaginal levels of the pro-inflammatory cytokine TNF-α decreases with VET.

Therefore, for aim 2, we plan to compare vaginal and urinary fluid from before versus after 12 weeks of VET for soluble mediators of inflammation (SMIs), including those associated with neutrophil and macrophage activity and those associated with Th1, Th2 and Th17 pathways.

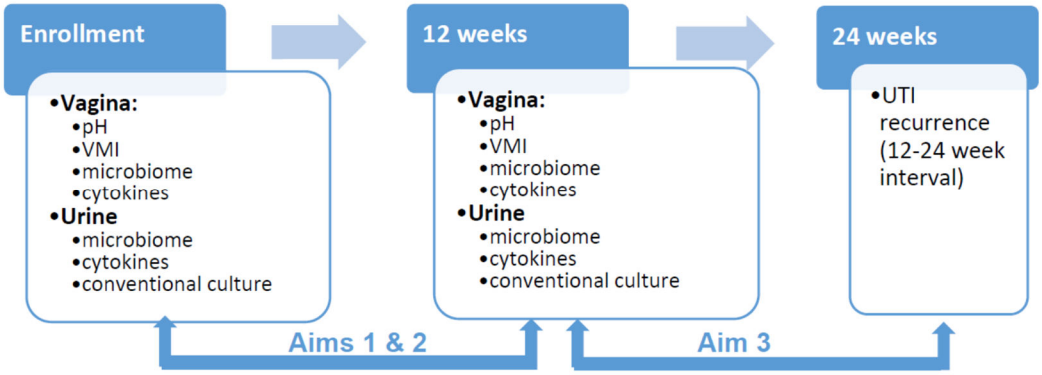
Aims 1 and 2 will identify "biomarkers" that are significantly impacted by VET. **The goal of Aim 3 is to compare these biomarkers between women who continue to experience rUTI versus those who remain UTI-free on VET.** These comparisons will provide preliminary data to support a future RCT of VET in women with rUTI. Specifically, those bio-markers which show the greatest promise to distinguish successful rUTI

prevention will then be rigorously evaluated in a future larger and adequately powered clinical trial to definitively identify the predictors of and the biological conditions associated with successful prevention of UTI recurrence among susceptible women.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

All three aims of this proposal will be accomplished via a **single-arm**, **open label clinical trial** of transvaginal estrogen to reduce UTI recurrence among menopausal women with confirmed rUTI (see figure below). Briefly, 24 weeks of vaginal estrogen treatment will be administered to 50 eligible postmenopausal women with a history of rUTI. Aims 1 and 2 will be accomplished after 12 weeks of VET (because 12 weeks may be required for adequate estrogenization. Aim 3 will be accomplished during the second 12-week interval of VET.



There is only one arm in this trial -- all participants will receive VET (which meets the standard of care for therapy in women with rUTI). A placebo arm is not considered because it would not be relevant to our scientific aims. A strength of the study design is that for Aim 1-2 and Aim 4 participants are compared to themselves, minimizing the effect of potential confounders. For Aim 3, the operative question is what distinguishes women who respond to VET vs. those who do not. These scientific reasons justify the single arm study design. This study meets the NIH definition for “Clinical Trials”, defined as a clinical research study (a) in which human participants are assigned to an intervention, (b) the study evaluates the effect(s) of the intervention on the participants, and (c) the effect being evaluated is a health-related biomedical outcome.

The vaginal estrogen treatment (VET) to be administered in this study is a conventional treatment that is commercially available. In general, VET may be administered as a cream, vaginal tablet or silicone ring. This study will use vaginal estradiol tablets (10mcg), administered daily for two weeks, followed by twice weekly.

