CLINICAL RESEARCH PROTOCOL

Title: Role of hormonal status on vascularization and vaginal tissue in women with pelvic organ prolapse

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***This research is part of the UTMB Claude Pepper Pilot Funded-Project*:**
Role of Connective Tissue Degradation in the Development of Pelvic Floor Dysfunction in the Elderly: Identifying Targets for Medical Management

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1.0 BACKGROUND

Pelvic floor dysfunction (PFD), disproportionately affecting older women, involves loss of pelvic floor support and urinary and fecal incontinence. Medical options are limited and up to 11% of women will require surgery, with increasing risk due to surgery with advancing age. The cause of PFD is multi-factorial and complex, and although risk factors are known, precise mechanisms are yet to be elucidated. Our primary aim in the current proposal is to determine important tissue differences, including muscle and connective tissue changes, between postmenopausal women and reproductive age women with PFD to help develop targeted treatments. In addition, we propose to develop noninvasive methods to evaluate future treatments for PFD. These goals will be complementary to provide preliminary data for submission for NIH funding for preclinical or clinical (FDA approved drugs) studies of potential medical treatments for PFD.

A. Significance

A1. PFD is increased in aging women.

Pelvic floor dysfunction affects up to 50% of elderly women.[1,2] Pelvic floor function involves the interplay between muscles, nerves and connective tissue, and dysfunction results in pelvic organ prolapse, urinary complaints (incontinence and voiding disorders) and defecatory dysfunction (constipation, incomplete emptying, and fecal incontinence). In addition to the discomfort of this medical condition, falls and fractures are more common in women with PFD,[7] suggestive that a sarcopenic condition is present. Psychosocial dysfunction, including depression and isolation are more common in women with this socially embarrassing disorder.[8]

Current treatments for PFD include pelvic floor muscle exercise and topical estrogen. However, most symptomatic women present with advanced cases of pelvic organ prolapse where pessary (vaginal support device) and surgery are the only options for relief of symptoms. Some types of prolapse are not responsive to pessary use or women may not be comfortable with the use of this device, therefore surgical management is common for treatment of PFD, with an 11% lifetime risk of surgery.[9,10] Unfortunately, recurrence of prolapse after surgical management is common,[11,12] and surgical risks are increased in older women. Women undergoing urogynecologic procedures after the age of 80 have a 13.6 times increased risk of death after the procedure compared with their younger counterparts.[13] Thus, one goal of this project is to identify the inflammatory and pelvic tissue metabolic dysregulation that occurs with PFD to better identify targeted nonsurgical treatment options for symptom relief in older women with limited treatment options.

A2. Multi-factorial cause of PFD

Because pelvic floor support depends on a complex interplay of factors, the cause of PFD is multi-factorial. Three proven risk factors for the development of this common disorder have been identified and include vaginal delivery, obesity, and age.[14-16] Since normal aging also induces wasting of the limb muscles, and vaginal and pelvic floor atrophy is commonly seen in PFD, we surmised that similar inducers of sarcopenia (loss of hormones, diminished blood flow, and inflammation) may be influencing PFD in older women along with other known factors listed below.

Tissue Injury from childbirth and pregnancy as precipitating factor - Mechanical injury to the pelvic floor is the precipitating factor for PFD, beginning in the reproductive years, with multiple births and

delivery of large babies increasing this risk.[17,18] In addition, continued mechanical stress on the pelvic floor, including chronic cough or chronic straining in constipation, increases the risk for PFD.

Hormonal Effects on Tissue - The most dramatic aging effects on the health of the vaginal wall and support structures occurs at the time of menopause, coinciding with an increase in symptomatic PFD. While vaginal estrogen use may assist some women with urinary urgency, recent data shows that it is not a successful treatment for urinary incontinence and prolapse.[19] Additionally, the vaginal estrogen response is blunted in chronically estrogen-deprived cells.[20] Estrogen is not an option for all women due to breast cancer or fear of estrogen supplementation.

The impact of progesterone and testosterone deprivation and supplementation on the health of pelvic support has not been well studied. Treatment of rats with testosterone improved urinary incontinence, presumably due to anabolic effects on the supportive pelvic floor muscles.[21] However, testosterone supplementation in ovariectomized (simulating menopause) mice has shown thinned vaginal epithelium, therefore further study of testosterone's effects on the entire pelvic floor should be studied.[22]

