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Original Research

Vestibular tissue changes following administration of intravaginal prasterone: a vulvoscopic open-label pilot study in menopausal women with dyspareunia

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Abstract

Background: Prasterone, an intravaginal dyspareunia treatment in menopausal women, improves vaginal health through intracellular conversion of dehydroepiandrosterone into androgens and estrogens. Phase 3 trials for prasterone showed significant improvement in vaginal tissue health and reduction of pain.
Aim: To assess vestibular changes with daily use of intravaginal prasterone in menopausal women with moderate to severe dyspareunia.
Methods: This open-label prospective pilot study was conducted over 20 weeks. It included 11 menopausal women (median age, 56 years) who were treated daily with intravaginal inserts of 6.5-mg prasterone and assessed monthly. During vulvoscopy, vestibular pain was assessed by cotton-tipped swab testing, and vestibular and vaginal health was independently assessed with the Visual Scale (VS). In addition, vulvoscopic photographs were obtained and assessed via the Vulvoscopic Genital Tissue Appearance (VGTA) scale to evaluate overall genital tissue health. Mean changes from baseline for genital tissue health and pain assessments were analyzed by repeated measures 1-way analysis of variance, followed by a Dunnett post hoc test. Sexual event diaries were completed and adverse events recorded.
Outcomes: Outcomes included indices of genital tissue health: pain assessment by cotton-tipped swab testing, VS of the vestibule and vagina, VGTA, and sexual event diary.
Results: Aggregate scores from the cotton-tipped swab test progressively improved, reaching statistical significance at week 16, which was maintained through week 20 (−7.27, $P = .019$). VS scores significantly improved from baseline by week 4 and were maintained through week 20 for the vestibule (−3.00, $P = .004$) and vagina (−4.00, $P = .002$). An overall 1607 vulvoscopic photographs were examined; all showed reduction in vestibular erythema and pallor at the end of the study. The mean change from baseline at week 20 for the VGTA score was −7.9 ($P = .0016$). Intercourse associated with pain was reduced from 81.3% of initiated events during the first month of the study to 8.3% during the last month. Sexual activities that were discontinued due to discomfort were reduced from 45.8% to 6.3%. No prasterone-related serious adverse events were reported.
Clinical Implications: Prasterone, a safe and effective intravaginal hormone treatment, significantly improves vestibular health parameters.
Strengths and Limitations: Strengths are the prospective study design and the use of multiple outcome measures to assess vestibular tissue health and pain associated with sexual activity. Limitations are the small study cohort and use of nonvalidated outcome measures.
Conclusion: Our findings suggest that intravaginal prasterone exerts biologic activity on the androgenic endodermal vestibule, as the medication passes from vagina to vestibule, resulting in amelioration of pain associated with sexual activity.

Keywords: prasterone; vestibule; genitourinary syndrome of menopause; vulvoscopy; clinical trial; intravaginal.

Introduction

Signs and symptoms of genitourinary syndrome of menopause (GSM) are associated with decreased hormone levels in menopause that can adversely affect genitourinary tissues, including the labia majora, labia minora, vestibule, clitoris, vagina, urethra, urethral meatus, and periurethral anterior vaginal wall prostate, as well as bladder and pelvic floor muscles.[1] It is estimated that from 1 to 6 years after menopause, 65% to 84% of women experience vulvovaginal atrophy (VVA), one of the signs of GSM.[2,3] With menopause occurring on average at age 51 years and a life expectancy of approximately 80 years in the United States, women can expect to spend approximately one-third of their lives in menopause.[4] A common bothersome symptom of women with GSM is painful intercourse, or dyspareunia, which is chronic, progressive, and unlikely to resolve spontaneously.[1,2,5-7] Adverse changes to the vagina in GSM that play a role in dyspareunia include shortening and narrowing of the vaginal lumen, loss of rugae in the vaginal epithelium, pallor and dryness of the vaginal mucosa, decrease in vaginal epithelial superficial cells, increase in vaginal epithelial parabasal cells, and increase in vaginal pH.[1,2]

Intravaginal prasterone (Intrarosa; Millicent Pharma) was approved by the US Food and Drug Administration (FDA) in 2016 for the safe and effective treatment of moderate to severe dyspareunia, a symptom of VVA, due to menopause.[8] There are multiple government regulatory agency–approved treatments for VVA, changing the quality of tissue in the vagina, with clinical trial outcomes consisting of change in

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vaginal pH, superficial and parabasal cells, and dyspareunia score.[9-13]

These treatments include intravaginal estradiol rings,[9] estradiol tablets,[9,10] and estradiol and conjugated equine estrogen creams.[11-13] In contrast, prasterone is dehydroepiandrosterone (DHEA), administered as a 6.5-mg vaginal insert with a concentration of 0.50%, and used once daily at bedtime.[8] Multiple randomized controlled trials have demonstrated that treatment with prasterone in women with GSM significantly improved the signs and symptoms of VVA.[14,15] In 2 pivotal phase 3 randomized double-blind trials of women (n = 253, n = 554) with complaints of moderate to severe dyspareunia, prasterone significantly decreased the percentage of vaginal epithelial parabasal cells, increased the percentage of vaginal epithelial superficial cells, decreased vaginal pH, and improved the dyspareunia score by 0.40 and 0.36 severity score units, respectively, as compared with placebo after 12 weeks of daily treatment.[14-16]

There are nonvaginal genitourinary tissue adverse changes associated with GSM: erythema of minor vestibular glands, fissures at the posterior fourchette, tenderness and prolapse of the urethral meatus, and atrophy of the clitoris.[1,17] These genital tissues have not yet been prospectively examined after daily administration of intravaginal 6.5-mg prasterone. Although prasterone is a vaginal insert, the significant reduction of pain in the phase 3 trials suggests improvement of vestibular endodermal genital tissue health, which is associated with dyspareunia in androgen- and estrogen-deficient states such as menopause.[1,18,19] To the best of our knowledge, there are no peer-reviewed prospective publications documenting visible changes to the vestibule with daily administration of 6.5-mg prasterone in menopausal women with VVA and moderate to severe dyspareunia. It is the aim of this study to investigate whether an intravaginal therapy, prasterone, could affect the vestibular tissue, by assessing for changes in visual appearance, pain, and responses on sexual event diaries over the 20-week administration of intravaginal 6.5-mg prasterone.

Methods

This open-label prospective vulvoscopic pilot study was conducted at a single research center under approval of an independent review board following the principles of the Helsinki Declaration. Study participants were recruited for this study through online listing, our database, and advertisement. They were women in the community with complaints of moderate to severe dyspareunia, who had not been previously or successfully treated for GSM. This 7-visit study focused, for the first time, on the vestibular health of women with moderate to severe dyspareunia who were treated daily with intravaginal inserts of 6.5-mg prasterone for 20 weeks. The following endpoints involving changes from baseline to end of study were used: pain scores on cotton-tipped swab testing, Visual Scale (VS) of the vestibule, VS of the vagina, changes in overall genital tissue health with the Vulvoscopic Genital Tissue Appearance (VGTA) scale via assessment of vulvoscopic photographs, and sexual event diary responses that recorded several parameters (eg, dryness and pain during penetrative sexual events).

