Treating Where it Hurts: A randomized blinded clinical trial of local estrogen to the vulvar vestibule for dyspareunia in postmenopausal women

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1. Protocol Title

Treating Where it Hurts: A randomized blinded clinical trial of local estrogen to the vulvar vestibule for dyspareunia in postmenopausal women

2. Objectives

Purpose:
To perform a pilot study to evaluate the efficacy and safety of local estrogen therapy applied to the vulvar vestibule in a cohort of postmenopausal women with moderate or severe dyspareunia, and to examine the microscopic features of this condition before and after local estrogen therapy.

Primary goals:

1. To compare serum levels of β-estradiol before and during paired dosing of the hormone to the vulvar vestibule nightly.
2. To assess the comparative treatment efficacy of two strengths of β-estradiol dosed at an increased frequency to the vulvar vestibule.
3. To assess and characterize the microscopic features of tender vulvar vestibule tissues in postmenopausal women who have moderate to severe dyspareunia acquired since menopause.
4. To assess whether β-estradiol cream applied nightly for three months to the vulvar vestibule normalizes the microscopic profile of vestibule findings in parallel with correction of dyspareunia.

Hypotheses:

1. Topical estrogen to the vulvar vestibule is safe
    a. β-estradiol serum levels associated with nightly estrogen applications to the vulvar vestibule will not be significantly different than levels published in studies of vaginal applications.
2. This condition is a form of vestibulodynia and will regress with topical estrogen therapy
    a. For postmenopausal women with significant coital pain, vestibule biopsies will show neural hyperplasia and an infiltrate of lymphocytes, hallmarks of localized provoked vulvodynia, and doses of topical β-estradiol will be effective when applied to the zone of tenderness, the vestibule.
3. Nightly therapy to the vestibule will be more efficacious than published results of standard semi-weekly vaginal therapy.
    a. Nightly therapy will effect a correction of moderate/severe dyspareunia in greater than 62% of subjects, a published rate of correction using the standard treatment frequency to the vagina.

Primary endpoints:

1. Serum levels of β-estradiol before and during treatment with two strengths of cream applied locally to the vestibule.
2. Measurements of endometrial thicknesses in subjects with a uterus at study end, comparing results to baseline measurements.
3. Changes in tissue lymphocyte densities and neuronal hyperplasia comparing baseline to study end.
4. Levels of dyspareunia measured by the Numerical Rating Scale (NRS) during regimens of nightly estradiol therapy applied to the vulvar vestibule, determining how soon and how uniformly this schedule corrects painful penetration in study subjects.

Secondary endpoints:

1. Pain descriptors as measured by the modified Vulvar Pain Assessment Questionnaire–screen (VPAQ-screen) (Dargie, 2016)
2. Sexual function evaluated using the validated but modified VPAQ-Screen.
3. Sexual distress as measured by the (VPAQ-Screen).
4. Lower Urinary Tract symptoms (LUTS) of menopause as measured by a set of validated questions.
5. Vestibular tenderness as measured clinically by the cotton swab touch test and the lidocaine test.
6. Vaginal and vestibular atrophy as measured by visual examination and pH of the vaginal secretions.
7. Characterization of the urogenital microbiome via swabs collected from the vestibule and vagina.

3. Background

Pain with sex is a common problem experienced by women after the age of menopause, and it is commonly termed "vulvovaginal atrophy" or "vaginal dryness" by most clinicians (ACOG, 2008). Genitourinary Syndrome of Menopause (GSM) is the new term put forth by two professional societies (Portman, 2014). Vulvar complaints develop in association with loss of ovarian estrogens, and the prevalence of sexual pain in women over 50 is 50% (ACOG, 2008). Symptoms are chronic and tend to worsen over time (Gass, 2013). The relationship of vulvar symptoms to sexual difficulties is well established (Simon, 2014). However, the research into this problem is variable in its focus, and there are many gaps.

For mild symptoms, sexual lubricants have proven to be of help. For significant vulvovaginal symptoms, estrogen therapy has been found to be the most effective therapy according to the Cochrane review of 2006 (Suckling, 2006). But even in well-designed studies of symptomatic women, the array of symptom complaints is broad and includes annoying rather than significant symptoms. Studies that include many women with mild complaints like "dryness" or 'itching,' are not fully representative of women with actual intimacy pain. Allodynia is pain from touch that normally would not hurt, and therefore it is an appropriate term with which to characterize dyspareunia.

