Official title: Investigation to Minimize Prolapse Recurrence Of the Vagina using Estrogen (IMPROVE)

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INVESTIGATION TO MINIMIZE PROLAPSE RECURRENCE OF THE VAGINA USING ESTROGEN (IMPROVE)

A randomized, double-blind clinical trial of conjugated estrogen vaginal cream compared with placebo given peri-operatively in 222 postmenopausal women with symptomatic pelvic organ prolapse undergoing a standardized transvaginal native tissue surgical repair.

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Study Title

Investigation to Minimize Prolapse Recurrence Of the Vagina using Estrogen (IMPROVE)

Objectives

Aim 1: Determine if adjunctive vaginal estrogen given pre- and postoperatively with standardized native tissue transvaginal prolapse repair surgery results in improved objective and patients' subjective sense of pelvic organ support, retreatment rate, postoperative satisfaction, and quality of life one year postoperatively with continued annual surveillance up to 3 years.

Aim 2: Determine if perioperative vaginal estrogen therapy in this same population impacts other pelvic floor disorder symptoms. Specifically, we will quantify the effects of vaginal estrogen on symptoms of overactive bladder and urinary incontinence (Subaim 2a), sexual function and dyspareunia (Subaim 2b), postoperative cystitis (Subaim 2c), and symptomatic vulvovaginal atrophy (Subaim 2d).

Aim 3: Quantify histomorphology and connective tissue homeostasis of the vaginal wall in postmenopausal women with symptomatic prolapse undergoing surgical repair with or without pre- operative intravaginal estrogen therapy. We will determine the potential mechanisms by which local estrogen treatment alters pelvic organ support and quantify the effects of vaginal estrogen on synthesis and degradation of extracellular matrix proteins and its impact on smooth muscle of the vaginal wall.

Design and Outcomes

Design: Randomized, double-blind, placebo-controlled trial of pre- and postoperative intravaginal estrogen therapy in 222 postmenopausal women with symptomatic prolapse undergoing a standardized surgical repair using native tissue.

Primary outcome: prolapse recurrence as defined by a composite of objective prolapse beyond the hymen, subjective/symptomatic prolapse (feeling of a bulge or *no change* or *worse* on the patient's global impression of improvement), or retreatment (e.g., reoperation or pessary use) assessed 1 year postoperatively. *Time to failure* will be compared in the two groups.

Important secondary outcomes assessing for changes in overactive bladder and urinary incontinence symptoms will be made by comparing symptoms (validated questionnaires) from Baseline to Pre-op/time of surgery. Aim 3 (see above, histomorphology, connective tissue studies) will be assessed from full thickness anterior apical vaginal wall biopsies taken at the time of surgery.

Interventions and Duration

Intervention: 1g conjugated estrogen (CE) cream (Premarin®, Pfizer Inc.) given nightly for 2 weeks then twice-weekly to complete planned 6-8 weeks (5 weeks minimum) therapy before a standardized uterosacral or sacrospinous ligament suspension transvaginal prolapse repair surgery. Those with a uterus will undergo concomitant total vaginal hysterectomy first. Twice-weekly CE cream application is then resumed upon discharge and continued for 1 year postoperatively.
Comparator: placebo vaginal cream, same schedule
Duration: The primary outcome of prolapse recurrence is assessed at one year postoperatively with continued surveillance of participants until 3 years postoperatively.

Sample Size and Population

Total sample size: N=222; 111 per treatment group

Target population: Women ≥48 years and at least 1 year postmenopausal (or status post BSO) with vulvovaginal atrophy; no oral, vaginal, or transdermal medication containing estrogens (including selective estrogen receptor modulators, or SERMs), androgens, or progestins within 8 weeks of screening; with symptomatic pelvic organ prolapse (≥stage 2 anterior/apical prolapse) fit for elective transvaginal native tissue repair will be included. Exclusions are BMI >35, current tobacco or steroid use, any other medications affecting vaginal milieu, vaginal infection, prior vaginal apical repair surgery or surgery using mesh for prolapse, and contraindications to estrogen therapy (e.g., stroke, prior thromboembolism, estrogen-responsive tumor, postmenopausal vaginal bleeding).

Other: randomization will be stratified by site (3 clinical sites participating), hysterectomy status, and duration since menopause (<10 and ≥10 years). The randomization lists will be distributed to each site's research pharmacy. Outcome assessors and patients will be blinded to assignment.

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1.1 Primary Objective

Determine if the experimental treatment (adjunctive vaginal estrogen given pre- and postoperatively with standardized native tissue transvaginal prolapse repair surgery) results in improved objective and patients' subjective sense of pelvic organ support, retreatment rate, postoperative satisfaction, and quality of life one year postoperatively.

1.2 Secondary Objectives

- Determine how perioperative vaginal estrogen therapy in this same population impacts other pelvic floor disorder symptoms. Specifically, we will quantify the effects of vaginal estrogen on…
  (a) symptoms of overactive bladder and urinary incontinence
  (b) sexual function and dyspareunia
  (c) postoperative cystitis
  (d) symptomatic vulvovaginal atrophy

- Quantify histomorphology and connective tissue homeostasis of the vaginal wall in this same population and determine the potential mechanisms by which local estrogen treatment alters pelvic organ support and quantify the effects of vaginal estrogen on synthesis and degradation of extracellular matrix proteins and its impact on smooth muscle of the vaginal wall.

2 BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

Pelvic organ prolapse (POP) is important from individual and public health perspectives. Although *some* degree of loss of vaginal or uterine support is present in most adult women, 3-6% have descent to the hymen or beyond, which corresponds to clinically significant disease and bother for patients.[1,2] POP is the most common reason for hysterectomy among postmenopausal women and is an indication for more than 338k inpatient surgical procedures each year.[3] The lifetime risk of surgery for POP and stress urinary incontinence is *20%*, and for POP surgery alone, *13%.*[4] There are substantial social, psychological and financial implications for women with POP.[5-7] For society, the estimated annual direct costs are more than $1 billion (for 226k surgeries)[8] and costs for POP surgeries in coming decades are expected to grow at twice the rate of population growth due to our aging population.[9]

Aging is a known risk for POP. The etiology of POP is multifactorial, and 3 risks are well-established: vaginal delivery, aging, and obesity.[10] Among ambulatory women presenting for yearly examination, the relative prevalence of POP increased by 40% with each decade of life.[1] As our population ages, projections for 2050 are that the number of US women with prolapse will increase 46% from 3.3 to 4.9 million, underscoring the substantial burden that POP represents for our healthcare system.[11]

Prolapse progression and recurrence after primary surgical repair are common in older women. While there are limited data describing the natural history of POP

progression, a single-site observational study of women over 2-8 years from within the Women's Health Initiative (WHI) reported incidence of grades 1-3 cystocele (i.e. bladder prolapse from just inside to just outside the hymen) to be 9.3/100 women-years and 5.7/100 women-years for rectocele.[12] Progression from grade 1 (generally asymptomatic) to grades 2 to 3 (i.e. symptomatic) prolapse was seen in 9.5% of patients with cystoceles and 14% of those with rectoceles. Some remissions occurred for grade 2-3 cystoceles and rectoceles, but, of note, there were no observed remissions for women with grade 2 or 3 uterine/apical prolapse. Another study from the WHI by Bradley et al described 1-year and 3-year incidence rates of prolapse at the hymen or beyond as 26% and 40%, respectively.[13] Importantly, older parous women were more apt to develop new or progressive prolapse than to regress.

Traditionally, reconstructive surgeries for anterior and apical vaginal prolapse are performed transvaginally using native tissue (e.g. using uterosacral or sacrospinous ligaments as fixation points), although transvaginal or abdominal/ laparoscopic approaches with augmentation by synthetic graft materials are also common. Reoperation *for recurrent prolapse* is common, and rates are highest in the traditional vaginal surgery group.[14] Denman et al reported 17% of patients needing repeat operations within 10 years, which likely represents an underestimate of the true rate.[15] Indeed, in a cross-sectional analysis of women in a private HMO in the Northwest undergoing POP or incontinence surgery, 29% of cases were reoperations.[16]

New surgical strategies may improve the efficacy of repair but with new risks. To address these unacceptably high failure and reoperation rates, surgical techniques have evolved; the addition of synthetic transvaginal mesh was first approved by the FDA in 2004. Since then, hundreds of thousands of mesh systems have been implanted, in particular for recurrent or advanced prolapse.[17] While it appears that mesh placed transvaginally to augment prolapse repairs may decrease the risk of recurrent objective anterior vaginal wall prolapse[18-20], there are significant trade-offs, including risks of visceral and vaginal erosion of mesh, chronic granulation tissue with discharge/drainage, de novo pain, worsening stress incontinence, and dyspareunia.[20-23] Mesh exposure has become a significant problem requiring surgical excision in 7-10% of cases.[20,24] Although synthetic mesh placed abdominally or laparoscopically for sacral-colpopexy (i.e. lifting the vagina to the sacrum via mesh) is generally considered to have an acceptable risk-benefit ratio, compared to vaginal procedures, there is longer operating time, increased cost, and a non-negligible risk of serious complications. All graft materials for prolapse repairs are under increased scrutiny, highlighted by recent advisories from the FDA warning about the risks associated with mesh use in pelvic surgery.[24-26]

***Summary*:** Given an aging population with increasing prevalence of POP, more women will require surgical management. Procedures utilizing graft materials, particularly if introduced transvaginally, are associated with complications that may represent an unacceptable risk for many women. Therefore, there is an urgent need to identify adjuncts to native tissue prolapse repair that may prevent or minimize recurrent disease.

The utility of estrogen in POP management is uncertain. Both basic and clinical investigations indicate that estrogen plays an important role in the function of supportive

connective tissues of the pelvic floor. Estrogen and progesterone receptors have been identified in the nuclei of connective tissue and smooth muscle cells of both the levator ani stroma and uterosacral ligaments.[27,28] The ratio of collagen I (generally consisting of well-organized fibers and associated with ligamentous tissue) to collagen III and IV (more common in loose areolar tissue) is decreased in pelvic connective tissues of postmenopausal women not on hormone replacement therapy.[29] This relative decrease in well-organized dense collagen is believed to contribute to weakening of vaginal tensile strength thereby leading to increased susceptibility to anterior vaginal wall prolapse. Further, estrogen is thought to play a role in inhibiting expression of collagen- and elastin-degrading proteins in the vagina and fibroblasts of uterine supportive tissue.[30,31] *In contrast*, other research suggests that matrix metalloproteinases (MMPs) are upregulated by estrogen and that estrogen-induced suppression of tissue inhibitors of metalloproteinases (TIMPs) is involved in *negative effects* on the pelvic floor.[32,33]

Clinical research regarding the effect of *systemic* hormone therapy on prevention or treatment of POP has also led to contradictory findings. Different estrogen receptors in the urogenital tract have been studied with various selective estrogen receptor modulators (SERMs). A meta-analysis of the effects of raloxifene (a SERM used for osteoporosis treatment and prevention) identified an apparent protective effect on the pelvic floor with reduction in the need for prolapse surgery at a 3-years (OR 0.50, 95% CI 0.31-0.81).[34] Importantly, however, the total number of women having prolapse surgery was small; there was no standardized evaluation for POP in any study; and the baseline obstetrical histories were not known or reported. Two ancillary studies from the WHI found no difference between women treated with *systemic* estrogen and progesterone compared with those treated with placebo in terms of percent of participants with prolapse ≥ stage 2, and duration of hormone therapy did not appear to affect rate of prolapse.[7,35] It should be noted, however, that progesterone often opposes the effect of estrogen in the female reproductive tract. A case-control study by DeLancey et al examining levator ani defects in women with and without prolapse noted higher rates of prolapse in women using hormone therapy.[36] The most recent Cochrane review of estrogens for the treatment or prevention of POP suggests that evidence is too limited and that there is a need for studies randomizing to estrogen preparations for the "prevention and management of POP, particularly as an adjunctive treatment for women using pessaries and also before and after prolapse surgery."[37]

The role of vaginal estrogen in other PFDs is better characterized. As both the lower female genital and urinary tracts arise from the primitive urogenital sinus, it is not unexpected that estrogen receptors are found in tissues of the vagina, urethra, bladder, and surrounding pelvic floor musculature.[38-40] Contrary to the limited information of estrogen's effects on POP, the effect of estrogen on urinary incontinence in postmenopausal women is more understood, although effects differ depending on *systemic* vs. *local* estrogen delivery.[41] Six trials of systemic estrogen demonstrated *worse* incontinence (urge, stress, or mixed) compared with placebo, in both systemic conjugated estrogens alone (RR 1.32, 1.17-1.48) or combined with progesterone (RR 1.11, 1.04-1.18). However, with *local* application of estrogen, *incontinence rates improved* (RR 0.74, 0.64-0.86), and urinary urgency (RR 0.38, 0.15-0.99) and frequency also improved. A recent randomized trial of *local* estrogen vs. an oral anticholinergic medication (oxybutynin) for treatment of overactive bladder in postmenopausal women demonstrated similar effectiveness in

decreasing number of daily voids and improvements in disease-specific quality of life.[42] Of note, the Cochrane review's summaries of improvements with local estrogen were based on just 2-4 trials, and further study was recommended. Effects on stress urinary incontinence are not as well-characterized, but local estrogen has been shown to decrease the pulsatility index in the urethral vascular network (indicating greater blood flow) and improve subjective stress incontinence bother in 60% of patients.[43]

Local intravaginal estrogen in postmenopausal women by any delivery system (creams, tablets, estrogen-containing rings) is known to decrease vaginal pH, increase blood flow, improve tissue compliance, and promote vaginal cell maturity.[44] Reviews and studies of other pelvic floor disorders commonly linked with loss of estrogen and urogenital atrophy observed in postmenopausal women (dryness, pruritus, pain/ dyspareunia[45-47], recurrent cystitis[48,49]) generally demonstrate improvement in symptoms and quality of life using local estrogen.[44,46,50,51] However, these conditions are not well-characterized in women also presenting with POP or for perioperative complaints such as early postoperative cystitis.