Inclusion and exclusion criteria

Participants were required to be female, aged 21 to 80 years, have a body mass index <37 kg/m^2, be menopausal, have VVA with moderate to severe dyspareunia, and have at least 1 score ≥2 (moderate pain) in 1 location on cotton-tipped swab testing of the vestibule during vulvoscopy at the screening visit. Moderate to severe dyspareunia was assessed by patient self-report at baseline as having pain with sexual activity, recorded as *none*, *mild*, *moderate*, or *severe*. This methodology has been standard in clinical trials for dyspareunia and is in compliance with FDA guidelines.[16,20,21] Participants were excluded for any of the following criteria: hypersensitivity to DHEA, use of prasterone within the last 6 months, documented or suspected breast cancer, undiagnosed genital bleeding, clinically significant findings on physical examination, uncontrolled hypertension, a medical condition or psychological disorder that made the individual ineligible for the study, use of local or systemic estrogen or androgen therapy, use of a selective estrogen receptor modulator, use of products with estrogenic or antiestrogenic effects, history of substance abuse or excessive alcohol use, or receipt of an investigational drug within 30 days prior to signing consent. After signing consent, participants who met the inclusion and exclusion criteria were enrolled.

Study assessments

During the screening visit (baseline), medical history, vital signs, and height and weight were obtained from each participant before undergoing a physical examination. In addition, participants had blood drawn for estradiol, testosterone, sex hormone–binding globulin, DHEA, DHEA sulfate, luteinizing hormone, and follicle-stimulating hormone to confirm that the levels were consistent with menopause.

The vulvoscopic examination was performed in a standardized manner, from lateral to medial and from external to internal visualization at every study visit, including cotton-tipped swabbed testing of the vestibule. Detailed examination of the labia majora, labia minora, vestibule, clitoris, urethral meatus, vagina, and periurethral anterior wall prostate[22,23] was performed at 4× magnification with a ZoomScope vulvoscope (Wallach Surgical Devices) with an attached foot pedal–controlled EOS XSi Digital SLR camera (Cannon) that was linked to an LED monitor, allowing the participant and the health care provider to observe vulvoscopic findings concomitantly in real time.

Cotton-tipped swab testing was performed at 1:00, 3:00, 5:00, 6:00, 7:00, 9:00, and 11:00 at each study visit to assess level of provoked pain[24] (Figure 1). Each participant was asked to rate her pain on a scale from 0 to 3 at each location, with 0 being no pain and 3 being severe pain; therefore, the range for the aggregate score for each participant was 0 to 21, with a lower score corresponding to less pain.

At each study visit, live assessments for genital tissue appearance were performed by a provider (nurse practitioner) blinded to the participant visit. The principal investigator did not participate in this assessment. The 5-item VS for the vestibule and the vagina was completed as each participant was examined. Each item of the VS (petechiae, pallor, friability, dryness, and redness) was rated as none to severe on a scale of 0 to 3 with a possible total score of 15.[21,25-27] A lower VS score corresponded to a better vulvoscopic vestibular or vaginal tissue appearance.

In addition, vulvoscopic photographs were taken. These were labeled by date and participant number only, transferred to a dedicated file on an encrypted computer, and assessed after the completion of the trial by a sexual medicine physician (IG) who had expertise in this instrument and had not

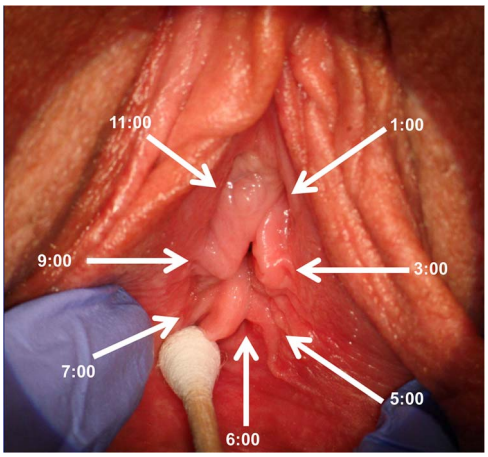


Figure 1. Vestibular locations for cotton-tipped swab testing, with swab at 7:00 shown here. The vestibule surrounds the vagina and lies between the vulva and the vagina.

examined any of the participants in person. All deidentified photographs were assessed at the end of the study, with the examiner blinded to participant and study visit, and rated as *normal* to *severe* (0-3, respectively) for each parameter via the VGTA scale. The VGTA is composed of 10 parameters: loss of labia majora, loss of labia minora, decreased glans clitoris, prominence of the urethral meatus, stenosis of the introitus, vestibular pallor, vestibular erythema, loss of vestibular moisture, loss of vaginal rugae, and loss of prominence of the anterior vaginal wall. The range for each participant was therefore 0 to 30 points for each visit. A lower VGTA score corresponded to a better vulvoscopic genital tissue appearance. The VGTA has been used multiple times in other studies to assess overall treatment-related changes in the health of genital tissue.[25-28]

At day 0, each participant was dispensed sufficient vaginal inserts and applicators for daily use until the next study visit and instructed to complete the sexual event diary after each attempt at sexual activity. The sexual event diary consisted of 7 questions about dryness and pain, to be answered *yes* or *no* after each attempt at sexual activity, whether partnered or alone. The questions of the sexual event diary were as follows: If you experienced dryness before, were you less dry? Did you use lubricant during this activity? Did you experience pain/discomfort during foreplay? Did you experience pain during masturbation? Did you experience pain during oral sex? Did you experience pain during intercourse? Did you stop early because of discomfort? The sexual event diary has not been validated but has been previously reported.[26,28] Adverse events were collected at every visit.

Data analysis

Missing data were handled by using the method of last observation carried forward. For cotton-tipped swab assessments, VS scores for the vestibule and vagina, and VGTA, the mean changes from baseline were analyzed by repeated measures 1-way analysis of variance with the Geisser-Greenhouse correction, followed by Dunnett multiple-comparison post hoc tests. Statistical analyses were performed with Prism version 9.5.1 for Windows (GraphPad Software).

Table 1. Demographics and relevant medical information.

	Median	IQR	Range
Age, y	56	52-60	44-69
Time since last menstrual period, y	10.5	6-13	1-31
Body mass index	30	24.5-35.0	18.2-36.8
	Mean	**SD**	**Reference range**
Hormone blood level			
Testosterone, ng/dL	21.4	18.3	20-75
SHBG, nmol/L	60.0	30.9	16.8-125.2
DHEA, ng/mL	3.1	2.9	1.3-9.8
DHEA-S, μg/dL	70.9	69.0	8-188
Estradiol, pg/mL	14.8	8.9	20-400
LH, mIU/mL	30.6	11.4	10.9-58.6
FSH, mIU/mL[a]	77.7	25.2	16.7-113.6
	No.	**%**	
Total patients	11	100	
Relevant medical history[b]			
Disc disease	5	45.5	
HSDD	4	36.4	
Urinary incontinence	4	36.4	
Hypothyroidism	3	27.3	
Depression	3	27.3	
Relevant surgical history[b]			
Hysterectomy	6	54.5	
Spine surgery	4	36.4	
Concomitant medications[b]			
Antidepressant	5	45.5	
Asthma medication	3	27.3	
Steroid	3	27.3	

Abbreviations: DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; HSDD, hypoactive sexual desire disorder; LH, luteinizing hormone; SHBG, sex hormone–binding globulin. [a]Only determined in women who had hysterectomy. [b]Conditions, procedures, or medications for ≥3 patients; those applying to ≤2 patients are not listed.