Muscle atrophy - Loss of pelvic floor support and muscular tone plays a critical role in PFD. Age has been associated with decreased pelvic floor muscle strength and increased size of the levator hiatus.[23] Women with PFD have decreased muscle contraction and tone in the levator ani muscles. Pelvic floor physical therapy, including biofeedback and Kegel exercises are helpful in improving mild urinary incontinence. A study in surgical patients showed 74% degeneration of striated muscles in levator ani muscles of women with urinary incontinence when compared with controls (0%).[24] Dysregulation of the vaginal smooth muscle content has an age dependent effect in women with pelvic organ prolapse.[25]

Vasculature - Decreased hormonal influence on the vagina and reproductive organs from lack of estrogen after menopause leads to decreased blood flow to pelvic organ structures including the uterus and vagina. This decreased blood flow, which can be observed during a vaginal exam and appears white and avascular in nature, can also be easily measured with ultrasound by our group, and may play a role in the atrophy of the uterus and vagina occurring after menopause; similar to the role skeletal muscle perfusion plays in age-related sarcopenia.[22] Testosterone may independently play a role in blood flow to the vagina.[26] A study of ovariectomized rats showed normalization of vaginal blood flow after treatment with testosterone.[27]

Elastin and Collagen Structure - Women with PFD have been noted to have a vaginal connective tissue balance shifted toward *degradation* rather than *regeneration* as compared to women without prolapse. Animal studies show increased matrix metalloproteinases MMPs (proteins which break down connective tissue elements) and abnormal collagen/elastin in a mouse model of prolapse, with few confirmatory human studies.[6]

Inflammation may play a role in the development of PFD - Two of the three major risk factors for PFD, age and obesity, are associated with inflammatory changes of the pelvic wall tissue. Additionally, postmenopausal women are more likely to have atrophic vaginitis related to decreased hormones and epithelial barrier function.[28] The pH and vaginal microflora change, increasing the risk of infection and further inflammation. The role of inflammation in the development of PFD has not been well studied,

though increased inflammation and resulting elevated MMPs is thought to effect the support of the pelvic organs.[5,6] Determining the important factors in development of PFD will allow us to strategize for optimal medical management of this disease and to target reversible tissue changes before they become permanent.

A3. Scientific innovation to improve health
We will utilize knowledge gained from this proposal in humans to direct future research projects to test targeted topical treatments in a mouse model of PFD and then translate these targeted treatments to clinical research.

Our long term goal is to develop drug combinations to be delivered topically (e.g. intravaginal rings). Finally, further refining of our pelvic imaging techniques to noninvasively monitor pelvic floor health would greatly improve our ability to individualize treatment of women with PFD and to monitor them after treatment and for early signs of progression of disease.

2.0 Rationale and Specific Aims

Aim: To determine the effect of aging, vaginal wall inflammatory markers, and macro- and microcirculatory flow on PFD.

1. Full thickness vaginal wall specimens and vaginal fluid collected during surgery will be evaluated for the primary outcome of tissue inflammatory cytokines.
2. Secondary outcomes will include evaluation of tissue MMPs, collagen/elastin structure, microvasculature, and muscle thickness.
3. To evaluate macrocirculatory blood flow in pelvic organs, women will be assessed with noninvasive imaging before and after randomization to vaginal estrogen in the postmenopausal group in the perioperative period.
4. Microcirculation will be measured by histologic staining of tissue vasculature

Data from this project will increase our understanding of the mechanisms of loss of pelvic floor muscle support, identify therapeutic targets and improve our ability to medically treat women with PFD, providing a safer, nonsurgical, and noninvasive option of diagnosis and treatment, and providing the necessary preliminary data for a NIH grant application.

We propose a 1-month peri-operative study in older women with PFD to assess inflammatory markers and blood flow in pelvic wall tissues. Younger women with PFD will serve as a positive control.

3.0 Study Population and Inclusion/Exclusion

It is anticipated that up to 30 female patients with Stage II or greater pelvic organ prolapse with planned gynecologic surgery at UTMB will be involved in this study with at least 15 participants completing the study, including at least 10 postmenopausal women, and at least 5 reproductive age women. Patients planning surgery for Stage II or greater pelvic organ prolapse may be eligible to participate. The study team will provide information about the study, including inclusion and exclusion, to clinic providers who may then inform their eligible patients about the study. If interested in learning more about the study, patients will be asked to speak with a study team member. The informed consent process will be completed in the clinic with a study team member authorized to

obtain study consent. During the consent process, patients are reminded of the voluntary nature of research participation; patients wanting to take part in the study are asked to sign the current approved study consent form. The completed written study consent form must be on file prior to a patient's participation in any study procedure.

Subjects must meet all of the inclusion criteria in order to participate in the study.

Inclusion criteria are:

- Females, 21 to 70 years of age, at the time of consent.
- Stage II or greater pelvic organ prolapse.
- Individuals who desire surgery to treat their PFD.
- Willing and able to comply with study procedures.
- Willing and able to provide written informed consent.