Perioperative use of vaginal estrogen is inconsistent & patient adherence is poor. Although some gynecologists may anecdotally recommend pre- and postoperative vaginal estrogen empirically for postmenopausal patients undergoing surgery, there are few studies that directly examine local estrogen use and perioperative outcomes.[52,53] Estrogens may affect cutaneous wound healing by modulating cytokine expression, inflammatory response, and matrix deposition. Stimulation of angiogenesis and regulation of proteolysis may accelerate re-epithelialization.[54,55] These effects have been studied in dermatologic *but not gynecologic* populations. A study that provides compelling evidence *to recommend* perioperative vaginal estrogen use will provide a strong incentive for women to remain adherent to vaginal estrogen. Otherwise, anecdotal or empiric prescriptions are likely to be unsuccessful because 9% of menopausal women receiving a vaginal estrogen prescription never fill it, and discontinuation occurs by most after just 3 months.[56,57]

***Summary*:** Menopause and loss of estrogen contribute to the decompensation of pelvic organ support. Overall, basic science and clinical investigations clearly demonstrate an important role for reproductive hormones in the maintenance of connective tissues and the extracellular matrix necessary for pelvic visceral support and healthy urogenital tract function, but the precise role for vaginal estrogen in POP management is uncertain. A scientifically rigorous study examining the impact of pre- and postoperative vaginal estrogen on maintenance of support after surgical repair of POP is needed and may provide, for the first time, evidence for a safe, low-cost adjunct for prevention of recurrent disease.

2.2 Study Rationale

2.2a Preliminary Studies: Studies in preclinical animal models (mice, guinea pigs, and rats) indicate that estrogen is important in elastic fiber renewal and collagen homeostasis in the fibromuscular vaginal wall. In this protocol, there is the suggestion of a unique adaptation to synthesize and assemble *new* elastic fibers allowing the vagina to respond to local estrogen treatment with increased synthesis of collagen and elastic fibers. To follow are summaries of studies in mice, guinea pigs[58], rats, and a pilot study in humans[59] that provide support and a rationale for the proposed specific aims.

***Mice*: Estrogen alters vaginal epithelium, muscularis, protease activation, and matrix synthesis.** Mice were ovariectomized to study the effect of menopause and later estrogen replacement (added after 14d) on proteins important in elastic fiber cross-linking, i.e. lysyl oxidase (LOX), lysyl oxidase-like 1 (LOXL1), and fibulin-5 (FBLN-5), and elastogenesis in the vaginal muscularis. The data indicate that, like bone, early after menopause there is rapid induction of protease activity and loss of matrix proteins. Thereafter, a slower decline in matrix synthesis results in a thinner and perhaps, even less resilient organ. The loss of both vaginal epithelial and muscularis thickness was fully reversed by estrogen replacement. This suggests that the baseline characteristics of the vaginal matrix at the time of menopause, together with the rate and severity of estrogen withdrawal, may compromise the ability of the vagina and its suspensory connective tissue to maintain pelvic organ support after menopause and with aging.

***Guinea pigs*: Systemic estrogen alters wound healing of the vaginal wall.**[58] Nulliparous ovariectomized guinea pigs treated with estradiol (or vehicle) replacement were used to determine effects of estrogen on biomechanical properties, LOX, collagen content, and histomorphology of the vagina with or without surgical injury to the perineum. Estradiol resulted in (i) significant growth, increased smooth muscle, and increased thickness of the vagina, (ii) increased distensiblity without compromise of maximal force at failure, and (iii) increased total and cross-linked collagen. The results suggest prolonged estradiol-induced increases in LOX and collagen crosslinks may sustain a matrix environment optimizing long term wound healing in the vagina.

***Rats*: Local estrogen cream improves vaginal function and connective tissue.** The effects of systemic and local estrogen on (i) uterine and vaginal weight, (ii) serum estradiol (E2) levels, (iii) collagen content and expression of proteins involved in collagen assembly, and (iv) biomechanical properties of the vaginal wall were studied using ovariectomized rats treated with systemic estradiol, vaginal conjugated estrogens cream, or vaginal placebo cream. Vaginal estrogen treatment increased total and cross-linked collagen content and markedly stimulated collagen mRNA expression in an inverse dose-effect relationship. The results suggest that high-dose vaginal estrogen results in down-regulation of ERα and loss of estrogen-induced increases in vaginal collagen. Low-dose vaginal estrogen treatment resulted in significant vaginal growth and increased distensiblity without uterine hyperplasia.

***Humans*: Effects of Preoperative Estrogen Treatment (the "PET" study) on connective tissues of the pelvic floor[59]**: Results from these preclinical animal models, therefore, led to a pilot trial examining the effects of vaginal estrogen on the biochemistry, molecular profile, & histology of the vaginal wall.
Population: 30 women ≤10yr post-menopausal & ≥stage 2 symptomatic anterior or apical prolapse (i.e., leading edge of bulge to the hymen or beyond) planning repair involving hysterectomy. Exclusions: BMI >35kg/m^2, current tobacco or steroid use, vaginal infection, and contraindications to estrogen therapy.
Intervention: Participants were randomized to 6 weeks of preoperative vaginal estrogen (Premarin® cream 0.625mg/gm, 1gm applied nightly for 2 weeks then 2 times per week)

or identical placebo cream. A standardized full-thickness biopsy was obtained from the apical anterior vaginal wall at the time of surgery.

Outcomes: Vaginal biopsies were analyzed for histological differences in tissue thickness and composition. Assays were also performed to assess mRNA (quantitative PCR), cross-linked collagen (hydroxyproline assays), collagen types I and III (immunoblots), and matrix metalloprotease (MMP) activity (zymography). Serum estrone (E1) and estradiol (E2) were assessed at baseline and the day of surgery using highly sensitive liquid chromatography-tandem mass spectrometry assays[60], and surgical specimens were also examined for endometrial & myometrial thickness and pathology.

Results: Demographics of Estrogen & Placebo groups were similar with no significant differences in age, BMI, parity, baseline prolapse, race/ethnicity, or route of hysterectomy. Two patients per group did not have surgery (unrelated to study medications) and were not analyzed in the final comparisons as they had no tissue biopsy. More patients in the Estrogen group (4 of 13) were non-adherent compared to Placebo (0 of 13), *P*=0.030, as assessed by a composite of patient diaries and tube weights at baseline and at surgery.

Histologic changes & collagen synthesis: Analyzed per-protocol and in those with sufficient tissue for analysis (Estrogen=8; Placebo=12), epithelial thickness was increased 1.8-fold in the Estrogen group (551±45 vs. 314±43 μm, *P*=0.002). Consistent with our studies in the 3 animal models, thickness of the muscularis was increased 2.7-fold (7571 vs. 2807 μm, *P*=0.088). Levels of collagen types *1α1*, *1α2*, & *3α* mRNA were increased 6.0-, 1.8-, & 2.5-fold in the vaginal muscularis of the Estrogen group (p<0.05 for *1α1* & *1α2*) (Fig.1A). Immunoblot analysis was completed in 4 patients in each group with sufficient tissue for protein analysis. Collagen type Ia protein increased 9-fold in the muscularis from women treated with Estrogen (*P*=0.012) (Fig. 1B). In contrast, differences in collagen III protein were not significant (*P*=0.27). Although similar in the vaginal *mucosal* layer, total cross-linked collagen content as determined by hydroxyproline assays was increased 3.2-fold in the muscularis of Estrogen patients (*P*=0.10). These studies, therefore, indicate that collagen type I is preferentially enhanced by vaginal estrogen treatment and that the increase in collagen content is localized to the underlying fibromuscular layer.

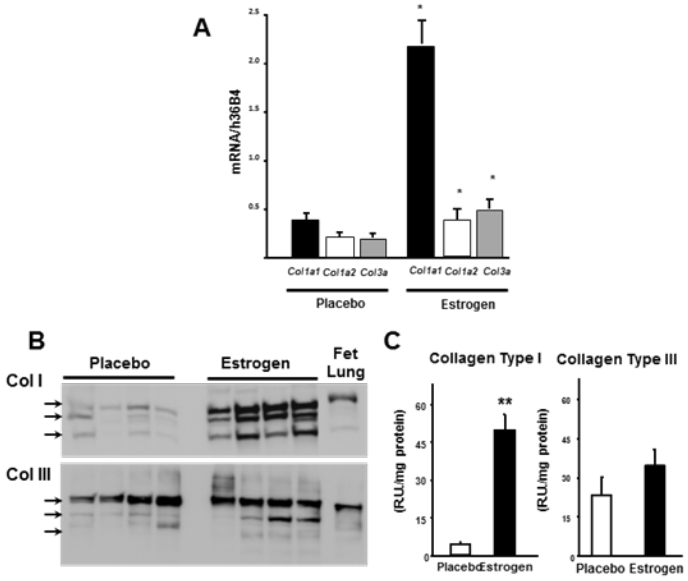


Fig. 1. Effect of vaginal estrogen on collagen types I and III in the vaginal muscularis. A. Levels of *Col1α1*, *1α2*, and *3α* mRNA in tissues from women treated with Placebo (n=12) or Estrogen (n=8). Immunoblot analysis (B) and densitometry (C) of collagen type I and III (n=4 per group). *P < 0.05; **P < 0.01

Collagenase and elastolytic activity: Since vaginal epithelium is known to be a rich source for protease inhibitors[61], the mucosa was evaluated for expression of these molecules. Although highly expressed in vaginal mucosa, mRNA levels of 3 serine protease inhibitors (*elafin*, *secretory leukocyte protease inhibitor*, and *α1-antitrypsin*) and two MMP inhibitors (*TIMP1* and *2*) were similar between Estrogen and Placebo. Interestingly, however, *MMP12* mRNA (aka human macrophage elastase, a protease important for collagen degradation) was suppressed in the vaginal mucosa from women treated with Estrogen compared to Placebo. The most striking effects of Estrogen were found using quantitative gelatin zymography in which MMP9 activity was down-regulated 6-fold in the mucosa and 4-fold in the muscularis (*P*=0.02) in Estrogen-treated women (Fig. 2).

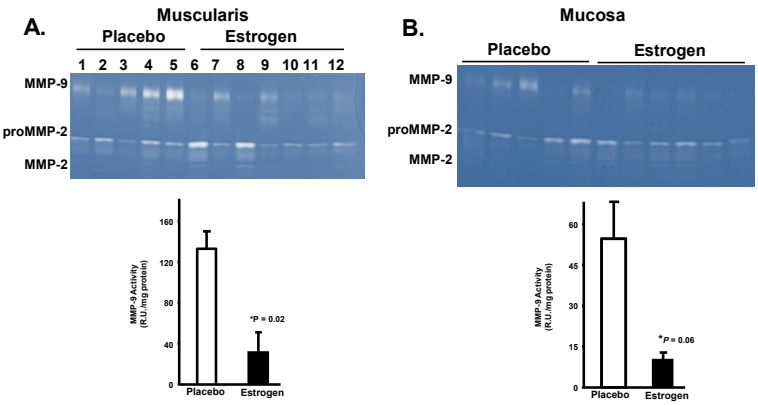


Fig. 2. Vaginal estrogen inhibits MMP-9 activity. Quantitative gelatin zymography was conducted in vaginal muscularis (A) or mucosa (B) from women treated with placebo or estrogen vaginal cream. Bars represent mean ± SEM

Tolerability & Safety: There were no differences in thickness of endometrium (1.8±1.2 vs. 1.4±0.7 mm) or myometrium (1.7±0.6 vs. 2.2±1.6 cm) for Estrogen vs. Placebo, and no cases of proliferative, secretory or hyperplastic endometrium. Consistent with menopausal norms, serum estrone and 17*β*-estradiol were low and did not differ among the two groups. Median (IQR) postoperative anterior wall prolapse stage 6mo postop was 0.5 (0.5,0.8) vs. 1 (0,1.3), *P*=0.56, in Estrogen vs. Placebo. However, this pilot study was not intended nor powered to examine anatomic endpoints, and the study drug was not administered postoperatively.

***Summary*:** Preoperative local vaginal estrogen application results in increased thickness of both the vaginal epithelium and muscularis in adherent postmenopausal women with prolapse. Increases in collagen type I mRNA and protein were disproportionate to that of collagen type III with type I-enriched muscularis. Further, there was down-regulation of MMP1, MMP9 and MMP12 in the vaginal wall with no change in expression of several protease inhibitors. Taken together, findings from this pilot trial suggest that six weeks of preoperative vaginal estrogen in postmenopausal women with prolapse may improve the substrate for suture placement at time of repair while mitigating surgical induction of several degradative enzymes, thereby improving long-term maintenance of connective tissue integrity in the pelvic floor.

2.2b Rationale for use of CE cream (vs. other vaginal estrogens): While adherence to prescription medications is often poor and hindered by increasing age, minimally-symptomatic conditions, polypharmacy, and labor-intensive therapy such as application of

a vaginal cream[62], in the setting of other rigorous clinical trials, exceptionally good adherence rates have been observed: CE cream 2x/wk discontinuation *over 1 year* was just 9.8% in a recent study.[63] Theoretically, adherence could be improved over cream using an estradiol ring (Estring®), which is replaced every 3 months, but users are sometimes bothered by difficulty with ring removal, concern over vaginal infection risk, bother with partner feeling the ring, or difficulty with insertion.[64] Further, a trial that randomized women undergoing reparative surgery immediately postoperatively to 12 weeks of vaginal estradiol or placebo rings found *increased granulation tissue* attributed to a foreign body reaction/ inflammation with the placebo (12 of 21) compared to the estradiol ring (3 of 22), $P<0.01$.[53] Another alternative, the vaginal estradiol tablet (Vagifem®), is only available as a 10 μg tablet, which is not equivalent to the 1g CE cream demonstrated as effective in matrix synthesis and decreased protease activity in the pilot study referenced above.[59] In addition, severely atrophic patients sometimes report failure of the tablet to dissolve and/or the tablet falling out with the emergence of the POP. Finally, there are other potential benefits stemming from the unique "Ring B" unsaturated equine estrogens, including inhibition of LDL and HDL oxidation and apoptosis in neurons.[65]

2.2c Rationale for timing & duration postoperative CE cream: Prior studies using a murine model of menopause demonstrated a "burst" in pro- and active MMP9 activity for 1-7d after withdrawal of estrogen; *LOX* and *LOXL1* mRNA were simultaneously decreased as were overall protein content and mature elastic fibers, indicating that remodeling occurs rapidly with withdrawal of estrogen (see 2.2a *Mice* above). Hence, it is a priority to resume local estrogen as soon as safely feasible after prolapse repair and to continue its use until mature scar formation has completed. Completion of connective tissue remodeling after wound healing occurs from 21d *up to 1 year after injury*.[66,67] This duration is necessary for collagen III to be replaced with stronger collagen I.[66] Therefore, this protocol calls for continued estrogen therapy (avoiding the potential consequences of withdrawal) throughout this remodeling process.