Results

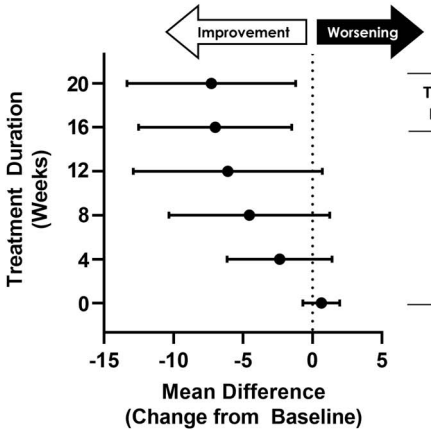
Demographics

Forty women were screened to reach the enrollment target of 15 participants meeting inclusion and exclusion criteria. Eleven participants completed the study, with 1 withdrawing consent at visit 4 and 3 lost to follow-up at visits 4, 5, and 6. Five were White, 3 were White/Hispanic/Latinx, 2 were Black, and 1 was Asian. Baseline characteristics of the study population are presented in Table 1. Baseline hormone levels were all consistent with menopausal blood test values. Medical history most commonly included disc disease, hypoactive sexual desire disorder, urinary incontinence, hypothyroidism, and depression. Antidepressants were the most common concomitant class of medication at baseline.

Assessments for vestibular pain and genital tissue health

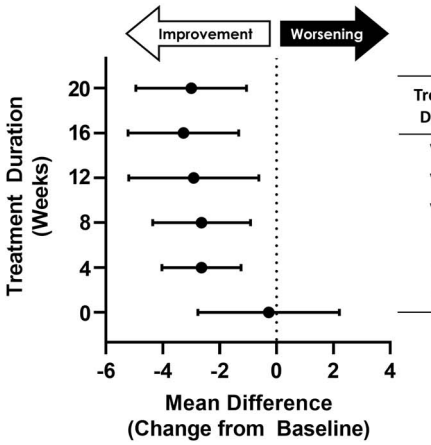
Cotton-tipped swab test scores on the vestibule were combined (Figure 1). As shown in Figure 2, these aggregate scores were progressively reduced from baseline through week 20. Changes reached statistical significance at week 16 (−7.00; 95% CI, −12.51 to −1.49), and these were maintained through week 20 (−7.27; 95% CI, −13.34 to −1.20).

Changes on the VS of the vestibule (Figure 3) and the vagina (Figure 4) indicated that by week 4 of active treatment, both



Treatment Duration	Mean Difference	95% CI	p value
Wk 20	-7.27	-13.34, -1.20	0.019
Wk 16	-7.00	-12.51, -1.49	0.013
Wk 12	-6.09	-12.89, 0.71	0.083
Wk 8	-4.55	-10.33, 1.24	0.142
Wk 4	-2.36	-6.14, 1.42	0.294
Wk 0	0.64	-0.69, 1.96	0.523

Figure 2. Effect of intravaginal prasterone therapy on vestibular pain assessment. Cotton-tipped swab testing was performed on various regions of the vestibule (1:00, 3:00, 5:00, 6:00, 7:00, 9:00, 11:00) to determine levels of pain. Pain scores for all tested regions were combined into an aggregate score. Data are presented as mean (95% CI) change in aggregate score from baseline for the vestibule at each clinic visit after study entry.



Treatment Duration	Mean Difference	95% CI	p value
Wk 20	-3.00	-4.94, -1.06	0.004
Wk 16	-3.27	-5.22, -1.33	0.002
Wk 12	-2.91	-5.19, -0.62	0.013
Wk 8	-2.64	-4.36, -0.92	0.004
Wk 4	-2.64	-4.03, -1.25	0.001
Wk 0	-0.27	-2.76, 2.22	0.998

Figure 3. Effect of intravaginal prasterone therapy on the Visual Scale (VS) scores for the vestibule. Scores for the 5-item VS were determined at each clinic visit after study entry. Data are presented as mean (95% CI) change in total vestibular VS score from baseline.

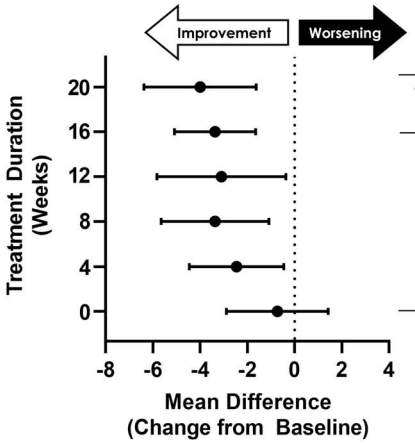
were significantly reduced from baseline. The mean change on the VS from baseline to week 4 was −2.64 (95% CI, −4.03 to −1.25) for the vestibule and −2.46 (95% CI, −4.46 to −0.45) for the vagina. These statistically significant improvements were maintained through week 20 in the vestibule (−3.00; 95% CI, −4.94 to −1.06) and vagina (−4.00; 95% CI, −6.38 to −1.62).

An overall 1607 vulvoscopic photographs were graded per the VGTA. From baseline to end of study, on average, each participant had 146 vulvoscopic photographs (mean, 21 per study visit per participant). Statistically significant changes were noted at weeks 4, 8, and 20 (Figure 5). Specifically, after prasterone treatment for 20 weeks, the mean change in VGTA score was −7.9 (95% CI, −12.4 to −3.5; $P = .0016$).

Photographs of the vestibule for all 11 participants at baseline and after 20 weeks of intravaginal prasterone treatment are shown in Figure 6. As can be seen on the vulvoscopic photographs, there was improvement of vestibular erythema and pallor in all cases.