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation.

Exclusion Criteria are:

- Contraindication for estrogen use.
- Any medical condition that, in the opinion of the investigator, would place the subject at increased risk for participation.
- History of connective tissue disease
- Previous hysterectomy or pelvic organ prolapse surgery.
- Known allergic reaction to any agent under investigation or required by the protocol.
- Use of hormone therapy in postmenopausal women within the last 90 days.
- History of prior non-compliance or the presence or history of psychiatric condition (including drug or alcohol addiction) that would, in the opinion of the investigator, make it difficult for the subject to comply with the study procedures or follow the investigators instructions.
- Females who are pregnant or lactating.

4.0 Treatment Assignment/Randomization

Study participants will be divided into three groups, appropriately equal in number:

1. Post-menopausal women with PFD on topical vaginal estrogen cream
2. Postmenopausal women with PFD on topical vaginal cream placebo (non-estrogen)
3. Premenopausal women with PFD

The postmenopausal women will be randomized to use topically applied vaginal estrogen or placebo using a random number generator.

5.0 Study Procedures

Postmenopausal Women

Study Visit 1

Within approximately 30 days of the scheduled surgery date, the postmenopausal subjects will come to the Pelvic Health Clinic at Victory Lakes to have an optical coherence tomography (OCT) scan and pelvic floor (endovaginal) ultrasound.

Both of these tests involve a vaginal probe being inserted in the vagina to collect pelvic organ and tissue images. The OCT produces higher resolution 3-dimensional images and takes 10-15 minutes or less and the endovaginal ultrasound takes about 30-45 minutes.

At this visit, up to 20ml of blood may be drawn for serum hormone levels of estradiol and testosterone, DHEA and androstendione.

Subjects will complete Uro Gyn Clinical Intake Form if not previously within 3 months. The Intake Form includes the following questionnaires: UDI-6, POPDI-6, CRADI-8, PFIQ, PISQ-12 (see protocol appendix for questionnaries).

Subjects will be randomized to placebo or estrogen vaginal cream.

Subjects will be provided with a topical vaginal cream to use daily until 2 days prior to your scheduled surgery date (4-6 weeks). Subjects will apply 0.5 gram of the study vaginal cream daily. Subjects will be given a study diary as a reminder and to record usage and will return their spent tube of cream for weighing as a measure of compliance.

Study Visit 2

Subject will return within a week prior to schedule surgery. Subjects will repeat the OCT, pelvic floor ultrasound and the questionnaires as done in Study Visit 1. Up to 20ml of blood may be drawn to repeat serum hormone levels of estradiol and testosterone, DHEA and androstendione.

Premenopausal Women

Study Visit 1

Premenopausal subjects will have their studies visits 1 scheduled in order to coincide with the subject's follicular or luteal phase of their menstrual cycle. Study visit 2 will be scheduled in order to coincide with opposite phase from study 1.

Premenopausal subjects will come to the Pelvic Health Clinic at Victory Lakes to have an optical coherence tomography (OCT) scan and pelvic floor (endovaginal) ultrasound.

Both of these tests involve a vaginal probe being inserted in the vagina to collect pelvic organ and tissue images. The OCT produces higher resolution 3-dimensional images and takes 10 minutes or less and the endovaginal ultrasound takes about 30 minutes.

At this visit, up to 20ml of blood may be drawn for serum hormone levels of estradiol and testosterone, DHEA and androstendione. Urine or serum pregnancy test will be performed for patients at risk of pregnancy at the discretion of the study gynecologist.

Subjects will complete Uro Gyn Clinical Intake Form if not previously within 3 months. The Intake Form includes the following questionnaires: UDI-6, POPDI-6, CRADI-8, PFIQ, PISQ-12 (see protocol appendix for questionnaries).

Study Visit 2

Subject will return within a week prior to schedule surgery. Subjects will repeat the OCT, pelvic floor ultrasound and the questionnaires as done in Study Visit 1. Up to 20ml of blood may be drawn to repeat serum hormone levels of estradiol and testosterone, DHEA and androstendione.

Surgical Tissue Collection

After anesthesia and prior to vaginal preparation for surgery, vaginal fluid (secretions) will be collected similar to getting a pap smear and vaginal rinse with saline will be performed and up to 30ml of the rinse material or lavage will be collected for research to look at cytokines and vaginal microflora.

The surgical tissue that is excised and removed is standard for the surgery performed, and typically this tissue is discarded after surgery. The tissue collected for this research is discarded surgical tissue, about 2-4 postage stamps in size, depending on whether anterior and/or posterior surgical repair is performed.