3 STUDY DESIGN

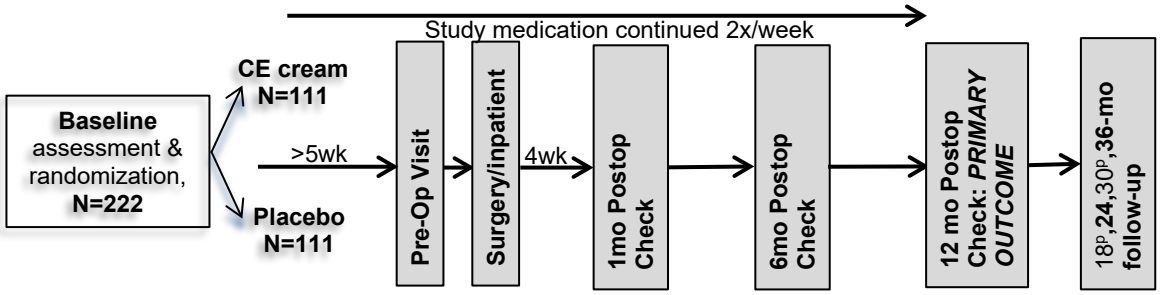
DESIGN: Randomized, double-blind, placebo-controlled trial (See Fig.3 "Design Schema")

POPULATION: Women ≥48 years and at least 1 year postmenopausal (or status post BSO) with vulvovaginal atrophy [no oral, vaginal, or transdermal medication containing estrogens (including SERMs), androgens, or progestins within 4 weeks of screening] with symptomatic POP (≥stage 2 anterior/apical prolapse) fit for elective transvaginal native tissue repair will be included. Exclusions are BMI >35, current tobacco or steroid use, any other medications affecting vaginal milieu, vaginal infection, prior vaginal apical repair surgery or surgery using mesh for prolapse, and contraindications to estrogen therapy (e.g., stroke, prior thromboembolism, estrogen-responsive tumor, postmenopausal vaginal bleeding).

INTERVENTION: **Conjugated estrogens (CE) cream 0.625mg/g (Premarin®) 1g** or **identical placebo**, inserted vaginally nightly for 2 weeks, then twice weekly for 4-6 more weeks before surgery (i.e. planned 6-8 weeks total of preoperative use, 5 weeks minimum).

CE (or placebo) cream is then resumed before hospital discharge and continued 2-times per week for one year. The planned **surgery will be standardized across sites**, as has been achieved previously[68,69] (see description below).

Figure 3. Design Schema



Questionnaires & Exam:								
▪ PFD questionnaires[#]	**X**	**X**			**X**		**X**	**X**
▪ PGI-S[70] (symptom severity)	**X**				**X**		**X**	**X**
▪ PGI-I[70] (symptom improvement)					**X**		**X**	**X**
▪ POP-Q prolapse exam[71]	**X**			**X**	**X**		**X**	**X**
Study drug dispensed:	**X**(1)		**X**(2)		**X***(3)	**X**(4)	**X***(5)	
Document tube weight**	**X**(1)		**X**(1,2)		**X***(3)	**X**(2-4)	**X***(5)	**X**(5)
Specimens & biopsies:								
▪ Vaginal maturation index[72]	**X**		**X**				**X**	
▪ Serum estrone & estradiol[60]	**X**						**X**	
▪ Whole blood, banked for later DNA/ genetic assessment (optional)	**X**							
▪ Vaginal wall biopsy[†]			**X**[†]					

[#] 3 validated short-form (in English and Spanish) Pelvic Floor Disorder (PFD) questionnaires (already used as standard of care at patient intake): **PFDI-20**[73], **PFIQ-7**[73], **PISQ-IR**[74] *plus* a 6-question survey of **vaginal atrophy symptomatology**[75], a general QoL instrument (**SF-12**), and a **body image questionnaire**. Total estimated time burden for participants: 45 min

[†] Optional full thickness vaginal wall biopsy from anterior apical vagina; this will be shipped to UTSW in buffered solution (RNAlater®) for histologic assessment & qPCR for mRNA testing. Tissue collected locally at UTSW will also be analyzed by zymography (protease enzyme activity), immunoblotting, and hydroxyproline assay for cross-linked collagen content.

***** At these time points at 3 and 9-mo postoperatively, new study drug will be shipped (Fed-Ex) to the patient (or she may choose to present to the research clinic to collect a new tube of study drug). Coordinator calls are also planned at these times to remind patients of new study cream coming and/or to confirm receipt.

** At these times of study cream dispensing, new and used tube weights will be documented. For example, at time of the 6 mo postoperative check, spent tubes #2 and #3 (which were given at time of surgery/ hospitalization and Fed-Ex'ed 3mo postop, respectively) are collected and weighed *and* the new tube #4 is weighed before being dispensed to the patient. Weight of new tubes may be recorded in the Investigational Drug Services pharmacies or in clinic.

[p] At these times 18 and 30-mo postoperatively, these are phone call "visits" only with no questionnaires or exam. However, the coordinator will phone the participant to query and document: (a) if she has symptoms of a bulge or, "something falling out that you can see or feel in your vaginal area"; (b) she has had treatment for pelvic organ prolapse since the last visit date; (c) she is experiencing new medical problems or complications; (d) she has been hospitalized, had surgery, been to the emergency department, or visited a doctor for urologic or gynecologic conditions since the last visit date; and (e) she has had any change in her medications. A reminder of the next in-person study visit is also given.

Randomization & Blinding: SAS V9.3 will be used to generate stratified randomization lists using a permuted block factor of size 4 and 6. Patients will be stratified by site, hysterectomy status, and duration since menopause (<10 & ≥10 years). The randomization lists will be distributed to each site's research pharmacy. Outcome assessors and patients will be blinded to assignment.

Standardization of surgical procedure: All patients will undergo either a uterosacral ligament suspension ("USLS") similar to that described by Shull[76] or a unilateral sacrospinous ligament fixation ("SSLF") in the same fashion described previously[68] as an inpatient surgical procedure under general anesthesia; these will align with a prior NICHD-sponsored Pelvic Floor Disorders Network (PFDN) surgical trial[68] with agreement on placement and type of sutures: if USLS, 1-2 permanent sutures and 1 delayed absorbable suture are placed bilaterally (i.e., 4-6 total) beginning at or above the level of the ischial spine, with the delayed absorbable suture caudad and the permanent monofilament suture(s) ~1 cm cephalad. If the SSLF is selected, 2 permanent and 2 delayed absorbable sutures (4 total) are placed unilaterally in the sacrospinous ligament. Based on level I evidence, *neither USLS nor SSLF is superior to the other for anatomic, functional, or adverse event outcomes*.[69] Participating study surgeons must have performed ≥20 of each vault suspension procedure with ≥5 in the 12 months before subject enrollment. Anterior/posterior colporrhaphies, vaginal hysterectomy, and midurethral slings are allowed *but not transvaginal mesh* for prolapse.

4 SELECTION AND ENROLLMENT OF PARTICIPANTS

Participants (N=222) in this trial must be postmenopausal women with symptomatic pelvic organ prolapse (POP). Eligible patients must be suitably healthy to undergo elective, major surgery. To be considered post-menopausal, women will need at least one year without menses or be surgically menopausal (i.e. status-post bilateral oophorectomy). Anticipated age range of participants will be from 48 years (the minimum allowed) to mid 70s, although there is not a strict age limit. Complete inclusion and exclusion criteria are summarized below.

4.1 Inclusion Criteria

- Postmenopausal, meeting criteria a, b, c, or d:
  a. Still with uterus and no menses for >1 year

b. Status-post bilateral oophorectomy (i.e., surgically menopausal)
c. Status-post hysterectomy (with or without oophorectomy) and >55 years
d. Vaginal pH ≥5 *if* ≤55 years and status-post hysterectomy but still with one or both ovaries

- Minimum age: 48 years
- Symptomatic apical and/or anterior vaginal wall prolapse, stage 2 or greater
- No estrogen or progesterone replacement within the last month (may come off current treatment, i.e. wash out, to join the study)
- Medically fit for elective surgery
- Physically able to apply/insert the study drug
- Available for clinic follow-up for minimum 1 year

4.2 Exclusion Criteria

- Concurrent use of steroid creams for other indications (e.g. lichen sclerosis)
- BMI >35 kg/m^2
- Recent history (within last month) of vaginal infection or vaginitis
- Contraindications to estrogen therapy (e.g. spontaneous DVT, stroke, breast or endometrial/ hormone-responsive cancer, genital bleeding of unknown cause)
- History of connective tissue disease
- Any estrogen, progesterone, SERM, or other medication impacting vaginal milieu, by any route (oral, transdermal, subdermal/ implantable, intravaginal, intrauterine); may come off current treatment (>1 month), i.e. wash out, to join the study
- History of vaginal irradiation
- Allergy to Premarin® or its constituents
- Prior apical repair or use of mesh for prolapse repair
- Current tobacco use

4.3 Study Enrollment Procedures

Experience from past clinical trials at each of the participating sites is that recruitment generally has not required special advertisements or alterations in existing referral patterns. Eligible patients undergo examination and evaluation for treatment in specialty urogynecology (rather than general gynecology) clinics at each site: UT Southwestern (UTSW), University of Alabama at Birmingham (UAB), and Brown University/ Women and Infants Hospital (WIH) of Providence, RI. The Research Coordinator nurse will review chief complaints and referral notes of new/ referred patients to screen for eligible study participation. A Screening Log will be maintained by the Research Coordinator or PI at each site to document identification of subjects who enter pretrial screening and reasons for ineligibility and for non-participation of eligible candidates. Weekly team research meetings also serve to remind clinic providers (residents, fellows, nurse practitioners, and faculty) of inclusion/exclusion criteria and to screen for eligible patients that may benefit from this trial.

Involvement of vulnerable populations: Fetuses, neonates, pregnant women, children, prisoners, and institutionalized individuals are not involved in this research protocol.

Consent procedure: After confirmation of study eligibility, potential subjects will be approached by the Research Coordinator, PI, or other study personnel (Co-Investigators) to describe the study in detail and obtain an informed consent from the patient. Patients will be afforded ample time to ask questions and may even take home study literature for review to return later for consent and completion of "Baseline" study evaluations. Each participating clinical site has access to in-person and telephone (i.e. "Language Line") interpreter services should patients benefit from/ prefer study description in a language other than English. A witness will be required to co-sign the consent form.

Assigning a Subject ID: After informed consent is obtained, the Research Coordinator will assign each participant a 6-digit Subject ID number. The first 2 digits identify the clinical site, e.g. "01" for UTSW, followed by the number unique to the participant, encountered consecutively; thus, patient #1 at UTSW would receive the Subject ID 010001. Patient #7 at UAB may be 020007.

Randomization: The Data Coordinating Center (DCC) of the primary site (UTSW) will generate stratified randomization lists. Patients will be stratified by site, hysterectomy status, and duration since menopause (<10 & ≥ 10 years). The randomization lists with randomization codes will be distributed to each site's research pharmacy. After informed consent is obtained, the Research Coordinator will alert the research pharmacy, and the pharmacist will assign and record the participant's unique Randomization Code. He/she then dispenses the appropriate study cream (active CE cream vs. placebo cream). Those conducting the primary and secondary measurements as well as the patients will be blinded to treatment assignment. These data will *not* be housed in the study's database (i.e., REDCap®). For medical reasons, there will be a protocol for breaking this blinding for providers needing to understand the medication that the patient is receiving.

5 STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Drug administration and dosing schedule: CE cream application dose and frequency will follow typical recommendations and the protocols observed in other clinical trials of CE cream, i.e. 1g applied vaginally nightly for 2 weeks followed by twice-weekly application. The research pharmacy at each site will house and dispense study medication. At Baseline, Surgery, and 6-mo postoperatively (i.e., Tubes #1, #2, and #4, respectively), the study cream will be dispensed directly to the patient (via Research Coordinator). At 3- and 9-mo postoperatively (Tubes #3 and #5, respectively), the study cream will be sent by the Research Coordinator to the participant via FedEx (or similar) once dispensed by the pharmacist.

Dose adjustments or escalation, changes in formulation: not applicable

Potential adverse effects:

Premarin® vaginal estrogen cream may cause some, all, or none of the side-effects listed below.
Minor side effects: vaginal itching (2-3%), breast tenderness (2-3%), vaginal discharge (6-11%)*, joint aches (7-9%)*, headache (10-13%)*.
Severe side effects: stroke (<1%), blood clots in the legs or lungs (<1%)

* = in clinical studies, the rates of these side-effects were comparable in patients treated with estrogen and placebo vaginal creams.

5.2 Handling of Study Interventions

Pfizer, Inc has agreed to provide active CE cream (Premarin® vaginal cream, PVC) for the study. A separate, central pharmacy has been identified that is able to produce *placebo cream* and receive/procure the CE cream from Pfizer. Pfizer will provide identical tubes to the PVC for the placebo cream; all tubes will be over-labeled with an opaque label to mask active vs. placebo cream. This central pharmacy, *Particle Sciences, Inc. (PSI)*, was established in 1991 as a Contract and Research Development company. It has extensive experience in pharmaceutical formulation development and characterization, especially in the area of nanoparticle suspensions and emulsions. It has substantial prior experience in production and stability testing of complex formulations—including hormone creams—under good manufacturing practices (cGMPs).

Placebo development: PSI guarantees development of placebo cream that is comparable in appearance, pH, and viscosity to the active study cream and test it for stability in batches at 0, 3, and 6 months, including under conditions designed to simulate 2-year stability, i.e. "accelerated stability" testing at 40ºC and 75% relative humidity (in addition to standard testing at 25ºC and 60% relative humidity). PSI is then able to provide the appropriate oversight and quality assessments of placebo cream manufacture once a satisfactory recipe is generated.