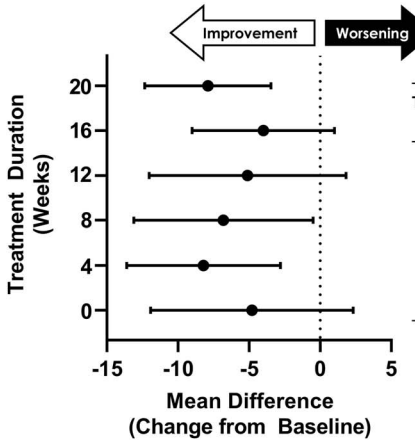
Diary data

After 4 weeks of prasterone treatment, 62.5% of the sexual activity events were reported on the sexual event diary as being "less dry" than before the start of prasterone (Figure 7). Between 12 and 20 weeks of treatment, >90% of sexual activity events were less dry than before. The number of sexual activity events that were initiated increased from 24 during the first 4 weeks of prasterone treatment to 32 during the final 4 weeks of the study (week 20; Figure 8). Use of lubrication was similar throughout each 4-week period during the 20-week study, ranging from 30.6% to 38.2% of sexual events initiated. Over the 5-month study period, 164 sexual events were initiated, averaging 15 events per participant (3 events per month per participant), including 110 intercourse events. During the first 4 weeks of prasterone treatment, 81.3% of intercourse events were associated with pain, whereas 8.3% were associated with pain during the last 4 weeks. Regarding penetrative self-pleasure events, 100% were associated with pain during the first 4 weeks of prasterone treatment, in contrast to 0% during the last 4 weeks. During the first 4 weeks of prasterone treatment, 45.8% of sexual activity events were discontinued due to discomfort, but only 6.3% of sexual activity events were discontinued due to discomfort in the last 4 weeks. Figure 8 does not include pain associated with masturbation or oral sex, since pain was seldom reported for these activities: 8.3% of masturbation events were associated with pain during the first week of prasterone treatment, while 2.8% of oral sexual events were associated with pain during weeks 8 to 12. All other diary data related to masturbation or oral sex indicated no pain.



Treatment Duration	Mean Difference	95% CI	p value
Wk 20	-4.00	-6.38, -1.62	0.002
Wk 16	-3.36	-5.08, -1.64	0.001
Wk 12	-3.09	-5.82, -0.36	0.026
Wk 8	-3.36	-5.64, -1.09	0.005
Wk 4	-2.46	-4.46, -0.45	0.017
Wk 0	-0.73	-2.88, 1.43	0.796

Figure 4. Effect of intravaginal prasterone therapy on Visual Scale (VS) scores for the vagina. Scores for the 5-item VS were determined at each clinic visit after study entry. Data are presented as mean (95% CI) change in total vaginal VS score from baseline.



Treatment Duration	Mean Difference	95% CI	p value
Wk 20	-7.90	-12.35, -3.45	0.001
Wk 16	-4.00	-9.01, 1.01	0.130
Wk 12	-5.10	-12.02, 1.82	0.173
Wk 8	-6.80	-13.10, -0.49	0.034
Wk 4	-8.20	-13.61, -2.79	0.005
Wk 0	-4.80	-11.93, 2.33	0.231

Figure 5. Effect of intravaginal prasterone therapy on Vulvoscopic Genital Tissue Appearance (VGTA) scores. Scores for the 10-item VGTA were graded at the end of the study by a reviewer blinded to participant and clinic visit. Data are presented as mean (95% CI) change in total VGTA score from baseline.

Adverse events

No prasterone-related serious adverse events were reported. Adverse events probably related to prasterone use included brief episodes (several seconds) of intermittent vaginal pain (n = 1) and urinary tract infection (n = 1). Adverse events not related to prasterone use included cold/flu symptoms (n = 3), bronchitis/bronchial asthma (n = 2), breast tenderness and fullness (n = 1), abnormal urinalysis result (n = 1), keratoconjunctivitis (n = 1), ear infection (n = 1), temporomandibular joint disorder (n = 1), rheumatoid arthritis (n = 1), lupus (n = 1), ankylosing spondylitis (n = 1), psoriasis flare (n = 1), cataract removal (n = 1), and shoulder spur/rotator cuff tear (n = 1).

Discussion

This prospective pilot study showed, for the first time, that intravaginal prasterone resulted in statistically significant improvement of vestibular tissue health on multiple outcome measures. Vestibular cotton-tipped swab testing, which assessed pain exclusively in the vestibule, showed a progressive decrease in pain throughout the study and reached statistical significance at 16 weeks. In addition, the improvement in VS scores for the vestibule and vagina was statistically significant, as observed at 4 weeks and maintained throughout the study. While it is expected that VS scores for the vagina would improve, as prasterone is approved by multiple government regulatory agencies for treatment of VVA, the finding of positive VS scores of the vestibule is new for this intravaginal medication. The VGTA scores showed statistically significant improvement from baseline at weeks 4 and 8 and at end of study. The photographs of all 11 participants showed decreased vestibular pallor and vestibular erythema from baseline to end of study.

We believe that the robust data of the diary supported these positive changes in vestibular health, as pain symptoms resolved, frequency of intercourse increased, and sexual activity was stopped less often. Thus, while the reduced pain could be attributed to prasterone treating the vaginal atrophy, our data suggest that the pain reduction is in part reflective of improvement of vestibular tissue health. To the best of our knowledge, prior to this prospective study, there has not been a published report of an intravaginal treatment for vaginal atrophy showing benefit to the adjacent vestibular tissue based on objective measures.

Clinical trial data show that there is no systemic change in sex steroid hormone blood levels with intravaginal prasterone use[29]; therefore, we presume the improvement in vestibular pain to be due to the medication passing over the nonkeratinized stratified squamous epithelium of the vestibule as

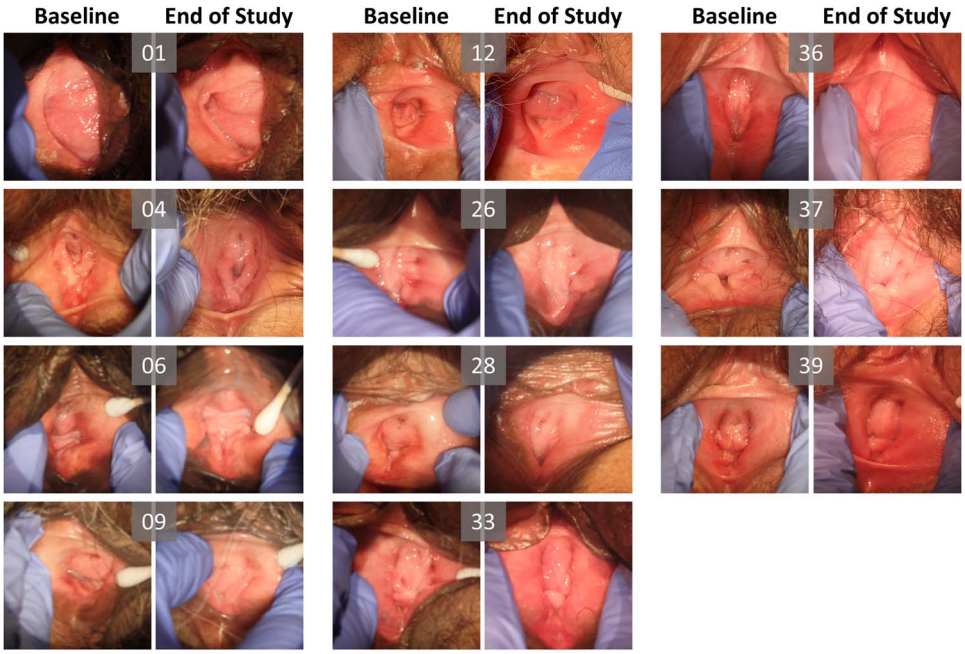


Figure 6. Representative vulvoscopic photographs from each participant (N = 11) taken at baseline and after 20 weeks of daily 6.5-mg prasterone intravaginal therapy (end of study). Photographs were graded by the Vulvoscopic Genital Tissue Appearance scale, demonstrating reduction in vestibular erythema and pallor.