Nastri, in her 2013 Cochrane review of estrogen and sexual function in menopause, found that no study of estrogen therapy was focused solely on women with sexual dysfunction in postmenopause. Only five of 27 studies evaluated sexual function as a primary outcome among other menopause symptoms. In those women, estrogen had only a "small to moderate" benefit (Nastri, 2013). One multi-country study of 4100 women has documented that 38% of those using estrogen products found them to be inadequate to correct genital pain (Nappi, 2013). Kao found that the mean pain score in postmenopausal women with penetration pain was 7 on a scale of 0-10 (SD 2.5) and 30% of her cohort were using estrogen products (Kao, 2014). General population studies indicate that a significant proportion of older women value their continued sexual activity (Schneidewind-Skibbe 2008).

Survivors of breast cancer are warned not to use estrogen products, the only effective therapy for the dyspareunia resulting from estrogen loss. Our previous randomized controlled trial (RCT) in this population explored the question of the location of this pain condition, finding it to be isolated to the vestibule in 90% of cases (Goetsch, 2014). The project that we are now submitting follows on our RCT findings because we believe the findings are relevant beyond the population of cancer survivors and pertain to postmenopausal dyspareunia generally. After ascertaining that the location of pain was the vulvar vestibule in women with severe atrophy, we found that on examination, pain evoked by light touch could be extinguished by application of 4% aqueous lidocaine (Goetsch, 2014). In order to judge whether therapy to the vulvar vestibule could prevent coital pain, the same group of subjects was taught home use of topical lidocaine applied to the vestibule prior to intimacy. Half of the group had previously abandoned having intercourse, but once they learned to pre-treat the vulvar vestibule for 3 minutes before touch, 95% of the study patients could have comfortable intercourse as often as they desired without having changed the atrophy (Goetsch, 2015).

Whereas our prior RCT provided convincing data that the location of pain in postmenopausal dyspareunia is the vulvar vestibule, and that a non-hormonal therapy can be an effective palliative option, there is a major unmet need for data about corrective therapy. The only curative therapy for noxious postmenopausal vulvar symptoms is estrogen (Gass, 2013). Various female populations are in need of more complete information about risks and benefits of local estrogen therapy, as safety is a concern for both women who have no contra-indication to estrogen and those who must balance risks of cancer recurrence. Herbenick documented that most young survivors of breast cancer wish for effective sexual enhancement products, demonstrating an unmet need in this population (Herbenick, 2008). That population presently numbers over 3 million women in the US (Natl Cancer Institute, 2013). A very recent ACOG Committee Opinion suggesting that vaginal estrogen therapy should be considered in survivors of estrogen-dependent breast cancer whose pain is refractory to non-hormonal remedies, because data does not show that use of local estrogen elevates rates of breast cancer recurrence (ACOG, 2016). This expert opinion, drawn from limited data on post-cancer vaginal estrogen, is an acknowledgement of the magnitude of the unmet need for effective therapy for dyspareunia in this breast cancer population. Our study will compare the serum levels achieved by vestibule dosing to serum levels from existing publications on the safety profile of estrogen products applied to the vagina.

Women's search for estrogen products that promise safe risk profiles is indicated by the number of women ordering estrogen products from compounding pharmacies. This has bearing on our proposal because we plan to work through the OHSU Research Pharmacy with Lloyd Center Pharmacy to formulate the intervention medications in this study. Lloyd Center Pharmacy has years of experience with compounded formulations of local estrogens. The North American Menopause Society Advisory Panel published data from a national population study demonstrating that 28% of US estrogen-users obtain their medications from compounding pharmacists. Women perceived the compounded versions as safer because they are "natural" and "bio-identical" (Pinkerton, 2015). In our practice we also encounter many women who feel they cannot afford the FDA-approved estrogen products, and they are open to getting a less expensive prescription from a compounding pharmacy.

There are many remaining gaps in current knowledge regarding postmenopausal dyspareunia. Our RCT was the first to draw attention to the vulvar vestibule in women with postmenopausal dyspareunia. We chose to study the population of women with no estrogen because they would represent the purest examples of estrogen lack for reasons of ovarian senescence and their cancer history. This project will focus on a larger population of postmenopausal women who can use estrogen. This project will add additional needed data regarding the prime location of tenderness. We aim to ascertain whether local estrogen therapy works more effectively when applied to the actual location of allodynia. By studying a postmenopausal population, we can advance knowledge about the vulvar vestibule and efficacy of local estrogen therapy.