Study drug distribution & storage: PSI will coordinate with research pharmacists at each clinical site to assure a reliable supply of study creams are shipped to the research pharmacies. It is anticipated that batches will be shipped approximately quarterly. CE cream and its placebo are stable for storage and shipment at room temperature.

Unused cream: Study cream tubes will all be weighed before being dispensed, and study participants will be asked to return their used tubes to the Research Coordinator (upon receipt of their next tube) in order to weigh these spent tubes and calculate total volume/grams cream used. This will be one metric of participant adherence to study medication. Unused cream will be destroyed by the Research Coordinator or the research pharmacist of the Investigative Drug Services pharmacies.

Accountability records: Study participants will be provided a "Medication Calendar" and given instructions for marking the days that they apply their study cream. Subjects will be asked to return their Medication Calendar along with the remaining study cream tube at their follow-up visits. Additional detail is provided in the Manual of Operations & Procedures.

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

- *Medications*: If vaginal discharge is encountered during the setting of study cream use, e.g. vaginal *Candidiasis* or bacterial vaginosis, appropriate treatment may be provided. For simplicity of not having to coordinate application of multiple vaginal creams (e.g., study cream and anti-fungal cream), oral treatments are preferred, e.g. diflucan or metronidazole pills for *Candidiasis* or bacterial vaginosis, respectively.
- *Surgical*: in addition to the required surgical intervention of uterosacral or sacrospinous ligament suspension (see 5.3.2), anterior/ posterior colporrhaphies, vaginal hysterectomy, and midurethral slings are allowed.

5.3.2 Required Interventions

- *Surgical*: All patients will undergo uterosacral ligament suspension ("USLS") similar to that described by Shull[76] and a prior network (PFDN) surgical trial[68] with agreement on number, placement, and type of sutures: 2-3 sutures placed bilaterally (4-6 total) beginning at or above the level of the ischial spine, with 1 delayed absorbable suture caudad and 1 or 2 permanent monofilament suture(s) ~1 cm cephalad. If uterosacral ligaments are deemed unusable due to scarring or inaccessibility—or by surgeon preference—the surgeon may instead perform a unilateral sacrospinous ligament fixation ("SSLF") in the same fashion described previously[68] also with 2 permanent and 2 delayed absorbable sutures (4 total). Based on level I evidence, *neither USLS nor SSLF is superior to the other for anatomic, functional, or adverse event outcomes*.[69] Participating study surgeons must have performed ≥20 of each vault suspension procedure with ≥5 in the 12 months before subject enrollment.

5.3.3 Prohibited Interventions

- *Medications:* Any oral or transdermal estrogen, Selective Estrogen Receptor Modulator (SERM), or other medication impacting vaginal milieu
- *Surgical*: Any placement of transvaginal mesh for prolapse

5.4 Adherence Assessment

The planned analyses will be according to a *modified intention-to-treat principle (mITT)*: i.e., all participants that are randomized *and* undergo a surgical prolapse repair *and* have at least one postoperative assessment will be analyzed according to their randomized assignment group. All eligible subjects who consent to the study and are enrolled will be available for analysis of baseline characteristics, and those that are randomized and use their study drug will be monitored for adverse events, but they must meet the additional qualifications above (i.e., undergo surgery and have a postoperative assessment) to be analyzed for the primary study outcome.

Various *per-protocol* analyses will also be considered, the first being an analysis similar to the *modified ITT* above but for only those participants receiving the *per-protocol* surgical repair (USLS or SSLF, see 5.3.2). Other *per-protocol* analyses will be comparisons of the

subset of participants in the two randomized groups who are *adherent* to their study medications. Therefore, defining *drug adherent* is critical. This will be defined in three ways: "strict", "per pivotal study", and objectively using VMI.

"Strict" Adherence Definition
Drug adherent will be defined as **>80% compliant to expected dosing using the subject's returned tubes** (i.e., comparing returned tube weights to that expected with at least 80% adherence) and/or **Medication Calendar**. Should there be a discrepancy in adherence designation between tube weight and medication calendar (e.g., using tube weights, the participant appears to have been non-adherent to study protocol but her calendar suggests she was adherent with at least 80% of planned doses recorded as completed), we will defer to the more objective tube weight.

"Per Pivotal Study" Adherence Definition
One of the 3 recommended dosage and administration schedules per Pfizer's package insert for Premarin® cream is twice-weekly administration of 0.5g intravaginally. This was based on a pivotal study by Bachmann et al in 2009.[63] In this study, the twice-weekly CE group of participants was defined as adherent with their drug if they self-reported, on average, at least *once-weekly* (and not more than three times weekly) application of the cream on their paper diary; medication tube weights were not collected. Similarly, in IMPROVE, if participants report via their Medication Calendar that they have applied their cream at least 50% of what is expected, this will be considered adherent.

Alternative Definition: using VMI
Cytomorphologically, vaginal atrophy can be defined by high numbers of (para)basal and intermediate cells and very low numbers of superficial cells. In a prolonged low-estrogen state, superficial cells are no longer produced, leaving essentially only parabasal and basal cells lining the vaginal wall. The Vaginal Maturation Index (VMI) will serve as an important *objective tool* to assess patient adherence with local estrogen application in those participants randomized to vaginal estrogen. This is a quantifiable measure of the proportion of vaginal epithelial superficial cells to parabasal and intermediate cells. While there is not a universally agreed-upon formula to calculate VMI or a cutoff to absolutely define "atrophic" vs. "estrogenized", several studies point toward the following[77]:

0 x % parabasal cells + 0.5 x % intermediate cells + 1.0 x % superficial cells.

A cutoff of 50 or less is indicative of atrophy.[78] Therefore, a **score >50 is indicative of study drug adherence in those randomized to active CE cream.**

The ThinPrep® system and its alcohol-based fixative allows for immediate cell fixation after swabbing of the upper vagina with a plastic spatula so the specimen is stable for transport to UT Southwestern for analysis, and the automated system assures greater uniformity and consistency in slide preparation. The VMI assessment will be conducted by a blinded examiner (Dr. Thibodeaux and/or his trained designee).

The VMI will be collected at Baseline, at time of surgery (or Pre-op clinic visit), and at 12

months postoperatively; if the participant is withdrawing or withdrawn before 12 months postoperatively, a final visit VMI will be collected. While this will provide objective assessment of adherence in those patients randomized to active CE cream, it will not suffice for those randomized to placebo cream (in which case, even adherent patients are expected to demonstrate objective atrophy on VMI) or when participants are unwilling to have VMI collected or if the specimen is deemed uninterpretable by the blinded pathologist.

6 STUDY PROCEDURES

See next page for **Schedule of Evaluations (6.1)**

PFD (Pelvic Floor Disorder) Questionnaires consist of 3 validated short-form (in English and Spanish) PFD questionnaires (already used as standard of care at patient intake): Pelvic Floor Distress Inventory (**PFDI-20**[73]**)**, Pelvic Floor Impact Questionnaire (**PFIQ-7**[73]**)**, Pelvic Organ Prolapse/ Urinary Incontinence Sexual Function Questionnaire (**PISQ-IR**[74]**)** *plus* a 6-question survey of **vaginal atrophy symptomatology**[75], a general quality-of-life (QoL) instrument (**SF-12**), and a **body image questionnaire**.

Schedule of Evaluations (Table 1)**Assessment**	**Baseline (≥5wk before Surgery)**	**Preop Visit** (w/in 2wk of surgery)	**Surgery/ Hospitalization**	**1mo Postop Visit** (±2wk)**	**3mo Postop *Call*** (±2wk)	**6mo Postop Visit** (5-7mo)	**9mo Postop *Call*** (±2wk)	**12mo Postop Visit** (10-14mo)	**18&30mo Postop *Calls*** (±2wk)	**24&36mo Postop Visits** (±2mo)
Informed Consent Form, Inclusion/Exclusion, Randomization	X									
Demographics	X									
Medical History	X									
Exam: POP-Q	X			X		X		X		X
Exam: Levator muscle strength assessment	X	X		X		X		X		X
Exam: Vaginal atrophy assessment	X	X	X	X		X		X		X
Exam: vaginal epithelium/ granulation tissue assessment	X	X		X		X		X		X
Medication Review	X	X		X	X	X	X	X	X	X
PFD Questionnaires	X	X			X*	X	X*	X	X*	X
PGI-I and/or PGI-S	X					X		X		X
Vaginal Maturation Index	X		X					X		
Blood Serum (E1, E2)	X							X		
Blood (whole), storage for DNA/ genetics assessment (optional)	X									
Vaginal Wall Biopsy (optional)			X							
Study Cream[Tube No.] delivery/receipt	X[1]		X[2]		X[3]	X[4]	X[5]			
Medication Calendar[Tube No.] collection		X[1]			X[2]	X[3]	X[4]	X[5]		
Preop History/Exam		X								
Postop Med/Surg update				X	X*	X	X*	X	X*	X
Postop Extra Treatment				PRN	PRN	PRN	PRN	PRN	PRN	PRN

Adverse Event, Protocol Deviation forms	PRN	PRN	PRN	PRN	PRN	PRN	PRN	PRN	PRN	PRN

POP-Q, pelvic organ prolapse quantification examination; PFD, Pelvic Floor Disorder; PGI-I and –S, Patient Global Impression of Improvement, - Severity; E1, estrone; E2, estradiol

*, At these times (3, 9, 18 and 30-mo postoperatively), these are phone call "visits" only with no questionnaires or exam. However, the coordinator will phone the participant to query and document: (a) if she has symptoms of a bulge or, "something falling out that you can see or feel in your vaginal area", which is Question #3 of the PFDI-20; (b) she has had treatment for pelvic organ prolapse since the last visit date; (c) she is experiencing new medical problems or complications; (d) she has been hospitalized, had surgery, been to the emergency department, or visited a doctor for urologic or gynecologic conditions since the last visit date; and (e) she has had any change in her medications. A reminder of the next in-person study visit is also given.

**, Clarification of timing of postoperative visits: the 1-month postop visit is defined as one calendar month after surgery. For example, if surgery is on July 1, one month postop is Aug 1, then ±2 week. This is to differentiate from surgery date + *4 weeks* or surgery date + *30 days*. Likewise, the 6-month postop visit is January 1, ±1 month; and the 12-month postop is July 1 of the following year, ±2 months.

6.2 Description of Evaluations

6.2.1 Consent/Screening Evaluation

Consenting Procedure

At the same patient encounter, both the consenting process and patient screening will occur. Generally, this will be the patient's initial encounter in the Urogynecology clinic but it may at any visit at which the patient selects surgical repair of symptomatic pelvic organ prolapse; for example, perhaps she previously had been managed with conservative measures such as pessary placement. The informed consent and the history and exam findings will be collected in a private room. The informed consent document will be written and provided in English or Spanish. Translator services are available at all study sites. Either the research coordinator or another member of the research team (PI or co-investigator) will be obtaining the consent, and the patient will sign and date/time her name to indicate understanding, which will be observed and countersigned by a witness.

Annual review of the consent document will be done in conjunction with continuing review by each clinical site's Institutional Review Board (IRB), and updates will be made as deemed appropriate. If other modifications to the document are deemed necessary by the research team (e.g., a change in protocol or known risk of study intervention), these modifications will be communicated to the IRB for approval.

Screening

Screening for eligibility will be relatively simple. Most inclusion/exclusion criteria are readily assessed from the patient's *routine intake history and physical examination* completed as part of usual care upon presentation to a Urogynecology clinic. For example, this history/exam would have already identified a symptomatic postmenopausal patient with prolapse not using any form of estrogen supplementation and an exam consistent with the eligibility criteria: stage 2 or greater anterior and/or apical prolapse and BMI $\leq$35 kg/m^2. No unique laboratory, radiology, or other invasive examination or testing is required to determine study eligibility beyond the history and examination performed as part of routine care in a Urogynecology clinic.

There is no set maximum allowable time between which study eligibility may be determined and beginning study participation. The visit at which randomization occurs and study drug is dispensed (Tube #1) is deemed the Baseline visit, and eligibility should be re-confirmed at this time.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

In this study, a single informed consent will encompass both screening and study procedures (as screening procedures are a part of routine care). Enrollment and randomization will occur on the same day all screening criteria are met and the individual agrees to participate. This enrollment/randomization date will be captured on a case report form at the *Baseline assessment*.

Baseline Assessments

- Contact Information (**Form 01**): name, address, phone, email data from patient and an alternative contact. Confirm patient's assent to being contacted by text and/or email.
- Demographics (**Form 02**): racial/ethnicity data and type of health insurance payer
- Medical History (**Form 03**): smoking history, confirmation of postmenopausal status, hysterectomy status, and assessment for history of recurrent urinary tract infection, diabetes, connective tissue disease, and various medical conditions
- Baseline Physical Exam (**Form 04**): gather height, weight, Pelvic Organ Prolapse Quantification (POP-Q) exam, uterine size, levator muscle tone and strength, investigator assessment of vaginal atrophy, and vaginal maturation index (VMI) specimen collection (see below).
- Medication Review (**Form 05**): list all medications related to treatment of incontinence and overactive bladder
- Pelvic Floor Disorder (PFD) Questionnaires (**Form 10**): *see description above in 6.1*
- Patient Global Impression of Severity (PGI-S) (**Form 11**): 1-question tool validated for urinary incontinence and modified for POP. Assesses overall impression of symptom severity related to POP.
- Vaginal Maturation Index (VMI): See also Section 5.4. A standard plastic spatula (similar to those used for a Pap smear and readily available in any gynecology clinic) will be used to sample the upper vaginal walls for 15-30 seconds. The spatula will be swirled in a ThinPrep® container (labeled with a preprinted barcode label with a Specimen ID linked with the participant's Subject ID number), which is then stable when stored at room temperature or 4ºC and shipped with cold packs in a cold shipping box for storage at UTSW.
- Blood serum collection: Blood will be drawn into a standard red top tube. 5mL (1 teaspoon) is gathered to assess baseline estrone (E1) and estradiol (E2) levels.
    - Tube: Red top glass, no additive, no clot activator with uncoated interior, vacutainer tubes (BD366430 - 16 x 100 mm x 10.0 ml)
    - Do not draw from an IV or shunt.
    - Post-collection: allow to clot for 20-50min (room temperature)
    - Centrifuge (after balancing) at 1000-2000 g (RCF) for 15 minutes. Although most clinical blood centrifuges only spin at one speed, one will need to compute the rpm's of the specific centrifuge to give 1000-2000 g. The method to convert RCF in g's to rpms is based on the centrifuge's radius of rotation.
    - Using a transfer pipette, the serum is removed from the red top tube (being careful not to disturb the clot) and dispensed into a screw-on top plastic tube for transport.
    - The transport tube is capped securely. A preprinted barcode label with the Specimen ID is affixed.
    - The stopper is replaced in the original red top tube, which is discarded with the transfer pipette in a biohazard container.
    - Serum may either be shipped within 24 hr of collection on cold packs in

a cold shipping box or stored at -20ºC and shipped on dry ice to UTSW (e.g., if bundling with other samples for efficiency).