The surgery will proceed as scheduled and tissue from the vagina will be collected and processed for further tissue analysis. The tissue collected will be the tissue that is typically discarded during the surgery.

Vaginal Secretion and Vaginal Fluid Analysis

Vaginal secretions and lavage fluid will be transported to Dr. Rick Pyles' lab and to Oak Crest Institute of Science and will be evaluated for cytokines, including IL-6, TNF-α, TNF-β, evidence of inflammatory cells, and vaginal microflora using an extended qPCR panel developed with an ITS Novel Methods Grant (Pyles, Vincent, 2012).

Vaginal Tissue Analysis

After excision, vaginal tissue will be sectioned into ~3mm pieces and preserved for analysis in formalin, RNALater, and frozen at -80C for future analysis. Formalin preserved sections will be paraffin-embedded and stained with H&E (epithelium, stroma, muscularis assessment), Masson's trichrome (collagen, vessel endothelium), Hart's (elastin), or IHC stained for MMPs, estrogen, progestin, and testosterone receptors and CD68 (macrophage). A trained pathologist will interpret the images and provide grading of findings of epithelium, muscularis, collagen, elastin, and IHC findings for presence of MMPs, hormone receptors, and macrophages. Fresh tissue will also be imaged using noninvasive imaging with optical coherence tomography (epithelial and stromal thickness) and multiphoton/SHG imaging (collagen structure) through collaboration with the CBME. Samples in RNALater will be shipped to Oak Crest Institute of Science for evaluation of the transcriptome.

Oak Crest Institute of Science will provide testing and analysis for this study. In addition, coded specimens and data (not having personal identifiers) may be used in ongoing collaborative research with Dr. Marc Baum of Oak Crest.

Ultrasound and OCT
Pelvic floor ultrasound will be performed using a standard clinical BK ultrasound system with abdominal, endovaginal, and 3D rotational (vaginal) probes. OCT imaging will be performed using an FDA-approved Niris OCT system (Imalux, Cleveland, OH). OCT images will be collected by direct tissue contact with the 2.7mm diameter probe that will be covered with a sterile disposable sheath and cleaned between use with Cidex. OCT images will be obtained using guidance with a colposcope and a magnetic (GPS) positioning system to record the location of the images collected. Digital images of the cervix and vagina will also be obtained with a digital camera for localization of images.

Parameters measured by ultrasound will include: vaginal thickness, muscularis thickness/volume, vaginal and uterine blood flow (flow and resistive index), levator ani muscle thickness/volume, uterine/cervical descent. Parameters measured by OCT will include vaginal epithelial and stromal thickness. Women will be measured before vaginal treatment with estrogen and 4 weeks after estrogen (or no estrogen) treatment.

The excised tissue will also be evaluated with high resolution imaging methods of OCT and multiphoton/second harmonic generation.

Medical Record Review
The medical record will be reviewed for demographics, medical/surgical history, hormonal status, medications, BMI, and tobacco status.

Tissue Banking
A portion of the subject's blood sample and surgical specimen may be banked at the Biorepository on the UTMB campus for future analysis or used in other approved research at UTMB. Specimen storage or banking involves transferring specimens into several smaller coded sample tubes for freezing and storage in the research.

6.0 Study Product Description

Formulation, Packaging, and Labeling
FDA-approved estradiol or placebo vaginal cream will be provided. The product will be labeled as to "Topical Vaginal Cream for Use on Study" and will be identifiable as to estrogen or placebo with a specific code number. Participants and the study physicians are blinded as to estrogen or placebo until data analysis completion.

Product Storage and Stability
Store at room temperature. Protect from temperatures in excess of 40° C (104° F). Store in original packaging until just before use. Store away from heat, moisture and light. Once product is opened, the product should be used within 90 days.

Dosage, Preparation and Administration
Estradiol vaginal cream 0.5 g vaginal daily or placebo vaginal cream will be applied vaginal daily from study day 1 every day until 2 days before scheduled surgery.

Topical estradiol is poorly absorbed systemically. In this study, subjects will be on a small topical dose of estradiol for a limited time period. In addition, the use of topical estrogen in this study is consistent with common clinical practice for treatment of vaginal atrophy before pelvic organ prolapse surgery.

In order to maintain double-blind, the estradiol and placebo cream will be formulated by a compounding pharmacy. The estradiol vaginal cream will contain 0.01% of estradiol and the placebo vaginal cream will contain the excipients only that are included in the estradiol vaginal cream.

Modification of Study Intervention for Participant
We don't anticipate any dose adjustments due the minimal time of study use.