For those who enroll, study procedures will include the collection of urinary and vaginal specimens (see below) at enrollment (before VET initiation) and after 12 weeks of therapy. The primary difference between this study and our routine clinical care is specimen collection. We will also track rUTI episodes during the first 24 weeks of VET (but the evaluation and treatment of those episodes will follow best practices in clinical care and will not be impacted by participation in this study).

b. If your study involves data/biospecimens from participants enrolled under other research studies with a written consent or under a waiver of consent, please list the IRB application numbers for those studies. Please note: Certificate of Confidentiality (CoC) protections applied to the data in source studies funded by NIH or CDC will extend to this new study if the funding was active in 2016. If this situation applies, Section 36, question 6 in the application will need to be answered "Yes" and "Hopkins Faculty" should be selected in question 7. No other documents are required.

Not applicable.

c. Study duration and number of study visits required of research participants.

The study will last 24 weeks. Study procedures will be timed to coincide with routine clinical visits (baseline, 12 weeks, 24 weeks). Participation in this study will not require additional visits (outside of what is required for clinical care).

At baseline, women will be screened for eligibility at a routine clinical visit. Those found to be eligible will be consented and enrolled. Women who initiate VET for UTI prevention are typically seen for clinical care 3 and 6 months after VET initiation (e.g., 12 and 24 weeks). For this proposed research, we will collect study specimens at the visit prior to VET initiation and at the 12-week follow up visits. No specimens will be collected at the 24 week visit.

d. Blinding, including justification for blinding or not blinding the trial, if applicable.

Not applicable. All participants and investigators will be aware of the study treatment (vaginal estradiol tablets).

e. Justification of why participants will not receive routine care or will have current therapy stopped.

Participants will receive the same VET regimen that would be prescribed as part of clinical care, including standard dosing. VET complies with best practices for rUTI prevention, as recommended by the American Urologic Association.

f. Justification for inclusion of a placebo or non-treatment group.

A placebo arm is not considered because it would not be relevant to our specific aims. The rationale is discussed above (see 4a).

g. Definition of treatment failure or participant removal criteria.

When VET is recommended in clinical practice, a 6-month trial is typical (to assess response). This study is limited to that 6-month period. Of course, some women may elect to stop VET during the observation period, possibly due to intolerance or dissatisfaction with the treatment. (Similarly, women receiving VET in clinical practice may elect to stop this Rx.) Participation in this study will not impact a decision to stop VET before the 6-month period is completed. Women who elect to stop VET will be encouraged to continue in the trial (to allow data collection as proposed).

h. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

At the conclusion of this trial, women will have the opportunity to continue VET as part of their clinical care. They will be informed that their prescription insurance coverage may or may not cover this treatment. Those who are not satisfied with VET will be offered alternative prevention options (such as daily antibiotic suppression).

i. If biological materials are involved, please describe all the experimental procedures and analyses in which they will be used.

Study procedures will be the same for the enrollment and subsequent visits. All study specimens will be labeled with a sample ID barcode, the visit type (enrollment, 12-week) and the study ID assigned to the participant.

1. Vaginal pH: A mid-vaginal swab will be used to measure pH using Nitrazine pH test paper.
2. Vaginal Maturation Index (VMI): A spatula will be used to collect a specimen from the lateral vaginal wall. The tip of the spatula will be placed in a SurePath Pap Collection vial and transported to the Pathology department for processing
3. Specimens to be frozen* and stored for later analysis:
    i. Vaginal microbiota: a mid-vaginal swab will be obtained and placed in 1ml stabilizing buffer solution.
    ii. Vaginal SMI and D/L lactic acid: a mid-vaginal swab will be obtained and stored dry.
    iii. Urinary microbiota and SMI: A urine specimen will be obtained with a 14F catheter. 10cc will be obtained and aliquoted (4ml reserved for microbiota and 2ml for SMI analysis). Note: We do not expect a *clinical* indication for urinary catheterization at the 24 week visit. Therefore, a catheterized urine specimen will not be collected at 24 weeks unless clinically indicated.