- Whole blood collection (optional): Blood will be drawn into a standard yellow top tube. 5mL (1 teaspoon) is gathered at baseline. After appropriate processing and shipment, these specimens will be aliquoted and stored at UT Southwestern for possible future aims to include DNA analysis and studies examining for genetic predictors of surgical success/failure.

Randomization (**Form 06**)

Randomization to either active CE cream or placebo cream will be assigned by the research pharmacist using the allocation tables provided to him/her previously by the DCC. Stratification will be by site, hysterectomy status, and years since menopause (<10 and ≥10). The pharmacist is alerted to the subject once she has completed the consent process and has been assigned a Subject ID number. The 4-digit Randomization Number will be given by the pharmacist to the Research Coordinator. At this time, study drug may either be dispensed directly to the patient (via the Research Coordinator) or, if timing is preferable, may be shipped to the patient via FedEx. The patient is to begin study cream application immediately (that evening) once obtaining the cream and document use on the Medication Calendar.

- Dispensation of study drug (Tube #1) is documented (**Form 07**)
- Protocol Deviation (**Form 21**): as needed

6.2.3 Follow-up Visits

Preoperative Visit (within 2wk of Surgery):

- Medication Review (**Form 05**): *updated from Baseline*
- Medication Calendar documentation (**Form 07**) of study drug use Baseline-to-Pre-op
- Pelvic Floor Disorder (PFD) Questionnaires (**Form 10**)
- Preop Visit and Physical Exam (**Form 12**): assesses for any possible complaints or complications related to study cream application and re-assesses levator muscle tone and strength, and investigator assessment of vaginal atrophyVaginal Maturation Index (VMI) for "Surgery" time point *may* be collected at this visit if it falls within 7 days of surgery.
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed

Surgery/Hospitalization:

- Surgeon's Report (**Form 13**): confirmation of VMI collection, investigator assessment of atrophy, surgeon assessment of tissue quality, confirmation of prolapse repair per protocol or reasons for deviation from protocol (if any), intraoperative complications.
- Hospitalization (**Form 14**): days hospitalized, bladder voiding status at discharge, immediate postoperative complications
- Return of study drug (Tube #1) and dispensation of Tube #2 is documented (**Form 07**). Tube #2 is dispensed to the subject with instructions to resume twice-weekly use once discharged home. The weight of Tube #1 is documented

and the tube appropriately disposed/destroyed.

- Medication Calendar (**Form 07**) documenting use of Tube #1 (Baseline-to-Surgery) is collected (if not already completed at Pre-op).
- Vaginal wall biopsy: an optional small <u>full-thickness biopsy</u> (triangle approximately 1.5x1 cm) will be obtained from the apical/ cephalad anterior vaginal wall (shown in figure below from an abdominal perspective). This is tissue that ordinarily would be discarded in the course of an anterior vaginal wall repair (colporrhaphy) procedure. This will be placed in buffered solution (RNAlater®). A preprinted barcode label with a Specimen ID is affixed. This is stable when stored at 4ºC. It is shipped to UTSW on cold packs for later histologic assessment and qPCR for mRNA testing. Tissue collected locally at UTSW will initially be delivered for processing to the lab in phoshate-buffered saline (PBS), allowing additional analysis by zymography (protease enzyme activity), immunoblotting, and hydroxyproline assay for cross-linked collagen content.

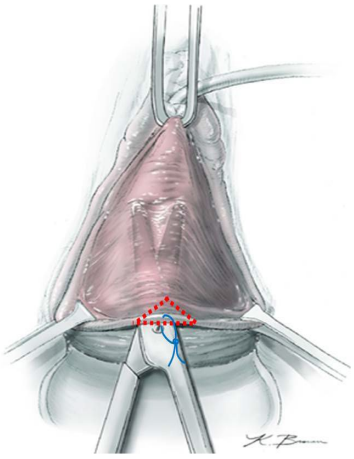


Figure 4. Vaginal wall biopsy (For orientation, a suture is placed loosely on the mid-portion of the apical margin of the triangular specimen)

- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed

<u>1-month Postoperative Visit (2-6wk Postop)</u>:

- Medication Review (**Form 05**): *updated from Preop Visit*
- Surgical pathology review is confirmed (**Form 14b**) and, if abnormal, prompts completion of adverse event documentation (**Form 09**)
- Postop Medical/Surgical History update (**Form 15**): documents any interval treatments for prolapse or urinary symptoms, interval medical history.
- Postop Exam (**Form 16**): re-assesses levator muscle tone and strength, investigator assessment of vaginal atrophy, examination for vaginal suture or mesh exposure/ ulcerations/ granulation tissue, and repeat POP-Q examination
- Postop Extra Treatments (**Form 17**): as needed
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed

<u>3-month Postoperative Phone Call (±2wk)</u>:

- Postop phone call documentation (**Form 18**) completed, with follow-up questions about feelings of vaginal pressure/ bulging and if there has been treatment for prolapse since the last study visit.
- Study cream Tube #3 is dispensed to the subject via FedEx with instructions to continue twice-weekly application.
- Medication Calendar (**Form 07**) documenting use of Tube #2 (Surgery-to-3mo) is collected either by mail or fax (or reminder to bring calendar to 6mo Postop Visit). Reminder to bring Study cream Tubes #2 and #3 to next appointment.
- Medication Review (**Form 05**): *updated from 1-month Postop Visit*
- Postop Extra Treatments (**Form 17**): as needed
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed

<u>6-month Postoperative Visit (±1mo)</u>:

- Study cream Tube #3 is collected (and #2, if not previously collected). Study cream Tube #4 is dispensed to the subject with instructions to continue twice-weekly application (**Form 07**). The weight of Tubes #2 and #3 are documented and the tubes appropriately disposed/destroyed.
- Medication Calendar documenting use of Tube #3 (3mo-to-6mo) is collected (**Form 07**).
- Medication Review (**Form 05**): *updated from 3-month Postop Call*
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed
- Pelvic Floor Disorder (PFD) Questionnaires (**Form 10**)
- Patient Global Impression of Severity (PGI-S) and Patient Global Impression of Improvement (PGI-I) (**Form 11**): 1-question tools assessing overall impression of symptom severity and improvement related to POP.
- Postop Medical/Surgical History update (**Form 15**)
- Postop Exam (**Form 16**)
- Postop Extra Treatments (**Form 17**): as needed

<u>9-month Postoperative Phone Call (±2wk)</u>:

- Postop phone call documentation (**Form 18**) completed, with follow-up questions about feelings of vaginal pressure/ bulging and if there has been treatment for prolapse since the last study visit.
- Study cream Tube #5 is dispensed to the subject via FedEx with instructions to continue twice-weekly application.
- Medication Calendar (**Form 07**) documenting use of Tube #4 (6mo-to-9mo) is collected either by mail or fax (or reminder to bring calendar to 12mo Postop Visit). Reminder to bring Study cream Tubes #4 and #5 to next appointment.
- Medication Review (**Form 05**): *updated from 6-month Postop Visit*
- Postop Extra Treatments (**Form 17**): as needed
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed

<u>12-month Postoperative Visit (±2mo)</u>:

- Study cream Tube #5 is collected (and #4, if not previously collected) (**Form 07**). The weight of Tubes #4 and #5 are documented and the tubes

appropriately disposed/destroyed.

- Medication Calendar documenting use of Tube #5 (9mo-to-12mo) is collected (**Form 07**).
- Medication Review (**Form 05**): *updated from 9-month Postop Call*
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed
- Pelvic Floor Disorder (PFD) Questionnaires (**Form 10**)
- Patient Global Impression of Severity (PGI-S) and Patient Global Impression of Improvement (PGI-I) (**Form 11**)
- Postop Medical/Surgical History update (**Form 15**)
- Postop Exam (**Form 16**)
- Postop Extra Treatments (**Form 17**): as needed
- Specimen collection, preparation, and shipment to UTSW: blood/serum (sent on cold packs within 24 hr of collection *or* store at -20ºC and ship on dry ice) and VMI (store at 4ºC and ship on cold packs)

<u>18-month Postoperative Phone Call (±2wk)</u>:

- Postop phone call documentation (**Form 18**) completed, with follow-up questions about feelings of vaginal pressure/ bulging and if there has been treatment for prolapse since the last study visit.
- Medication Review (**Form 05**): *updated from 12-month Postop Visit*
- Postop Extra Treatments (**Form 17**): as needed
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed

<u>24-month Postoperative Visit (±2mo)</u>:

- Medication Review (**Form 05**): *updated from prior call or visit*
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed
- Pelvic Floor Disorder (PFD) Questionnaires (**Form 10**)
- Patient Global Impression of Severity (PGI-S) and Patient Global Impression of Improvement (PGI-I) (**Form 11**)
- Postop Medical/Surgical History update (**Form 15**)
- Postop Exam (**Form 16**)
- Postop Extra Treatments (**Form 17**): as needed

<u>30-month Postoperative Phone Call (±2wk)</u>:

- Postop phone call documentation (**Form 18**) completed, with follow-up questions about feelings of vaginal pressure/ bulging and if there has been treatment for prolapse since the last study visit.
- Medication Review (**Form 05**): *updated from 12-month Postop Visit*
- Postop Extra Treatments (**Form 17**): as needed
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed

<u>36-month (Final) Postoperative Visit (±2mo)</u>:

- Medication Review (**Form 05**): *updated from prior call or visit*
- Adverse Events (**Form 09**) and Protocol Deviation (**Form 21**): as needed
- Pelvic Floor Disorder (PFD) Questionnaires (**Form 10**)

- Patient Global Impression of Severity (PGI-S) and Patient Global Impression of Improvement (PGI-I) (**Form 11**)
- Postop Medical/Surgical History update (**Form 15**)
- Postop Exam (**Form 16**)
- Postop Extra Treatments (**Form 17**): as needed

6.2.4 Completion/Final Evaluation

See 36-month Postoperative Visit above.

At the completion of 36 months or at the participant's final visit (if early withdrawal), "Final Status" documentation (**Form 20**) will be completed. This gives the following possible reasons for withdrawal: ineligible/ screen failure (few, if any, of these are anticipated); subject never began Study Cream; subject did not complete/undergo surgery; subject voluntarily withdrew from study (reason specified); investigator withdrew patient from study (reason specified); lost to follow-up; death; transition to long-term resident or skilled nursing facility; study termination; other (reason specified).

Possible reasons for early withdrawal by the subject may include intolerance of the study medication, patient preference, or inability to continue with follow-up examinations and assessments. Early withdrawal of the participant by the investigator may also be for participant's inability to adhere to study medication or visits.

If a participant withdraws (or is withdrawn) after randomization but before 12 months postoperatively, there is a prompt to the research coordinator/ physician examiner to collect a final VMI specimen.

It is not anticipated that participants will need to continue to be monitored after study withdrawal and discontinuation of the study medication.

7 SAFETY ASSESSMENTS

All participants will be monitored for safety and the emergence of adverse events. As detailed in Section 6.2.3, adverse events (**Form 09**) are queried and inventoried at all follow-up appointments. Of note, the *surgery is not the study intervention*—although adverse events encountered with surgery will be documented and followed. With respect to the actual study intervention, i.e., application of vaginal estrogen cream, these are the expected adverse experiences/toxicities documented in prior studies:

Minor side effects: vaginal itching (2-3%), breast tenderness (2-3%), vaginal discharge (6-11%)*, joint aches (7-9%)*, headache (10-13%)*.
Severe side effects: stroke (<1%), blood clots in the legs or lungs (<1%)

* = in clinical studies, the rates of these side-effects were comparable in patients treated with estrogen and placebo vaginal creams

If minor side effects such as those listed above are encountered early in the study (e.g., in the preoperative time period between baseline and surgery), the participant will be encouraged to try to continue the study medication with reassurance and as-needed therapies such as acetaminophen for management of headache or body aches or breast tenderness. Vaginal discharge—if related to *Candidiasis* or bacterial vaginosis—can be treated with oral medication as detailed in 5.3.1. Frequently, these minor side effects are found to be transient. If at any time these effects are more bothersome to the patient and persist despite attempts at management, the study medication will be discontinued, but the patient will continue to be monitored if she has not withdrawn from the study.

If severe side effects such as those listed above are encountered and thought possibly due to study medication, the study medication will be discontinued.

7.1 Specification of Safety Parameters

No particular safety measures or laboratory parameters are pre-specified to direct the investigators to discontinue the study medication in a participant.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

As detailed in Item 6.2.3, possible safety issues or adverse events (**Form 09**) are queried and inventoried at all follow-up appointments.