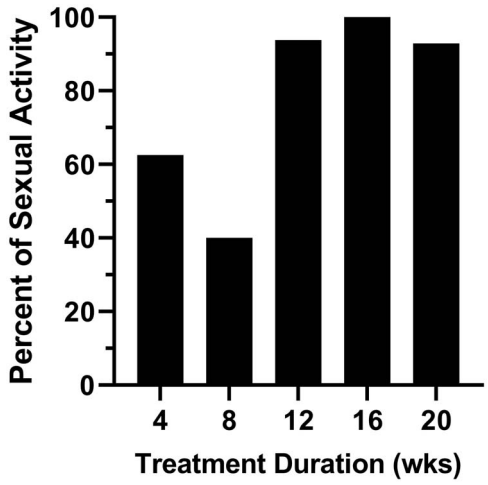


Figure 7. Effect of intravaginal prasterone therapy on patients' perception of "dryness." After each attempted sexual activity event, patients were instructed to record in a sexual event diary whether they were "less dry" than before the initiation of intravaginal prasterone therapy.

gravity drains the prasterone from the vagina to the vestibule. The vestibule, which surrounds the vagina, is a unique surface of endodermal tissue between the vulva (ectoderm) and the vagina (mesoderm). The lateral border of the vestibule is the Hart line at the inner aspect of the labia minora, while the medial border is the hymenal ring at the distal end of the vagina. The vestibule also extends superiorly from above the urethral meatus to just below the clitoris and right and left frenulum and inferiorly to the posterior fourchette (Figure 9). Anything passing into or out of the vagina must go through the vestibule. Under the nonkeratinized stratified squamous epithelium layer of the vestibule, the lamina propria contains blood vessels, nerves, smooth muscle, collagen, elastin, and lubrication glands, which are hormonally mediated in part by testosterone. Evidence for the role of testosterone in vestibular lubrication is derived from the observation of a high density of androgen receptors in the vestibular epithelium, lamina propria, and vestibular gland epithelium.[17,18] Vestibular pain has been reported to be ameliorated through local treatment with testosterone in women with hormonally mediated vestibulodynia.[30] As previously discussed, prasterone converts intracellularly, in part, to testosterone and dihydrotestosterone.[29]

Intravaginal prasterone use does not result in systemic changes in sex steroid hormone blood levels because prasterone has a unique mechanism of action that involves intracrinologic synthesis of androgens and estrogens within vaginal cells.[31,32] Prasterone enters vaginal cells as a DHEA substrate, where it is metabolized by 17-beta and 3-beta hydroxysteroid dehydrogenase, 5-alpha reductase, and aromatase enzymes intracellularly into testosterone, dihydrotestosterone, estradiol, and estrone.[33,34] These intracellular sex steroid hormones act on intracellular androgen and estrogen receptors to restore vaginal health.[33,34] Subsequently, these 4 intracellular sex steroids are transformed into inactive intracellular metabolites and are then excreted from the vaginal epithelial cells into the systemic circulation.[33,34] Thus, unlike all approved estradiol-based medications for the treatment of menopausal symptoms, the intracrinologic vaginal administration of prasterone does not result in any increase in circulating levels of testosterone, estradiol, and DHEA.[29,35] As a result, intravaginal prasterone inserts are the only FDA-approved treatment for menopausal women that does not carry a box warning for estrogen side effects and has no restrictions on duration of use.[8]

Prior to prasterone, GSM treatments were exclusively estrogen based. Prasterone, which acts intracrinologically, allows for the intracellular synthesis of androgens and estrogens in the vaginal epithelium. We have observed improvement in vestibular health and reduction in vestibular pain from

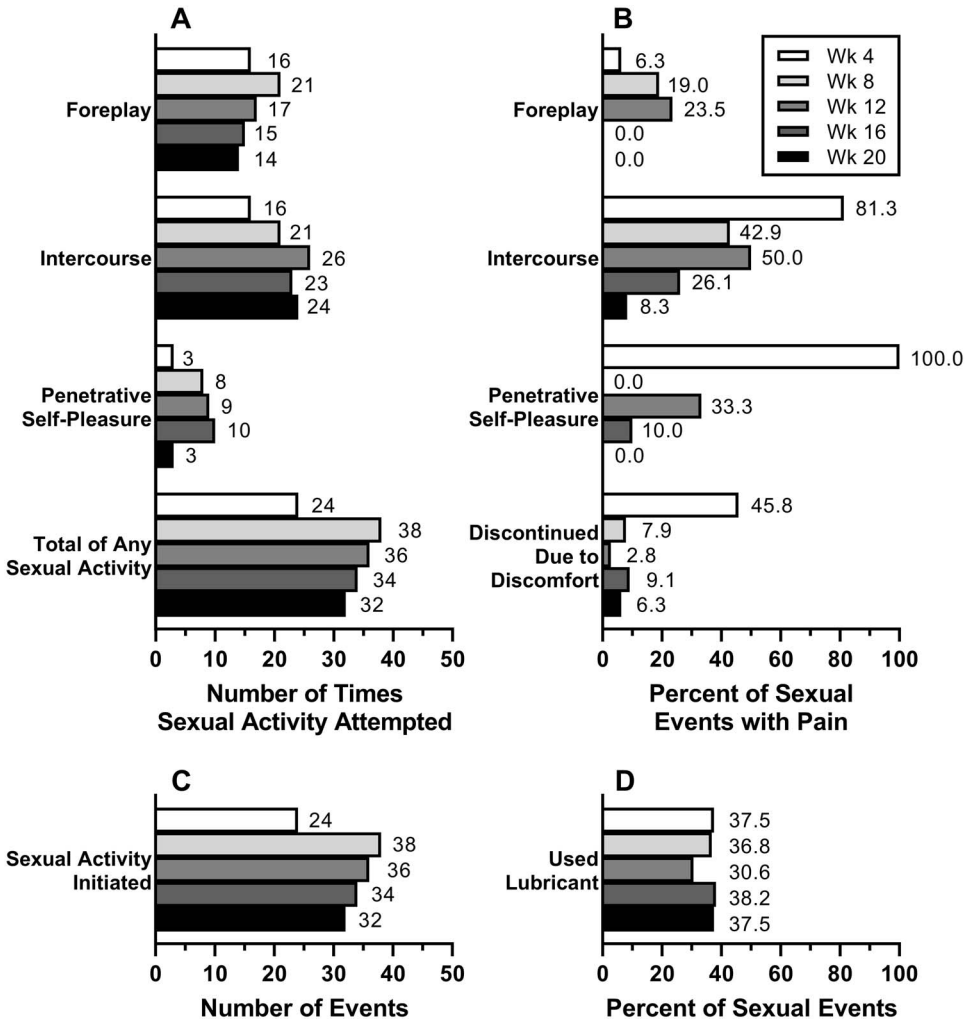


Figure 8. Effect of intravaginal prasterone therapy on pain during sexual activity. (A, C) The total number of times that sexual activity was attempted and the number of attempts at specific types of sexual activity for each month of the treatment period. (B, D) The percentage of sexual events associated with pain and the percentage of sexual events when lubrication was used for each month of the treatment period.

prasterone, a government regulatory agency–approved treatment for VVA. It is likely that because the vestibule is adjacent to the vagina, prasterone also acts intracellularly in vestibular epithelium, which has the same intracellular enzymes.[36] This replicates other reports[37] as well as the experience in our sexual medicine facility among patients who continue to have GSM symptoms despite management with traditional estradiol-only treatment and experience symptom improvement with use of prasterone.