Another research issue relates to the historical focus by clinicians and researchers on atrophy as the causative process that explains coital discomfort and pain after menopause. Our clinical experience leads us to a different hypothesis because of decades as experts in dyspareunia care and vulvar health. Our overarching hypothesis is that postmenopausal dyspareunia is a localized pain condition of the vestibule and is caused by neural hyperplasia such as is found in the chronic condition of localized provoked vulvodynia. This condition can be caused by low or absent circulating estrogen, and therefore in postmenopause it occurs in parallel with the tissue thinning that characterizes atrophy. However, whereas atrophy occurs in all genital tissues from uterus to vulva, the allodynia of neural hyperplasia is localized to the vulvar vestibule. Our research group has published work demonstrating neural hyperplasia in the vestibular tissues of postmenopausal women who still had dyspareunia despite use of systemic and/or local vaginal estrogens (Leclair, 2013). This project will explore the historic paradigm of atrophy as the cause of dyspareunia. The standard therapeutic dosage and timing regimen for local estrogen was derived from previous studies that used atrophy rather than pain as the primary endpoint. We will analyze tissue biopsies from the vulvar vestibule before and after the intervention

of vestibule estrogen, comparing neural hyperplasia, lymphocyte and mast cell infiltrate densities, which are all hallmarks of localized provoked vestibulodynia. (Leclair, 2013)

Vaginal atrophy has a well-established set of findings as defining features. These include the maturation index, which is a microscopic evaluation of squamous cells gathered from the mid-vaginal walls by a swipe of a cotton-tipped applicator. Cells indicative of normal maturation include a preponderance of mature superficial squames rather than intermediate or basal cells. Another indicator of a non-atrophic vaginal milieu is a pH under 5.0 as judged by color change on litmus paper. In the absence of confounding vulvovaginal conditions, these clinical tests assess vaginal atrophy. There is not a similar test for atrophy of the vulvar vestibule, and it is assessed visually and assumed to be atrophic when vaginal atrophy is present.

The standard regimen for local estrogen supplementation was approved by the FDA for the primary outcome of vaginal atrophy (Suckling 2006). The regimen that lowers pH and converts an immature maturation index to a mature index is the following: two weeks of daily intravaginal estrogen by any of several low-dose products such as cream or tablets. Once the atrophy is corrected, the maintenance regimen doses the same estrogen product twice weekly. The vaginal mucosa responds quite quickly to this dosing schedule of estrogen. After 2005 the FDA began requiring that products correct more than atrophy. They required that products also assess “the most bothersome symptom” (Ettinger, 2008). These symptoms could include irritation, itching or a sense of dryness and perhaps dyspareunia. No studies to date have assessed the effect of estrogen on pain at the vestibule, which is the likely site of tenderness, nor what dosing regimen is required to address pain instead of atrophy.

Urinary symptoms are part of the Genitourinary Syndrome of Menopause. For this reason, we are collaborating with our urogynecology colleagues to explore some bladder issues that our cohorts may exhibit. Evolving research has pointed to the role that microorganisms play, and hormone status has a strong relationship to urinary tract health. It has become possible to evaluate microbial communities with techniques that are culture-independent using a DNA-based approach (Brubaker, 2015). From locally collected swabs from the vestibule and vagina we will characterize the resident microbial community using a DNA-based approach by amplifying the bacterial 16S ribosomal RNA (rRNA) gene via PCR. We will perform next-generation sequencing using Illumina MiSeq (Metzker 2010). This will be analyzed with the help of the urogynecology biostatistician using QIIME bioinformatics software.

Because dyspareunia is a pain condition whose provocation is vaginal intercourse, it has been important to have a proxy maneuver that does not involve a sexual partner. Foster and colleagues tested and validated the Tampon Test using a hygiene item with which most women have had experience (Foster, 2009). It was validated in pre-menopausal women with dyspareunia and Goetsch used it in her postmenopause study (Goetsch, 2015). Not all subjects had tampon pain despite all having intercourse pain. It is the only proxy test accepted by experts in vulvar pain.

This will be a novel study in several ways. It will ascertain the absorption of low dose estrogen from the vulvar vestibule. It will study whether there is a relative therapeutic benefit to applying local estrogen to the location that hurts in cases of postmenopausal dyspareunia. It will be the first study to gauge therapeutic efficacy of local estrogen by paring physical findings in the pain location with validated measures of dyspareunia. It will be the first study to evaluate paired microbiome samples of the vulvar vestibule and vagina in postmenopausal women. It will be the first study to vary the estrogen dosing to specifically reduce pain rather than reduce measures of atrophy. It will be the first study to assess vestibule histology before and after a therapeutic intervention known to correct dyspareunia.

4. Study Design

This will be a prospective, randomized, double blind intervention pilot study that will compare two strengths

of β-estradiol applied nightly to the vulvar vestibule for three months in women with moderate or severe postmenopausal dyspareunia. Treatment efficacy and safety parameters as well as tissue histology will be assessed.