The actual risks of the intervention in this study, conjugated estrogen (CE) cream (i.e., Premarin cream), at the planned dose and frequency, are minor. In a well-done, large study comparing CE cream to placebo cream (Bachmann 2009, total n=423)[63] with 12mo follow-up, these adverse events were reported; there were no statistically significant differences between CE cream and placebo cream:

- vaginal discharge or leukorrhea: CE cream 6.1%, placebo 10.5%
- headache: CE cream 11.5%, placebo 12.8%
- arthralgia: CE cream 7.7%, placebo 8.3%
- any adverse event leading to drug discontinuation (12 wk): CE cream 3.5%, placebo 2.1%

In a different study comparing vaginal CE cream (also 0.625mg/1 g) to an oral synthetic estrogen (Botsis 1997, total n=72, 6mo follow-up)[79], these adverse events were reported; as above, they were not statistically different between treatment arms:

- vaginal itching: CE cream 2.8%, Synthetic PO estrogen: 2.8%
- breast tenderness: CE cream 2.8%, Synthetic PO estrogen: 2.8%

Finally, in another larger study comparing CE cream to placebo cream (Freedman 2009, total n=305, 12-wk follow-up)[80], ANY adverse event was reported in 55% of patients using CE cream and 47% using placebo cream, but AE's bothersome enough for withdrawal from the study occurred in just 3 of 150 (2%) CE cream patients and 2 of 155 (1.3%) placebo cream patients.

Risk of a *severe* side effect related to this dosing of CE cream (i.e., stroke, deep vein thrombosis, or pulmonary embolism) is less than 1%.

Although systemic absorption of vaginal estrogen has been documented, in this trial we will use low dosage vaginal CE cream (1g of 0.625mg/g cream) because this therapy does not involve significant increases in estrogen in blood and has been shown to have positive effects on postmenopausal vaginal atrophy. Women at high risk for potential adverse effects from systemic absorption of estrogen (specifically stroke, blood clots in the lungs or deep veins of the legs, or breast, ovarian or endometrial cancer) are excluded from study participation.

Any hypothetical adverse event involving endometrial pathology related to estrogen exposure (i.e., hyperplasia, cancer) is not applicable in this study as all participants will either be status-post hysterectomy or undergoing total vaginal hysterectomy at the time of the planned prolapse repair surgery.

7.3 Adverse Events and Serious Adverse Events

Definitions:

- An **adverse event (AE)** is defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events will be recorded regardless of their relationship to the study intervention (**Form 09**).

- A **serious adverse event (SAE)** is defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly (**Form 09b**).

No pre-specified laboratory measures will be collected to assess safety.

These AE's will be collected as *solicited events* (**Forms 09, 12, 13, 14, 15**):

- Vaginal or vulvar complaints possibly associated with vaginal estrogen cream: itching, irritation, vaginal or vulvar rash, yeast infection or other discharge.
- Non-vaginal complaints anticipated with the use of vaginal estrogen cream: breast tenderness, join aches, headache.
- Intraoperative AE's: bladder perforation, ureteral injury, urethral injury, rectal injury, other bowel injury, major vascular injury, anesthesia-related complications.
- AE's encountered during hospitalization after surgery: blood transfusion, wound complications, febrile or dermatologic complications, organ damage complications, cardiovascular complications, pulmonary complications, gastrointestinal complications, neurological complications, other.
- AE's encountered or reported at postoperative visits: vaginal or vulvar complaints (as above)/ non-vaginal complaints (as above) possibly related to estrogen

application, wound complications, febrile or dermatologic complications, neurological complications, other.

Case report forms (**Forms 09, 13, 14, 15**) all offer lines for data entry for "other" events, and Form 09 gives a reminder as to events triggering a SAE designation (**Form 09b**).

Possible safety issues or adverse events are queried and inventoried at all follow-up appointments and calls.

7.4 Reporting Procedures

Each clinical investigator is responsible for reporting serious or unexpected adverse events to the IRB at their institution and to the Safety Monitor. The Safety Monitor/Officer (currently DSMB Chair, Dr. Thomas Gregory), DCC Biostatistician (currently Dr. Linda Hynan), and NIA Project Scientist or Program Officer are notified by email when a report of any serious adverse event is entered into the REDCap database. The Safety Monitor summarizes the case; if it is potentially related to the intervention, the Safety Monitor reports it to the NIA Program Officer and the remaining DSMB members in an expedited manner (i.e., within 24 hours of learning of these events). The Safety Monitor (currently Dr. Thomas Gregory) will determine whether the case should be reported to the IRBs at all the institutions participating in the trial. The DCC summarizes all SAE's, including those that are definitely not related to the intervention, for the DSMB at each DSMB meeting.

Approximately every 6 months, or more frequently upon request of the Chair of the DSMB, the DCC will summarize all adverse events (including serious, unexpected and expected) by randomization group in a report to the DSMB. In addition, the report will contain accrual and drop-out rates. The DSMB will summarize their findings to the NIA with approval to continue the clinical trial, or a recommendation to modify the trial or terminate the trial.

Further, in accord with Investigator-Initiated Research (IIR) clinical studies with Pfizer, all serious adverse events will also be submitted to Pfizer via their IIR SAE form; this applies to all subjects receiving a Pfizer product (i.e. Premarin cream) or blinded therapy and also includes those SAEs not study-drug-related. The clinical study reporting period begins from the time that the subject receives her first dose of Pfizer product (or blinded therapy) through and including 28 days after the last administration of the Pfizer product (or blinded therapy). The time frame for reporting the SAE to Pfizer is immediately upon awareness (if fatal or life-threatening SAE) or within 24 hours of first awareness (if not fatal or life-threatening). The PI will include a rationale and medical justification as an assessment of causality, i.e., the determination of whether there is a reasonable possibility that the Pfizer product (or blinded therapy) caused or contributed to an AE. Finally, new, updated, or corrected information about a previously-reported SAE will also be communicated to Pfizer in a follow-up report.

7.5 Follow-up for Adverse Events

The PI and biostatistician at UTSW will be responsible for:

- maintaining an appropriate log of data regarding these events
- monitoring the course of the event to resolution or the end of the trial

- notifying all site investigators of all serious adverse events for appropriate reporting to their local Institutional Review Boards
- reviewing updated blinded, aggregate data summarizing participant safety. These reports will include incidence, severity, and relatedness to treatment of all reported and treatment emergent Adverse Events and withdrawals from the trial due to Adverse Events.

7.6 Safety Monitoring

The NIA has appointed a Data Safety Monoitring Board (DSMB), which will regularly evaluate the safety of the participants in IMPROVE. The IMPROVE UTSW PI and Biostatistician will be blinded to treatment allocation using dummy treatment group assignments while the DSMB and statistician/ programmer will be unblinded. The DSMB will consist of a chairperson and 2 other individuals who have expertise in clinical study design/ conduct and biostatistics. All board members will be independent of the institutions and investigators participating in the trial and have no financial ties to Pfizer (maker of Premarin® vaginal cream) or to the outcome of the trial.

The DSMB will review the study design and plans for recruitment, adherence, interventions, data quality and safety monitoring. The responsibilities of the DSMB are to:

- evaluate the safety of trial participants
- ensure confidentiality of data and the results of monitoring
- report to the UTSW PI and biostatistician on the safety of participants
- make recommendations on continuation, termination, or modifications to the trial

DSMB Process: There will be 2 sections of the DSMB meeting: open and closed. In the open section of the meeting, the PI and Biostatistician will present the report to the DSMB and answer questions. The closed section of the meeting will include the members of the DSMB only.

Reports to the DSMB, DSMB Review and Recommendations: The format of the report will be developed by the UTSW PI and biostatistician and approved by the DSMB. Additions and other modifications to the report may be directed by the DSMB. Interim DSMB reports generally consist of baseline characteristics, adverse events, serious adverse events and listings of extended evaluations by urogynecologists, if indicated, presented by dummy treatment group assignment. Adverse events will be categorized by seriousness and investigator-determined relationship to study drug. Data files to be used for interim analyses will have undergone established editing procedures to the extent possible, but all data, both edited and unedited, will be used to prepare interim reports. An unblinded statistician/ programmer will provide the dummy treatment group assignments to the IMPROVE biostatistician. In addition, the unblinded statistician/ programmer will prepare a confidential, sealed envelope to be included with the DSMB report using the real treatment assignment and an administrative assistant not associated with IMPROVE will mail the report one week in advance of the DSMB meeting. No other personnel at UTSW will have access to the real treatment assignment codes. The reports to the DSMB will be numbered and provided in sealed envelopes within an express mailing package to maintain confidentiality. All DSMB reports are confidential. A copy of the report will be retained in

a locked, confidential file at the Coordinating Center. After each review, the DSMB will send a brief letter to the UTSW PI and biostatistician that contains a recommendation to continue, terminate, or alter the study.

Access to Interim Data: Access to the accumulating safety and outcome data will be limited to as small a group as possible.

Confidentiality: All materials and proceedings of the DSMB are completely confidential.

8 INTERVENTION DISCONTINUATION

Condition for which the study intervention (CE cream) will be discontinued:

- Intolerance of the study cream by the participant related to developing toxicity (e.g., allergy to conjugated estrogen or the vehicle in the active or placebo creams). This is determined by patient report and/or signs on physical examination, e.g. vulvovaginal skin irritation or inflammation: erythema, ulcerations, lesions, heat, tenderness, edema.

Temporary study medication discontinuation:
If symptoms or signs of vulvovaginal irritation exist but it is unclear whether this is related to study medication (e.g., vaginal discharge perhaps due to other conditions such as *Candidiasis* or bacterial vaginosis), these conditions may be treated with vaginal therapies (e.g. clotimazole or metronidazole or clindamycin creams) at the discretion of the clinical provider. In these scenarios, *temporary discontinuation* of the study medication cream may be deemed appropriate. The study cream should be resumed as soon as reasonably appropriate clinically.

Elective study medication discontinuation by the participant:
Subjects may, of course, withdraw voluntarily from participation in the study at any time and for any reason. Participants will continue to be followed, with their permission, even if the study intervention is discontinued. There are no planned modifications to the schedule and duration of continued follow-up after study medication discontinuation.

There currently are not plans for replacement of subjects who discontinue early.

If voluntary withdrawal occurs, the subject will be asked to continue scheduled evaluations, complete an end-of-study evaluation, permit collection of a VMI specimen, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable.

9 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

A large simple trial (randomized, controlled) design was selected to evaluate for possible superiority of vaginal estrogen cream use compared to placebo. The primary outcome is a comparison by treatment group of time-to-failure of the prolapse repair at 1 year after surgery with "failure" defined by the *any* of these outcomes occurring:

- Recurrent prolapse *beyond the hymen* of the anterior or posterior vaginal wall *or* apical descent >1/3 into the vaginal canal based on continuous measurements of the Pelvic Organ Prolapse Quantification (POP-Q)[71] examination
- Report of any *symptoms* of bulge: "yes" to question #3 of the Pelvic Floor Distress Inventory, "Do you usually feel a bulge or something falling out that you can see or feel in your vaginal area?" or *no change* or worse on the Patient Global Impression of Improvement (PGI-I).
- Retreatment of prolapse with either pessary or surgery

The null hypothesis for this study is that 12 months following transvaginal native tissue repair for prolapse with or without preoperative and continued postoperative local estrogen treatment, women will have the same rate of recurrent prolapse. The current definition of success in a surgical trial used by NICHD's Pelvic Floor Disorders Network (PFDN) is this same composite of no prolapse beyond the hymen, no symptomatic recurrence, and no retreatment.[81,82]

9.2 Sample Size and Randomization

The literature reports failure by the above definition occurring in ~40% of patients with a native tissue USLS (i.e., ~60% "success").[69] A 2-sided logrank test with an overall sample size of **188 subjects** (94 each group) achieves 80% power at a 0.05 significance level to detect a hazard ratio of 0.5188 when the proportion of those "surviving" (i.e. "successes") in the control group is **65% compared to 80%** in the treated group, **measured at 1 year**. This presumes the accrual pattern across the study time period (3 years of accrual) is uniform. This N=188 already accounts for **25%** either lost to follow-up *or non-adherent to study cream* in the control and treatment groups. Allowing for an additional **15% loss of participants** from baseline to surgery, **222 total participants** are needed.

Potential problems & alternatives:

Differences in baseline characteristics between the groups may occur by chance, but we will mitigate that risk by stratifying by clinical site, hysterectomy status, and duration since menopause (<10y & ≥10y).

9.2.1 Treatment Assignment Procedures

A randomized, double-blind, placebo-controlled trial design (CE cream versus placebo) will be used in this study. SAS V9.3 will be used by the study biostatistician to generate the stratified, randomization lists. Patients will be stratified by site, hysterectomy status, and duration since menopause (<10 and ≥10 years). The randomization lists will be distributed to each site's research pharmacy (or their designee). Those conducting the primary and secondary measurements as well as the patients will be blinded to treatment assignment. These data will *not* be housed in study's database (i.e. REDcap®).

At the discretion of the primary investigators at any of the clinical sites, they may request the primary site PI to have the blind broken. The primary site PI—likely in consultation

with the Safety Monitor, as needed—will then request this information of the DCC study database manager (permitting Dr. Hynan to remain blinded) if it is clinically needed to understand the medication that the patient is receiving. It is anticipated that this would only be requested for important medical reasons, e.g., a serious adverse event or significant allergic reaction to study cream. In most cases, patient skin irritation with cream application could prompt discontinuation of the study medication without unmasking. There is no plan for clinical site research pharmacists to unmask participants, research coordinators, or local site PIs directly. Planned breaking of the randomization codes will only be after all participants have reached the primary study outcome time point one year postoperatively; there are no plans for interim/ early data analyses.

9.3 Interim analyses and Stopping Rules

No analyses of efficacy are planned to be presented until the end of the trial. Additional DSMB review may be requested by the UTSW PI and biostatistician, and an emergency review by the DSMB requested at any time if questions of participant safety arise. Between-groups comparisons of safety variables will be presented to the DSMB.

Should the DSMB decide to issue a recommendation to terminate or alter the study protocol based on safety, futility, or poor study performance (e.g., slow accrual, high loss-to-follow-up, poor quality control) a full vote of the 3 site PI's will be required. Examples of findings that might trigger a safety review are the number of SAEs overall, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events. Such findings are presented to the study biostatistician or to the Data and Safety Monitoring Board (DSMB) statistician to review the events by group to determine whether there are statistical as well as clinical concerns. The statistician reports the findings to a closed session of the DSMB or to the Safety Officer and/or NIA. The findings are used to determine what steps will be taken.

9.4 Outcomes

9.4.1 Primary outcome

Aim 1. The primary outcome is dichotomous success/failure of prolapse repair (see Item 9.1 above) as measured 12 months postoperatively. This is a composite outcome with failure defined by objective prolapse beyond the hymen, subjective sense of bulge by the patient, or retreatment by any intervention. It is not anticipated that any component of this primary outcome will require adjudication by committee.