Menopause is classically associated with depression and/or anxiety,[38-42] hypoactive sexual desire disorder,[43,44] cardiovascular risk factors,[45] obesity,[45] hypothyroidism,[46] and urinary incontinence.[47-49] Our study population of menopausal women exhibited classic history, physical, and hormonal findings of menopause.[1] They had poor genital tissue health consistent with pallor; erythema; thin, pale, less hydrated mucosa; shorter, narrowed vaginal canal; absent vaginal rugae; friable mucosa with petechiae; ulcerations and bleeding with minimal trauma; increased vaginal pH; and vaginal discharge with odor.[2] Our study population had hormonal blood levels consistent with menopausal values.

The use of objective and subjective outcome measures to assess overall genital tissue health as well as vestibular tissue health is one of the strengths of the study. Photographs were rated with the VGTA by a reviewer experienced with the scale. Another strength of this study is that the VGTA confirms that nonadjacent tissues (labia minora, labia majora, clitoris) were not affected, as the effect of prasterone on the vestibule is not consistent with a systemic drug effect. The diary gave an insight into the individual sexual experiences of the study cohort. The study participants were volunteers from the local community, representing diverse ethnicities, and were not preselected from our sexual medicine clinic. This is the first time that an androgen/estrogen-responsive organ, the vestibule, was examined after treatment with an intravaginal drug, prasterone, that converts intracellularly to androgens and estrogens. Limitations of this study include the small pilot study size and the fact that the study was open label without placebo. In addition, none of the outcome measures have yet been validated, although they have been used in multiple studies.[21,25,26,28] To the best of our knowledge, there are no established and validated outcome measures to assess vestibular tissue health.

Future research might involve a prospective, randomized, placebo-controlled clinical trial studying the use of intravaginal prasterone to assess the health of the vestibule in

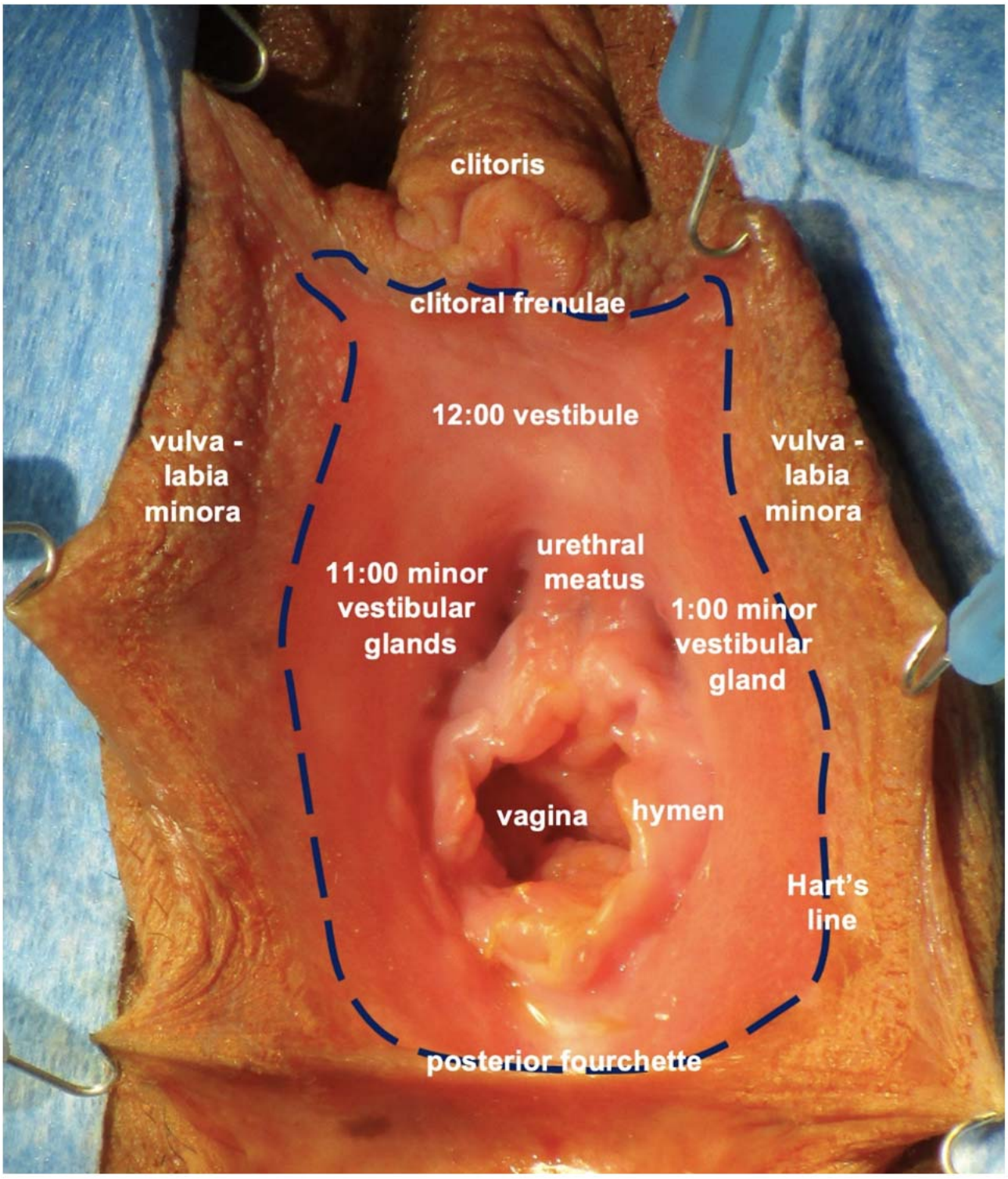


Figure 9. The vestibule in relation to other genital tissues and structures, reprinted with permission from the *Journal of Sexual Medicine*.[33]

a larger cohort. Since the urethral meatus lies within the vestibule (Figure 9), another future parameter to study might be changes in urinary tract infections with the use of intravaginal prasterone.[50]

Conclusion

In this open-label prospective pilot study, we utilized a government regulatory agency–approved intravaginal treatment whose mechanism of action involves synthesis of androgens and estrogens, rather than an estrogen-only treatment. Using objective and subjective outcome measures, we have shown, for the first time, that a safe and effective treatment for the mesodermal vagina can also treat the endodermal vestibule. We hypothesize that due to gravity, the medication enters the nonkeratinized stratified squamous epithelium of the vestibule. These vestibular cells contain a high density of androgen receptors, as well as critical enzymes that

convert the substrate DHEA intracellularly into testosterone, dihydrotestosterone, estradiol, and estrone, resulting in improvement in vestibular tissue health.[17,36] Although more research is needed, we propose that menopausal women with persistent pain, despite local vaginal estradiol-only treatment, should consider the use of prasterone to ameliorate their dyspareunia symptoms.