Aim 1:
MICROBIOTA ANALYSIS OF VAGINAL AND URINARY SPECIMENS:
The vaginal and urinary microbiota will be characterized through molecular techniques (e.g., sequencing amplicons of the V3-V4 region of the bacterial 16S rRNA gene, shot gun or whole genome metagenomics, RNA-seq, functional analyses including metabolomics and proteomic analyses).

Vaginal D- and L-lactic acid assays: Measuring vaginal L- and D-isomers separately allows us to assess the association between VET and the concentration of these individual isomers. ***Vaginal "estrogenization"***: This will be assessed with the Vaginal Maturation Index, a standard cytologic measure of epithelial cellular maturation.

Aim 2:

SOLUBLE MEDIATORS OF IMMUNITY

Concentrations of soluble biomarkers will be determined. We currently plan to use the fluorescent bead based Luminex multiplex system, but specific methods may be modified according to laboratory standards.

5. Inclusion/Exclusion Criteria

Eligibility: Participants in this study will be postmenopausal women (menopausal for at least 1 year) with a minimum age of 55 years. Participants will have documentation of recurrent UTI, defined as follows

- History of treatment for at least 3 UTIs in the past year or 2 episodes within 6 months AND
- At least one positive urine culture during an acute symptomatic episode.

Exclusion criteria:

- Women receiving antibiotic prophylaxis to prevent UTI recurrence;
- Women with contraindications to vaginal estrogen (as indicated on the FDA-mandated package insert) and those who have used vaginal or systemic estrogen within the past 6 months;
- Women with an active UTI and those who have received antibiotics within the prior 2 weeks;
- Women with complicated rUTI, defined by immune compromise, anatomic or functional abnormalities of the urinary tract, indwelling catheterization, those performing self-catheterization, and those with neurological disease or illness relevant to the lower urinary tract;
- Women with only asymptomatic bacteriuria (rather than recurrent symptomatic UTI)
- Women who have had UTI diagnosed only in the setting of institutionalization, including hospitalization
- Women with a blood clotting disorder (e.g., Factor V Leiden, prothrombotic mutation, protein C, protein S or antithrombin deficiency)

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.
b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.
c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

The vaginal estrogen therapy to be used in this study is vaginal estradiol tablets (10mcg), administered daily for two weeks, followed by twice weekly. This product is FDA approved and marketed for treatment of menopausal vaginal atrophy and are commonly used in post-menopausal women for a variety of related indications.

7. Study Statistics

a. Primary outcome variable.
b. Secondary outcome variables.
c. Statistical plan including sample size justification and interim data analysis.
d. Early stopping rules.

For our first aim (to compare the vaginal and urinary microbiota before and after VET in women with rUTI), the primary outcome will be the relative abundance of Lactobacilli in the vagina and in urine. We hypothesize that after VET, the vaginal and urinary microbiota will exhibit significantly increased relative abundance of Lactobacillus and of key Lactobacillus spp. (L. crispatus, L. gasseri, L. jensenii, L. iners). Separately for each taxon and species, we will determine whether the pre-to-post change in relative abundance is significantly different from zero by paired t-test and Wilcoxon signed-rank sum test where appropriate. We will investigate the effect of VET on vaginal and urinary microbiota taxa separately. We will repeat these analyses using absolute taxa abundances estimated by pan-bacterial 16S qPCR, with a focus on key lactobacilli (e.g. *L. crispatus* and *L. iners*), and, where possible, for other bacteria of interest, including those responsible for most community-acquired UTI (*Escherichia*, *Klebsiella* and other uropathogens).

Related to the primary aim is the concentration of lactic acid in the vagina, which reflects synthesis by Lactobacilli. Therefore, we will also compare D-lactic acid concentrations in the vagina before and after VET in women with rUTI. We hypothesize that in the vagina, D-lactic acid concentration will increase. We will use similar statistical methods (as described above for aim 1) for this additional comparison.