Accountability Procedures for the Study Intervention
After randomization to vaginal cream vs. placebo, subjects will be given cream in an amount anticipated to last the duration of the study period. If the subject needs a replacement cream, that will be provided. Unused product will be stored during active shelf life and then disposed of.

Assessment of Subject Compliance
Compliance with the study intervention will be determined by study diary.
Patients will be asked to returned unused cream at end of study period to further measure compliance.

Concomitant Medications/Treatments
Any medications the subject takes are permitted with the exception of sex hormone replacement in postmenopausal women.

7.0 Potential Risks and/or Discomforts of Participation

Topical Vaginal Cream**:** Allergic reaction; Irritation or other discomfort; Vaginal burning; Vulvar-vaginal infection; Vaginal bleeding; Increased vaginal discharge; Trauma from applicator; Headache; Breast tenderness.

OCT – Possible discomfort with the probe in connection with the evaluation. Some women have reported minor irritation, redness and vaginal bleeding.

Pelvic floor (endovaginal) ultrasound – The test is usually painless although some women have reported mild discomfort from the pressure of the probe. There are no known harmful effects on humans.

Blood Draw – The discomfort associated with drawing blood from a vein is a light pinch or pinprick at the puncture site. The risks include possible bruising and swelling around the puncture site; less commonly, infection, small blood clot or bleeding at the puncture site and faintness from the procedure.

8.0 Adverse Events or Unanticipated Problems

Any adverse event (AE) or unanticipated problems considered serious by the PI or study clinician will be reported to the IRB in accordance with IRB policies and procedures.

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the Serious Adverse Event (SAE) Form and sent by fax within 24 hours of site awareness.
- SAEs or unanticipated problems other than death and immediately life-threatening events, regardless of relationship, will be reported via fax by the site within 72 hours of becoming aware of the event.

AEs will be graded for severity and relationship to study participation.

Severity of Event: All AEs will be assessed by the clinician using the following guidelines to quantify intensity.

- Mild: events require minimal or no treatment and do not interfere with the patient's daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Life threatening: any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Relationship: The clinician's assessment of an AE's relationship to the study article is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: associated or not associated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Associated – The event is temporally related to the administration of the study product and no other etiology explains the event.
- Not Associated – The event is temporally independent of study product and/or the event appears to be explained by another etiology.

Serious Adverse Event (SAE): An SAE is defined as an AE that meets one of the following conditions:

- Death during the period of protocol defined surveillance
- Life-threatening event (defined as a subject at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical

intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

- recorded and reported appropriately
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

9.0 Study Withdrawal/Discontinuation

A study subject will be discontinued from participation in the study if any of the following occur:

- Unacceptable toxicity or serious adverse event
- Intercurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject in the opinion of the treating investigator
- Development of any exclusion criteria
- Subject withdrawals consent to continue in the research for any reason
- Vaginal bleeding of postmenopausal women on treatment cream

Handling of Withdrawals

Subjects who are agreeable may either undergo an exam and exit interview or complete the study procedures as previously described and completed in Study Visit 1 at the time of withdrawal from the study.

If a subject withdraws, a replacement subject may be recruited.

10. Statistical Considerations

This study will be a placebo-controlled, single center study with 3 arms of women planning on undergoing surgery for pelvic organ prolapse.

The primary outcome will be vaginal epithelial thickness based on OCT measures. Findings of collagen and elastin scores, epithelial/stromal/muscularis thickness, cytokines, MMPs, inflammatory cells, and receptors will be also compared between groups. Appropriate statistical analysis for ordinal (e.g. scores) and continuous (e.g. thickness) data will be applied, including tests to determine normality (Shapiro-Wilk) and analysis with ANOVA if criteria are met (e.g. normal, continuous). If ANOVA criteria are not met, then Kruskal-Wallis will be performed for non-normal, ordinal (e.g. scores) data sets.

Power Analysis

Preliminary and historical data are available for OCT thickness, therefore power analysis was based on differences in the OCT thickness mean difference between groups of 40 micrometers with standard deviation 18, and 80% power. Based on T-test and Bonferonni correction (alpha/number of tests = 0.025) for 2 comparisons (1. postmenopausal treatment vs placebo and 2. reproductive age women at 2 different stages of the cycle), five subjects were calculated to be needed per arm to be able to reject

the null hypothesis that the population means of the groups are equal. For example, the postmenopausal group with no estrogen is expected to have epithelial thickness of 100um, the postmenopausal group that receives estrogen is expected to have epithelial thickness of 180um, and the reproductive age women are expected to have epithelial thickness of 240um at follicular phase and 200um at the luteal phase.