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References

1. Portman D, Gass M, Kingsberg S, *et al.* Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the International Society for the Study of Women's Sexual Health and the North American Menopause Society. *Menopause*. 2014;**21**(10):1063–1068.
2. Nappi RE, Martini E, Cucinella L, *et al.* Addressing vulvovaginal atrophy (VVA)/genitourinary syndrome of menopause (GSM) for healthy aging in women. *Front Endocrinol (Lausanne)*. 2019;**10**:561. https://doi.org/10.3389/fendo.2019.00561
3. Palma F, Volpe A, Villa P, Cagnacci A, Writing Group of AGATA Study. Vaginal atrophy of women in postmenopause: results from a multicentric observational study—the AGATA study. *Maturitas*. 2016;**83**:40–44. https://doi.org/10.1016/j.maturitas.2015.09.001
4. Dennerstein L, Alexander JL, Kotz K. The menopause and sexual functioning: a review of the population-based studies. *Annu Rev Sex Res*. 2003;**14**:64–82.
5. Simon JA, Davis SR, Althof SE, *et al.* Sexual well-being after menopause: an International Menopause Society white paper. *Climacteric*. 2018;**21**(5):415–427. https://doi.org/10.1080/13697137.2018.1482647
6. Avis N, Colvin A, Karlamangla A, *et al.* Change in sexual functioning over the menopausal transition: results from the study of women's health across the nation. *Menopause*. 2017;**24**(4):379–390. https://doi.org/10.1097/GME.0000000000000770
7. Kingsberg S, Wysocki S, Magnus L, Krychman ML. Vulvar and vaginal atrophy in postmenopausal women: findings from the REVIVE (Real Women's Views of Treatment Options for Menopausal Vaginal Changes) survey. *J Sex Med*. 2013;**10**(7):1790–1799.
8. Intrarosa. Package insert. *Millicent Pharma*. 2016.
9. Speroff L. Efficacy and tolerability of a novel estradiol vaginal ring for relief of menopausal symptoms. *Obstet Gynecol*. 2003;**102**(4):823–834. https://doi.org/10.1016/s0029-7844(03)00764-6
10. Constantine GD, Simon JA, Pickar JH, *et al.* Estradiol vaginal inserts (4 μg and 10 μg) for treating moderate to severe vulvar and vaginal atrophy: a review of phase 3 safety, efficacy and pharmacokinetic data. *Curr Med Res Opin*. 2018;**34**(12):2131–2136. https://doi.org/10.1080/03007995.2018.1527578
11. Crandall C. Vaginal estrogen preparations: a review of safety and efficacy for vaginal atrophy. *J Womens Health (Larchmt)*. 2002;**11**(10):857–877. https://doi.org/10.1089/154099902762203704
12. Biehl C, Plotsker O, Mirkin S. A systematic review of the efficacy and safety of vaginal estrogen products for the treatment of genitourinary syndrome of menopause. *Menopause*. 2019;**26**(4):431–453. https://doi.org/10.1097/gme.0000000000001221
13. Ibe C, Simon JA. Vulvovaginal atrophy: current and future therapies (CME). *J Sex Med*. 2010;**7**(3):1042–1050. https://doi.org/10.1111/j.1743-6109.2009.01692.x
14. Labrie F, Archer DR, Koltun W, *et al.* Efficacy of intravaginal dehydroepiandrosterone (DHEA) on moderate to severe dyspareunia and vaginal dryness, symptoms of vulvovaginal atrophy, and of the genitourinary syndrome of menopause. *Menopause*. 2016;**23**(3):243–256.
15. Labrie F, Archer DF, Bouchard C, *et al.* Prasterone has parallel beneficial effects on the main symptoms of vulvovaginal atrophy: 52-week open-label study. *Maturitas*. 2015;**81**(1):46–56. https://doi.org/10.1016/j.maturitas.2015.02.005
16. Labrie F, Archer DF, Martel C, Vaillancourt M, Montesino M. Combined data of intravaginal prasterone against vulvovaginal atrophy of menopause. *Menopause*. 2017;**24**(11):1246–1256. https://doi.org/10.1097/gme.0000000000000910
17. Traish A, Vignozzi L, Goldstein I, Simon JA, Kim NN. Role of androgens in female genitourinary tract structure and function: implications in the genitourinary syndrome of menopause. *Sex Med Rev*. 2018;**6**(4):558–571. https://doi.org/S2050-0521(18)30046-5
18. Traish AM, Kim N, Min K, Munarriz R, Goldstein I. Role of androgens in female genital sexual arousal: receptor expression, structure, and function. *Fertil Steril*. 2002;**77**(suppl 4):11–18. https://doi.org/10.1016/s0015-0282(02)02978-3
19. Maseroli E, Vignozzi L. Testosterone and vaginal function. *Sex Med Rev*. 2020;**8**(3):379–392. https://doi.org/10.1016/j.sxmr.2020.03.003
20. Labrie F, Archer D, Bouchard C, *et al.* Intravaginal dehydroepiandrosterone (prasterone), a physiological and highly efficient treatment of vaginal atrophy. *Menopause*. 2009;**16**(5):907–922.
21. Simon JA, Archer DF, Kagan R, *et al.* Visual improvements in vaginal mucosa correlate with symptoms of VVA: data from a double-blind, placebo-controlled trial. *Menopause*. 2017;**24**(9):1003–1010. https://doi.org/10.1097/gme.0000000000000880
22. Tomalty D, Giovannetti O, Hannan J, *et al.* Should we call it a prostate? A review of the female periurethral glandular tissue morphology, histochemistry, nomenclature, and role in iatrogenic sexual dysfunction. *Sex Med Rev*. 2022;**10**(2):183–194. https://doi.org/10.1016/j.sxmr.2021.12.002
23. Tomalty D, Giovannetti O, Gaudet D, *et al.* The prostate in women: an updated histological and immunohistochemical profile of the female periurethral glands and their relationship to an implanted midurethral sling. *J Sex Med*. 2023;**20**(5):612–625. https://doi.org/10.1093/jsxmed/qdac046
24. Winter A, Rubin RS. Vulvoscopic evaluation of vulvodynia. In: Goldstein A, Pukall CF, Goldstein I eds. *Female Sexual Pain Disorders*. John Wiley & Sons Ltd; 2021: 125–131.
25. Goldstein I, Simon JA, Kaunitz AM, *et al.* Effects of ospemifene on genitourinary health assessed by prospective vulvar-vestibular photography and vaginal/vulvar health indices. *Menopause*. 2019;**26**(9):994–1001. https://doi.org/10.1097/gme.0000000000001350
26. Goldstein SW, Winter AG, Goldstein I. Improvements to the vulva, vestibule, urethral meatus, and vagina in women treated with ospemifene for moderate to severe dyspareunia: a prospective vulvoscopic pilot study. *Sex Med*. 2018;**6**(2):154–161. https://doi.org/10.1016/j.esxm.2018.03.002
27. Archer DF, Simon JA, Portman DJ, Goldstein SR, Goldstein I. Ospemifene for the treatment of menopausal vaginal dryness, a symptom of the genitourinary syndrome of menopause. *Expert Rev Endocrinol Metab*. 2019;**14**(5):301–314. https://doi.org/10.1080/17446651.2019.1657008
28. Goldstein SW, Goldstein I, Kim NN, Kellogg-Spadt S, Murina F. Safety and efficacy of fractional CO2 laser treatment to the vestibule: a randomized, double-blind, sham-controlled, prospective 3-site clinical study in women with vestibular pain. *J Sex Med*. 2023;**20**(6):800–812. https://doi.org/10.1093/jsxmed/qdac053
29. Labrie F, Martel C, Bérubé R, *et al.* Intravaginal prasterone (DHEA) provides local action without clinically