5. Study Population Number of Subjects

The study will include 50 subjects affected by dyspareunia and 5 reference cases (Total N=55). We estimate a need to screen 60 women in order to enroll 50 patients and we arrived at this estimate using the following considerations. We assume that all potential subjects who pass the phone screening and come for a screening examination having the complaint of postmenopausal dyspareunia will be found to have tenderness in the vulvar vestibule, as found in 100% of screened women in our previous study to locate the site of genital tenderness in a similar population (Goetsch, 2014). The prediction of how many women may be eliminated at screening comes from cautions regarding uterine issues. Although prior studies have indicated that use of local estrogen is without risk of stimulation to the uterus, we will be studying doses to an anatomic area with no prior data on absorption. We therefore feel it is important to screen candidates with a uterus to ensure that they enter the study with no evidence of thickening of the uterine lining, which can predispose to endometrial cancer. In women with a uterus, we will assess the width of endometrial stripes by transvaginal ultrasound. A 2011 British study of transvaginal endometrial screening found that 10-20% of asymptomatic women had a stripe width of 5 or more. (Jacobs, 2011) Those with a stripe ≥5mm will be excluded. The Oregon obesity prevalence is 29% (StateofObesity.org), defining a population at increased risk for endometrial thickening from adipose-related estrogen unopposed by any progestin. From a 2012 publication reporting the number of women in a Kaiser Permanente Northwest population who have had hysterectomies, we hypothesize that 26% of women screened will not have an intact uterus. (Lacie, 2012) Calculating the excess screens needed due to thickened endometrium in obese women and considering the proportion likely to have had hysterectomies, an estimated 60 women will be screened to enroll 50 subjects. All will be in the OHSU cohort.

A sub-study of patients will receive vulvar biopsies at baseline and again after the 3-month intervention. A total of 20 paired biopsies (one at baseline and one after the 3-month intervention) will complete this sub-study. The number has been estimated using a power analysis based on our previous histopathologic findings (see section 12). Subjects completing both biopsies will be remunerated with $100. If a subject only completes the baseline biopsy she will not count toward the total as the before and after intervention analysis of the biopsy samples is critical. As such, more than 20 women may enroll in the sub-study, but if any participants discontinue prior to the post-intervention biopsy they will be replaced so a total of 20 paired biopsies will be collected.

Reference patients will be 5 women who are not using estrogen supplements and do not have dyspareunia. They will be recruited from an OHSU urogynecology study of postmenopausal women who have urinary complaints and control women who do not. Among those screened or who have finished the study, we anticipate being able to approach women who are not using estrogen products but who have no urinary complaints and no dyspareunia. We would ask them to fill out the entry questionnaire and have a 4mm mucosal biopsy in the right or left vestibule adjacent to a Bartholin's duct orifice. This is the equivalent location of worst tenderness in women with localized provoked vestibulodynia of postmenopause. Subjects who agree to a biopsy will be remunerated with $100.

Inclusion and Exclusion Criteria

Potential subjects will first be screened during a phone conversation with the Women's Health Research Unit (WHRU) recruitment coordinator or study staff (see phone screen form). After pre-screening eligibility is confirmed, if subjects want to screen for study participation they will be scheduled to be seen in the WHRU clinic with the study coordinator and study clinician.

In the event of screen failure, data collected by phone and at screening will be retained for separate analysis.

Inclusion Criteria

1. Postmenopausal women aged at least 40 years old.
2. Postmenopausal, defined by at least one of the following:
    i. Amenorrhea ≥1 year in women > age 50 with a previously functioning uterus and presence of 1 ovary
    ii. Bilateral oophorectomy
    iii. FSH >30 in women with at least 1 ovary who are age 40-50 with >1 year amenorrhea
    iv. A history of peak menopausal symptoms >2 year prior in women age>51 with no functioning uterus (previous ablation or hysterectomy)
    v. FSH >40 in women of age >51 if they have no prior history of classic menopausal symptoms (hot flashes, night sweats) and have 1 ovary but amenorrhea that was surgically induced (by ablation or hysterectomy)
3. Onset of dyspareunia after menopause but not before.*
4. Stable heterosexual partnership ≥1 years (or by investigator discretion if less than 1 years) and both partners want to have more satisfying penetrative intimacy.
5. No estrogen product use, local or systemic, for 6 months.
6. More than 6 months of consistent insertional pain with intercourse (may have stopped having intercourse due to this consistent experience of pain).*
7. Willingness to enter a study where she will receive low-dose local estrogen.*
8. Willingness to enter a study that requires application of cream on a frequent schedule for 3 months. *
9. Willingness to evaluate the progress of therapies by use of the Tampon Test as many as 2 times per week, and willingness to attempt intercourse if the Tampon Test indicates tolerable penetrative pain.*