11.0 Ethics and the Protection of Human Subjects

The PI will ensure that this study is conducted in full conformity with applicable regulations and UTMB IRB policies and procedures. Any amendments to the protocol or consent materials will be approved by the IRB before they are placed into use.

Potential candidates will be asked to read and review the approved study consent form. The study clinician will review the consent document with the candidate. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. Potential candidates will have the opportunity to take the consent form home to discuss the study with family members, friends or other providers. Study candidates will sign the informed consent document prior to any procedures being done specifically for the study. Subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Study participants will be informed of any abnormality that may be discovered upon the study ultrasound and referred for further evaluation and testing.

Data and Safety Monitoring Plan

Oversight of internal monitoring of the participants' safety and data quality will be conducted by the PI, along with members of the research team. The PI will assume primary responsibility for monitoring the safety of the study participants. If any safety concerns are identified, then reasonable and appropriate actions to be taken will be discussed with the investigative team.

Data integrity and security is the responsibility of all study personnel and will be carefully monitored by the investigators. All study paperwork will be stored in a locked file cabinet accessible only to designated research staff. Electronic data (i.e., computer files) will be created and accessed solely by project personnel and be password protected to guard against loss.

Study investigators are instructed to report adverse events to the PI and study coordinator. All serious adverse events and unanticipated problems will be reported to the UTMB IRB immediately by telephone and by written report within 24 hours of receipt of notice. Non-serious events requiring reporting to the IRB will be done in a prompt manner, within the 10-working day window. The research team members will review adverse events, SAEs and unanticipated problems as they are arise and make modifications to the protocol and consent form as warranted.

Privacy/Confidentiality Issues

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and any sponsor. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Principal investigator.

Individuals authorized to view study records are identified to the study subject on the informed consent. Study records may also be made available for internal compliance reviews and quality assurance representatives.

In accordance with the Food and Drug Administration Amendment Act of 2007 (FDAAA) and The International Committee of Medical Journal Editors (ICMJE) member journals trials-registration policy as a condition for publication, this study will be registered in the public trials registry ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Results will be published to clinicalTrial.gov when available but will not identify individual subjects.

12.0 Record Retention

The study shall last appr*oximately 1 year.*

Research records will be maintained in accordance with applicable IRB policies and federal/state regulations.

References

1. Hendrix SL, Clark A, Nygaard I, et al. Pelvic organ prolapse in the Women's Health Initiative: gravity and gravidity. Am J Obstet Gynecol 2002;186(6):1160–6.
2. Bradley CS, Zimmerman MB, Qi Y, et al. Natural history of pelvic organ prolapse in postmenopausal women. Obstet Gynecol 2007;109(4):848–54
3. Tinelli A, Malvasi A, Rahimi S, Negro R, Vergara D, Martignago R, et al. Age-related pelvic floor modifications and prolapse risk factors in postmenopausal women. Menopause 2010;17:204-12.
4. Sartori MGF, Feldner PC, Jarmy-Di Bella ZIK, Castro A, Baracat EC, Rodrigues de Lima G et al. Sexual steroids in urogynecology. Climateric 2011;14:5-14.
5. Connell KA. Elastogenesis in the vaginal wall and pelvic-organ prolapse. N Engl J Med 2011;364:2356-58.
6. Budatha M, Roshanravan S, Zheng Q, Weislander C, Chapman SL, Davis EC, et al. Extracellular matriz proteases contribute to progression of pelvic organ prolapse in mice and humans. J Clin Invest 2011;121:2048-59.
7. Pal L, Hailpern SM, Santoro NF, Freeman R, Barad D, Kipersztok S, et al. Increased incident hip fractures in postmenopausal women with moderate-to-severe pelvic organ prolapse. Menopause 2011;18:967-73.
8. Nygaard I, Turvey C, Burns TL, Crischilles E, Wallace R. Urinary incontinence and depression in middle-aged United States women. Obstet Gynecol 2003;101:149-56
9. Olsen AL, Smith VJ, Bergstrom JO, Colling JC, Clark AL. Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet Gynecol 1997;89:501-6.
10. Fialkow MF, Newton KM, Lentz GM, Weiss NS. Lifetime risk of surgical management for pelvic organ prolapse or urinary incontinence. Int Urogynecol J 2008;19:437-440.