significant changes in serum concentrations of estrogens or androgens. *J Steroid Biochem Mol Biol*. 2013;**138**:359–367. https://doi.org/10.1016/j.jsbmb.2013.08.002
30. Burrows L, Goldstein AT. The treatment of vestibulodynia with topical estradiol and testosterone. *Sex Med*. 2013;**1**(1):30–33.
31. Labrie F. Extragonadal synthesis of sex steroids: intracrinology. *Ann Endocrinol (Paris)*. 2003;**64**(2):95–107.
32. Labrie F, Martel C, Balser J. Wide distribution of the serum dehydroepiandrosterone and sex steroid levels in postmenopausal women: role of the ovary? *Menopause*. 2011;**18**(1):30–43.
33. Bertin J, Dury AY, Ouellet J, Pelletier G, Labrie F. Localization of the androgen-synthesizing enzymes, androgen receptor, and sex steroids in the vagina: possible implications for the treatment of postmenopausal sexual dysfunction. *J Sex Med*. 2014;**11**(8): 1949–1961. https://doi.org/10.1111/jsm.12589
34. Labrie F. Intracrinology in action: importance of extragonadal sex steroid biosynthesis and inactivation in peripheral tissues in both women and men. *J Steroid Biochem Mol Biol*. 2015;**145**: 131–132. https://doi:10.1016/j.jsbmb.2014.09.012
35. Martel C, Labrie F, Archer DF, *et al*. Serum steroid concentrations remain within normal postmenopausal values in women receiving daily 6.5mg intravaginal prasterone for 12 weeks. *J Steroid Biochem Mol Biol*. 2016;**159**:142–153. https://doi.org/10.1016/j.jsbmb.2016.03.016
36. Labrie F. All sex steroids are made intracellularly in peripheral tissues by the mechanisms of intracrinology after menopause. *J Steroid Biochem Mol Biol*. 2015;**145**:133–138. https://doi.org/10.1016/j.jsbmb.2014.06.001
37. González S, Mainar LB, Campo RZ. Effectiveness, safety and tolerability of intravaginal Prasterone for the treatment of genitourinary syndrome in postmenopausal women in Spain: the Estip-Es study. *Am J Biomed Sci Res*. 2021;**12**(6):600–606.
38. Mulhall S, Andel R, Anstey KJ. Variation in symptoms of depression and anxiety in midlife women by menopausal status. *Maturitas*. 2018;**108**:7–12. https://doi.org/10.1016/j.maturitas.2017.11.005
39. Clayton AH, Ninan PT. Depression or menopause? Presentation and management of major depressive disorder in perimenopausal and postmenopausal women. *Prim Care Companion J Clin Psychiatry*. 2010;**12**(1):PCC.08r00747. https://doi.org/10.4088/PCC.08r00747blu
40. Georgakis MK, Thomopoulos TP, Diamantaras AA, *et al*. Association of age at menopause and duration of reproductive period with depression after menopause: a systematic review and meta-analysis. *JAMA Psychiatry*. 2016;**73**(2):139–149. https://doi.org/10.1001/jamapsychiatry.2015.2653
41. Avis NE, Brambilla D, McKinlay SM, Vass K. A longitudinal analysis of the association between menopause and depression: results from the Massachusetts Women's Health Study. *Ann Epidemiol*. 1994;**4**(3):214–220. https://doi.org/10.1016/1047-2797(94)90099-x
42. Moyneur E, Dea K, Derogatis LR, Vekeman F, Dury AY, Labrie F. Prevalence of depression and anxiety in women newly diagnosed with vulvovaginal atrophy and dyspareunia. *Menopause*. 2020;**27**(2):134–142. https://doi.org/10.1097/gme.0000000000001450
43. Nappi RE, Wawra K, Schmitt S. Hypoactive sexual desire disorder in postmenopausal women. *Gynecol Endocrinol*. 2006;**22**(6): 318–323. https://doi.org/10.1080/09513590600762265
44. Nappi RE, Lachowsky M. Menopause and sexuality: prevalence of symptoms and impact on quality of life. *Maturitas*. 2009;**63**(2): 138–141. https://doi.org/10.1016/j.maturitas.2009.03.021
45. Trémollières FA, Pouilles JM, Cauneille C, Ribot C. Coronary heart disease risk factors and menopause: a study in 1684 French women. *Atherosclerosis*. 1999;**142**(2):415–423. https://doi.org/10.1016/s0021-9150(98)00252-4
46. Bates JN, Kohn TP, Pastuszak AW. Effect of thyroid hormone derangements on sexual function in men and women. *Sex Med Rev*. 2020;**8**(2):217–230. https://doi.org/10.1016/j.sxmr.2018.09.005
47. Balzarro M, Rubilotta E, Mancini V, *et al*. Impact of overactive bladder-wet syndrome on female sexual function: a systematic review and meta-analysis. *Sex Med Rev*. 2019;**7**(4):565–574. https://doi.org/10.1016/j.sxmr.2019.05.002
48. Padoa A, McLean L, Morin M, Vandyken C. The overactive pelvic floor (OPF) and sexual dysfunction part 1: pathophysiology of OPF and its impact on the sexual response. *Sex Med Rev*. 2021;**9**(1):64–75. https://doi.org/10.1016/j.sxmr.2020.02.002
49. Padoa A, McLean L, Morin M, Vandyken C. The overactive pelvic floor (OPF) and sexual dysfunction. Part 2: evaluation and treatment of sexual dysfunction in OPF patients. *Med Rev*. 2021;**9**(1): 76–92. https://doi.org/10.1016/j.sxmr.2020.04.002
50. Rubin R, Moyneur R, Tjoa ML, Sadiq A, Labrie F, Dury A. Prevalence of urinary tract infections in women with genitourinary syndrome of menopause and the impact of vaginal prasterone on urinary tract infections. *Presented at: American Urological Association Annual Meeting*; 2020; session MP30-15.