9.4.2 Secondary outcomes

Aim 2: Quantify the effects of vaginal estrogen on symptoms of overactive bladder and urinary incontinence (**Subaim 2a**) and sexual function and dyspareunia (**Subaim 2b**) using validated questionnaires at regular intervals (Fig.3, Table 2) and assess for differences in rate of postoperative cystitis (**Subaim 2c**), and symptomatic vulvovaginal atrophy (**Subaim 2d**)

TABLE 2. Questionnaires Description

Pelvic Floor Distress Inventory (PFDI-20)[73] (**Aim 2a**)	There are 3 scales (urinary, POP, and colorectal-anal symptom distress inventories) including questions about incomplete bladder emptying, urinary frequency, urgency, and urge- and stress-associated incontinence
Pelvic Floor Impact Questionnaire (PFIQ-7)[73] (**Aim 2a**)	3 scales measure disease-specific impact on QoL (Incontinence, POP, and Colorectal-Anal) with questions about effect on physical activity, social activities, and emotional health.
Pelvic Organ Prolapse/Urinary Incontinence Sexual Function Questionnaire (PISQ-IR)[74] (**Aim2b**)	Specifically assesses the impacts of PFDs (urinary incontinence and POP) on sexual health and includes questions quantifying sexual desire, arousal, orgasm, satisfaction, and pain associated with intercourse.
SF-12	Validated, commonly-used QoL questionnaire assessing overall mental and physical health
Patient Global Impression of Improvement & Severity (PGI-I, PGI-S)[70]	1-question tools validated for urinary incontinence and modified for POP. Assess overall impression of resolution of symptom bother and symptom severity related to POP.

Subaim 2a (changes in overactive bladder symptoms) will draw from the relevant questions on from the PFDI-20 and PFIQ-7. Information on stress urinary incontinence will stem from the relevant questions (PFDI-20 and PFIQ-7) completed at baseline and at the Pre-op visit.

Use of CE cream and conversion of inelastic, avascular, thin epithelium to a thicker, well-rugated epithelium corresponding to decreased pH could reduce the incidence of postoperative cystitis (**Subaim 2c**), which is common after genitourinary surgery (14% after USLS, defined by +culture within 1mo post-op)[84]. This study will use a more liberal definition of post-op cystitis to include any prescription of antibiotics (culture-driven or empiric) for cystitis symptoms in the first postoperative month.

Atrophy (and its resolution, **Subaim 2d**) will be queried at baseline, Pre-op, 6- and 12-mo postoperatively using standardized questions about bother related to vaginal dryness, irritation/itching, soreness/pain, discharge, and dyspareunia (if sexually active); these symptoms are each scored as 1=not at all, 2=somewhat, 3=moderately, or 4=quite a bit bothersome.[75] The most bothersome symptom (MBS) is also queried. On exam, a modification of the vaginal health composite score[85] will have the examiner grade vaginal mucosal dryness, pallor, presence of rugae, and presence/absence of petechiae. The vaginal maturation index objectively categorizes percent of superficial, intermediate, and parabasal cells to be used as an index of patient adherence and to examine for correlation with symptom bother from other PFDs.

Aim 3: Quantify histomorphology and connective tissue homeostasis of the vaginal wall in postmenopausal women with symptomatic prolapse undergoing surgical repair with or without pre- operative intravaginal estrogen therapy.

***Aim 3 Methodology*:**

a. Vaginal wall biopsy: Standardized full-thickness anterior apical vaginal wall biopsies will be obtained from all consenting participants at the time of prolapse repair. Biopsies from secondary clinical sites will be received in RNA preservative (RNAlater®). Although this buffer is excellent for preservation of RNA and facilitates dissection of biopsy material into various tissue components (e.g., muscularis from mucosa) and fixation of tissues after receipt, immunoblot analysis and enzymes assays are not possible. Thus, tissues obtained from only the primary site (UTSW) will be used to quantify protein and enzyme activity in addition to molecular analysis. Biopsies from all sites will be used for molecular and histopathology analyses.

b. Rationale for study of candidate genes and proteins in the vaginal wall: The pelvic viscera are supported anatomically by the levator ani muscle complex and connective-tissue attachments of the pelvic organs (aka the "endopelvic fascia").[86] The vagina's support is commonly divided into apical (Level I), mid- (Level II), and distal (Level III) regions.[87] The cardinal and uterosacral ligaments (Level I) have long been regarded as integral to the system of support of the pelvic organs[88], and despite the name *utero*-sacral, this ligament interlaces and is contiguous with the upper vagina in more than two-thirds of women.[89,90] Further, the connective tissue sheet enveloping the vagina suspends it to the levator ani via the arcus tendineous fascia pelvis (Level II).[91] Dysfunction or disruption of one or more of these muscular, ligamentous, or connective tissue components leads to loss of support and, eventually, to POP. Importantly, the fibromuscular layer of the vaginal wall coalesces and is contiguous with the endopelvic fascia, and, thus, plays a role in the suspension of the vagina to the pelvic floor musculature. Work from the Word laboratory and others provides compelling evidence that the vaginal muscularis is abnormal in women with prolapse compared with age- and parity-matched asymptomatic controls with respect to smooth muscle proteins[92], cellular composition[93,94], ultrastructural abnormalities, and protease activity.[95] Findings from other laboratories[96-99], together with the known association between inherited elastinopathies and POP[100-102], suggest that defective collagen or elastic fiber assembly (or increased degradation) is a primary and pivotal event in the pathophysiology of POP.

Synthesis and assembly of collagen and elastin.

a. Relative levels of mRNA for collagen types *1α1*, *1α2*, & *3*, *tropoelastin*, *LOX*, *LOXL-1*, and *TGFβ* will be quantified using qPCR and gene-specific primers which have been validated in the Word laboratory.
b. Immunoblot analysis for Collagen types I and III will be conducted using samples from CE- and placebo-treated women and compared with expression in vaginal tissues from premenopausal women with normal vaginal support and premenopausal women with prolapse (samples in our existing biorepository). Likewise, levels of tropoelastin, LOX, LOXL-1 and fibulins-3 and -5 will be quantified in urea-extracted matrix fractions from the mucosa (lamina propria + epithelium) and muscularis. This analysis will allow us to determine (i) the effect of menopause on expression of these matrix proteins in the vaginal wall, and (ii) if vaginal estrogen alters the connective tissue matrix.
c. Collagen and elastin content will be analyzed using hydroxyproline and desmosine assays, respectively. The distribution of collagen (newly synthesized, denatured, or mature) will be analyzed in fractions of tissue extracts (i.e. supernatant of NaOH

extraction as newly synthesized uncrosslinked or weakly crosslinked collagen; extraction of insoluble residue with 0.5 M acetic acid in which the supernatant represents denatured collagen and insoluble residue as crosslinked collagen).

d. Histomorphology will be analyzed in detail. Specifically, we will use Hart's staining to analyze elastic fiber morphology and content[103], picrosyrius red stain to analyze collagen morphology and content[103], thicknesses of epithelium, lamina propria, and muscular wall, and CD68 immunostaining to quantify macrophages. Macrophages may be important in POP since our pilot data indicate estrogen-induced downregulation of MMP12 (i.e., human macrophage elastase) in the vaginal wall.

Matrix degradation. We hypothesize that certain collagenases (MMP-1 and -13), trypsinogens (PRSS3)[104], and elastases (MMP-2, -9 and -12) and their respective inhibitors may be regulated by vaginal estrogen. We propose that postmenopausal women with POP will respond to estrogen therapy with decreased levels of MMPs (collagenases and elastases), decreased NTx (elastin degradative product) and decreased collagenase associated with collagen/elastin deposition relative to placebo-treated controls. Further, we propose that *failure* of reconstructive surgery will be associated with increased markers of collagenase and elastase activity.

a. Collagenase activity will be determined using collagenase activity assays with FITC-labeled soluble type-I collagen as substrate (Chondrex Inc. Redmond, WA). MMP-9 and MMP-2 will be analyzed using quantitative gelatin zymography and endogenous active MMPs (-2,-9, -13) will be measured using active MMP fluorescent immunoassays (R&D Systems).
b. The serine protease, PRSS3, will be analyzed using casein zymography, IHC, and immunoblot analysis.
c. Levels of cross-linked collagen-type I N-telopeptides (NTx) will be used as an index of collagen degradation in biological fluids and tissues. NTx will be quantified in homogenates of mucosa and muscularis using competitive inhibition enzyme linked immunosorbent assays normalized to total protein (Oswteomark NTx Urine, Wampole Laboratories, Princeton, NJ).
d. A cassette of signature protease inhibitors (cystatin C, SLPI, TIMP-1 and -2, and elafin) will be analyzed using qPCR and immunoblot analysis.
e. Elastase activity in vaginal muscularis and mucosa will be determined using BODIPY-FL-labeled DQ elastin conjugate that is highly labeled so that the fluorescence signal is quenched until enzymatic digestion yields highly fluorescent fragments (Molecular Probes). We have experience with preparation of vaginal tissue for these assays.

Vaginal smooth muscle.

a. Relative levels of mRNA for smooth muscle *α actin* will be used as a marker for smooth muscle cells and myofibroblasts. Smooth muscle myosin heavy chain and h- and l-caldesmon will be assessed as markers for the fully differentiated smooth muscle phenotype.[92]
b. Immunoblot analysis for myosin heavy chain, caldesmon, and *α* actin will be conducted using samples from CE- and placebo-treated women and compared with expression in vaginal tissues from premenopausal women with normal vaginal support and others with prolapse (samples in our biorepository).[92]

c. Histomorphology will be analyzed in detail. Specifically, we will use immunostaining for α actin and smooth muscle myosin heavy chain to analyze smooth muscle morphology and content.[93,94]

Molecular profile of vaginal wall tissues (all clinical sites). High quality RNA from tissue samples obtained in this trial will facilitate a comparative analysis of gene expression related to matrix synthesis and degradation. It is not possible to analyze hundreds of genes involved in these pathways especially in ~392 (196 ea of muscularis and mucosa) tissues. We, therefore, choose to quantify mRNA levels of select genes related to matrix synthesis and degradation to determine a molecular profile of estrogen action in vaginal tissues.

Analysis of tissues from women enrolled in our pilot trial indicated that, of 7 genes important in collagen and elastin synthesis, the magnitude of estrogen-induced change was greatest and most consistent in *Cola1* (6-fold). With increased numbers of subjects, we expect that vaginal estrogen will also result in increased *LOX* gene expression (which correlates with enzyme activity) in the vaginal muscularis. Hence, we will utilize qPCR to measure relative levels of *LOX* and *Cola1a* mRNA as markers of collagen synthesis in all tissue samples from this trial. *Col3* and *tropoelastin* will be used as potential negative controls and at least two housekeeping genes will be used as normalizers (*h36B4* and *GAPDH*).

Our pilot trial indicated that mRNA levels for serine protease and MMP inhibitors were not regulated by vaginal estrogen. The single exception was the serine protease inhibitor, *α1-antitrypsin,* which tended to be increased in mucosal samples from women treated with estrogen (P=0.27). In contrast with protease inhibitors, 2 protease genes (*MMP1* and *MMP12),* were significantly decreased in tissues from CE-treated women, and MMP9 activity was also downregulated dramatically in CE-treated women. Thus, *MMP1*, *MMP12,* and *α1-antitrypsin* will be quantified in all tissue samples using *MMP2* and *TIMP1* as negative controls.

This molecular profile will determine if collagen synthesis and degradation is regulated by vaginal estrogen. Nonetheless, *MMP1* gene expression remained elevated in 1 of 7 mucosal samples from women treated with CE. As shown in Fig.2 of this protocol, MMP9 was not universally downregulated in women treated with CE. These studies illustrate that estrogen-induced downregulation of matrix proteases may vary significantly among subjects. We will also quantify ERα and ERβ mRNA to test whether responses correlate with expression of estrogen nuclear hormone receptors in the vaginal wall and will use the well-known estrogen-responsive gene, *VEGF*, as an index of estrogen action in these tissues.

Future Aim.

From those participants consenting to an optional additional blood draw, 5mL (1 teaspoon) of whole blood will be obtained at baseline and stored in a typical yellow-top tube. This will be stored for possible future DNA analysis such as genetic analyses looking for genetic predictors of surgical success or failure.

9.5 Data Analyses

Aim 1. The **primary outcome** will be analyzed according to the assigned treatment group (modified intention-to-treat): i.e., all participants that are randomized *and* undergo a surgical prolapse repair *and* have at least one postoperative assessment will be analyzed according to their randomized assignment group. All eligible subjects who consent to the study and are enrolled will be available for analysis of baseline characteristics, and those that are randomized and use their study drug will be monitored for adverse events, but they must meet the additional qualifications above (i.e., undergo surgery and have a postoperative assessment) to be analyzed for the primary study outcome. At baseline, continuous measures will be compared between groups with Student's t-test and chi-square (or Fisher's exact test) for categorical measures. Scores from repeated surveys are continuous outcomes data and will be analyzed using repeated measures analysis of variance. We will test for interactions between time effect and treatment effect. For categorical data (e.g. prolapse stage), the analysis will be by categorical data modeling using weighted least squares and mixed logit models, with cross-sectional assessments (at specific time intervals) by chi-square. For missing follow-up points there are two possible approaches: (i) if there are few missing follow-up points, then their values may be imputed through multiple imputation methods, in which case repeated measures analysis of variance may be used following multiple imputation; (ii) more likely, we will make time a random effect and a mixed effects model with repeated measures will be applied. Time-to-event or event-free-time will be analyzed using survival methods to include the log-rank test and Cox regression. For measures that may not be assumed normal, the data will be transformed using a rank transformation prior to analysis.

In accordance with NIH policy, we will include an analysis of the intervention effect (CE cream) performed by racial/ethnic subgroups, recognizing that the analyses will not have high statistical power for detecting clinically meaningful differences between races/ ethnicities.

Aim 2 Outcomes. As above, continuous scores from repeated surveys will be analyzed using repeated measures analysis of variance with appropriate corrections made, as necessary, for missing data.