11. Clark AL, Gregory T, Smith VJ, Edwards R. Epidemiologic evaluation of reoperation for surgically treated pelvic organ prolapse and urinary incontinence. Am J Obstet Gynecol 2003;189:1261-67.
12. Fialkow MF, Newton KM, Weiss NS. Incidence of recurrent pelvic organ prolapse 10 years following primary surgical management: a retrospective cohort study. Int Urogyn J 2008;19:1483-87.
13. Sung VW,Weitzen S,Sokol ER, Rardin CR, Myers DL. Effect of patient age on increasing morbidity and mortality following urogynecologic surgery. Am J Obstet Gynecol 2006;194:1411-7.
14. Pierce CB, Hallock JL, Blomquist JL, Handa VL. Longitudinal changes in pelvic organ support among parous women. Female Pelvic Med Reconstr Surg 2012;18:227-32.
15. Nygaard I, Barber MD, Burgio KL, Kenton K, Meikle S, Schaffer J, et al. Prevalence of symptomatic pelvic floor disorders in US women. JAMA 2008;300:1311-16.
16. Kepenekci I, Keskinkilic B, Akinsu F, Cakir P, Elhan AH, Erkek AB, et al. Prevalenc of pelvic floor disorders in the female population and impact of age, mode of delivery and parity. Dis Colon Rectum 2011;54:85-94
17. Helibrun ME, Nygaard IE, Lockhart ME, Richter HE, Brown MB, Kenton KS et al. Correlation between levator ani muscle injuries on MRI and fecal incontinence, pelvic organ prolapse, and urinary incontinence in primarous women. Am J Obstet Gynecol 2010;202:488e1-6.
18. Diez-Itza I, Arrue M, Ibanez L, Paredes J, Murgiondo A, Sarasqueta C. Postpartum impairment of pelvic floor muscle function: factors involved and association with prolapse. Int Urogynecol J 2011;22:1505-11.
19. Townsend MK, Curhan GC, Resnick NM, Grodstein F. Postmenopausal hormone therapy and incident urinary incontinence in middle-aged women. Am J Obstet Gynecol 2009;200:86e1-6.
20. Bachmann G. Quantifying estrogen treatment effect on vaginal tissue: cellular age matters Menopause;12:656-7.
21. Mammadov R, Simsir A, Tuglu I, Evren V, Gurer E, O¨ zyurt C. The effect of testosterone treatment on urodynamic findings and histopathomorphology of pelvic floor muscles in female rats with experimentally induced stress urinary incontinence. Int Urol Nephrol (2011) 43:1003–1008.
22. Kim NN, Min K, Pessina MA, Munarriz R, Goldstein I, Traish AM. Effects of ovariectomy and steroid hormones on vaginal smooth muscle contractility. Int J of Impotence Research 2004;16:43–50.
23. Weemhoff M, Shek KL, Dietz HP. Effect of age on levator function and morphometry of the levator hiatus in women with pelvic floor disorders. Int Urogynecol J 2010;21:1137-42.
24. Zhu L, Lang J, Chen J, Jie Chen (2005) Morphologic study on levator ani muscle in patients with pelvic organ prolapse and stress urinary incontinence. Int Urogynecol J 16: 401–404.
25. Bortolini MAT, Shynlova O, Crutz HP, Castro RA, Birao MJBC, Lye S, Alarab. Expression of genes encoding smooth muscle contractile proteins in vaginal tissue of women with and without pelvic organ prolapse Neurourol Urodynam 2012;31:109-14.
26. Berman JR, Almeida FG, Jolin J, Raz S, Chaudhuri G, Gonzalez-Cadavid NF. Correlation of anterogen receptors, aromatase, and 5-alpha reducatase in the human vagina with menopausal status. Fertil Steril 2003;79:925-31.
27. Traish AM, Kim SW, Stankovic M, Goldstein I, Kim NN. Testosterone increases blood flow and expression of androgen and estrogen receptors in the rat vagina. J Sex Med 2007;4:609-19
28. Farage M, Maibach H. Lifetime changes in the vulva and vagina. Arch Gynecol Obstet 2006;273:195-202.
29. Moss JA, Malone AM, Smith TJ, Kennedy S, Nguyen C, Vincent KL, Motamedi M, Baum MM. Pharmacokinetics of a mutipurpose pod-intravaginal ring simultaneously delivering five drugs in the ovine model. *AIDS* (undergoing review).

APPENDIX

NOTE: The following questionnaires are included in the UroGyn Intake Form; Pelvic Health clinic patients are asked to complete the Intake packet at the time of their initial clinic visit. If the study patient completed these Intake questionnaires less than 3 months prior to taking part in this study, data from the questionnaires will be used for this research. If the clinical intake questionnaires were completed 3 or more months prior to taking part in this study, participants will be asked to complete the questionnaires again at Study Visit 1; in addition, questionnaires will be repeated as part of Study Visit 2.