*n/a for reference group

Exclusion Criteria

1. Consistently has pain in the pelvis or low abdomen (chronic pelvic pain)
2. Negative cotton-swab touch test in the vulvar vestibule* or vestibular tenderness that is not extinguishable by application of lidocaine 4% topical solution applied for 3 minutes.
3. Partner with sexual dysfunction limiting his performance or making it inconsistent. (The use of male therapy for erectile dysfunction is acceptable.)
4. Diagnosis by a physical therapist or clinician of significant pelvic floor muscle tension causing pain (pelvic floor myalgia) or has been found on screening examination to have pelvic floor tenderness or bladder tenderness.
5. Constant burning pain localized to the vulva.
6. Allergy to local estrogen products or lidocaine numbing agents.*
7. Previous estrogen receptor positive breast cancer or endometrial cancer or has been cautioned about estrogen because of any other cancer
8. Endometrial thickness ≥5mm on screening via transvaginal ultrasound.
9. Vulvovaginitis assessed at screening exam.

*n/a for reference group

Vulnerable Populations

This study will not include special populations. The criteria for the intended population will exclude groups designated as special populations.

Setting

Research will be conducted in the Women's Health Research Unit or the Center for Women's Health clinics in the Department of Ob/Gyn at OHSU.

Recruitment Methods

Flyers will invite potential subjects to call the Women's Health Research Unit to be pre-screened for this study. Flyers will be posted in clinics where women are seen at OHSU and in satellite clinics. We will also reach out to OHSU patients previously diagnosed via EPIC Cohort Discovery search. Additionally, potential subjects who are seen by providers in the Program in Vulvar Health at the Center for Women's Health or in the "over-45" clinic in the generalist division at the Center for Women's Health may be invited to call the Women's Health Research Unit. Many postmenopausal women with the complaint of dyspareunia present for care in these clinics. There will be monetary remuneration for subjects who undergo vulvar biopsies. Recruitment of the five reference subjects without dyspareunia is supported by our urogynecology colleagues.

8. Consent Process

After a potential subject is pre-screened by phone and pre-screening eligibility are confirmed she will be given an appointment at the WHRU to meet with a research assistant who will explain all details of the study. Once the nature of the study is clear, all questions are answered and the subject agrees to the protocol, the research assistant will obtain written consent. A nurse practitioner or study physician will perform a screening examination to ensure that the woman meets all inclusion criteria and has no exclusion criteria. There will be clarification that the participant can decide with no negative consequences to decline to participate and that she can withdraw from study participation at any point. She will be told that she can contact a representative of the WHRU at any time for any questions or worries and will be given contact information.

Modifications to the Consent Process

Potential subjects who call the posted number that they see on ads will speak to the WHRU recruitment coordinator or study coordinator(s) working on the study. Study staff will interview the caller using the phone screen checklist to ascertain that the woman meets the inclusion criteria and does not have excluding attributes. Approval to discuss participant's medical history and other inclusion/exclusion criteria prior to consent will be allowed through approval of the Waiver of Authorization form through the OHSU IRB.

Non-English Speaking Subjects

Subjects who do not speak English will not be enrolled.

9. Procedures Involved

This study includes up to 7 visits as outlined below:

<u>Visit 1 (Screening Visit)</u>

- Consent
- Review of inclusion/exclusion criteria
- Medical/surgical and medication history
- Pelvic examination to assess genital atrophy and vestibule tenderness

    - Vestibule tenderness assessment will include the cotton-swab touch test (a test of mucosal sensitivity) and the lidocaine test (application of 4% aqueous lidocaine to tender mucosa for 3 minutes with reassessment of the cotton swab test to assess by what percentage the mucosal pain has been extinguished).
    - The initial cotton swab used to map the tenderness in the vestibule and one from a vaginal side wall sample will be labeled, frozen and stored for later batched analysis of the microbiome composition.
- Transvaginal ultrasound to assess endometrial stripe thickness
    - Since subjects will have been selected because of having pain with vaginal penetration, it will be assumed that the vaginal probe used for the ultrasound examination will not be comfortable for the subject to insert without some preventative treatment. The study clinician will pre-treat the location of tenderness with 4% aqueous lidocaine for several minutes prior to insertion of the vaginal probe. This should prevent or minimize local pain from the vaginal probe.
- Blood sample collection for serum β-estradiol
- Completion of questionnaires
    - Demographics
    - Vulvar Pain Assessment Questionnaire (VPAQ), modified, #1
    - Lower Urinary Tract Symptoms (LUTS) questionnaire
- Randomization to study drug by Research Pharmacy, after all eligibility criteria confirmed by study staff
- Vulvar biopsy (for those subjects enrolling in the sub-study)
    - If participant agrees to biopsy, the baseline sample can be collected at either V1 or V2, dependent on subject preference
    - 4mm vestibule punch biopsy at the 4 or 8 o'clock site next to one of the Bartholin duct orifices after the zone has been pretreated with topical 5% lidocaine ointment for 10 minutes