Our second aim is to compare host immune responses, by measuring vaginal and urinary soluble mediators of inflammation (SMIs), before and after VET in women with rUTI. The specific SMIs of interest are shown in the table that follows:

Th1 associated (IFN-ɣ, IL-12)	Th2 associated (IL-4, IL-6, IL-10)
Th17 associated (IL-17, IL-23)	Inflammation/macrophage activating (TNF-α, IL-8, IL-1β)

We hypothesize that after VET, vaginal and urinary samples will show decreased SMIs associated with neutrophil and macrophage activity and Th2-associated cytokines, with concomitant increases in Th1 and Th17 related pathways. Separately for each SMI, we will test whether these pre-to-post VET changes are significantly different from zero using paired t-tests or Wilcoxon signed-rank sum tests as appropriate. SMI values will be natural log-transformed. SMI values below the lower limit of detection will be imputed using the lower limit of detection of the laboratory assay divided by the square root of 2. As the SMI expression in the vagina and bladder are not well-characterized, we will also perform descriptive and exploratory analyses of the distribution of each SMI, by pre-post status, with percentile plots and generalized gamma models using graphical approaches. Use of the generalized gamma models will facilitate a more complete exploration of these highly regulated SMI markers.

All of these outcomes will then be compared to observed rates of UTI recurrence in the study population. This comparison will provide preliminary data to support a future RCT of VET in women with rUTI. For this analysis, observed UTI events will be considered as a function of “biomarkers”, including vaginal and urinary microbiota and SMIs, as well as

vaginal lactic acid. We will compare women who remain UTI free versus those who experience recurrence with respect to differences in microbiota taxa abundances (relative and absolute) and SMI concentrations. These comparisons will be performed with t-tests, Mann-Whitney test, or Chi-squared tests as appropriate.

We will recruit 50 women for this trial. With loss to follow-up, we conservatively estimate that 40-45 women will provide complete data. At 80% power and alpha of 0.05, we anticipate that we will have sufficient power to detect a 17% to 19% change in *Lactobacillus spp* relative abundance in the vaginal microbiota pre-to-post VET. Prior research suggests that we are likely to observe much larger changes in vaginal samples (>30%) with VET. Furthermore, with a sample size of 40 to 50 women, we will be able to estimate the mean change in *Lactobacillus spp* relative abundance from pre-to-post VET to within ±11.6% to ±12.9% with 95% confidence interval.

We are not planning interim analyses. Early stopping rules do not apply.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.
b. Steps taken to minimize the risks.
c. Plan for reporting unanticipated problems or study deviations.
d. Legal risks such as the risks that would be associated with breach of confidentiality.
e. Financial risks to the participants.

Participation in this study is felt to represent minimal risk to participants. All women enrolled in this trial will be receiving a medication that is commonly prescribed in clinical practice. This medication (vaginal estrogen) is felt to be safe and effective in the treatment of postmenopausal vaginal atrophy and is currently recommended by the American Urologic Association as a best practice for preventing recurrent UTI.

The collection of specimens (vaginal swabs and urine specimens) represents minimal harm to participants. Such specimens are often collected for clinical care in the setting of the evaluation and management of recurrent UTI.

To protect participant privacy and confidentiality, data management will be via Red Cap and CRMS. All collected specimens will be labeled with the participant's study number. The log that links each participant to the assigned study number will be kept electronically in CRMS.

Review of the EMR will be required to gather data regarding patients' clinical course and observed UTI recurrences. Data from chart abstraction will be collected via RedCap. Only IRB-approved members of the study team will have access to these data. No identifiable information will be stored on private devices or directly on computers or other electronic devices."Paper documents containing patient information (such as signed consent documents) will be uploaded electronically but the original hard copies will also be retained in accordance with institutional policy."

9. Benefits

a. Description of the probable benefits for the participant and for society.

The participants will receive study medications at no cost. This might represent a significant savings to some participants, as out of pocket costs for VET may be up to $150 per month.