Participant's Study Number: _____ Initials: _____ Date Completed: _____

4. Urinary Distress Inventory/UDI-6

Do you experience…

4a. frequent urination? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

4b. urine leakage associated with a feeling of urgency, that is a strong sensation of need to go to the bathroom? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

4c. urine leakage related to coughing, sneezing or laughing? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

4d. small amounts of urine leakage (that is, drops)? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

4e. difficulty emptying your bladder? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

4f. pain or discomfort in lower abdomen or genital region? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

5. Pelvic Organ Prolapse Distress Inventory/POPDI-6

Do you experience…

5a. pressure in your lower abdomen? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

5b. heaviness or dullness in the pelvic area? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

5c. a bulge or something falling out that you can see or feel in your vaginal area? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

5d. having to push on the vagina or around the rectum to have or complete a bowel movement? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

5e. a feeling of incomplete bladder emptying? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

5f. having to push up on the bulge of the vaginal area with your fingers to start or complete urination? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

9. Colorectal-Anal Distress Inventory 8/CRADI-8

Do you experience…

9a. the feeling you need to strain too hard to have a bowel movement (constipation)? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

9b. the feeling you have not completely emptied your bowel at the end of a bowel movement?
☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

9c. losing stool beyond your control if stool is well formed? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

9d. losing stool beyond your control if your stool is loose? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

9e. losing gas from your rectum beyond your control? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

9f. pain when you pass gas? ☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

9g. a strong sense of urgency and have to rush to the bathroom to have a bowel movement?
☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

9h. part of your bowel ever passing through the rectum and bulging outside during or after a bowel movement?

☐ Yes ☐ No
If Yes, how much does this bother you? ☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

19. PELVIC FLOOR IMPACT QUESTIONNAIRE/PFIQ

Some women find that bladder, bowel or vaginal symptoms affect their activities, relationships and feelings. For each question below, place an X in the response that describes **how much YOUR ACTIVITIES, RELATIONSHIPS OR FEELINGS HAVE BEEN AFFECTED BY YOUR BLADDER, BOWEL OR VAGINAL SYMPTOMS OR CONDITIONS over the LAST 3 MONTHS.**

	Please mark an answer IN ALL 3 COLUMNS for each question.		
	Bladder/Urine	**Bowel/Rectum**	**Vagina/Pelvis**
1. ability to do household chores (cooking, laundry, housecleaning?	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit
2. ability to do physical activities such as walking, swimming or other exercise?	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit
3. entertainment activities such as going to a movie or concert?	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit
4. ability to travel by car or bus for a distance greater than 30 minutes away from home?	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit
5. participating in social activities outside your home?	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit
6. emotional health (nervousness, depression, etc.)?	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit
7. feeling frustrated?	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit	☐ Not at all ☐ Somewhat ☐ Moderately ☐ Quite a bit

20. PELVIC ORGAN PROLAPSE/URINARY INCONTINENCE SEXUAL FUNCTION QUESTIONNAIRE/PISQ-12

Following is a list of questions about you and your partner's sex life. Your confidential answers will be used to help understand what is important to patients about their sex lives. If no sexual activity, ☐ check here and answer only question #1.

Please check the box that best answers the question for you. While answering the question, consider your sexuality over the past 6 months.	**Always**	**Usually**	**Some times**	**Seldom**	**Never**
1. How frequently do you feel sexual desire? This feeling may include wanting to have sex, planning to have sex, feeling frustrated to lack of sex, etc.	☐	☐	☐	☐	☐
2. Do you climax (have an orgasm) when having sexual intercourse with your partner?	☐	☐	☐	☐	☐
3. Do you feel sexually excited (turned on) when having sexual activity with your partner?	☐	☐	☐	☐	☐
4. How satisfied are you with the variety of sexual activities in your current sex life?	☐	☐	☐	☐	☐
5. Do you feel pain during sexual intercourse?	☐	☐	☐	☐	☐
6. Are you incontinent of urine (leak urine) with sexual activity?	☐	☐	☐	☐	☐
7. Does fear of incontinence (either stool or urine) restrict your sexual activity?	☐	☐	☐	☐	☐
8. Do you avoid sexual intercourse because of bulging in the vagina (either the bladder, rectum or vagina falling out)?	☐	☐	☐	☐	☐
9. When you have sex with your partner, do you have negative emotional reactions such as fear, disgust, shame or guilt?	☐	☐	☐	☐	☐
10. Does your partner have a problem with erections that affects your sexual activity?	☐	☐	☐	☐	☐
11. Does your partner have a problem with premature ejaculation that affects your sexual activity?	☐	☐	☐	☐	☐
12. Compared to orgasms that you have had in the past, how intense are the orgasms you have had in the past 6 months? ☐ Much less intense ☐ Less intense ☐ Same intensity ☐ More intense ☐ Much more intense					