Visit 2 (Enrollment/Randomization) – ***__**THIS VISIT MAY BE COMBINED WITH VISIT 1***__**

- Confirmation of inclusion/exclusion criteria
- Update to medical and medication history
- Dispensing of study drug with review of how to use/apply by study staff
- Dispensing of diary forms and review of instructions for documentation
- Dispensing of Tampons
    - Diary questions include pain scoring of either a Tampon Test (a validated test used to assess vestibular tenderness), or a sexual penetration encounter

Visit 3 (12 hour blood draw +/- 60 minutes)

- Blood *sample collection for serum β-estradiol* [12 hours after the initial study drug dose (8 or 9am after an initial dosing at 8 or 9pm the evening before)]

Visit 4 (week 4 +/- 3 days)

- Update to medical and medication history
- Review of possible symptoms associated with study drug
- Collection and review of diary, additional pages dispensed as needed
- Pelvic examination to assess genital atrophy and vestibule tenderness
- Blood sample collection for serum β-estradiol
- Completion of questionnaire, VPAQ #2

Visit 5 (week 8 +/- 3 days) – telephone or email contact only

- Update to medical and medication history
- Review of possible symptoms associated with study drug
- Review of diary

Visit 6 (week 12 +/- 3 days)

- Update to medical and medication history
- Review of possible symptoms associated with study drug
- Collection and review of diary
- Pelvic examination to assess genital atrophy and vestibule tenderness
- Collection of swabs from the vestibule and from the vaginal side walls for later analysis of the microbiome composition.
- Repeat transvaginal ultrasound to assess endometrial stripe thickness
- Blood sample collection for serum β-estradiol
- Completion of questionnaires: VPAQ #3 and LUTS questionnaire
- Remaining study medication collected from participant by study staff
- Repeat vulvar biopsy (for those subjects enrolled in the sub-study)

Visit 7 (follow-up)

- Subjects will be contacted by phone, mail or email 4 months after study completion to ask the status of their dyspareunia and estrogen regimens and to administer follow-up questionnaires (VPAQ modified + LUTS questionnaire).

Long-term follow up

Study staff may request permission to contact subjects by telephone or email for up to 5 years to ask the status of their pain and any medication they are using to treat their pain.

Duration of Participation:

The duration of active participation will be 12 weeks and limited further data will be obtained from a phone call, mail, or electronic communication 7 months after enrollment into the study. Participants may be asked to allow additional follow-up contact for up to 5 years after their last study visit.

Participation for reference group subjects will last the duration of Visit 1. There will be no follow up of reference subjects.

Genetic Testing:

No human genetic testing or collection of human genetic information is involved in this study. Ribosomal RNA will be analyzed from resident vaginal and vestibular microbes.

10. Study Drug

The study drug will be compounded β-estradiol, an FDA approved medication. It will be compounded by Lloyd Center Pharmacy, which has a long history of collaboration with the OHSU Research Pharmacy. They adhere to the standards of the US Pharmacopeial Convention (USP), a 194 year-old scientific organization that sets standards for medication purity; in the US the FDA enforces these standards. Lloyd's adheres to Chapters 797 (sterile medications) and 795 (non-sterile medications) of the USP, and the β-estradiol cream, being a non-sterile product, will adhere to standards set out in Chapter 795.

Subjects will be randomized to receive one of two strengths of compounded β-estradiol cream: 100mcg or 50mcg/0.5 gram. Each subject will be issued a pump that will dispense 0.5cc (0.5gm) of study drug with each pump action. They will be instructed in application of the cream to the vulvar vestibule on a nightly basis. They will be asked to keep a diary record of drug applications.

The β-estradiol cream will be twice the strength of the recommended FDA-approved strength so as to be able to dose half the amount for purposes of patient acceptability. Too much cream would likely elicit complaints of messiness and excess discharge. The base study cream will be Versabase, a well-known and well-absorbed base for compounded vaginal medications. It is free of propylene glycol, a preservative found in the base used in the proprietary vaginal creams, known to be an irritant to the vestibule.