Subaim 2a & 2b. Assuming a baseline UDI-6 score (*obtained from the PFDI-20*) of 7.90±1.91 for the stress-incontinent subpopulation of IMPROVE (expected to be at least 60%, or *67* participants per study arm)[105], in order to demonstrate a clinically meaningful improvement (decrease) of 0.5(SD)[106] to 6.95±1.91 using CE cream from baseline to time of surgery (preop), with 80% power and α 0.05, 65 participants are needed per arm. Further, to demonstrate an improvement in a baseline IIQ-7 score (*obtained from the PFIQ-7*) of 9.67±7.14[105] to 6.12±7.1, 64 participants are needed per arm (**Subaim 2a**). Similarly, to find a clinically important improvement (increase) in overall PISQ-12 score from baseline 25.0±4.95 (women with prolapse[107]) to 27.48±4.95 at preop (or any postoperative time point), 64 participants are needed per arm (**Subaim 2b**). Thus, enrollment goals designed to address the primary aim (Aim 1) are sufficient to address these important secondary aims.

Because midurethral slings and anticholinergic or beta-3 agonist medications are not excluded in study participants, these other interventions will clearly impact upon

incontinence rates and will be controlled for in statistical comparisons between the two groups. Gross estimates of overactive bladder in the 2 groups will also be assessed by rates of anticholinergic or beta agonist use, as medication history will be queried at each patient encounter. Modest improvements in *stress* and overall urinary incontinence will be assessed from baseline to Pre-op (i.e. approximately 6-8wks local estrogen) using the relevant PFDI-20 and PFIQ-7 questions (see above Analyses) at both time points.

Subaim 2c. Proportions of participants with postoperative cystitis will be compared using chi-square. Using the liberal definition of post-operative cystitis to include any patient prescribed antibiotics for cystitis symptoms, this study will have sufficient power to demonstrate an improvement from a 22% cystitis rate to 10% (80% power,α 0.05). The postoperative "MedSurg" history form (**Form 15**) queries whether there has been any interval occurrence of urinary tract infection (Item B4) and whether symptoms were treated empirically (i.e., no urine culture) or if culture-proven.

Subaim 2d. Comparisons of vaginal maturation index and atrophy symptoms will use analysis of covariance, with treatment and study center as factors and baseline values as the covariate.

Aim 3 Outcomes: If there is 10% loss of patients from randomization (baseline) to surgery, there will still be 100 patients per arm eligible for the standardized intraoperative apical anterior vaginal wall surgical biopsy. Our pilot trial (Section 2.2a, *Humans*) indicates sufficient power to determine 2-fold differences in collagen subtypes and MMP activity using tissues from UTSW. Further, with ~42 samples from UTSW (21 in each group), this will provide 80% power to also detect 2-fold differences in LOX protein and mRNA expression. All Aim 3 outcomes of interest (e.g. histological wall thicknesses, qPCR-determined relative mRNA levels, collagen subtypes and elastic fiber content, collagenase and protease activity) will be summarized using the following statistics: mean, standard deviation, median, minimum and maximum. Additionally, because most biomarkers are not normally distributed, the geometric mean and standard deviation based on natural log transformed values will also be calculated. Summaries will be constructed by treatment group (CE vs. placebo) and comparisons conducted using 2-tailed Student's *t*-tests comparing natural log transformed levels. Because this translational study (Aim 3) is considered to be descriptive and a relatively large number of markers are being considered, all p-values will be presented without adjustment for multiple comparisons and should be interpreted accordingly. We will correlate any treatment-group differences in vaginal wall or smooth muscle histomorphology, ERs, collagen gene expression, and protease downregulation with *clinical outcomes*, namely, objective or subjective "success" as outlined in Aims 1 and 2.

Ideally, CE cream application will support an improvement in prolapse recurrence success/failure rates 1y postop, but even if CE cream has a null or non-significant effect, the entire population will be divided into prolapse repair failures (*cases*) and successes (*controls*), and, controlling for CE cream exposure, univariate comparisons will be made of the continuous outcomes from Aim 3 (2-tailed Student's *t*-tests). Those variables that attain a significance level of 0.1 or less in univariate analyses will be considered jointly in a multivariable backward stepwise logistic regression model. An accepted "rule of thumb"

is that one needs 10 events (i.e. 10 cases of prolapse repair failure) for each predictor added to the model (i.e., for each degree of freedom).[108,109] If a total of 67 cases are identified (i.e., 40% repair failure in 222 participants and 25% patient loss), this would be sufficient to analyze up to at least 7 variables in the multivariable model. Thus, we will identify important biomarkers that predict greater risk for failure of native tissue repair. These findings could theoretically be used to tailor the choice of surgical approaches for these higher-risk patients.

10 DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Nurse research coordinators from each clinical site (UTSW, UAB, and WIH) will collect patient data captured by clinic providers and surgeons all blinded to study intervention using case report forms. Specimens (serum, whole blood, cytology specimens for vaginal maturation index, and standardized vaginal wall biopsies in women undergoing vaginal hysterectomy) will be processed by the research coordinators for secure shipment to UTSW, which will serve as the biorepository and site of analysis. Other data will be entered at each site using REDcap®, a Web-based, HIPAA-compliant tool used by many major medical institutions.

A set of electronic case report forms will be developed in REDCap® following the order on existing paper forms. Dropdowns or radio buttons based on allowable responses will be defined where possible. Ranges will be set for numeric values for notification of outliers. Each paper form will provide space for the following identifiers: Site ID, Participant ID (anonymous patient study number), Study Visit Identifier, and Visit Date. The combination of Site / Participant ID will become the unique identifier for each subject in IMPROVE. When a visit packet is complete, the research coordinator will check each paper form for accuracy and completeness. A designated person will enter the data into REDCap®. If any data are missing or questionable, the data entry operator will set the form status to "incomplete." Once entry is complete, the entry operator will set the form status to "unverified." A second person will compare the entered data against the paper form. When all are correct, the reviewer will set the form status to "complete."

Refer to Manual of Procedures (MOP) for a complete inventory of Case Report Forms.

10.2 Data Management

REDCap® has a data quality module, which will automatically report missing values, validation errors, and outliers. Additional project specific cross-checks defined by the PI will be designed and added to the REDCap® data quality checks. Each site will be able to execute the rules and receive a list of discrepancies. Each error in the list has a direct link to the item in question, where it can be corrected or set to "exclude" from future reports.

REDCap® facilitates electronic form building and data collection. It has an automatic audit trail, report builder capability and data quality module. REDCap® can export data to SAS, SPSS, R, STATA or Microsoft Excel. The IMPROVE REDCap® database will reside on

a secure UTSW server with continuous backup. Only authorized personnel with designated rights can access this study, and that at various levels. For example, a coordinator will be able to enter and check data, but cannot design screens. When used for a multisite project as with IMPROVE, an institution can be restricted to entering and viewing only its own data. REDCap® provides a protocol event schedule where specific forms are linked to events (visits) in the protocol. Authentication used in the REDCap® application is compliant with the UT Southwestern Medical Center's Health System password and security standards.

Data Export: In the Data Export Tool menu, REDCap® allows for selection of forms and/or specific data items to be exported for analysis.

10.3 Quality Assurance

10.3.1 Training

Participating study surgeons must have performed ≥20 of each vault suspension (uterosacral ligament suspension and sacrospinous ligament fixation) procedure with ≥5 in the 12 months before subject enrollment.

All research personnel from the 3 clinical sites have completed the Collaborative IRB Training Initiative (CITI) Basic Course in Human Research Protections (or its equivalent), teaching in human subjects protection, and education in Good Clinical Practices (GCP) with maintenance certification of this training approximately every 3 years.

10.3.2 Quality Control Committee

A *quality control committee* (as a function of the Steering Committee) will consist of the 3 clinical site PI's and DCC biostatistician, which will convene at least twice annually to address any issues of study or data quality that emerge.

10.3.3 Metrics

Data Auditing: After data are audited (10% of the data packets selected randomly, more if the error rate is greater than 0%) by the study coordinator or designated individual, the status of the form will be set to "locked," preventing further edits. REDCap® maintains an audit trail of all changes to the database.

Tracking Reports: A set of reports will be implemented in REDCap® to track accrual, withdrawals, adverse events, and overdue visits.

10.3.4 Protocol Deviations

Protocol deviations, i.e. unplanned instances of protocol noncompliance, will be captured using a report form (**Form 21**), which could be linked—as needed or appropriate—to any scheduled study visit. Potential deviations will be listed for the research coordinator to select (e.g., error in consenting, eligibility, randomization, study treatment administration, participant missing a scheduled visit, incomplete visit

assessment, or *other*). Deviations will be reported to the site's IRB. The circumstances of the deviation will also be captured on Form 21.

Protocol deviations will be formally reviewed annually at each site's IRB continuing review and also reviewed by the Quality Control Committee (10.3.2).

10.3.5 Monitoring

Local Institutional Review Board: Each site's local IRB is charged in an annual continuing review process to assess for updates on the following:

- reportable local and non-local AE's and whether these should prompt updates to the informed consent document
- participant tolerance of study procedures
- participant accrual vs. withdrawal rates
- errors in obtaining informed consent
- rate and nature of protocol deviations and violations
- participant complaints (if any)
- changes in or new financial interests (and other potential conflicts of interest) of study personnel
- DSMB reports
- new or significant preliminary observations or descriptions of reports from outside trials that could be relevant to continuation of the study

Further, each IRB may then request a plan to address these items including plans to increase enrollment, if necessary, in order to meet study objectives.

Data Quality Monitoring: *see also 10.2* and *10.3.3*. REDCap® has a data quality module, which will automatically report missing values, validation errors, and outliers. Additional project specific cross-checks defined by the PI will be designed and added to the REDCap® data quality checks. Each site will be able to execute the rules and receive a list of discrepancies. Each error in the list has a direct link to the item in question, where it can be corrected or set to "exclude" from future reports.

Other Quality Monitoring:

- The DSMB will meet semi-annually (*see 7.6*) largely to evaluate the safety of trial participants but also with these broader charges: ensure confidentiality of data and the results of monitoring, report to the UTSW PI and biostatistician on the safety of participants, and make recommendations on continuation, termination, or modifications to the trial.

- A Quality Control Committee (*see 10.3.2*) of the 3 clinical site PI's will convene at least twice yearly to address issues of protocol noncompliance and data quality at the clinical sites.

11 PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study.

11.2 Informed Consent Forms

The informed consent and the history and exam findings as detailed above will be collected in a private room. The informed consent will be written and provided in English or Spanish. A common template for the research informed consent form will be used by all of the clinical sites, modifying the content or format as necessary to meet the requirements of their respective institutional human subjects committees. This protocol must be approved by the IRBs at the clinical sites and DCC before implementation.

Translator services are available at all study sites. Either the research coordinator or another member of the research team (PI or co-investigator) will be obtaining the consent, and the patient will sign and date/time her name to indicate understanding, which will be observed and countersigned by a witness.

The consent form describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

Included in the informed consent process is the addition of participant education as to procedures for maintaining confidentiality according to the Health Insurance Portability and Accountability Act (HIPAA).

Any data, specimens, forms, reports, and other records that leave a clinical site will be identified only by a subject identification number (Subject ID) to maintain confidentiality. Only the research coordinators and the site PI will have access to the list linking medical record number to Subject ID. The investigational pharmacy staff at each clinical site will have knowledge of whether a patient received CE (Premarin®) or placebo cream. The research coordinator and site PI will have the responsibility for securing original source documents containing any identifiable patient data. Each patient's study documents will be kept in an individual binder, and these will be stored in a locked cabinet and office. Electronically, once these data are entered into REDCap® (see above), only authorized personnel with designated rights can access this study. The IMPROVE REDCap® database will reside on a secure UTSW server with continuous backup. Access to specimens collected locally or that arrives from consortium sites and stored at UTSW will be monitored and granted only by Drs. Rahn and Word.

Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NIA, and the OHRP.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NIA, the OHRP, the FDA, or

other government agencies as part of their duties to ensure that research participants are protected.

12 ETHICAL CONSIDERATIONS

This study will be conducted in accord with the U.S. Department of Health and Human Services' (HHS) Office for Human Research Protections (OHRP) and the Human Subject Research (HRP) Code of Federal Regulations Title 45, Part 46 (i.e., 45 CFR 46). The current U.S. system for protection of human research subjects is heavily influenced by the Belmont Report of 1979, which outlines the basic ethical principles in research involving human subjects: (a) *respect for persons*, (b) *beneficence*, and (c) *justice*.

Respect for persons respects an individual patient's autonomy and clarifies that those with diminished autonomy require special protections. The IMPROVE study will require consent of subjects to volunteer for participation after adequate discussion of risks and benefits. If the autonomy of a participant comes into question (e.g., a previously autonomous participant develops diminished autonomy in the setting of delirium or dementia in an elderly patient), the participant may either be withdrawn from the study or continue only with the assent of the patient's legal medical decision-maker (e.g., person with power of attorney).

Beneficence describes an obligation to (i) do no harm, and (ii) maximize possible benefits/ minimize possible harms. The potential harms in IMPROVE are modest/ minimal as detailed in Section 7, but there will be vigilant surveillance for these potential adverse events, and treatments will be provided (or the study cream discontinued) when events occur.

Justice addresses who ought to receive the benefits of research and bear its burdens. Importantly, as the IMPROVE study has the potential for *benefit* to participants, no restrictions are being made based on race, ethnicity, or language/ literacy. Further, in the selection of clinical sites to participate in IMPROVE, efforts were made to have a wide geographical, socioeconomic, and racial/ ethnic distribution of participants.

13 COMMITTEES

Steering Committee: The IMPROVE Steering Committee has the primary responsibility to guide further revisions to the IMPROVE protocol and, ultimately, to insure that the protocol and study are conducted satisfactorily. This committee also oversees preparation of publications and will establish guidelines for authorship. Its membership is limited to the Principal Investigators from each clinical site, the primary site (DCC) biostatistician, and, at the discretion of the NIA, a representative program scientist. The Steering Committee will convene in person twice per year with additional teleconferences throughout the year.

14 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies and procedures developed by the Steering Committee. Any presentation, abstract, or manuscript will be made available for review by the sponsor and the NIA prior to submission.

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