We anticipate no additional direct benefit to the participants, other than personal satisfaction in knowing that they are helping with biomedical research that can potentially improve the health of other women.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants will receive remuneration to compensate them for the time and effort required for compliance and completion of study events. Specifically, we plan to offer each participant $100 to compensate her for participating in this study. Each participant will receive $50 at the end of the first 12 weeks of the study and a second $50 payment at the conclusion of the study. Women who cannot complete the study (such as due to medication intolerance) will be paid when they drop out of the study, assuming they return for specimen collection at that interval.

In addition, study medications (24 weeks of vaginal estrogen therapy) will be provided at no cost to the participants.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

The participants will receive study medications at no cost.

12. Transfer of Materials

Transfer of biospecimens from Johns Hopkins to another organization for research purposes and receipt of biospecimens from an outside organization for your research must adhere to JHU policies for material transfer (https://ventures.jhu.edu/faculty-inventors/forms-policies/) and biospecimen transfer (https://hpo.johnshopkins.edu/enterprise/policies/176/39187/policy_39187.pdf?_=0.622324232879).

Please complete this section if your research involves transfer or receipt of biospecimens.

a. Will you **receive** biospecimens from an external entity for this research? [Yes/No].
If "Yes", please confirm you will secure an MTA/research agreement from the appropriate office (JHTV/ORA) prior to transfer.
See: https://ventures.jhu.edu/technology-transfer/material-transfer-agreements/ .

We will not receive biospecimens for this research.

b. Will you **transfer** biospecimens to an external entity as part of this research? [Yes/No]

If "Yes", please address each of the following:

1) Describe the nature of the research collaboration with the external entity and the rationale for the transfer. (Include an explanation of your intellectual contribution to the design of the research study, resulting data and sharing, and participation in the planned publications.)

   We plan to transfer biospecimens for this research. Specifically, the microbiome and lactic acid analyses will be performed in the laboratory of Dr. Rebecca Brotman at the University of Maryland School of Medicine. Specifically, she will supervise these analyses in the Microbiome Service Lab of the Institute for Genome Sciences. Dr. Brotman's laboratory will receive the (de-identified) vaginal and urinary specimens and will perform the microbiome analyses. She will then return data to our Johns Hopkins team regarding microbiome analyses. She will not receive data from Johns Hopkins. Dr. Brotman will be included as a co-author on publications and other scholarly products from this work.

   Until we reach the desired sample size for this research, specimens will be frozen and stored at Johns Hopkins Bayview campus in a facility maintained by the division of Infectious Disease.

2) Please confirm you will secure an MTA through the appropriate office (JHTV or ORA) prior to transfer.
   (See: https://ventures.jhu.edu/technology-transfer/material-transfer-agreements/.)

   A material transfer agreement will be obtained prior to transfer.

3) If the biospecimens you intend to transfer were obtained through clinical or research procedures at Johns Hopkins and "Other" is selected in Item 4, Section 23, please submit the following items in that Section:

   a. A pdf version of a completed JHTV Online "Material Transfer Agreement Request Form for Outbound Material" https://ventures.jhu.edu/technology-transfer/material-transfer-agreements/ **OR** a copy of the COEUS PD (Proposal Development Summary).
   b. A completed Biospecimen Transfer Information Sheet https://www.hopkinsmedicine.org/institutional_review_board/forms/.
   c. A signed and dated "De-identified Human Subject Certification" https://livejohnshopkins-my.sharepoint.com/:w:/g/personal/sdamare1_jh_edu/ETANthXrGPVBmYs-_UC59fUBu9b1An7tYUWh4GjiG2fH4Q?rtime=5kjTd-F410g
   d. Approval documents from recipient site, if applicable.
   e. Copies of the consent forms associated with the IRB protocols under which the biospecimens were collected, with language appropriate to this transfer highlighted.
   f. The name of the specialist you are working with in ORA to complete a contract/MTA.

Please see the following website for more information about transferring human biospecimens to outside entities:
https://www.hopkinsmedicine.org/institutional_review_board/news/announcement_transfer_human_biospecimens_outside_entities.html/ .