Subjects, investigators and personnel will be blinded as to the randomization, as the OHSU research pharmacy will make the assignments. We will request that subjects bring remaining study medication to their final study visit (Visit 6) for collection by study staff so dosing may be confirmed after analysis, if needed.

11. Data and Specimens

a. Handling of Data and Specimens

Data will be entered into REDCap from paper charting and/or via direct REDCap entry by study participants using laptop or iPad. Serum specimens will be stored in freezers in WHRU and sent for batched analysis at the Oregon National Primate Research Center (ONPRC). Ultrasound data and photos will be stored electronically and in charts in WHRU and REDCap.

Serum specimens will be stored in WHRU freezers and then batch shipped to the ONPRC for analysis. Biopsy specimens will be preserved in formalin and batched for staining and analysis by Dr. Terry Morgan in the OHSU pathology department. Data will be stored until the study has been published and then archived to Iron Mountain for indefinite storage. Any remaining serum or biopsy specimens will be stored for one year beyond completion of the study or until advised to discard by the PI.

Batched serum specimens will be sent to the ONPRC lab on dry ice. Swabs with samplings from vestibules and vaginas will be frozen and stored for later sequencing and analysis.

b. Sharing of Results with Subjects

Subjects with a uterus will undergo a screening pelvic ultrasound and an exit pelvic ultrasound to assess the endometrial stripe. If at screening the stripe is ≥5mm, they will be advised to consult with their personal gynecologist to consider a progestin challenge test and they will be excluded from study participation.

If at study exit the stripe is ≥5mm, they will be advised to consider oral Norethindrone for 10 days to see if any bleeding is provoked and follow-up with their personal gynecologist. In addition, they will be advised to consult with their personal gynecologist for advice on monitoring the endometrium in the future should they wish to continue using estrogen cream. It is not expected that use of study drug will stimulate endometrial thickening.

Serum β-estradiol results will not be shared with the participants.

c. Data and Specimen Banking

Specimens and data may be placed in the WHRU repository for future research. No genetic research will be done.

Blood samples will be sent for analysis to the ONPRC. Tissue biopsies will be analyzed and then stored by the Department of Pathology, OHSU. Swabs collecting material from vestibule and vagina will be frozen and stored for later sequencing and analysis as batched samples.

12. Data Analysis

Fifty women who are postmenopausal, have moderate or severe entry dyspareunia and meet Friedrich's criteria for vestibulodynia will be recruited into the study. We are assuming a baseline pain score of 70 ± 21.5 (mean ± std) from a previously reported study of a mixed group of treated and untreated postmenopausal patients with dyspareunia, (Kao, 2012), and we assume a 30% treatment effect after four weeks with the lower-dose intervention drug. A sample size of 50, divided into two arms, allowing for a 10% drop out rate in each of two arms, will achieve >85% power to detect at least 20 points mean difference in dyspareunia pain scoring between the two intervention drug strengths at 4 weeks, with an alpha level of 0.05 for two-sample t-test.

The tissue biopsy sub-group will be 10 subjects from each intervention arm (n=20), and using a one-sided exact test with alpha level of 0.05, this number of subjects will achieve 80% power to detect a difference of 15.6% in inflammation, assuming the baseline proportion of moderate or strong inflammation is 95%. (Leclair, 2013). Patients' biopsies at 3 months will be compared to their baseline biopsy.

To establish a reference group of biopsy specimens, we wish to seek out 5 subjects to fulfill the exploratory aim of assessing the histologic profile of vestibule tissues in postmenopausal women who are not on estrogen and do not have penetrative pain. The prevalence of this type of patient has not been established. We would expect them to have atrophy but no vestibular inflammation or neural hyperplasia. They will not technically be "controls" for purposes of this study. As there are no published studies analyzing the histologic character of the vestibule in such women, the small exploratory number (n=5) does not allow a power analysis. We will recruit these subjects with the help of our Urogynecology colleagues who are doing a study on the vaginal/bladder microbiome and are screening postmenopausal patients.

Swabs with material for microbiome analysis will be analyzed with the help of the Urogyn bioinformatics specialist using QIIME bioinformatics software.

13. Privacy, Confidentiality, and Data Security

Confidentiality of personal health information will be maintained according to HIPAA requirements for research. All subjects will receive a study number to which all subsequent data will refer. Personal identifiers will not be on questionnaires, data, abstract sheets, or in the main database. All data will be kept in locked files or a password protected computer in the Principal Investigator's (PI) office. We will do our best to keep information confidential by keeping it coded and on password-protected computer.

14. **Provisions to Monitor the Data to Ensure the Safety of Subjects**
See Data Safety Monitoring Plan (DSMP) document.

15. **Risks and Benefits**

a. Risks to Subjects

Risks to subjects include:

- Breach of confidentiality
  - We will do our best to avoid this risk as outlined above and in the DSMP
- Inconvenience of coming in for several visits
- Inconvenience of keeping a study diary
- Psychological discomfort from filling out questionnaires to assess pain and distress
- Psychological stressors related to a study which focuses on sexual function and pain since these issues may have created stresses in their relationship
- Discomfort of ultrasound
  - There are no known harmful effects of transvaginal ultrasound. Women may experience vaginal discomfort which we plan to minimize with the use of 4% aqueous lidocaine for several minutes prior to insertion of the vaginal probe
- Discomfort from blood draw
  - Women may feel some pain when their blood is drawn by venipuncture, but there is minimal risk involved. There is a small chance the needle will cause bleeding, a bruise, or an infection
- Discomfort from vestibule biopsies (this is optional)
  - A vestibule biopsy is not from a zone that produces hypertrophied scars, and from our prior experience patients grade the experience as painless, due to topical lidocaine followed by injected lidocaine preparation, with minor pain as they heal.

The subjects in this study will have been screened to be postmenopausal in hormone status, and therefore there will be no risk of pregnancy.

- Study Drug**:**
  - All medicines may cause side effects, but many people have no side effects or minor side effects. You may have some side effects we do not expect because we are still learning about estradiol vaginal cream applied to the vulvar vestibule.
  - The estrogen cream used in the study is similar to the FDA approved estradiol vaginal cream (ESTRACE). When estrogen cream is placed deep in the vagina in the same dose as the study cream, absorption is low and for most women blood levels remain the same as for those not using estrogen cream. If absorption takes place, side effects may include:
  - Most Common:
    - Headache (13%)
  - Less Common (1-10%):
    - Hot flushes
    - Breast tenderness
    - Vaginal infection (yeast infection, bacterial infection)

        - Vaginal pain/discomfort
        - Vaginal spotting
        - Gastrointestinal complaints (abdominal pain, nausea, diarrhea)
        - Urinary tract infection
        - Hair loss
        - Insomnia
        - Anxiety
        - Dizziness
        - Changes in libido
        - Back and joint pain
        - Respiratory tract infection
        - Discoloration of the skin
    - Rare but Serious Side Effects (<1%)*
        - Endometrial cancer
            - Estrogen therapy may cause the lining of the uterus to grow and can increase the risk of uterine cancer. Because the amount of estrogen absorbed is low when using topical estrogen cream, the risk for uterine cancer is lower than when taking oral estrogen. You will have pelvic ultrasounds throughout the study to monitor the lining of the uterus.
        - Breast cancer
            - Estrogen therapy is associated with a small increased risk of breast cancer.
        - Cardiovascular disease –
            - Estrogen therapy has been shown to increase the risk of heart disease including heart attack, stroke, and serious blood clots in some women.
        - Dementia
            - An increased risk of developing probable dementia in postmenopausal women 65 years of age or older has been reported in women receiving oral estrogen alone or estrogen combined with progestins.
        - As with any drug, allergic reactions may occur due to the study drug. These may range from mild to severe, can be life-threatening/fatal and include hives, difficulty breathing, and swelling of your face, lips, tongue, or throat. You should seek emergency medical treatment should you experience an allergic reaction.

b. Potential Benefits to Subjects

Subjects will be entering the study because they experience pain with sexual intimacy. There is a greater than 50% probability that they will experience lessening and even correction of their dyspareunia by study completion, since all will get study drug, which is the known therapy for this problem. All will apply study drug to the specific site of tenderness and is hypothesized to increase the likelihood of benefit. If the intervention is successful for them, they can continue to use the intervention regimen once the study is completed, providing a benefit. All subjects will be offered the option to receive information about the outcome of the study once it is completed, analyzed and published in a peer review journal and the information may benefit each of them.

It is possible that the gynecologic screening via ultrasound may uncover a condition for which timely intervention is beneficial to the subject. The possible findings would include intrauterine polyps or thickened endometrium that could be pre-cancerous. The potential benefit is great if cancer might be prevented.

Subjects may experience psychological benefits in relation to their sexual function and sexual relationship by exploring their issues and having a source of expert information about dyspareunia and vulvar pain in the study staff.

16. **Drugs or Devices**

There will be neither a new drug application nor a premarket approval application as we are using an FDA approved drug, Lidocaine 4% topical solution, NDC # 0054-3505-47.

We will follow Research Pharmacy policies & procedures for the compounding, randomization and dispensation of study drug.

Drug will be dispensed by the research pharmacy to study staff and study staff will dispense study drug directly to subjects. Subjects will be requested to return any unused drug.

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